EXPERIMENTAL MODELS

RD16/0008

INDEX

IOBA-Eye Institute, University of Valladolid, Valladolid (RD16/0008/0001):

1- Neuroretina organotypic culture

2- Double and triple layered co-culture model

3- Proliferative vitreoretinopathy (Intravitreal platelet-rich plasma)

4- Proliferative vitreoretinopathy (Intravitreal platelets)

5- Autologous translocation of retinal pigment epithelium-choroid graft

6- Endophthalmitis (Intravitreal S. aureus)

7- *Fresh retinal pigment epithelial (RPE) cells

8- *Fresh retinal stem cells (RSCs) from ciliary margin

9- *Chronic-Inflammatory Ocular Surface (SV-40) immortalized human corneal epithelial (HCE) cell line

10- Limbal stem cell deficiency (Pig)

11- Partial limbal stem cell deficiency (Rabbit)

12- Total limbal stem cell deficiency (Rabbit)

13- Wound healing in vivo model (Rabbit)

14- *Inflammatory diseases of the ocular surface (3D model of human conjunctiva)

15- *Corneal wound healing under inflammatory conditions

16- *Oxidate stress based cell culture (ARPE19)

Laboratory of Neurophysiology, Faculty of Medicine, University of Barcelona-IDIBAPS, Barcelona (RD16/0008/0014):

17- Dry eye (Removing lacrimal gland)

18- Allergic keratoconjunctivitis

19- *Trabecular meshwork cell lines

20- Corneal pain and ocular sensitivity

21- *Schlemm's canal endothelium cell lines

Experimental Ophthalmology, Hospital Reina Sofía-University of Murcia, Murcia (RD16/0008/0026):

22- Transient retinal ischemia by selective ligature of the ophthalmic vessels

*Cell Lines

23- Transient retinal ischemia by increasing the intraocular pressure

24- Selective motoneuron degeneration by injection of substances in oculomotor muscles

25- Selective axotomy-induced RGC death by microcrush lesion of optic nerve

26- Selective axotomy-induced RGC death by crush lesion of optic nerve

27- Selective axotomy-induced RGC death by intraocular transection of the optic nerve

28- Regenerative responses of injured neurons in the central nervous system through peripheral nerve grafts

29- Degenerative retinal conditions by inherited retinal dystrophy (RCS and P23H-1)

30- Laser-induced ocular hypertension

31- Light-induced retinal degeneration

32- Model of induced-phototoxicity by focal light emitting diode

Ophthalmology Department, Hospital Vall Hebron, Barcelona (RD16/0008/0023):

33- Laser-induced choroidal neovascularization (C57BL)

34- Retinal toxicity after inner limiting membrane (ILM) dyeing

University of Navarra, Pamplona (RD16/0008/0011):

35- Laser-induced choroidal neovascularization

36- Factor H (CFH) and Apolipoprotein E (apoE) deficiency (C57BL6/J)

37- Choroidal neovascularisation

38- Corneal neovascularisation

39- Light induced retinal damage

40- Acute conjunctivitis

IIS Aragón (Aragon Research Institute), LIM (*Laboratorio de Investigación Molecular*), Hospital Miguel Servet, Zaragoza (RD16/0008/0029):

41- Retinitis pigmentosa model (P23H-1)

Hospital General de Guadalajara-University of Alcala de Henares, Guadalajara-Madrid (RD16/0008/0020):

42- Retinal degeneration model (Rd10)

*Cell Lines

43- Achromatopsia model

44- Retinal degeneration by excitatory aminoacids intraocular injection

University of Alicante, Alicante (RD16/0008/0016):

45- Experimental Glaucoma (DBA/2J mouse)

46- Ischemia-reperfusion

47- Retinitis pigmentosa (P23H-3)

48- Retinitis pigmentosa (Rd10)

49- *Retinal cells signalling pathways and efficacy/toxicity of compounds (661W, RGC5, R28 and MU-PH1 cell lines)

50- Rotenone and MPTP-induced Parkinson disease

Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid (RD16/0008/0017):

51-*Achondroplasia (RCJ3.1C5.18 cell line)

52- *Achondroplasia (Ach G380 cell line human cell line)

53- Achondroplasia (B6; 129s-Fgfr3tm1Dor/J)

54- Alzheimer´s disease (B6C3Tg(APPswe,PSEN1dE9)85Dbo/J)

55- Corneal wound healing

56- *Corneal wound healing (Rabbit corneal cell line)

57- Glaucoma

58- *Glaucoma (Rabbit non-pigmented ciliary epithelium cell line)

59- *Glaucoma (Human non-pigmented ciliary epithelium cell line(HCE))

60- *Glaucoma (Human crystalline lens cell line(HCL))

61- Huntington´s disease (Tg B6CBA-Tg(Hdexon1)61Gpb/J)

62- *Ocular Infection (Inmortalized Human Corneal Limbal Epithelial Cells (HCLEC))

63- *Ocular infection (Immortalized Human Conjunctival Epithelial Cell Lines (HCjE))

64- Retinal ganglion cells (RGC) degeneration (DBA/2J)

65- *Retinal degeneration/Glaucoma (RGC-5 cell line)

66- *Retinal degeneration/Glaucoma (R28 cell line)

*Cell Lines

Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, University Complutense of Madrid, Madrid (RD16/0008/0005):

67- Experimental glaucoma

68- Chorioretinal ischemia induced by hypercholesterolemia

69- Laser photocoagulation of the retina and choroid

70- Organotypic retinal cultures

71- Experimental glaucoma

Ophthalmic Research Unit "Santiago Grisolía", Faculty of Medicine, University of Valencia; and Universitary and Polytechnic Hospital La Fe; Valencia (RD16/0008/0022):

72- Chronic Glaucoma induction by a series of intracamerular injections of hyaluronic acid (Wistar rat)

73- Proliferation, apoptosis and angiogenesis in ophthalmic diseases (C57BL/6 mouse)

74- Neuroprotection and apolipoprotein E (apoE$^{-/-}$ mouse)

75- Rat model of glaucoma by intracamerular injections of sodium hyaluronate

76- *Inflammation and immune response in retinal pigment epithelial cell cultures [immortalized human retinal pigment epithelial cell line (ARPE19)]: Immune aggression in vitro model

77- Development of the visual system

78- Development of the visual system

79- Preclinical safety of intravitreal docosahexaenoic acid in a rabbit model

Human Molecular Genetics Laboratory, School of Medicine, University of Castilla-La Mancha; Albacete (RD16/0008/0019):

80- Genetic glaucoma (lrp2 associated)

*Cell Lines

Experimental Model 1	**Neuroretina organotypic culture**
Target Disease	Retinal degeneration
Species	Human, Pig (*Sus scrofa domestica*)
Short Description	Organotypic retina culture, from human and other mammals, has been demonstrably useful in improving our knowledge of retinal physiology and pathobiology. Furthermore, in organotypic cultures the morphology and functionality of the organ is temporarily retained, and experimental conditions are under control. In brief, eyes are dissected to remove the iris and the lens. The vitreous is then remove from the posterior eyecup by cotton swabs. The neuroretina is detached as a whole by paintbrushing and cutting the optic nerve. Finally, the neuroretina is unrolled in a Petri dish and cut into 5x5 mm explants. Samples are explanted on Transwell culture dishes with the photoreceptor layer facing the membrane, and cultured in Neurobasal A medium supplemented with B-27.
Scientific Publications	- Fernandez-Bueno I, Pastor JC, Gayoso MJ, Alcalde I, Garcia MT. **Müller and macrophage-like cell interactions in an organotypic culture model of porcine neuroretina**. Mol Vis 2008; 14:2148-56. - Fernandez-Bueno I, Fernandez-Sanchez L, Gayoso MJ, Garcia-Gutierrez MT, Pastor JC, Cuenca N. **Time course modifications in organotypic culture of human neuroretina**. Exp Eye Res 2012; 104:26-38. - Fernandez-Bueno I, Garcia-Gutierrez MT, Srivastava GK, Gayoso MJ, Gonzalo-Orden JM, Pastor JC. **Adalimumab (TNF-blocker) reduces the expression of GFAP immunoreactivity increased by exogenous TNFα in an organotypic culture of porcine neuroretina**. Molecular Vision 2013. Epub ahead of print. - Rodriguez-Crespo D, Di Lauro S, Singh A, Garcia-Gutierrez MT, Garrosa García M, Pastor JC, Fernandez-Bueno I, Srivastava GK. **Triple-layered mixed co-culture model of RPE cells with neuroretina for evaluating the neuroprotective effects of adipose-MSCs**. Cell Tissue Res 2014; 358:705-16. - Di Lauro S, Rodriguez-Crespo D, Gayoso MJ, Garcia-Gutierrez MT, Pastor JC, Srivastava GK, Fernandez-Bueno I. **A novel co-culture model of porcine central neuroretina explants and retinal pigment epithelium cells**. Molecular Vision 2016;22:243-53
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 2	Double and triple layered co-culture model
Target Disease	Retinal degeneration
Species	Human, Pig (*Sus scrofa domestica*)
Short Description	Retina is a complex structure consisting neuroretina and RPE layers. There are onsets of retinal diseases if these layers lose their structures and functions. There are many treatment strategies based on drugs as well as cells. Nevertheless it lacks appropriate in vitro models to test these treatment strategies before performing in vivo test. Experimental animal model development and testing a treatment strategy has many hurdles, hence, it could be interesting to develop co-culture models which can mimic, at least partially, in vivo retinal condition. Thus, it could solve many pre-clinical issues including minimizing number of experimental animals. Due to these reasons, it had been developed double and triple layered co-culture models. Double layered co-culture model consists two layers; a neuroretina organotypic culture and a monoculture of RPE cells or MSCs. Triple layered co-culture consists all these three layers. Important; These layers are physically separated. They are in comunications only through biomolecules which they secret. Cell secreted biomolecule profile, co-culture microenvironment condition could be modified using a drug property. RPE could be treated for degeneration. Neuroretina is under spontaneous degeneration.
Scientific Publications	- Rodriguez-Crespo D, Di Lauro S, Singh AK, Garcia-Gutierrez MT, Garrosa M, Pastor JC, Fernandez-Bueno I, Srivastava GK. **Triple-layered mixed co-culture model of RPE cells with neuroretina for evaluating the neuroprotective effects of adipose-MSCs**. Cell Tissue Res. 2014;358(3): 705-16. - Singh AK, Srivastava GK, García-Gutiérrez MT, Pastor JC. **Adipose derived mesenchymal stem cells partially rescue mitomycin C treated ARPE19 cells from death in co-culture condition**. Histol Histopathol. 2013;28(12): 1577-83. - Di Lauro S, Rodriguez-Crespo D, Gayoso MJ, Garcia-Gutierrez MT, Pastor JC, Srivastava GK, Fernandez-Bueno I. **A novel co-culture model of porcine central neuroretina explants and retinal pigment epithelium cells**. Molecular Vision 2016;22:243-53
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 3	**Proliferative vitreoretinopathy (Intravitreal platelet-rich plasma)**
Target Disease	Proliferative vitreoretinopathy (PVR)
Species	Rabbit (*Oryctolagus cuniculus*)
Short Description	Intravitreal injection of 0.15ml of PRP, transconjuctival cryotherapy and vitrectomy are performed. PRP is prepared from fresh citrated rabbit blood and injected into the vitreous cavity through the equator, using a 25g needle after pupillary dilatation.
Scientific Publications	- Piñon RM, Pastor JC, Saornil MA, Goldaracena MB, Layana AG, Gayoso MJ, Guisasola J. **Intravitreal and subretinal proliferation induced by platelet-rich plasma injection in rabbits**. Curr Eye Res 1992; 11:1047-55 - Goldaracena MB, Pastor JC, Saornil MA, Garcia-Layana A, De la Fuente LF. **[An effective model of PVR]**. Arch. Soc. Esp. Oftal. 1994; 67:127-34 - Goldaracena MB, Garcia-Layana A, Pastor JC, Saornil MA, de la Fuente F, Gayoso MJ. **The role of retinotomy in an experimental rabbit model of proliferative vitreoretinopathy**. Curr Eye Res 1997; 16:422-7
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 4	**Proliferative vitreoretinopathy (Intravitreal platelets)**
Target Disease	Proliferative vitreoretinopathy (PVR)
Species	Pig (*Sus scrofa domestica*)
Short Description	Animals receive four 3-mm-long retinectomies in the posterior retina, a partial mechanical vitectomy (about 1ml of central vitreous), and six cryoapplications at the retinal periphery. In addition, platelet concentrated plasma (10^6 platelets/mm^3) is injected intravitreally.
Scientific Publications	- Garcia-Layana A, Pastor JC, Saornil MA, Gonzalez G. **Porcine model of proliferative vitreoretinopathy with platelets**. Curr Eye Res 1997; 16:556-63
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 5	**Autologous translocation of retinal pigment epithelium-choroid graft**
Target Disease	Age-related macular degeneration (AMD)
Species	Pig (*Sus scrofa domestica*)
Short Description	Autologous translocation of the choroid and RPE is carried out in porcine eyes closely following a technique described by van Meurs & Van Den Biesen (2003). Briefly, a standard 20G pars plana lensectomy and a pars plana vitrectomy are performed. The central retina is detached by the subretinal injection of Ringer's lactate solution through a retinotomy performed with a 39G angled rigid microinjection cannula. Recipient RPE and Bruch's membrane are left intact. Afterwards, demarcation of a rectangular area of 2x2 mm, using an endolaser probe, at the donor nasal midperiphery is performed. Using a bimanual technique with vitreous forceps and scissors, a full-thickness graft of neuroretina, RPE and choroid is carefully separated from the sclera. The graft, then consisting of the RPE and choroid, is translocated to the recipient bed in the central and a bubble of perfluorooctane is injected. Finally, after fluid⁄air exchange, the eye is filled up with 1000 Cst silicone oil.
Scientific Publications	- Fernandez-Bueno I, Rodriguez de la Rua E, Hileeto D, Parrado ML, Regueiro-Purriños M, Sala-Puigdollers A, Srivastava GK, Gonzalo-Orden JM, Pastor JC. **Histology and immunochemistry evaluation of autologous translocation of RPE-choroid graft in porcine eyes**. Acta Ophthalmol Scan 2012. Epub ahead of print.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 6	**Endophthalmitis (Intravitreal S. aureus)**
Target Disease	Endophthalmitis
Species	Rabbit (*Oryctolagus cuniculus*)
Short Description	Rabbits were injected with approximately 200 CFU of a typed strain of S. aureus intravitreally.
Scientific Publications	- Del Nozal MJ, Bernal JL, Pampliega A, Marinero P, López MI, Coco R. **High-performance liquid chromatographic determination of vancomycin in rabbit serum, vitreous and aqueous humour after intravitreal injection of the drug**. J Chromatogr A 1996; 727:231-8 - Coco RM, López MI, Pastor JC, Nozal MJ. **Pharmacokinetics of intravitreal vancomycin in normal and infected rabbit eyes**. J Ocul Pharmacol Ther 1998; 14:555-63. - Coco RM, Lopez MI, Pastor JC. **Pharmacokinetics of 0.5 mg of a single and multiple dose of intravitreal vancomycin in infected rabbit eyes.** J Ocul Pharmacol Ther 2000;16(4):373-81.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 7	**Fresh retinal pigment epithelial (RPE) cells**
Target Disease	Retinal degeneration
Species	Human, Pig (*Sus scrofa domestica*)
Short Description	Retinal diseases such as dry AMD, retinitis pigmentosa and many others are still non-curable or currently used therapeutic approaches are insufficiently effective. Their pathogenesis, likely multifactorial, involving a complex interaction of metabolic, functional, genetic and environmental factors, remain poorly understood. Although major abnormalities are seen in four functionally interrelated tissues, i.e., photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaries, the impairment of RPE cell functions is an early and crucial event in the cellular and molecular pathways leading to clinically relevant retinal changes. Detecting changes in RPE cells and repairing the pathways involved by using cellular and molecular based strategies are crucial for developing an effective therapy. For this reason, our laboratory is involved in investigation based on human RPE cells. We develop collaboration with the Universitary Hospital of Valladolid for obtaining human eye globes for isolating, cultivating pure fresh human RPE cells using our established protocols (Immunol Methods. 2013 Jan 11). Furthermore, porcine eye resembles with human eye in many properties such as similar size, anatomy and histology. Furthermore, retinal development in pig eye shows substantial similarity to human retinal development. These characteristics make pig eyes and their RPE cells an ideal model for performing pre-clinical tests. For this reason, our laboratory is involved in investigation based on pig RPE cells. We develop a collaboration with slaughter-house, Valladolid for obtaining pig eye globes for isolating, cultívating pure fresh pig RPE cells using our established protocols (Exp Eye Res. 2011;93:956-62.). In summary, we offer fresh pure human and porcine RPE cells for investigation purpose.
Scientific Publications	- Srivastava GK, Reinoso R, Singh AK, Fernandez-Bueno I, Martino M, Garcia-Gutierrez MT, Pastor JC, Corell A. **Flow cytometry assessment of the purity of human retinal pigment epithelial primary cell cultures**. J Immunol Methods. 2013 Jan 11. - Srivastava, G., Martin, L., Singh, A., Rodriguez-Cabello, J. and Pastor, J. **Evaluation of human retinal pigment epithelial cells growth on elastin-like recombinamers substrates**. Acta Ophthalmologica, 2011;89: 0. - Srivastava GK, Reinoso R, Singh AK, Fernandez-Bueno I, Hileeto D, Martino M, Garcia-Gutierrez MT, Merino JM, Alonso NF, Corell A, Pastor JC. **Trypan Blue staining method for quenching the autofluorescence of RPE cells for improving protein expression analysis**. Exp Eye Res. 2011;93:956-62.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 8	**Fresh retinal stem cells (RSCs) from ciliary margin**
Target Disease	Retinal degeneration
Species	Human, Pig (*Sus scrofa domestica*)
Short Description	Retinal diseases such as dry AMD, retinitis pigmentosa and many others are still non-curable or currently used therapeutic approaches are insufficiently effective. The pathogenesis remains poorly understood. Major abnormalities are seen in four functionally interrelated tissues, i.e., photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaries. Stem cell therapy showed favorable out comes. Retinal stem cells (RSCs) are present during embryonic development; they persist in quiescent forms in the adult mammalian eye in ciliary marginal zone. They have the ability to differentiate into different retinal cell type. Thus, they are promising for developing stem cell based treatments for retinal diseases. For this reason, our laboratory is involved in investigation based on human RSCs. We develop collaboration with Universitary Hospital of Valladolid for obtaining human eye globes for isolating, cultivating pure fresh human RSCs using our established protocols. Furthermore, porcine eye resembles with human eye in many properties such as similar size, anatomy and histology. Furthermore, retinal development in pig eye shows substantial similarity to human retinal development. These characteristics make pig eyes and their RSCs an ideal model for performing pre-clinical tests. For this reason, our laboratory is involved in investigation based on pig RSCs. We develop collaboration with slaughter-house, Valladolid for obtaining pig eye globes for isolating, cultivating pure fresh pig RSCs using our established protocols. In summary, we offer fresh pure human and porcine RSCs for investigation purpose.
Scientific Publications	- Srivastava GK, Rodríguez-Crespo D, Casado-Coterillo C, Singh AK, Garcia-Gutierrez MT., Coronas J, Pastor JC. **Evaluation of retinal stem cells adhesion, growth, viability and protein expression on chitosan surface**. ISER meeting, Berlin, July, 2012. - Srivastava GK, Rodríguez-Crespo D, Singh AK, Casado-Coterillo C, Fernandez-Bueno I, Garcia-Gutierrez MT, Coronas J, Pastor JC. **Chitosan feasibility to retain retinal stem cell phenotype and slow proliferation for retinal transplantation**. BioMed Research International 2014; Epub 2014 Feb 2.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 9	**Chronic-Inflammatory Ocular Surface (SV-40 immortalized human corneal epithelial (HCE) cell line)**
Target Disease	Chronic-inflammatory ocular surface diseases
Species	Human
Short Description (max. 250 words)	Although there are available therapies for acute-moderate ocular surface diseases, there is a lack of effective therapy for the treatment of more chronic-severe inflammatory diseases. These chronic-severe diseases are characterized for the possibility of corneal damage, that may lead to a sight-threatening outcome. It is clear that secreted molecules from inflammatory cells are responsible for many of the features observed in ocular inflammatory diseases. However, there is growing evidence showing that epithelial cells are active participants in inflammation, as they express and produce a large number of molecules implicated in the initiation and perpetuation of the inflammation process. For that reason, epithelial cells, and in our case corneal epithelial cells, are considered as a potential target for therapeutic interventions. This experimental model represents an in vitro model of ocular inflammation for the study of the implication of the corneal epithelium in the development of chronic-severe inflammatory diseases that may lead to visual loss. In this model, the SV-40 immortalized human corneal epithelial (HCE) cell line (Araki-Sasaki et al., IOVS 1995) has been exposed to different cytokines, including Th1 (INF-γ), Th2 (IL-4, IL-13) and Th17 (IL-17), and to TNF-α and TGF-β. HCE response in terms of cytokine/chemokine secretion and/or several receptor types' expression has been characterized.
Scientific Publications	- Enríquez de Salamanca A, Calder V, Gao J, Galatowicz B, Garcia-Vazquez C, Fernández I, Stern ME, Diebold Y, Calonge M. **Cytokine responses by conjunctival epithelial cells: an in vitro model of ocular inflammation**. Cytokine. 2008; 44:160-167 - Benito MJ, Calder V, Corrales RM, García Vázquez C, Narayanan S, Herreras JM, Stern ME, Calonge M, Enríquez de Salamanca A. **Effect of TGF-β on ocular surface epithelial cells**. Exp Eye Res. 2013; 107:88-100. - Arranz-Valsero I, Schulze U, Contreras-Ruiz L, García-Posadas L, López-García A, Paulsen F, Diebold Y. **Involvement of corneal epithelial cells in the Th17 response in an in vitro bacterial inflammation model**. Mol Vis. 2013; 19:85.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 10	**Limbal Stem Cell Deficiency**
Target Disease	Limbal Stem Cell Deficiency
Species	Pig (*Sus scrofa domestica*)
Short Description (max. 250 words)	An animal model of total LSCD was developed in pigs. 4-week-old male pigs were tranquilized with an intramuscular injection of Midazolam (0.35 mg/kg), Ketamine (5 mg/kg) and Atropine (0.02 mg/kg). Anesthesia induction was performed with an intravenous injection of Propofol (3-4mg/kg). Ocular surface was rinsed with sterile saline and 2.5% iodine solution. N-heptanol-based denudation of the corneal surface was done. Then, 360º cryogenic lesions were applied to the entire limbal area. A device was constructed to this end and the procedure was standardized. Intraorbital triamcinolone was immediately injected after surgery and systemic antibiotics were given for the 1st post-op week. After creation of the injury the eyelid was closed. Animals were weekly evaluated and scored by the same two independent researchers for: edema, opacification, neovascularisation, and re-epithelization. Photographs were taken at each evaluation point. Corneal impression cytologies were taken weekly to detect goblet cells. Histopathology analyses at the end of follow-up evaluated the degree of damage created in the limbal niche and the presence of inflammation and goblet cells (as a sign of conjuntival in-growth) in the central cornea. Additional evaluations were performed by laser scanning confocal microscopy (HRT-III Rostock Cornea Module) at the end of follow-up (12 weeks). Severe corneal scarring, neovascularization, opacification, and epithelial defects (fluorescein staining in green) were observed after 4 weeks and were maintained throughout the follow-up, resembling human LSCD. The presence of goblet cells in the central cornea was observed in corneal impression cytologies, resembling the conjunctival in-growth of human LSCD. Confocal microscopy demonstrated vascularization, inflammatory cells and goblet cells in the central cornea and complete (360º) limbal destruction, corroborated by pathology examination.
Scientific Publications	- Galindo S, Plata-Cordero M, Vuelta E, Iglesias J, Regueiro M, Gonzalo-Orden M, Hileeto D, Nieto-Miguel T, Herreras JM, Calonge M. **Ocular Surface Failure Due to Limbal Stem Cell Deficiency (LSCD): Development of Two Efficient Animal Models.** Invest Ophthalmol Vis Sci 2012; 53: E-Abstract 3516
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 11	**Partial limbal stem cell deficiency (Rabbit)**
Target Disease	Limbal stem cell deficiency
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description (max. 250 words)	A partial Limbal Stem Cell Deficiency (LSCD) model was created in rabbits. Rabbits (weighing 4-4.5 kg) were anesthetized with intramuscular injection of 50 mg/Kg ketamine (Imalgene 1000®, Merial, Lyon, France) and 7 mg/Kg xilasyn (Rompun®, Bayer AG, Leverkusen, Germany), complemented with topical ophthalmic anesthetic combined (Colircusí®, Alcon, Barcelona, Spain). Denudation of corneal surface was made by cotton swab soaked with n-hepanol for 1 minute, and then, cornea was stained with fluorescein sodium to ensure of the removal of corneal epithelium. Surgical 180º limbal peritomy (temporal limbus, from 7 to 1 o'clock) was performed by crescent knife. Topical anti-inflammatory and antibiotics (Maxitrol® and Tobrex®, Alcon), intramuscular analgesics (0.02 mg/Kg buprenorphine, Buprex®), and subcutaneous antibiotics (5 mg/Kg enrofloxacin, Alsir®, Esteve, Barcelona, Spain) were administered daily for 5 days. Corneal neovascularization, corneal opacity and epithelial defects were clinically scored weekly by two different researchers. At the end of follow-up (11 weeks), histopathology and immunofluorescence analyses evaluated the degree of damage created in the limbal niche and the presence of inflammation and goblet cells (as a sign of conjuntival in-growth) in the central cornea and limbus. Rabbit corneas developed neovascularization, opacification, and epithelial defects after 3 weeks, resembling mild human LSCD. Histopathology and immunoflourescence analyses showed complete destruction of the injured area (180º) and mild inflammation of the non-injured area. New ocular treatments could be applied in this model in order to evaluate their effect in a mild LSCD.
Scientific Publications	- Galindo S, Plata-Cordero M, Vuelta E, Iglesias J, Regueiro M, Gonzalo-Orden M, Hileeto D, Nieto-Miguel T, Herreras JM, Calonge M. **Ocular Surface Failure Due to Limbal Stem Cell Deficiency (LSCD): Development of Two Efficient Animal Models**. Invest Ophthalmol Vis Sci 2012; 53: ARVO E-Abstract 3516. - Galindo S, Herreras JM, López-Paniagua M, Rey E, de la Mata A, Plata-Cordero M, Calonge M, Nieto-Miguel T. **Regenerative effect of human adipose tissue-derived mesenchymal stem cells in experimental corneal failure due to limbal stem cell niche damage**. Stem Cells (under review). - Galindo S, Herreras JM, Plata-Cordero M, de la Mata A, López-Paniagua M, Rey E, Nieto-Miguel T, Calonge M. **Safety and preliminary efficacy of human adipose tissue mesenchymal stem cells (hAT-MSC) to restore the ocular surface in an in vivo experimental model of limbal stem cell deficiency (LSCD)**. Invest Ophthalmol Vis Sci 2014; 55: ARVO E-abstract 5162. - Calonge M, Galindo S, Nieto-Miguel T, López-Paniagua M, de la Mata A, Plata-Cordero M, Rey E, Herreras JM. **Ocular Surface Inflammation in a Rabbit Model of Partial and Total Limbal Stem Cell Deficiency**. Invest Ophthalmol Vis Sci 2015; 56: ARVO E-abstract 5638.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 12	**Total limbal stem cell deficiency (Rabbit)**
Target Disease	Limbal stem cell deficiency
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description (max. 250 words)	A total Limbal Stem Cell Deficiency (LSCD) model was created in rabbits. Rabbits (weighing 4-5 kg) were anesthetized with intramuscular injection of 50 mg/Kg ketamine (Imalgene 1000®, Merial, Lyon, France) and 7 mg/Kg xilazine (Rompun®, Bayer AG, Leverkusen, Germany), complemented with topical ophthalmic anesthetic combined (Colircusí®, Alcon, Barcelona, Spain). Denudation of corneal surface was made by cotton swab soaked with n-hepanol for 1 minute, and then, cornea was stained with fluorescein sodium to ensure of the removal of corneal epithelium. Surgical 360º limbal peritomy was performed by crescent knife. Topical anti-inflammatory and antibiotics (Maxitrol® and Tobrex®, Alcon), intramuscular analgesics (0.02 mg/Kg buprenorphine, Buprex®), and subcutaneous antibiotics (5 mg/Kg enrofloxacin, Alsir®, Esteve, Barcelona, Spain) were administered daily for 5 days. Corneal conjunctivalization, corneal neovascularization, corneal opacity and epithelial defects were clinically scored weekly by two different researchers. At the end of follow-up (11 weeks), histopathology and immunofluorescence analyses were performed to evaluate the degree of damage created in the limbal niche and the presence of inflammation and goblet cells (as a sign of conjuntival in-growth) in the central cornea and limbus. Rabbit corneas developed neovascularization, opacification, and epithelial defects, after 3 weeks, resembling moderate/severe human LSCD. Histopathology and immunofluorescence analyses showed complete destruction of the limbus and inflammation of corneal and limbal stroma. New ocular treatments could be applied in this model in order to evaluate their effect in a moderate/severe LSCD.
Scientific Publications	
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 13	**Wound healing in vivo model (Rabbit)**
Target Disease	Corneal wounds
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description (max. 250 words)	New Zealand white rabbits (2.5-3 kg) were anesthetized with intramuscular injection of ketamine (50 mg/kg) and xylazine (7 mg/kg), complemented with two drops of topical ophthalmic anesthesia (Combined double anesthetic, Colircusí®). In addition, systemic analgesics (0.02 mg/kg buprenorphine) and antibiotics (5 mg/kg enrofloxacin) were administered before performing corneal damage. A filter paper disc (6 mm in diameter) soaked with 1-heptanol, was positioned in the center of each right corneal surface and left in place for 1 minute. The disc was then removed, and the damaged corneal epithelium was washed gently with sterile saline. The epithelial defects were revealed with 5 μl of sodium fluorescein (Colircusí Fluotest®), after 1 minute the excess of fluorescein was washed with sterile saline and the ocular surface was photographed using cobalt blue light and yellow barrier filter, immediately after injury (t=0) and at 8, 24, 32, 48, 56 and 72 hours after injury. The stained area was measured by computerized planimetry with NIH Image (Image J software). At the end of the follow-up (72 h) the animals were euthanized by intravenous injection of pentobarbital sodium (Dolethal®) under anesthesia. Fluorescein staining revealed that the epithelial defect decreased along the time until wounds were completely closed. Topical ocular administration of new treatments could be applied in this model in order to evaluate the wound healing rate.
Scientific Publications	
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 14	**Engineered human conjunctival-like tissue to study ocular surface inflammatory diseases (3D model of human conjunctiva)**
Target Disease	Inflammatory diseases of the ocular surface
Species	Human
Short Description (max. 250 words)	Inflammatory ocular surface diseases are very prevalent among the global population. Patients demand more efficacious, new treatments for their diseases and, at the same time, governments and pharmaceutical companies are concerned about the cost of the research needed to develop new drugs. The increasing use of three-dimensional models has shown their utility in decreasing research costs by providing more reliable results and reducing the use of animals in research. The conjunctiva is involved in different ocular surface diseases, playing an active role in the pathophysiology of common conditions such as dry eye disease, Sjögren's syndrome, and allergic conjunctivitis, among others. To date, the majority of in vitro investigations concerning the conjunctival tissue have been carried out using monolayer culture techniques that do not recapitulate the complexity of the whole tissue. For those reasons, we have developed a three-dimensional model of the human conjunctiva that can be used to perform pathophysiology experiments and test drug response. Briefly, fibrin-based matrices (derived from human plasma or plasma cryoprecipitate) were used as scaffolds, and primary cells obtained from cadaveric conjunctival tissue were seeded (fibroblast inside and epithelial cells on the surface). Characterization of conjunctival constructs showed epithelial cell stratification, cell polarization and functionality. Conditions such as desiccation and exposure to IL-13 were used to in vitro mimic dry eye disease or allergy. In response to those stimuli, conjunctival constructs increased IL-6 and MUC5AC production. Therefore, this three-dimensional model can be used to study ocular inflammatory diseases and test novel therapies.
Scientific Publications	García-Posadas L, Soriano-Romaní L, López-García A, Diebold Y. **An Engineered Human Conjunctival-Like Tissue to Study Ocular Surface Inflammatory Diseases**. PLoS ONE 12(3): e0171099. doi:10.1371/journal.pone.0171099
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 15	**In vitro human corneal wound healing model**
Target Disease	Corneal wound healing under inflammatory conditions
Species	Human
Short Description (max. 250 words)	The ability of the cornea to heal and maintain its clarity has paramount importance in preserving the eyesight. Corneal opacity insults, such as trauma, eye surgery, or inflammatory diseases of the anterior part of the eye. In the ocular surface, a pathologic wound healing process, along with local inflammation and neovascularization can induce failure in the functional recovery of the ocular surface tissues, which can lead to corneal blindness. In particular, corneal healing process under inflammatory conditions is not fully understood. In this model, confluent monolayers of the SV-40 immortalized human corneal epithelial (HCE) cell line (Araki-Sasaki et al., IOVS 1995) are consistently wounded, creating a cell-free area, based on the technique described by Liang et al. (Liang et al., 2007). Cultures are gently washed with DMEM/F12 to remove loose cells. Cells are then exposed to different cytokines in culture medium. Cells for control conditions are also scratched, washed, and maintained in culture medium after the scratch. Immediately after the scratch and/or at different time points, at least four images of the scraped area are captured using phase contrast microscopy. The remaining wounded area and the scratch width at six different points per image are measured. The same scratched area is selected for the measurements at each time of study. Using this model, influence of inflammatory molecules or treatments in corneal wound closure can be studied, and relative contribution of cell proliferation and cell migration processes analyzed.
Scientific Publications	Arranz-Valsero I, Soriano-Romaní L, García-Posadas L, López-García A, Diebold Y. I**L-6 as a corneal wound healing mediator in an in vitro scratch assay**. Exp Eye Res 2014; 125: 183-192. DOI 10.1016/j.exer.2014.06.012
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 16	Oxidative stress based cell culture model (line ARPE19)
Target Disease	Retinal degeneration
Species	Human
Short Description (max. 250 words)	Retina is a principal anatomical structure for vision. The retina is exposed to oxidative stress along life span, which produce damage in retinal structure obstructing its proper function. The retinal pigment epithelial cells layer is a crucial part of retina. Our laboratory has established in vitro cell culture models applying oxidative stress. The Glucose oxidase (GOx) enzyme is applied for generation of H2O2 which produces continues oxidative stress in cell culture. The H2O2 is applied for acute oxidative stress. Depending on experimental requirements it can be selected an oxidative stress model. The developed oxidative stress based cell culture models can be used for testing anti-oxidant effects.
Scientific Publications	Rodriguez-Crespo D, Di Lauro S, Singh AK, Garcia-Gutierrez MT, Garrosa M, Pastor JC, Fernandez-Bueno I, Srivastava GK. **Triple-layered mixed co-culture model of RPE cells with neuroretina for evaluating the neuroprotective effects of adipose-MSCs**. Cell Tissue Res. 2014 Dec; 358(3):705-16. Rodriguez-Crespo D. **Evaluación del cocultivo de células madre mesenquimales y células del epitelio pigmentario de la retina en un modelo de cultivo organotípico de neurorretina de cerdo**. Master thesis. 2013. IOBA, Universidad de Valladolid.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 17	**Dry eye (Removing lacrimal gland)**
Target Disease	Dry eye
Species	Guinea Pig (*Cavia porcellus*), Rat (*Rattus norvegicus*)
Short Description	Experimental animal model of Dry eye by surgically removing main lacrimal gland from one eye. The model produces a significant reduction of tearing volume and characteristics of dry eye disease as measured up to four weeks in the ipsilateral eye. Some contralateral effects can be also present.
Scientific Publications	- Callejo G, Castellanos A, Castany M, Gual A, Luna C, Acosta MC, Gallar J, Gibin JP, Gasull X. **Acid-sensing ion channels detect moderate acidifications to induce ocular pain.** Pain. 2015 (in press) - **Effects of Long-Term Eye Dryness on Voltage-Gated Na+ Currents of Guinea-Pig Cold-Sensitive Ocular Trigeminal Ganglion Neurons**. *ARVO Meeting Abstracts March 26, 2012 53:1792* - **Cyclosporine A Decreases Hyperexcitability of Corneal Cold Receptor Terminals by Altering Action Potential Generation in Experimental Dry Eye**. *ARVO Meeting Abstracts March 26, 2012 53:1795* - *Article submitted to publication*
Institution	Laboratory of Neurophysiology, Faculty of Medicina, University of Barcelona-IDIBAPS, Barcelona

Experimental Model 18	**Allergic keratoconjunctivitis**
Target Disease	Dry eye
Species	Guinea Pig (*Cavia porcellus*), Rat (*Rattus norvegicus*)
Short Description	Experimental animal model of Allergic keratoconjunctivitis by animal sensitization with ovalbumin. Sensitivazion is induced on day 0 by i.p. injection of a solution of 100 ug ovalbumin + 20 mg Al(OH)3 in 1ml saline solution. On days 14-18, 10 ul drops of 10% ovalbumin in saline are applied daily to both eyes to induce ocular allergy.
Scientific Publications	- Callejo G, Castellanos A, Castany M, Gual A, Luna C, Acosta MC, Gallar J, Gibin JP, Gasull X. **Acid-sensing ion channels detect moderate acidifications to induce ocular pain.** Pain. 2015 (in press) - Acosta MC, Luna C, Quirce S, Belmonte C, Gallar J. **Changes in sensory activity of ocular surface sensory nerves during allergic keratoconjunctivitis.** Pain. 2013; 154:2353-2362.
Institution	Laboratory of Neurophysiology, Faculty of Medicine, University of Barcelona-IDIBAPS, Barcelona

Experimental Model 19	**Trabecular meshwork cell lines**
Target Disease	Glaucoma, aqueous humor outflow
Species	Human
Short Description	Lines of trabecular meshwork cells to study cellular aspects of the cells that form the trabecular meshwork tissue and regulate the passage of aqueous humour through the conventional route. Cell lines include TM5 (normotensive trabecular meshwork cells) and HTM-3 (human trabecular meshwork cells derived from a glaucoma patient).
Scientific Publications	- Li A, Leung CT, Peterson-Yantorno K, Stamer WD, Civan MM. **Cytoskeletal dependence of adenosine triphosphate release by human trabecular meshwork cells**. Invest Ophthalmol Vis Sci. 2011 Oct 10;52(11) - Pang IH, Shade DL, Clark AF, Steely HT, DeSantis L. **Preliminary charactarization of a transformed cell strain derived from human trabecular meshwork**. Curr Eye Res. 1994 Jan;13(1):51-63.
Institution	Laboratory of Neurophysiology, Faculty of Medicina, University of Barcelona-IDIBAPS, Barcelona

Experimental Model 20	**Ocular pain and itch**
Target Disease	Corneal pain, ocular sensitivity, peripheral innervation
Species	Mouse, Rat
Short Description	Animal models of itch and pain by applying painful or pruritic substances in the ocular surface. Measurement of blinking rate, lacrimation, ocular mechanical sensitivity.
Scientific Publications	- Callejo G, Castellanos A, Castany M, Gual A, Luna C, Acosta MC, Gallar J, Giblin JP, Gasull X. **Acid-sensing ion channels detect moderate acidifications to induce ocular pain**. Pain. 2015 Mar;156(3):483-95.
Institution	Laboratory of Neurophysiology, Faculty of Medicine, University of Barcelona-IDIBAPS, Barcelona

Experimental Model 21	**Schlemm's canal endothelium cell lines**
Target Disease	Glaucoma, intraocular pressure, aqueous humor outflow
Species	Human
Short Description	Lines of Schlemm's canal endothelial cells to study cellular aspects of these cells that participate and regulate the passage of aqueous humor through the conventional route. The cell line is derived from a human normotensive patient.
Scientific Publications	- Braakman ST, Pedrigi RM, Read AT, Smith JA, Stamer WD, Ethier CR, Overby DR. **Biomechanical strain as a trigger for pore formation in Schlemm's canal endothelial cells**. Exp Eye Res. 2014 Oct;127:224-35. - Stamer WD, Braakman ST, Zhou EH, Ethier CR, Fredberg JJ, Overby DR, Johnson M. **Biomechanical of Schlemm's canal endothelium and intraocular pressure reduction**. Prog Retin Eye Res. 2015 Jan;44:86-98.
Institution	Laboratory of Neurophysiology, Faculty of Medicine, University of Barcelona-IDIBAPS, Barcelona

Experimental Model 22	**Transient retinal ischemia by selective ligature of the ophthalmic vessels**
Target Disease	Ischemia-induced retinal ganglion cells (RGC) degeneration
Species	Rat (*Rattus norvegicus*)
Short Description	To investigated the pattern of RGC loss that follows transient ischemia of the retina induced by selective ligature of the ophthalmic vessels (SLOV). Ischemia-induced RGC death is a progressive event that takes place in at least two phases: an early rapid and a later more protracted period of cell loss. The amount and duration of these periods of cell loss are determined by the duration of the period of ischemia. In brief, the left ON head is exposed in the orbit, the superior aspect of its dural sheath is opened longitudinally, and a 10-0 nylon suture is introduced between the dural sheath and the ON and tied around the sheath, to interrupt blood flow through the ophthalmic vessels, which run in an inferior and nasal aspect within the sheath. Care is taken not to damage the ON. Interruption of retinal blood flow during ischemia is assessed by direct ophthalmoscopy of the eye fundus through the surgical microscope. The animals that do not show a complete interruption of retinal blood flow during the ischemic period are excluded. At the end of the ischemic period, the ligature is released, and retinal reperfusion is assessed through the surgical microscope. The animals that do not show a complete recovery of retinal blood flow within the first few minutes after the ligature is released are also excluded. Eye fundus inspection is facilitated, because most eyes appeared mydriatic after the induction of transient ischemia. When necessary, a drop of 1% tropicamide is applied topically to induce mydriasis.

Scientific Publications	- Jehle T, Dimitriu C, Auer S, Knoth R, Vidal-Sanz M, Gozes I, Lagrèze WA. **The neuropeptide NAP provides neuroprotection against retinal ganglion cell damage after retinal ischemia and optic nerve crush**. Graefes Arch Clin Exp Ophthalmol. 2008 Sep;246(9):1255-63. Erratum in: Graefes Arch Clin Exp Ophthalmol. 2008; 246(9):1355. - Lönngren U, Näpänkangas U, Lafuente M, Mayor S, Lindqvist N, Vidal-Sanz M, Hallböök F. **The growth factor response in ischemic rat retina and superior colliculus after brimonidine pre-treatment**. Brain Res Bull. 2006; 11;71(1-3):208-18. - Vidal-Sanz M, Lafuente M, Sobrado-Calvo P, Selles-Navarro I, Rodriguez E, Mayor-Torroglosa S, Villegas-Perez MP. **Death and neuroprotection of retinal ganglion cells after different types of injury**. Neurotox Res. 2000;2(2-3):215-27. - Mayor-Torroglosa S, De la Villa P, Rodríguez ME, López-Herrera MP, Avilés-Trigueros M, García-Avilés A, de Imperial JM, Villegas-Pérez MP, Vidal-Sanz M. **Ischemia results 3 months later in altered ERG, degeneration of inner layers, and deafferented tectum: neuroprotection with brimonidine**. Invest Ophthalmol Vis Sci. 2005; 46(10):3825-35. - Avilés-Trigueros M, Mayor-Torroglosa S, García-Avilés A, Lafuente MP, Rodríguez ME, Miralles de Imperial J, Villegas-Pérez MP, Vidal-Sanz M. **Transient ischemia of the retina results in massive degeneration of the retinotectal projection: long-term neuroprotection with brimonidine.** Exp Neurol. 2003; 184(2):767-77. - Lafuente López-Herrera MP, Mayor-Torroglosa S, Miralles de Imperial J, Villegas-Pérez MP, Vidal-Sanz M. **Transient ischemia of the retina results in altered retrograde axoplasmic transport: neuroprotection with brimonidine**. Exp Neurol. 2002; 178(2):243-58. - Lafuente MP, Villegas-Pérez MP, Mayor S, Aguilera ME, Miralles de Imperial J, Vidal-Sanz M. **Neuroprotective effects of brimonidine against transient ischemia-induced retinal ganglion cell death: a dose response in vivo study.** Exp Eye Res. 2002; 74(2):181-9. - Lafuente MP, Villegas-Pérez MP, Sellés-Navarro I, Mayor-Torroglosa S, Miralles de Imperial J, Vidal-Sanz M. **Retinal ganglion cell death after acute retinal ischemia is an ongoing process whose severity and duration depends on the duration of the insult**. Neuroscience. 2002;109(1):157-68. - Vidal-Sanz M, Lafuente MP, Mayor-Torroglosa S, Aguilera ME, Miralles de Imperial J, Villegas-Pérez MP. **Brimonidine's neuroprotective effects against transient ischaemia-induced retinal ganglion cell death**. Eur J Ophthalmol. 2001; 11 Suppl 2:S36-40. - Lafuente MP, Villegas-Pérez MP, Sobrado-Calvo P, García-Avilés A, Miralles de Imperial J, Vidal-Sanz M. **Neuroprotective effects of alpha(2)-selective adrenergic agonists against ischemia-induced retinal ganglion cell death**. Invest Ophthalmol Vis Sci. 2001; 42(9):2074-84. - Vidal-Sanz M, Lafuente MP, Mayor S, de Imperial JM, Villegas-Pérez MP. **Retinal ganglion cell death induced by retinal ischemia. neuroprotective effects of two alpha-2 agonists**. Surv Ophthalmol. 2001; 45 Suppl 3:S261-7; discussion S273-6.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 23	**Transient retinal ischemia by increasing the intraocular pressure**
Target Disease	Retinal ganglion cells (RGC) degeneration
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	To investigated the pattern of RGC loss that follows transient ischemia of the retina induced by elevation of intraocular pressure (IOP). Ischemia-induced RGC death is a progressive event that takes place in at least two phases: an early rapid and a later more protracted period of cell loss. The amount and duration of these periods of cell loss are determined by the duration of the period of ischemia. To increase the IOP, two 6-0 silk sutures are placed on the bulbar conjunctiva of the eye just below and above the corneoscleral limbus and are used to pull tangentially in opposite directions until the blood flow to the retina is interrupted completely. Sutures were tied to a metal frame designed for these experiments to maintain the IOP above systolic arterial levels throughout the transient ischemic period. Interruption of the blood flow to the retina is assessed constantly by examining the fundus of the eye through the operating microscope, and the sutures are retightened when needed to maintain interruption of the intraretinal blood flow. To permit microscopic observation of the eye fundus and, thus, of retinal blood flow, the pupil is dilated with a drop of 1% tropicamide, and the corneal surface is covered with a drop of 2% hydroxipropilmethylcellulose and a coverslip. During the periods of retinal ischemia, interruption of the blood flow to the iris also was observed. At the end of the transient ischemic period, the conjunctival sutures were released slowly, and this is followed by the complete restoration of the blood flow to the retina and the iris.
Scientific Publications	- Sellés-Navarro I, Villegas-Pérez MP, Salvador-Silva M, Ruiz-Gómez JM, Vidal-Sanz M. **Retinal ganglion cell death after different transient periods of pressure-induced ischemia and survival intervals. A quantitative in vivo study**. Invest Ophthalmol Vis Sci. 1996 Sep;37(10):2002-14. PMID: 8814140
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 24	**Selective motoneuron degeneration by injection of substances in oculomotor muscles**
Target Disease	Abducens motoneurons degeneration
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	To investigate in vivo the survival of abducens motoneurons (AMNs) at different periods of time after a single intramuscular injection of the neurotoxin botulinum toxin A (BTxA) or doxorubicin (DXR) in the abducens muscle. The AMNs were labeled with fluorogold (FG) applied intramuscularly in the lateral rectus muscle. The numbers of labeled neurons were determined in adult control animals, in young animals that had received BTxA and in adult rats that had received DXR, at various survival times. The intramuscular injection of BTxA in young animals does not induce significant motoneuron deat, while DXR injection causes variable amounts of motoneuron death that is related both to the survival period and to the amount of DXR injected
Scientific Publications	- Gómez-Ramírez AM, Villegas-Pérez MP, Miralles de Imperial J, Salvador-Silva M, Vidal-Sanz M. **Effects of intramuscular injection of botulinum toxin and doxorubicin on the survival of abducens motoneurons**. Invest Ophthalmol Vis Sci. 1999 Feb;40(2):414-24. PMID: 9950601
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 25	**Selective axotomy-induced RGC death by microcrush lesion of optic nerve**
Target Disease	Retinal ganglion cells (RGC) degeneration
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	Injury of the optic nerve has served as an important model for the study of cell death and axon regeneration in the CNS. Microcrush lesion of the optic nerve, which completely transects all RGC axons and minimizes the amount of secondary damage, creates a well-defined injury site. The microcrush lesion of the optic nerve is an excellent model to study strategies designed to promote axon regeneration in the inhibitory white matter environment. In brief, the left optic nerve is exposed through a superior temporal approach, and the dural sheath is slit longitudinally, taking care to avoid the ophthalmic artery. Microcrush lesions are made with 10-0 sutures used to completely constrict the optic nerve by holding a tight knot for 60 s and then releasing the suture. The fundis oculi is examined to verify the integrity of the retinal blood circulation. Animals whose retinas show ischemic damage are discarded.
Scientific Publications	- Ellezam B, Dubreuil C, Winton M, Loy L, Dergham P, Sellés-Navarro I, McKerracher L. **Inactivation of intracellular Rho to stimulate axon growth and regeneration**. Prog Brain Res. 2002; 137:371-80. - Ellezam B, Selles-Navarro I, Manitt C, Kennedy TE, McKerracher L. **Expression of netrin-1 and its receptors DCC and UNC-5H2 after axotomy and during regeneration of adult rat retinal ganglion cells**. Exp Neurol. 2001; 168(1):105-15. - Sellés-Navarro I, Ellezam B, Fajardo R, Latour M, McKerracher L. **Retinal ganglion cell and nonneuronal cell responses to a microcrush lesion of adult rat optic nerve**. Exp Neurol. 2001; 167(2):282-9. - Lehmann M, Fournier A, Selles-Navarro I, Dergham P, Sebok A, Leclerc N, Tigyi G, McKerracher L. **Inactivation of Rho signaling pathway promotes CNS axon regeneration**. J Neurosci. 1999; 1;19(17):7537-47.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 26	**Selective axotomy-induced RGC death by crush lesion of optic nerve**
Target Disease	Retinal ganglion cells (RGC) degeneration
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	Intraorbital nerve crush (IONC) is a type of injury in which RGC axons are interrupted by pressure without a physical gap among the edges of the injury. However, there is a high variability among the results reported using this kind of lesion which are mainly due to the diversity of tools utilized, to the distance from the eye where the ON is injured and to the pressure exerted, which, if not enough, results in a partial lesion with a variable number of axons spared. In our IONC model, pressing the ON at 3 mm from the eye with watchmaker's forceps during 10 s, resulted in complete interruption of the retinofugal projection, as demonstrated by their failure to transport CTB orthogradely and FG retrogradely, even several months after the lesion. In brief, to perform Intraorbital nerve crush (IONC) injury an incision is made in the superior orbital rim, the superoexternal orbital contents are dissected, and the superior and external rectus muscles are removed, then the optic nerve (ON) is crushed during 10 s at 3 mm from the optic disc using watchmaker's forceps. Before and after the procedure, the eye fundus is observed through the operating microscope to assess the integrity of the retinal blood flow.

Scientific Publications	- Agudo-Barriuso M, Lahoz A, Nadal-Nicolás FM, Sobrado-Calvo P, Piquer-Gil M, Díaz-Llopis M, Vidal-Sanz M, Mullor JL. **Metabolomic changes in the rat retina after optic nerve crush**. Invest Ophthalmol Vis Sci. 2013 Jun 21;54(6): 4249-59. - Parrilla-Reverter G, Agudo M, Nadal-Nicolás F, Alarcón-Martínez L, Jiménez-López M, Salinas-Navarro M, Sobrado-Calvo P, Bernal-Garro JM, Villegas-Pérez MP, Vidal-Sanz M. **Time-course of the retinal nerve fibre layer degeneration after complete intra-orbital optic nerve transection or crush: a comparative study**. Vision Res. 2009; 49(23):2808-25. - Parrilla-Reverter G, Agudo M, Sobrado-Calvo P, Salinas-Navarro M, Villegas-Pérez MP, Vidal-Sanz M. **Effects of different neurotrophic factors on the survival of retinal ganglion cells after a complete intraorbital nerve crush injury: a quantitative in vivo study**. Exp Eye Res. 2009; 15;89(1):32-41. - Nadal-Nicolás FM, Jiménez-López M, Sobrado-Calvo P, Nieto-López L, Cánovas-Martínez I, Salinas-Navarro M, Vidal-Sanz M, Agudo M. **Brn3a as a marker of retinal ganglion cells: qualitative and quantitative time course studies in naive and optic nerve-injured retinas**. Invest Ophthalmol Vis Sci. 2009; 50(8):3860-8. - Agudo M, Pérez-Marín MC, Sobrado-Calvo P, Lönngren U, Salinas-Navarro M, Cánovas I, Nadal-Nicolás FM, Miralles-Imperial J, Hallböök F, Vidal-Sanz M. **Immediate upregulation of proteins belonging to different branches of the apoptotic cascade in the retina after optic nerve transection and optic nerve crush**. Invest Ophthalmol Vis Sci. 2009; 50(1):424-31. - Agudo M, Pérez-Marín MC, Lönngren U, Sobrado P, Conesa A, Cánovas I, Salinas-Navarro M, Miralles-Imperial J, Hallböök F, Vidal-Sanz M. **Time course profiling of the retinal transcriptome after optic nerve transection and optic nerve crush**. Mol Vis. 2008; 3;14:1050-63. - Jehle T, Dimitriu C, Auer S, Knoth R, Vidal-Sanz M, Gozes I, Lagrèze WA. **The neuropeptide NAP provides neuroprotection against retinal ganglion cell damage after retinal ischemia and optic nerve crush**. Graefes Arch Clin Exp Ophthalmol. 2008; 246(9):1255-63. Erratum in: Graefes Arch Clin Exp Ophthalmol. 2008; 246(9):1355.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 27	**Selective axotomy-induced RGC death by intraocular transection of the optic nerve**
Target Disease	Retinal ganglion cells (RGC) degeneration
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	Death of retinal ganglion cells (RGCs) is the cause of blindness in several diseases, including glaucoma. In recent years, much effort has gone into attempting to elucidate the causes and mechanisms of RGC loss so that strategies can be developed to counteract the process. Intraorbital optic nerve transection (IONT) results in the loss of the injured neurons, the RGCs and within the following two weeks post-lesion, approximately 80% of the RGC population is lost, only approximately 15% of the RGCs remain in the retina. A variety of substances have been shown to attenuate RGC death after ONT, with brain derived neurotrophic factor (BDNF) receiving particular attention. This model has allowed quantitative studies on the capacity of axotomized RGCs for axonal regeneration. Moreover, IONT is a valuable model not only for investigation into pathways that contribute to RGC death but also as a model for neuronal apoptosis in the CNS. In experimental animals, the left optic nerve (ON) is cut close to its origin in the optic disc to axotomize the entire population of RGCs. To access the ON at the back of the eye, an incision is made in the skin overlying the superior orbital rim, the supero-external orbital contents were dissected, and the superior and external rectus muscles are sectioned. The dura mater of the ON is opened longitudinally, and the ON is transected completely as close as possible to the eye. Care is taken not to damage the retinal blood supply, which enters the eye separately in the inferonasal aspect of the ON sheath.

Scientific Publications	- Galindo-Romero C, Valiente-Soriano FJ, Jiménez-López M, García-Ayuso D, Villegas-Pérez MP, Vidal-Sanz M, Agudo-Barriuso M. **Effect of brain-derived neurotrophic factor on mouse axotomized retinal ganglion cells and phagocytic microglia**. Invest Ophthalmol Vis Sci. 2013 Feb 1;54(2):974-85. - Galindo-Romero C, Avilés-Trigueros M, Jiménez-López M, Valiente-Soriano FJ, Salinas-Navarro M, Nadal-Nicolás F, Villegas-Pérez MP, Vidal-Sanz M, Agudo-Barriuso M. **Axotomy-induced retinal ganglion cell death in adult mice: quantitative and topographic time course analyses**. Exp Eye Res. 2011; 92(5):377-87. - Sánchez-Migallón MC, Nadal-Nicolás FM, Jiménez-López M, Sobrado-Calvo P, Vidal-Sanz M, Agudo-Barriuso M. **Brain derived neurotrophic factor maintains Brn3a expression in axotomized rat retinal ganglion cells**. Exp Eye Res. 2011; 92(4):260-7. - Alarcón-Martínez L, de la Villa P, Avilés-Trigueros M, Blanco R, Villegas-Pérez MP, Vidal-Sanz M. **Short and long term axotomy-induced ERG changes in albino and pigmented rats**. Mol Vis. 2009; 17;15:2373-83. - Chidlow G, Casson R, Sobrado-Calvo P, Vidal-Sanz M, Osborne NN. **Measurement of retinal injury in the rat after optic nerve transection: an RT-PCR study**. Mol Vis. 2005; 2;11:387-96. - Casson RJ, Chidlow G, Wood JP, Vidal-Sanz M, Osborne NN. **The effect of retinal ganglion cell injury on light-induced photoreceptor degeneration**. Invest Ophthalmol Vis Sci. 2004; 45(2):685-93. Peinado-Ramón P, Salvador M, Villegas-Pérez MP, Vidal-Sanz M. **Effects of axotomy and intraocular administration of NT-4, NT-3, and brain-derived neurotrophic factor on the survival of adult rat retinal ganglion cells. A quantitative in vivo study**. Invest Ophthalmol Vis Sci. 1996; 37(4):489-500. - Villegas-Pérez MP, Vidal-Sanz M, Rasminsky M, Bray GM, Aguayo AJ. Rapid and protracted phases of retinal ganglion cell loss follow axotomy in the optic nerve of adult rats. J Neurobiol. 1993; 24(1):23-36. - McKerracher L, Vidal-Sanz M, Essagian C, Aguayo AJ. **Selective impairment of slow axonal transport after optic nerve injury in adult rats**. J Neurosci. 1990; 10(8):2834-41. - Aguayo AJ, Bray GM, Carter DA, Villegas-Perez MP, Vidal-Sanz M, Rasminsky M. **Regrowth and connectivity of injured central nervous system axons in adult rodents**. Acta Neurobiol Exp (Wars). 1990; 50(4-5): 381-9. - Vidal-Sanz M, Villegas-Pérez MP, Bray GM, Aguayo AJ. **Persistent retrograde labeling of adult rat retinal ganglion cells with the carbocyanine dye dil**. Exp Neurol. 1988; 102(1):92-101. - Villegas-Pérez MP, Vidal-Sanz M, Bray GM, Aguayo AJ. **Influences of peripheral nerve grafts on the survival and regrowth of axotomized retinal ganglion cells in adult rats**. J Neurosci. 1988; 8(1):265-80.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 28	**Regenerative responses of injured neurons in the central nervous system through peripheral nerve grafts**
Target Disease	Retinal ganglion cell (RGC) axons regeneration
Species	Rat (*Rattus norvegicus*)
Short Description (max. 250 words)	The pattern of axonal regeneration, specificity of reinnervation, and terminal arborization in the brainstem by axotomized retinal ganglion cell axons is studied in rats with peripheral nerve grafts linking the retina with ipsilateral regions of the brainstem, including dorsal and lateral aspects of the diencephalon and lateral aspect of the superior colliculus. Functional studies on the restoration of the pupillary light reflex by regenerating retinal ganglion cell axons have been carried out with this experimental model. In brief, autologous segments of the left common peroneal nerve are used to link the retina with the brainstem. The optic nerve of the left eye is exposed intraorbitally, and after longitudinal incision of the meningeal sheath, the nerve is completely transected close to the sclera without affecting the retinal blood supply. One end of a 3-cm-long autologous common peroneal nerve segment is apposed to the ocular stump with three 10/0 monofilament sutures. At the same time, the distal end of the PN segment is inserted into the ipsilateral side of the brainstem.

Scientific Publications	- Vidal-Sanz M, Avilés-Trigueros M, Whiteley SJ, Sauvé Y, Lund RD. **Reinnervation of the pretectum in adult rats by regenerated retinal ganglion cell axons: anatomical and functional studies**. Prog Brain Res. 2002;137:443-52. - Avilés-Trigueros M, Sauvé Y, Lund RD, Vidal-Sanz M. **Selective innervation of retinorecipient brainstem nuclei by retinal ganglion cell axons regenerating through peripheral nerve grafts in adult rats**. J Neurosci. 2000; 1;20(1):361-74. - Whiteley SJ, Sauvé Y, Avilés-Trigueros M, Vidal-Sanz M, Lund RD. **Extent and duration of recovered pupillary light reflex following retinal ganglion cell axon regeneration through peripheral nerve grafts directed to the pretectum in adult rats**. Exp Neurol. 1998; 154(2): 560-72. - Vidal-Sanz M, Bray GM, Aguayo AJ. **Regenerated synapses persist in the superior colliculus after the regrowth of retinal ganglion cell axons**. J Neurocytol. 1991; 20(11):940-52. Erratum in: J Neurocytol 1992; 21(3):234. - Aguayo AJ, Rasminsky M, Bray GM, Carbonetto S, McKerracher L, Villegas-Pérez MP, Vidal-Sanz M, Carter DA. **Degenerative and regenerative responses of injured neurons in the central nervous system of adult mammals**. Philos Trans R Soc Lond B Biol Sci. 1991; 29;331(1261):337-43. - Bray GM, Villegas-Pérez MP, Vidal-Sanz M, Carter DA, Aguayo AJ. **Neuronal and nonneuronal influences on retinal ganglion cell survival, axonal regrowth, and connectivity after axotomy**. Ann N Y Acad Sci. 1991; 633:214-28. - Aguayo AJ, Bray GM, Rasminsky M, Zwimpfer T, Carter D, Vidal-Sanz M. **Synaptic connections made by axons regenerating in the central nervous system of adult mammals**. J Exp Biol. 1990; 153:199-224. - **Slow transport rates of cytoskeletal proteins change during regeneration of axotomized retinal neurons in adult rats**. McKerracher L, Vidal-Sanz M, Aguayo AJ. J Neurosci. 1990; 10(2): 641-8. - Vidal Sanz M. **[Regeneration of the visual system in the rat. Essay rewarded by the Premio de la Academia Curso 1989]**. An R Acad Nac Med (Madr). 1990;107(1):17-49. - Aguayo AJ, Bray GM, Carter DA, Villegas-Perez MP, Vidal-Sanz M, Rasminsky M. **Regrowth and connectivity of injured central nervous system axons in adult rodents**. Acta Neurobiol Exp (Wars). 1990; 50(4-5):381-9. - Keirstead SA, Rasminsky M, Fukuda Y, Carter DA, Aguayo AJ, Vidal-Sanz M. **Electrophysiologic responses in hamster superior colliculus evoked by regenerating retinal axons**. Science. 1989; 13;246(4927):255-7. - Villegas-Pérez MP, Vidal-Sanz M, Bray GM, Aguayo AJ. **Influences of peripheral nerve grafts on the survival and regrowth of axotomized retinal ganglion cells in adult rats**. J Neurosci. 1988; 8(1):265-80. - Vidal-Sanz M, Bray GM, Villegas-Pérez MP, Thanos S, Aguayo AJ. **Axonal regeneration and synapse formation in the superior colliculus by retinal ganglion cells in the adult rat**. J Neurosci. 1987; 7(9):2894-909. - Bray GM, Villegas-Pérez MP, Vidal-Sanz M, Aguayo AJ. **The use of peripheral nerve grafts to enhance neuronal survival, promote growth and permit terminal reconnections in the central nervous system of adult rats**. J Exp Biol. 1987;132:5-19. - Bray GM, Vidal-Sanz M, Aguayo AJ. **Regeneration of axons from the central nervous system of adult rats**. Prog Brain Res. 1987; 71:373-9. - Aguayo AJ, Vidal-Sanz M, Villegas-Pérez MP, Bray GM. **Growth and connectivity of axotomized retinal neurons in adult rats with optic nerves substituted by PNS grafts linking the eye and the midbrain**. Ann N Y Acad Sci. 1987;495:1-9. - Keirstead SA, Vidal-Sanz M, Rasminsky M, Aguayo AJ, Levesque M, So KF. **Responses to light of retinal neurons regenerating axons into peripheral nerve grafts in the rat**. Brain Res. 1985; 16;359(1-2):402-6. - Munz M, Rasminsky M, Aguayo AJ, Vidal-Sanz M, Devor MG. **Functional activity of rat brainstem neurons regenerating axons along peripheral nerve grafts**. Brain Res. 1985; 5;340(1):115-25.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 29	**Degenerative retinal conditions by inherited retinal dystrophy (RCS and P23H-1)**
Target Disease	Retinal degeneration
Species	Rat (*Rattus norvegicus*): Royal College of Surgeons (RCS) and P23H-1
Short Description	The dystrophic Royal College of Surgeons (RCS) and the P23H-1 rats are two well known animal models of photoreceptor inherited degenerative diseases. Both models suffer an almost complete photoreceptor degeneration due to a defect in the MERTK (RCS) or in the rhodopsin (P23H) gene. RCS-p^{+}- (dystrophic) and RCS-p^{+} rdy^{+}- (nondystrophic) pigmented rats and P23H-1 albino rats are available. These animals may be used to study the events taking place during retinal degeneration or to test strategies to prevent this degeneration, such as light exposure prevention, neurotrophic factor administration or intraocular cell transplantation.
Scientific Publications	- García-Ayuso D, Salinas-Navarro M, Nadal-Nicolás FM, Ortín-Martínez A, Agudo-Barriuso M, Vidal-Sanz M, Villegas-Pérez MP. **Sectorial loss of retinal ganglion cells in inherited photoreceptor degeneration is due to RGC death**. Br J Ophthalmol. 2014;98(3):396-401. - García-Ayuso D, Ortín-Martínez A, Jiménez-López M, Galindo-Romero C, Cuenca N, Pinilla I, Vidal-Sanz M, Agudo-Barriuso M, Villegas-Pérez MP. **Changes in the photoreceptor mosaic of P23H-1 rats during retinal degeneration: implications for rod-cone dependent survival**. Invest Ophthalmol Vis Sci. 2013;54(8):5888-900. - García-Ayuso D, Salinas-Navarro M, Agudo M, Cuenca N, Pinilla I, Vidal-Sanz M, Villegas-Pérez MP. **Retinal ganglion cell numbers and delayed retinal ganglion cell death in the P23H rat retina**. Exp Eye Res. 2010; 91(6): 800-10. - Marco-Gomariz MA, Hurtado-Montalbán N, Vidal-Sanz M, Lund RD, Villegas-Pérez MP. **Phototoxic-induced photoreceptor degeneration causes retinal ganglion cell degeneration in pigmented rats**. J Comp Neurol. 2006; 10;498(2):163-79. - Wang S, Villegas-Pérez MP, Holmes T, Lawrence JM, Vidal-Sanz M, Hurtado-Montalbán N, Lund RD. **Evolving neurovascular relationships in the RCS rat with age**. Curr Eye Res. 2003; 27(3):183-96. - Wang S, Villegas-Pérez MP, Vidal-Sanz M, Lund RD. **Progressive optic axon dystrophy and vacuslar changes in rd mice**. Invest Ophthalmol Vis Sci. 2000; 41(2):537-45. - Villegas-Pérez MP, Lawrence JM, Vidal-Sanz M, Lavail MM, Lund RD. **Ganglion cell loss in RCS rat retina: a result of compression of axons by contracting intraretinal vessels linked to the pigment epithelium**. J Comp Neurol. 1998; 2;392(1):58-77. - Villegas-Pérez MP, Vidal-Sanz M, Lund RD. **Mechanism of retinal ganglion cell loss in inherited retinal dystrophy**. Neuroreport. 1996; 12;7(12):1995-9.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 30	**Laser-induced ocular hypertension**
Target Disease	Retinal ganglion cells (RGC) degeneration
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	In adult rats and mice, perilimbar and episcleral vein photocauterization induces ocular hypertension, which in turn results in devastating damage of the RGC population. In wide triangular sectors, preferentially located in the dorsal retina, RGCs lose their retrograde axonal transport, first by a functional impairment and after by mechanical causes. This axonal damage affects up to 80% of the RGC population, and eventually causes their death, with somal and intra-retinal axonal degeneration that resembles that observed after optic nerve crush. Importantly, while ocular hypertension affects the RGC population, it spares non-RGC neurons located in the ganglion cell layer of the retina. In addition, functional and morphological studies show permanent alterations of the inner and outer retinal layers, indicating that further to a crush-like injury of axon bundles in the optic nerve head there may by additional insults to the retina, perhaps of ischemic nature. To induce ocular hypertension, the left eyes of anesthetized animal are treated during a single session with a series of diode laser (532 nm, Quantel Medical, Clermont-Ferrand, France) burns. The laser beam is delivered directly, without any lenses, aimed at the limbal and episcleral veins. The spot size, duration, and power are set up according to the experimental animal species used and pigmented condition (albino or pigmented).

Scientific Publications	- Dekeyster E, Aerts J, Valiente-Soriano FJ, De Groef L, Vreysen S, Salinas-Navarro M, Vidal-Sanz M, Arckens L, Moons L. **Ocular Hypertension Results in Retinotopic Alterations in the Visual Cortex of Adult Mice**. Curr Eye Res. 2015;23:1-15. - Ortín-Martínez A, Salinas-Navarro M, Nadal-Nicolás FM, Jiménez-López M, Valiente-Soriano FJ, García-Ayuso D, Bernal-Garro JM, Avilés-Trigueros M, Agudo-Barriuso M, Villegas-Pérez MP, Vidal-Sanz M. **Laser-induced ocular hypertension in adult rats does not affect non-RGC neurons in the ganglion cell layer but results in protracted severe loss of cone-photoreceptors**. Exp Eye Res. 2015;132C:17-33. - Rojas B, Gallego BI, Ramírez AI, Salazar JJ, de Hoz R, Valiente-Soriano FJ, Avilés-Trigueros M, Villegas-Perez MP, Vidal-Sanz M, Triviño A, Ramírez JM. **Microglia in mouse retina contralateral to experimental glaucoma exhibit multiple signs of activation in all retinal layers**. J Neuroinflammation. 2014;11:133. - de Hoz R, Gallego BI, Ramírez AI, Rojas B, Salazar JJ, Valiente-Soriano FJ, Avilés-Trigueros M, Villegas-Perez MP, Vidal-Sanz M, Triviño A, Ramírez JM. **Rod-like microglia are restricted to eyes with laser-induced ocular hypertension but absent from the microglial changes in the contralateral untreated eye**. PLoS One. 2013;8(12):e83733. - Agudo-Barriuso M, Villegas-Pérez M, de Imperial JM, Vidal-Sanz M. **Anatomical and functional damage in experimental glaucoma**. Curr Opin Pharmacol. 2013; 13(1): 5-11. - Gallego BI, Salazar JJ, de Hoz R, Rojas B, Ramírez AI, Salinas-Navarro M, Ortín-Martínez A, Valiente-Soriano FJ, Avilés-Trigueros M, Villegas-Perez MP, Vidal-Sanz M, Triviño A, Ramírez JM. **IOP induces upregulation of GFAP and MHC-II and microglia reactivity in mice retina contralateral to experimental glaucoma**. J Neuroinflammation. 2012; 14;9:92. - Vidal-Sanz M, Salinas-Navarro M, Nadal-Nicolás FM, Alarcón-Martínez L, Valiente-Soriano FJ, de Imperial JM, Avilés-Trigueros M, Agudo-Barriuso M, Villegas-Pérez MP. **Understanding glaucomatous damage: anatomical and functional data from ocular hypertensive rodent retinas**. Prog Retin Eye Res. 2012; 31(1):1-27. - Nguyen JV, Soto I, Kim KY, Bushong EA, Oglesby E, Valiente-Soriano FJ, Yang Z, Davis CH, Bedont JL, Son JL, Wei JO, Buchman VL, Zack DJ, Vidal-Sanz M, Ellisman MH, Marsh-Armstrong N. **Myelination transition zone astrocytes are constitutively phagocytic and have synuclein dependent reactivity in glaucoma**. Proc Natl Acad Sci U S A. 2011; 18;108(3):1176-81. - Cuenca N, Pinilla I, Fernández-Sánchez L, Salinas-Navarro M, Alarcón-Martínez L, Avilés-Trigueros M, de la Villa P, Miralles de Imperial J, Villegas-Pérez MP, Vidal-Sanz M. **Changes in the inner and outer retinal layers after acute increase of the intraocular pressure in adult albino Swiss mice**. Exp Eye Res. 2010; 91(2):273-85. - Ramírez AI, Salazar JJ, de Hoz R, Rojas B, Gallego BI, Salinas-Navarro M, Alarcón-Martínez L, Ortín-Martínez A, Avilés-Trigueros M, Vidal-Sanz M, Triviño A, Ramírez JM. **Quantification of the effect of different levels of IOP in the astroglia of the rat retina ipsilateral and contralateral to experimental glaucoma**. Invest Ophthalmol Vis Sci. 2010; 51(11):5690-6. - Salinas-Navarro M, Alarcón-Martínez L, Valiente-Soriano FJ, Ortín-Martínez A, Jiménez-López M, Avilés-Trigueros M, Villegas-Pérez MP, de la Villa P, Vidal-Sanz M. **Functional and morphological effects of laser-induced ocular hypertension in retinas of adult albino Swiss mice**. Mol Vis. 2009. 5;15:2578-98. - Salinas-Navarro M, Alarcón-Martínez L, Valiente-Soriano FJ, Jiménez-López M, Mayor-Torroglosa S, Avilés-Trigueros M, Villegas-Pérez MP, Vidal-Sanz M. **Ocular hypertension impairs optic nerve axonal transport leading to progressive retinal ganglion cell degeneration**. Exp Eye Res. 2010; 90(1):168-83.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 31	**Light-induced retinal degeneration**
Target Disease	Photorecetor and Retinal Degeneration
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	Light-induced retinal damage (phototoxicity) selectively brings about photoreceptor cell death. Thus, this model is useful for studying the potential mechanisms underlying photoreceptor death and the subsequent retinal degeneration processes, since it mimics the photoreceptor degeneration that forms the main characteristic of human diseases such as retinitis pigmentosa or age-related macular degeneration. Moreover, it is useful to investigate the anatomic and functional changes triggered by light exposure in the experimental animal retina. In brief, before light exposure, left pupil mydriasis is induced by topical application of a drop of 1% atropine. The right eye is not dilated to be used for comparison. Because the severity of retinal phototoxicity in rodents depends on the time of the day when exposure starts (circadian rhythm) and on the previous light exposure (rearing conditions and dark adaptation period), the exposure always began between 10 and 12 AM and after 12 h of dark adaptation. Animals are individually exposed to 24 h of continuous fluorescent cold white light. Fluorescent bulbs are situated in the ceiling right above the animal cages, which are transparent. Light intensity within the cages is 3,000±100lx. Mydriasis is inspected 12 h after the initiation of light exposure, and when necessary, a second drop of atropine is instilled in the left eye at this time.
Scientific Publications	- Montalbán-Soler L, Alarcón-Martínez L, Jiménez-López M, Salinas-Navarro M, Galindo-Romero C, Bezerra de Sá F, García-Ayuso D, Avilés-Trigueros M, Vidal-Sanz M, Agudo-Barriuso M, Villegas-Pérez MP. **Retinal compensatory changes after light damage in albino mice**. Mol Vis. 2012; 18:675-93. - García-Ayuso D, Salinas-Navarro M, Agudo-Barriuso M, Alarcón-Martínez L, Vidal-Sanz M, Villegas-Pérez MP. **Retinal ganglion cell axonal compression by retinal vessels in light-induced retinal degeneration**. Mol Vis. 2011; 17:1716-33. - Marco-Gomariz MA, Hurtado-Montalbán N, Vidal-Sanz M, Lund RD, Villegas-Pérez MP. **Phototoxic-induced photoreceptor degeneration causes retinal ganglion cell degeneration in pigmented rats**. J Comp Neurol. 2006; 10;498(2):163-79.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 32	**Model of induced-phototoxicity by focal light emitting diode**
Target Disease	Age-related macular degeneration
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	Animals are dark adapted overnight (at least 12 h) and subsequent manipulations were conducted under dim red light (λ>600 nm). One hour prior to light emitting diode induced-phototoxicity (LIP), animals are anaesthetized and pupil mydriasis is induced in the left eye with one drop of tropicamide (Tropicamida 1%; Alcon-Cusí, S.A., El Masnou, Barcelona, Spain). LIP is performed in the left eye between 10–12 am to avoid variations in the amount of retinal toxicity related to the time of light exposure. The animal's head is placed horizontal on a head-holder and the left eye is exposed to blue-light (400 nm) by a light emitting diode (LED) (emission spectrum 390–410; catalogue number 454–4405; Kingbright Elec. Co., Taipei, Taiwan) connected to a computer to control for duration of exposure (10 secs) and intensity of light (200 lux); lux intensity is measured with a luxometer (light meter TES-1330; TES Electrical Electronic Corp., Taipei, Taiwan) placed 1 mm below and perpendicular to the LED). The LED is held by a micromanipulator and placed 1 mm above and perpendicular to the corneal apex of the left eye.
Scientific Publications	- Ortín-Martínez A, Valiente-Soriano FJ, García-Ayuso D, Alarcón-Martínez L, Jiménez-López M, Bernal-Garro JM, Nieto-López L, Nadal-Nicolás FM, Villegas-Pérez MP, Wheeler LA, Vidal-Sanz M. **A novel in vivo model of focal light emitting diode-induced cone-photoreceptor phototoxicity: neuroprotection afforded by brimonidine, BDNF, PEDF or bFGF**. PLoS One. 2014;9(12):e113798.
Institution	Experimental Ophthalmology, University of Murcia, Murcia

Experimental Model 33	**Laser-induced choroidal neovascularization (C57BL)**
Target Disease	Age-related macular degeneration (AMD)
Species	Mouse (*Mus musculus*): C57BL
Short Description	Choroidal Neovascularization was induced by laser breakage (Diode laser) in mouse C57BL Bruch's membrane. Laser was used at several intensities (100-250mv/100ms), but the best results were obtained with 250mv/ms in five focus points. Neovessels were visualized by confocal microscopy, using endothelial cells labeling (Von Willembrand and DIL heart perfusion).
Scientific Publications	No scientific publications yet.
Institution	Ophthalmology Department, Hospital Vall Hebron, Barcelona

Experimental Model 34	**Retinal toxicity after inner limiting membrane (ILM) dyeing**
Target Disease	Traction maculopathies
Species	Pig (*Sus scrofa domestica*)
Short Description	Vital dyes facilitate vitreoretinal surgery by visualizing nearly transparent structures such the inner limiting membrane (ILM). It is beyond question that vital dyes being injected into human eye need to be safe and potential toxic effects have to be ruled out in advance. To perform a long term toxicity study, after general anesthesia 3 animals were submitted to dark adaptation for 30 min for electroretinography test. Before surgery animals were ophthalmologic examined by indirect ophthalmoscopy and slit lamp to evaluate any abnormality. It was performed vitrectomy and injection of the new ILM dye in vitreous cavity of the right eye. Left eye was used as control. Retinal toxicity was assessed by ERG, histology and retinal ganglion cell quantification 7 days after dye administration.
Scientific Publications	No scientific publications yet.
Institution	Ophthalmology Department, Hospital Vall Hebron, Barcelona

Experimental Model 35	**Laser-induced choroidal neovascularization**
Target Disease	Wet Age-related macular degeneration (AMD), High myopia
Species	Rat (Rattus norvegicus), Mouse (Mus musculus)
Short Description	Laser-induced murine coroidal neovascularization (LI-CNV) model. Eyes are dilated with tropicamide 1% and 4-12 laser photocoagulation sites are placed around the optic disc to induce CNV. A diode laser (810 nm) is used and bubble formation confirmed the rupture of Bruch's membrane. The development of de CNV process is controlled by periodical fluorescein angiographies and the atrophic area is measured by CD31 flat mount immunofluorescence. This animal model can be used to evaluate the activity of anti or pro-angiogenic substances administrated by intravitreal, subretinal or endovenous injections or oral administration.
Scientific Publications	- Recalde S, Zarranz-Ventura J, Fernández-Robredo P, García-Gómez PJ, Salinas-Alamán A, Borrás-Cuesta F, Dotor J, García-Layana A. **Transforming growth factor-β inhibition decreases diode laser-induced choroidal neovascularization development in rats: P17 and P144 peptides**. Invest Ophthalmol Vis Sci. 2011; 9;52(10):7090-7. - García-Layana A, Vásquez G, Salinas-Alamán A, Moreno-Montañés J, Recalde S, Fernández-Robredo P. **Development of laser-induced choroidal neovascularization in rats after retinal damage by sodium iodate injection**. Ophthalmic Res. 2009; 42(4):205-12.
Institution	University of Navarra, Pamplona

Experimental Model 36	**Factor H (CFH) and Apolipoprotein E (apoE) deficiency (C57BL6/J)**
Target Disease	Retinal degeneration
Species	Mouse (Mus musculus): C57BL6/J
Short Description	Animals deficient or partially deficient for complement factor H (CFH) and Apolipoprotein E (apoE) genes are models of retinal degeneration. All the animals are C57BL6/J background. These animals presented retinal alteration such as large amount of vacuoles, Bruch's membrane (BM) is swollen and deposits are found between BM and RPE. We are studying apoE-/-/ CFH -/-, apoE+/- /CFH+/- and apoE-/-/CFH+/- genotypes. These animal models could be used to study de influence of CFH and the complement system in retinal degeneration. Another application could be the combination of these mice with coroidal neovascularization process (i.e.) to investigate the implication of these genes in the development of wet AMD from the dry form of the pathology.
Scientific Publications	- Garcia-Garcia L, Fernandez-Robredo P, Recalde S, Moreno-Orduña M, Fernandez-Garcia V, Caire J, Marchena-Fernandez MA, de la Villa P, Garcia-Layana A. **Effects Of The Absence Of both Complement Factor H And Apolipoprotein E On Retinal Morphology In Mice**. ARVO May 2012.
Institution	University of Navarra, Pamplona

Experimental Model 37	**Choroidal neovascularisation**
Target Disease	Age-related Macular Degeneration, Diabetic retinopathy, High Myopia
Species	Rat (Rattus norvegicus), Mouse (Mus musculus)
Short Description	Choroidal neovascularization (CNV) refers to the formation of new vessels in the subretinal space that arise from the choriocapillaris extending through Bruch's membrane (BM) and into the subretinal space, the sub-retinal pigment epithelium (RPE), or both. The CNV destroys the macula, resulting in visual loss. CNV is most commonly diagnosed as an advanced stage of age-related macular degeneration (ARMD), ocular histoplasmosis, myopic degeneration, or choroidal rupture, and is one of the major causes of visual loss in Western countries. Laser is delivered through a slit lamp, with a handled coverslip (22 x 30 mm) serving as a contact lens and using gonioscopic contact solution. A pattern of 8 to 12 lesions was concentrically placed at approximately equal distance around the optic nerve. The purpose is to disrupt Bruch's membrane (BM), which is clinically evident by acute vapour bubble formation, with or without intraretinal or subretinal haemorrhage.
Scientific Publications	- Zarranz-Ventura J, Fernández-Robredo P, Recalde S, Salinas-Alamán A, Borrás-Cuesta F, Dotor J, García-Layana A. **Transforming growth factor-beta inhibition reduces progression of early choroidal neovascularization lesions in rats: P17 and P144 peptides**. PLoS One. 2013 May 31;8(5):e65434. doi: 10.1371/journal.pone.0065434. Print 2013. - Recalde S, Zarranz-Ventura J, Fernández-Robredo P, García-Gómez PJ, Salinas-Alamán A, Borrás-Cuesta F, Dotor J, García-Layana A. **Transforming growth factor-β inhibition decreases diode laser-induced choroidal neovascularization development in rats: P17 and P144 peptides**. Invest Ophthalmol Vis Sci. 2011;52(10):7090-7.
Institution	University of Navarra, Pamplona

Experimental Model 38	**Corneal neovascularisation**
Target Disease	Angiogenesis-related diseases
Species	Rat (Rattus norvegicus), Mouse (Mus musculus)
Short Description	The corneal neovascularisation model is useful to investigate antiangiogenic therapies, since cornea is an avascular tissue. It will be assessed by chemical damage and topical treatment administration of the compounds to be tested.
Scientific Publications	Currently there are no publications from the group with regards to this model; however, we are in the process of writing some data.
Institution	University of Navarra, Pamplona

Experimental Model 39	**Light induced retinal damage**
Target Disease	Age-related Macular Degeneration
Species	Rabbit (*Oryctolagus cuniculus*)
Short Description	Photochemical damage occurs after an exposure to high energy radiation within the visible spectrum of light, causing morphological changes in the retina and the formation of superoxide anion. In our laboratory we are able to create a model of phototoxicity in rabbits. Animals are exposed to a light source for 120 minutes and then immediately sacrificed or one week after exposure. We demonstrated that light damage produces an increase in retinal oxidative stress immediately after light exposure that decreases one week after exposure. However, some morphological alterations appear days after light exposure including apoptotic phenomena. This model may be useful in the future to study the protective effect of antioxidant substances or new intraocular lenses with yellow filters.
Scientific Publications	- Saenz-de-Viteri M, Heras-Mulero H, Fernández-Robredo P, Recalde S, Hernández M, Reiter N, Moreno-Orduña M, García-Layana A. Oxidative stress and histological changes in a model of retinal phototoxicity in rabbits. Oxid Med Cell Longev. 2014;2014:637137. Epub 2014 May 27
Institution	University of Navarra, Pamplona

Experimental Model 40	**Acute conjunctivitis**
Target Disease	Conjunctival inflammation
Species	Rat (Rattus norvegicus)
Short Description	Conjunctivitis is an inflammatory process of the conjunctiva and eyelids, and is usually associated with viral or bacterial infection. We can reproduce the acute conjunctivitis models by subconjunctival LPS injection.
Scientific Publications	- Fernandez-Robredo P, Recalde S, Moreno-Orduña M, García-García L, Zarranz-Ventura J, García-Layana A. **Azithromycin reduces inflammation in a rat model of acute conjunctivitis.** Mol Vis. 2013;19:153-65. Epub 2013 Jan 28.
Institution	University of Navarra, Pamplona

Experimental Model 41	**Retinitis pigmentosa model (P23H-1)**
Target Disease	Retinitis pigmentosa
Species	Rat (*Rattus norvegicus*): P23H line 1
Short Description (max. 250 words)	Autosomal Dominant Model of RP degeneration, discover by Mat Lavail, at the University of California, San Francisco, USA. The rat shows a progressive loss of rod photoreceptors with 40% diminish ONL at the first month.
Scientific Publications	- La Vail MM. **Survival of some photoreceptor cells in albino rats following long term exposure to continuous light**. Invest Ophthalmol 1976; 15:64-70. - Cuenca N, Pinilla I, Sauvé Y, Wang S, Lu B, Lund RD. **Regressive and reactive changes in the connectivity patterns of rod and cone pathways of P23H transgenic rat retina**. Neuroscience 2004; 127: 301-317. - Pinilla I, Sauvé Y, Lund RD. **Enhanced cone dysfunction in rat homozygous for the P23H rhodopsin mutation**. Neuroscience Letters 2005; 382: 16-21.
Institution	IIS Aragón (Aragon Research Institute), LIM (Laboratorio de Investigación Molecular), Zaragoza

Experimental Model 42	**Retinal degeneration model (Rd10)**
Target Disease	Photoreceptors Degeneration
Species	Mouse (*Mus musculus*): Rd10
Short Description (max. 250 words)	Rd10 mouse model of retinal degeneration. This animal consists on a mouse model of heredodegenerative disease. The animal express a mutation in the gene of the beta subunit of 6 phosphodiesterase, which determines a progressive loss of rod photoreceptor starting at 25 postnatal day (p25). By p40 more that 95 % of retinal photoreceptors have degenerated.
Scientific Publications	- Barhoum, R. Martínez-Navarrete, G; Corrochano, S.; Germain, F.; de la Rosa, E.J.; de la Villa, P. & Cuenca, N. **Time course of functional and structural modifications during retinal degeneration in the rd10 mouse**. Neuroscience, 155 :698-713 (2008)
Institution	Universidad de Alcala, Madrid (Hospital General de Guadalajara, Guadalajara)

Experimental Model 43	**Achromatopsia model**
Target Disease	Dysfunction of cone photoreceptors (achromatopsia)
Species	Mouse (*Mus musculus*)
Short Description (max. 250 words)	Gnat Mouse model of retinal disease. This animal consists on a mouse model of achromatopisa. The animal express a mutation in the gene the cone transducin, which determines an absolute lack of cone response.
Patent	- Patent: Zurita Redondo, E.; Montoliu José, L.; Fernandez López, A.; de la Villa Polo P.; Gonzalez-Neira, A.; Benitez Ortiz J. **Nuevo Modelo Animal de Acromatopsia**. P201231296. Spain. *Consejo Superior de Investigaciones Científicas, Universidad de Alcalá* and *Centro Nacional de Investigaciones Oncológicas, CIBER-Enfermedades Raras*.
Institution	Universidad de Alcala, Madrid (Hospital General de Guadalajara, Guadalajara)

Experimental Model 44	**Retinal degeneration by excitatory aminoacids intraocular injection**
Target Disease	Induced Retinal Degeneration
Species	Mouse (*Mus musculus*)
Short Description	Mouse model of retinal disease caused by excitotoxicity. This animal consists on a mouse model of retinal degeneration that includes cells of the inner retina. The disease is induced by eye injection of a mixture of excitatory aminoacid agonists. A significant loss of inner retinal cells is accounted according to the drug concentration. The degree of functional response of the retina and inner retinal degeneration is addressed by electrophysiological and structural methods.
Scientific Publications/ Patent	In progress
Institution	Universidad de Alcala, Madrid (Hospital General de Guadalajara, Guadalajara)

Experimental Model 45	**Experimental Glaucoma (DBA/2J mouse)**
Target Disease	Glaucoma
Species	Mouse (*Mus musculus*): DBA/2J
Short Description	DBA/2J mice develop progressive eye abnormalities that closely mimic human hereditary glaucoma. Defects include iris pigment dispersion, iris atrophy, anterior synechia (adhesion of the iris to the cornea), and elevated intraocularpressure (IOP). The onset of disease symptoms begins between three and four months of age with 56% of females and 15% of males showing signs of iris pigment epithelium loss and transillumination of the peripheral iris. By six to seven months of age, all mice demonstrate significant widespread transillumination and thickening of the iris border. Elevation of IOP is evident in some females by six months of age. By nine months of age, both sexes exhibit elevated IOP, with pressures higher in females (mean: 20.3 +/-79; 1.8 mmHg) compared to males (mean: 16.2 +/-79; 1.4 mmHg). Retinal histopathology reveals retinal ganglion cell, as well as GABAergic and cholinergic amacrine cell, loss. (Moon JI et al. 2005). Two alleles contribute to the eye phenotype, GpnmbR150X and Tyrp1isa; both are present in DBA/2J mice.
Scientific Publications	- Fernández-Sánchez L, Pérez de Sevilla-Müller L, Brecha N, Cuenca N. **Outer retinal degeneration in a DBA/2J mice, a model of ocular hypertension**. Póster. Sociedad de Investigación en Retina y Ciencias de la Visión (SIRCOVA) 2013.Valencia, Spain. - Fernández-Sánchez L, Perez De Sevilla-Müller L, Brecha N, Cuenca N. **Morphological and Functional Study of Retinal Astrocytes in DBA/2J Mice**. Póster 54:5096. Association for Research in Vision and Ophthalmology (ARVO) 2013. Seattle, Washington (USA). - Fernández-Sánchez L, de Sevilla Müller LP, Brecha NC, Cuenca N. **Loss of outer retinal neurons and circuitry alterations in the DBA/2J mouse**. Invest Ophthalmol Vis Sci 2014;55(9):6059-6072.
Institution	University of Alicante, Alicante

Experimental Model 46	**Ischemia-reperfusion**
Target Disease	Ocular hypertension
Species	Mouse (*Mus musculus)*
Short Description	The retinal ischemia-reperfusion model is used in the study of transient ischemia-related diseases, such as central retinal artery occlusion, angle-closure glaucoma, and others. The method for experimentally producing an ischemia-reperfusion model in the mouse retina is based on the augmentation of intraocular pressure by increasing the height of the infusion bottle connected with the needle in the anterior chamber.
Scientific Publications	- Fernández-Sánchez L., Ambrosio A., Martins T., Cuenca N. **Efecto neuroprotector del TUDCA en un modelo de muerte de células ganglionares inducida por isquemia-reperfusión**. Presentación oral XXV Congreso GENN 2014. Cullera, Valencia. - Qi Y, Zhao M, Bai Y, Huang L, Yu W, Bian Z, Zhao M, Li X. **Retinal ischemia/ reperfusion injury is mediated by Toll-like receptor 4 activation of NLRP3 inflammasomes**. Invest Ophthalmol Vis Sci 2014;55(9):5466-5475. - Gao S, Andreeva K, Cooper NG. **Ischemia-reperfusion injury of the retina is linked to necroptosis via the ERK1/2-RIP3 pathway**. Mol Vis 2014;20:1374-1387. - Fang IM, Yang CM, Yang CH. **Chitosan oligosaccharides prevented retinal ischemia and reperfusion injury via reduced oxidative stress and inflammation in rats**. Exp Eye Res 2015;130:38-50.
Institution	University of Alicante, Alicante

Experimental Model 47	**Retinitis pigmentosa (P23H-3)**
Target Disease	Autosomal Dominant Retinitis Pigmentosa (adRP)
Species	Rat (*Rattus norvegicus*): P23H line 3
Short Description	Mutant rhodopsin transgenic rat model of autosomal dominant retinitis pigmentosa. The P23H mutation is a substitution of proline by histidine at position 23 of the rhodopsin amino acid sequence, is the most frequent cause of RP in United States. In P23H rats, this mutation causes a misfolded rhodopsin protein variant that accumulates in the endoplasmic reticulum, which results in rod photoreceptor cell death.
Scientific Publications	- Noailles A, Fernández-Sánchez L, Lax P, Cuenca N. **Microglia activation in a model of retinal degeneration and TUDCA neuroprotective effects**. J Neuroinflammation 2014;11:186. - Fernández-Sánchez L, Lax P, Pinilla I, Martín-Nieto J, Cuenca N. **Tauroursodeoxycholic acid (TUDCA) prevent retinal degeneration in transgenic P23H rats**. Invest Ophthalmol Vis Sci. 2011; 52:4998-5008 - Fernández-Sánchez L, Lax P, Esquiva G, Martín-Nieto J, Pinilla I, Cuenca N. **Safranal, a saffron constituent, attenuates retinal degeneration in P23H rats**. PLoS One. 2012; 7(8):e43074
Institution	University of Alicante, Alicante

Experimental Model 48	**Retinitis pigmentosa (Rd10)**
Target Disease	Autosomal Recessive Retinitis Pigmentosa (arRP)
Species	Mouse (*Mus musculus*): Rd10
Short Description	The retinal degeneration 10 (Rd10) mouse is a model of autosomal recessive retinitis pigmentosa that carries a spontaneous mutation in exon 13 of the gene encoding the beta subunit of rod cGMP phosphodiesterase. This mutation leads to massive apoptotic death of photoreceptors in the early postnatal period. Morphological and physiological studies have shown that the rd10 mouse may be a good model to assess rescue approaches, since photoreceptor degeneration starts around P18 and, therefore, does not overlap with the period of postnatal retinal maturation. The timecourse of photoreceptor apoptosis peaks around P25 and is followed by a secondary degeneration of bipolar and horizontal cells, which are postsynaptic to degenerated rods. Indeed, electroretinogram studies have revealed that the alteration in the physiology of the inner retina parallels the structural degeneration.
Scientific Publications	- Barhoum R; Martínez-Navarrete G.C; Corrochano S; Germain F; Fernandez-Sanchez. L; De La Rosa E.J; De La Villa P; Cuenca N. **Functional and structural modifications and time course of retinal degeneration in the rd10 mouse**. Neuroscience. 2008; 155:698-713. - Pang JJ, Dai X, Boye SE, Barone I, Boye SL, Mao S, Everhart D, Dinculescu A, Liu L, Umino Y, Lei B, Chang B, Barlow R, Strettoi E, Hauswirth WW. **Long-term retinal function and structure rescue using capsid mutant AAV8 vector in the rd10 mouse, a model of recessive retinitis pigmentosa**. Mol Ther. 201; 19(2):234-42.
Institution	University of Alicante, Alicante

Experimental Model 49	**Retinal cells signalling pathways and efficacy/toxicity of compounds (661W, RGC5, R28 and MU-PH1 cell lines)**
Target Disease	Neurodegenerative retinal diseases AMD, Glaucoma, diabetic retinopathy.
Species	Mouse (*Mus musculus*): cell lines 661W and MU-PH1 Rat (*Rattus norvegicus*): cell lines RGC5 and R28
Short Description	Cone photoreceptor-derived cell line 661W was isolated from a retinal tumor of transgenic mice, expressing the SV40 T-antigen under the control of the photoreceptor specific promoter IRBP. This cell line homogenously expresses several cone markers including opsins, transducin and arrestin, and lacks rod-specific protein markers. The signalling pathways activated in 661W cells, in response to apoptosis, have been widely characterized and they have been successfully used as a high throughput screening tool in the search of new neuroprotective compunds for retinal dystrophies. R28 cell line was immortalized with the 12S portion of the E1A gene, as it contains the immortalizing but not the transforming functions of the gene. This cell line expresses several photoreceptor markers (IRBP, S-Ag and recoverin), as well as glial markers (vimentin). It has been used in many studies as a tool to evaluate the efficacy of different drugs in relation to retinal diseases. MU-PH1 is a novel spontaneously immortalized, Müller-derived cell line obtained from adult mice retina. Under standard culture conditions these cells demonstrated an epithelioid-like morphology and were able to form neurospheres. MU-PH1 cells express the Müller cell marker vimentin, and several neural and stem cell markers (nestin, Abcg2, alpha-tubulin, beta-III-tubulin and Ascl1). MU-PH1 cells also express stably the photoreceptor markers transducin, blue opsin and red/green opsin.
Scientific Publications	- Tan E, Ding XQ, Saadi A, Agarwal N, Naash MI, Al-Ubaidi MR. **Expression of cone-photoreceptor-specific antigens in a cell line derived from retinal tumors in transgenic mice**. Invest Ophthalmol Vis Sci. 2004;45(3):764-768. - Seigel GM, Mutchler AL, Adamus G, Imperato-Kalmar EL. **Recoverin expression in the R28 retinal precursor cell line.** In Vitro Cell Dev Biol Anim. 1997; 33(7):499-502 - Gómez-Vicente V, Flores A, Lax P, Murciano C, Yáñez A, Gil MI, Cuenca N, Gozalbo D, Maneu V. **Characterization of a new murine retinal cell line (MU-PH1) with glial, progenitor and photoreceptor characteristics**. Exp Eye Res. 2013
Institution	University of Alicante, Alicante

Experimental Model 50	**Rotenone and MPTP-induced Parkinson disease**
Target Disease	Parkinson disease
Species	Rat (*Rattus norvegicus*)
Short Description	The 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) neurotoxin induce the selective destruction of dopaminergic neurons, in brain and retina, reproducing the main symptoms of Parkinson disease in these tissues. It is also well-know that a wide variety of pesticides and metals, such as rotenone, aluminum and copper origin the main features of parkinsonism. Rotenone is dissolved in sterile natural oil Miglyol 812 at 3 mg/ml and administered subcutaneously every 48 h at 3 mg/kg for 60 days.
Scientific Publications	- Lax P, Esquiva G, Esteve-Rudd J, Otalora BB, Madrid JA, Cuenca N. **Circadian dysfunction in a rotenone-induced parkinsonian rodent model**. Chronobiol Int 2012;29(2):147-156. - Esteve-Rudd J, Fernández-Sánchez L, Lax P, De Juan E, Martín-Nieto J, Cuenca N. **Rotenone induces degeneration of photoreceptors and impairs the dopaminergic system in the rat retina**. Neurobiol Dis 2011; 44(1):102-115. - Ferro MM, Bellissimo MI, Anselmo-Franci JA, Angellucci ME, Canteras NS, Da Cunha C. **Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: histological, neurochemical, motor and memory alterations**. J Neurosci Methods 2005;148(1):78-87. - Cuenca N, Herrero MT, Angulo A, de Juan E, Martínez-Navarrete GC, López S, Barcia C, Martín-Nieto J. **Morphological impairments in retinal neurons of the scotopic visual pathway in a monkey model of Parkinson's disease**. J Comp Neurol 2005;493(2):261-273.
Institution	University of Alicante, Alicante

Experimental Model 51	**Achondroplasia (RCJ3.1C5.18 cell line)**
Target Disease	Achondroplasia
Species	Rat (*Rattus norvegicus*): RCJ3.1C5.18 cell line
Short Description	RCJ3.1C5.18 cells, a chondrogenic precursor cell line derived from multipotential mesenchymal rat stem cells were transfected with full-length human mutant FGF receptor type 3, ACH FGF receptor type 3 (FGF receptor type 3G380R). Expression of FGF receptor type 3 was regulated by a tetracycline suppression system, the receptor is expressed in the absence of tetracycline in the culture medium. We have tested the ability of pyridoxal-5'-phosphate-6-azophenyl-2', 4'-disulfonate (PPADS) to decrease the overactivation and signalling of FGF receptor type 3 in achondroplasic chondrocytes. PPADS reduced the tyrosine phosphorylation of FGF receptor type 3 triggered by fibroblast growth factor 9 (FGF9) (50% reduction), as well as the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) pathway. As a consequence of this inhibitory effect on ERK1/2 activity the loss of extracellular matrix was also reversed by PPADS. The action of PPADS seems to be due to a mechanism independent of P2 receptor antagonism.
Scientific Publications	- Guzmán-Aránguez A, Crooke A, Yayon A, Pintor J. **Effect of PPADS on achondroplasic chondrocytes: Inhibition of FGF receptor type 3 over-activity**. European Journal of Pharmacology. 2008; 584:72–77
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 52	**Achondroplasia (Ach G380 cell line)**
Target Disease	Achondroplasia
Species	Human Ach (G380R) cell line
Short Description	Immortalized human chondrocytes carrying the heterozygous achondroplasia mutation (G380R)
Scientific Publications	- Guzmán-Aránguez A, Legeai Mallet L, Pintor J. **Fibroblast growth factor receptor 3 inhibition by small interfering RNAs in achondroplasia** Anales de la Real Academia de Farmacia. 2011;77(1)
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 53	**Achondroplasia (B6; 129s-Fgfr3tm1Dor/J)**
Target Disease	Achondroplasia
Species	Mouse (Mus musculus): B6; 129s-Fgfr3tm1Dor/J
Short Description	Approximately 50% of mice that are homozygous for the Fgfr3 targeted mutation die between birth and 21 days of age. Surviving pups may live as long as 8 months. RNAase protection analysis of adult homozygous brain tissue indicates that an abnormal transcript may be generated, but that no functional protein results. Severe skeletal defects (kyphosis, scoliosis, crooked tails, curvature and overgrowth of long bones) are evident. Inner ear defects (lack of pillar cell differentiation and tunnel of Corti formation) resulting in profound deafness are also observed.
Scientific Publications	
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 54	**Alzheimer's disease (B6C3-Tg(APPswe,PSEN1dE9)85Dbo/J)**
Target Disease	Alzheimer's
Species	Mouse (*Mus musculus*): B6C3-Tg(APPswe,PSEN1dE9)85Dbo/J
Short Description	Double transgenic mice express a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) both directed to CNS neurons. Both mutations are associated with early-onset Alzheimer's disease. The "humanized" Mo/HuAPP695swe transgene allows the mice to secrete a human A-beta peptide. Both the transgenic peptide and holoprotein can be detected by antibodies specific for human sequence within this region (Signet Laboratories' monoclonal 6E10 antibody). The included Swedish mutations (K595N/M596L) elevate the amount of A-beta produced from the transgene by favoring processing through the beta-secretase pathway. This "humanized" Mo/HuAPP695swe protein is immunodetected in whole brain protein homogenates. The transgenic mutant human presenilin protein (PS1-dE9), which in high levels displaces detectable endogenous mouse protein, is also immunodetected in whole brain protein homogenates. The donating investigator reports that transgenic mice develop beta-amyloid deposits in brain by six to seven months of age.
Scientific Publications	- Bakalash S; Pham M; Koronyo Y; Salumbides BC; Kramerov A; Seidenberg H; Berel D; Black KL; Koronyo-Hamaoui M. **Egr1 expression is induced following glatiramer acetate immunotherapy in rodent models of glaucoma and Alzheimer's disease**. Invest Ophthalmol Vis Sci. 2011; 52(12): 9033-46. - Reiserer RS; Harrison FE; Syverud DC; McDonald MP. **Impaired spatial learning in the APP + PSEN1DeltaE9 bigenic mouse model of Alzheimer's disease**. Genes Brain Behav. 2007; 6(1):54-65.
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 55	**Experimental corneal wound healing**
Target Disease	Corneal wound healing
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description	Corneal wounds were made in both eyes by anaesthetising the animals. After topical anaesthesia with oxibuprocaine and tetracaine corneal wounds were made to the epithelia of both eyes by applying a 3-mm disc of Whatman no. 1 paper soaked in n-heptanol. Discs were placed in the centre of the cornea and left there for 30 s (Cintron et al., 1979). Eyes were washed repeatedly with isotonic saline solution. The wounds were stained with 2% fluorescein and eyes were examined with a slit lamp. Images were taken, managed and analysed with apropriated software.
Scientific Publications	- Pintor J, Bautista A, Carracedo G, Peral A. **UTP and diadenosine tetraphosphate accelerate wound healing in the rabbit cornea**. Ophthalmic and Physiological Optics. 2004; 24: 186–193 - Mediero A, Guzmán-Aranguez A, Crooke A, Peral A, Pintor J. **Corneal re-epithelialization stimulated by diadenosine polyphosphates recruits RhoA/ROCK and ERK1/2 pathways**. Invest Ophthalmol Vis Sci. 2008; 49(11):4982-92
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 56	**Corneal wound healing (Rabbit corneal cell line)**
Target Disease	Corneal wound healing
Species	Rabbit (*Oryctolagus cuniculus*): Corneal cell line, SIRC (Statens Seruminstitut Rabbit Cornea)
Short Description	Rabbit *$P2Y_2$-R* cDNA was cloned using a combination of degenerate reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE). To test the efficacy of synthesized siRNAs targeting *$P2Y_2$-R*, immunocytochemistry, immunohistochemistry, and quantitative RT-PCR (qRT–PCR) assays were performed. Migration assays were performed both in vitro and in vivo by wounding the epithelium with a pipette tip and n-heptanol, respectively. These wounds were performed 72 h after siRNA transfection either in the presence or the absence of the $P2Y_2$ agonist, 100 μM Ap_4A (diadenosine tetraphosphate).
Scientific Publications	- Crooke A, Mediero A, Guzmán-Aránguez A, Pintor J. **Silencing of P2Y2 receptor delays Ap4A-corneal re-epithelialization process**. Mol Vis. 2009; 15: 1169–1178.
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 57	**Experimental Glaucoma**
Target Disease	Glaucoma (IOP)
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description	New Zealand white rabbits IOP modificacions measured by Tonopen XL after aplying substances like Adenosine tetraphosphate or antagonists of adrenoceptors and cholinoceptors, Ap4.
Scientific Publications	- Pintor J, Peláez T, Peral A. **Adenosine Tetraphosphate, Ap4, a Physiological Regulator of Intraocular Pressure in Normotensive Rabbit Eyes**. J Pharmacol Exp Ther. 2004 308:468-473
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 58	**Glaucoma (Rabbit non-pigmented ciliary epithelium cell line)**
Target Disease	Glaucoma (PIO)
Species	Rabbit (*Oryctolagus cuniculus*)
Short Description	We have demonstrated that 5-methoxycarbonylamino-N-acetyltryptamine (5-MCA-NAT), reduces intraocular pressure (IOP) in rabbits. In addition, we have reported a link between hypotensive effect of 5-MCA-NAT and sympathetic nervous system. Moreover, it is known that aqueous humour production is controlled by the activation of adrenoceptors (ADRs) present in the ocular ciliary epithelium. Thus, the aim of this study is to investigate if the hypotensive effect of 5-MCA-NAT is due to a regulation of ciliary ADR genes expression. To confirm this we followed the effect of 5-MCA-NAT on rabbit IOP for 144 consecutive hours. A sustained IOP reduction for up to 72 h ($P<0.01$) was seen. In addition, changes in ADRB2 and ADRA2A mRNA were measured in cultured rabbit nonpigmented ciliary epithelial cells. After 5-MCA-NAT treatment, a significant downregulation of ADRB2 and upregulation of ADRA2A was observed. These results provide the regulation of ADRs mRNA by 5-MCA-NAT.
Scientific Publications	- Crooke A, Huete-Toral F, Martínez-Águila A, Alarma-Estrany P, Pintor J. **Regulation of ocular adrenoceptor genes expression by 5-MCA-NAT: implications for glaucoma treatment**. Pharmacogenetics and Genomics. 2011; 21(9):587-9
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 59	**Glaucoma (Human non-pigmented ciliary epithelium cell (HCE))**
Target Disease	Glaucoma
Species	Human
Short Description	The HCE immortalized NPE cell line was developed by M. Coca-Prados from primary cultures of human epithelium.
Scientific Publications	- Carré DA, Mitchell CH, Peterson-Yantoro K, Coca-Prados M, Civan MM. **Adenosine stimulates Cl- channels of non pigmented ciliary epithelial cells**. Cell Physiology 1997;273(4):1354-1361
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 60	**Glaucoma (Human cristaline cell (HCL))**
Target Disease	Glaucoma
Species	Human
Short Description	The HCL immortalized cell line was developed by M. Coca-Prados from primary cultures of human epithelium.
Scientific Publications	
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 61	**Huntington disease (Tg B6CBA-Tg(Hdexon1)61Gpb/J)**
Target Disease	Huntington disease
Species	Mouse (Mus musculus): Tg B6CBA-Tg(Hdexon1)61Gpb/J
Short Description	Mice have been generated that are transgenic for the 5' end of the human HD gene carrying (CAG)115-(CAG)150 repeat expansions. In both the 61Gpb and 62Gpb founder lines, the transgene is ubiquitously expressed. Transgenic mice exhibit a progressive neurological phenotype that mimics many of the features of HD, including choreiform-like movements, involuntary stereotypic movements, tremor, and epileptic seizures, as well as nonmovement disorder components, including unusual vocalization. They urinate frequently and exhibit loss of body weight and muscle bulk through the course of the disease. Neurologically they develop Neuronal Intranuclear Inclusions (NII) which contain both the huntingtin and ubiquitin proteins. These NII have also been identified in human HD patients. The age of onset of HD symptoms is reported to occur between 15 and 21 weeks for the 61Gpb line and between nine and 11 weeks for the 62Gpb line.
Scientific Publications	-Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW, Bates GP. **Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice**. Cell 1996;87:493-506. -Gimenez E, Montoliu L. **A simple polymerase chain reaction assay for genotyping the retinal degeneration mutation (Pdeb(rd1)) in FVB/N-derived transgenic mice**. Lab Anim 2001;35:153-156
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 62	**Ocular Infection (Inmortalized Human Corneal Limbal Epithelial Cells (HCLEC))**
Target Disease	Ocular Infection
Species	Human
Short Description	We utilized a stable tetracycline-inducible RNA interfering system targeting the core 1 ß1,3-galactosyltransferase (C1galt1 or T-synthase), a critical galactosyltransferase required for the synthesis of core 1 O-glycans, to explore the role of mucin-type carbohydrates in apical endocytic trafficking in human corneal keratinocytes. Using cell surface biotinylation and subcellular fractionation, we found increased accumulation of plasma membrane protein in endosomes after C1galt1 depletion. Confocal laser scanning microscopy and fluorometry revealed increased translocation of negatively charged fluorescent nanospheres after C1galt1 knockdown sustained by an active transport process and largely independent of apical intercellular junctions. Internalization of nanospheres could be blocked by dynasore, nocodazole, chlorpromazine, and hyperosmotic sucrose, suggesting a mechanism for clathrin-coated pit budding and vesicular trafficking. This possibility was supported by experiments showing nanosphere colocalization with clathrin heavy chain in the cytoplasm.
Scientific Publications	- Guzman-Aranguez A, Woodward AM, Pintor J, Argüeso P. **Targeted Disruption of Core 1 b1,3-galactosyltransferase (C1galt1) Induces Apical Endocytic Trafficking in Human Corneal Keratinocytes**. PLoS ONE. 2012; 7(5): e36628. doi:10.1371/journal.pone.0036628
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 63	**Ocular infection (Immortalized Human Conjunctival Epithelial Cell Lines (HCjE))**
Target Disease	Ocular infection
Species	Human
Short Description (max. 250 words)	The immortalized conjunctival (HCjE) cell lines exhibit the mucin gene expression repertoire of their native epithelia. These cell lines will be useful in determining regulation of ocular surface mucin gene expression and, potentially, goblet cell differentiation.
Scientific Publications	- Gipson IK, Spurr-Michaud S, Argüeso P, Tisdale A, Ng TF, Russo CL. **Mucin Gene Expression in Immortalized Human Corneal–Limbal and Conjunctival Epithelial Cell Lines**. Invest Ophthalmol Vis Sci. 2003; 44(6)
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 64	**Retinal ganglion cells (RGC) degeneration (DBA/2J)**
Target Disease	Degeneration of retinal ganglion cells
Species	Mouse (*Mus musculus*): DBA/2J
Short Description	DBA/2J is a widely used inbred strain that is valuable in a large number of research areas, including cardiovascular biology, neurobiology, and sensorineural research. Aging DBA/2J mice develop progressive eye abnormalities that closely mimic human hereditary glaucoma. Defects include iris pigment dispersion, iris atrophy, anterior synechia (adhesion of the iris to the cornea), and elevated intraocularpressure (IOP). The onset of disease symptoms begins between three and four months of age with 56% of females and 15% of males showing signs of iris pigment epithelium loss and transillumination of the peripheral iris. By six to seven months of age, all mice demonstrate significant widespread transillumination and thickening of the iris border. Elevation of IOP is evident in some females by six months of age. By nine months of age, both sexes exhibit elevated IOP, with pressures higher in females (mean: 20.3 +/-79; 1.8 mmHg) compared to males (mean: 16.2 +/-79; 1.4 mmHg). Retinal histopathology reveals retinal ganglion cell, as well as GABAergic and cholinergic amacrine cell, loss. (Moon JI *et al*. 2005). Two alleles contribute to the eye phenotype, *GpnmbR150X* and *Tyrp1isa*; both are present in DBA/2J mice.
Scientific Publications	- Anderson MG, Smith RS, Hawes NL, Zabaleta A, Chang B, Wiggs JL, John SWM. **Mutations in genes encoding melanosomal proteins cause pigmentary glaucoma in DBA/2J mice**. Nature Genetics. 2001; 30, 81-85 - John SWM, Smith RS, Savinova OV, Hawes NL, Chang B, Turnbull D, Roderick MDTH, Heckenlively JR. **Essential Iris Atrophy, Pigment Dispersion, and Glaucoma in DBA/2J Mice**. Invest Ophthalmol Vis Sci. 1998; 39(6) - Pérez de Lara MJ, Santano C, Guzmán-Aránguez A, Valiente-Soriano FJ, Avilés-Trigueros M, Vidal-Sanz M, de la Villa P, Pintor J. A**ssessment of inner retina dysfunction and progressive ganglion cell loss in a mouse model of glaucoma**. Exp Eye Res. 2014;122:40-9
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 65	**Retinal degeneration/Glaucoma (Ganglion cell line-RGC-5)**
Target Disease	Retinal degeneration and Glaucoma
Species	Rat (*Rattus norvegicus*): Transformed Rat Retinal Ganglion Cell Line
Short Description	Retinal cells were isolated from postnatal day 1 (PN1) rats and transformed with the c2 E1A virus. In order to isolate retinal ganglion cells (RGC), single cell clones were chosen at random from the transformed cells. Expression of Thy-1 (a marker for RGC), glial fibrillary acidic protein (GFAP, a positive marker for Muller cells), HPC-1/ syntaxin (a marker for amacrine cells), 8A1 (a marker for horizontal and ganglion cells) and neurotrophins was studied using reverse transcriptase-polymerase chain reaction (RT-PCR), immunoblotting and immunocytochemistry. One of the retinal cell clones, designated RGC-5, was positive for Thy-1, Brn-3C, Neuritin, NMDA receptor, GABA-B receptor, and synaptophysin expression and negative for GFAP, HPC-1, and 8A1, suggesting that it represented a putative RGC clone. The results of RT-PCR analysis were confirmed by immunocytochemistry for Thy-1 and GFAP. These cells may be valuable in understanding of retinal ganglion cell biology and physiology including in vitro manipulations in experimental models of glaucoma.
Scientific Publications	- Krishnamoorthy RR, Agarwal P, Prasanna G, Vopat K, Lambert W, Sheedlo HJ, Pang IH, Shade D, Wordinger RJ, Yorio T, Clark AF, Agarwal N. **Characterization of a transformed rat retinal ganglion cell line**. Molecular Brain Research. 2001; 86:1–12
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 66	**Retinal degeneration/Glaucoma (Retinal precursor cell line-R28)**
Target Disease	Retinal degeneration and Glaucoma
Species	Rat (*Rattus norvegicus*): Retinal precursor cell line-R28
Short Description	R28 is an adherent retinal precursor cell line derived from postnatal day 6 Sprague-Dawley rat retina immortalized with the 12S E1A gene of adenovirus. The 12S E1A gene was introduced via an incompetent retroviral vector; therefore, no infectious virus is produced by R28 cells. The cells have been passaged 200 times thus far, and show no signs of senescence.
Scientific Publications	- Krishnamoorthy RR, Agarwal P, Prasanna G, Vopat K, Lambert W, Sheedlo HJ, Pang IH, Shade D, Wordinger RJ, Yorio T, Clark AF, Agarwal N. **Characterization of a transformed rat retinal ganglion cell line**. Molecular Brain Research. 2001; 86:1–12 - Seigel GM, Mutchler AL, Adamus G, Imperato-Kalmar EL. **Recoverin expression in the R28 retinal precursor cell line**. In Vitro Cell Dev Biol 1997;33(7):499-502
Institution	Biochemistry and Molecular Biology IV Department, Faculty of Optics and Optometry, University Complutense of Madrid, Madrid

Experimental Model 67	**Experimental glaucoma**
Target Disease	Glaucoma
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description	Experimental glaucoma is produced by unilateral injection of 0.86 mg of α-chymotrypsin diluted in 0.13 of saline into the posterior chamber. This enzyme exerts effects on aqueous humor outflow routes and induces glaucoma in 82% of the animals. The animals show an IOP increase and glaucomatous cupping of optic disc. Elevated IOP is observed as soon as 72 h post injection and remain significantly higher than control 40 days after injection.
Scientific Publications	- Fernández R, Triviño A, Ramírez JM, García M, Ramírez AI, Salazar JJ, Fernández A, Gutkowska J. **Immunoreactive atrial natriuretic factor in aqueous humor: Its concentration is increased with high intraocular pressure in rabbit eyes**. Vision Res. 1990; 30: 1305-1310. - Fernandez R, Ramírez JM, Triviño A, Sánchez B, Paraiso P, García M, Ramírez AI, Salazar JJ, Fernández-Cruz A, Gutkowska J. **Experimental glaucoma significantly decreases atrial natriuretic factor (ANF) receptors in the ciliary proceses of the rabbit eye**. Exp Eye Res. 1991; 53: 591-596
Institution	Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, University Complutense of Madrid, Madrid

Experimental Model 68	**Chorioretinal ischemia induced by hypercholesterolemia**
Target Disease	Age-related macular degeneration (MAD), ischemia, atherosclerosis
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description	Laboratory rabbits have been widely used for the study of atherosclerosis for several reasons: they exhibit hypercholesterolemia within a few days of administration of a cholesterol-enriched diet and high sensitivity to the inducement of atheromatic lesions similar to human. The hypercholesterolemia is induced feeding the rabbits with a standard diet enriched with 0.5% cholesterol for 8 months. Weight and serum samples are recorded immediately before the experiment and monitored monthly thereafter. Serum values of total cholesterol, triglycerides, phospholipids, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL) are analyzed. Blood samples are taken from the marginal vein of the ear and analyzed with commercially available enzymatic kits following the manufacturer's instructions.
Scientific Publications	- Triviño A, Ramírez AI, Salazar JJ, De Hoz R, Rojas B, Padilla E, Tejerina T, Ramírez JM. **A cholesterol-enriched diet induces ultrastructural changes in retinal and macroglial rabbit cells**. Exp Eye Res. 2006; 83: 357-366 - Salazar JJ, Ramírez AI, De Hoz R, Ruiz E, Tejerina T, Triviño A, Ramírez JM. **Alterations in the choroid in hypercholesterolemic rabbits: reversibility after normalization of cholesterol levels**. Exp Eye Res. 2007; 84: 412-422. - Ramírez AI, Salazar JJ, De Hoz R, Rojas B, Ruiz E, Tejerina T, Ramírez JM, Triviño A. **Macroglial and retinal changes in hypercholesterolemic rabbits after normalization of cholesterol levels**. Exp Eye Res. 2006; 83: 1423-38. - Rojas B, Ramirez AI, Salazar JJ, de Hoz R, Redondo A, Raposo R, Mendez, MT, Tejerina MT, Triviño A, Ramirez JM. **Low-dosage statins reduce choroidal damage in hypercholesterolemic rabbits**. Acta Ophthalmol. 2011; 89: 660-669. - Salazar JJ, Ramírez AI**,** de Hoz R, **Rojas B,** Ruiz E, Tejerina T, Triviño A, Redondo AM, Ramírez JM. **A cholesterol-enriched diet induces ultrastructural changes in the retina and optic nerve of rabbits**. In*:* Glial Cells in Health and Disease (Proceedings of the The VIII European Meeting). Bologna; Medimond, eds. 2007; pages: 35-39. - Ramírez JM, Salazar JJ, de Hoz R, Rojas B, Gallego BI, Ramírez AI, Triviño A. **Choroidal vessel wall: hypercholesterolaemia-induced dysfunction and potential role of statins**. In: Current Basic and Pathological Approaches to the Function of Muscle Cells and Tissues - From Molecules to Humans. Haruo Sugi (Ed.) 2012; chapter 12, pages: 255-298. - Triviño A, de Hoz R, Rojas B**,** Gallego BI, Ramírez AI, Salazar JJ, Ramírez JM. **Effects of Hypercholesterolemia in the retina**. In: Ocular Diseases. Adedayo Adio (Ed.) 2012; chapter 1, pages: 1-42.

Institution	Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, University Complutense of Madrid, Madrid

Experimental Model 69	**Laser photocoagulation of the retina and choroid**
Target Disease	Sight-impairing retinal disorders, such as age-related macular degeneration (AMD), and advanced stages of this disease, including choroidal neovascular elements.
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description	Transpupillary photocoagulation is performed by diode laser emiting at a wavelength of 810nm. Laser parameters used are 200 µm spot size; 150 mW power and 0.2 sec exposure time. The laser can be applied as a single-pulse mode or as a micropulsed mode (frecuency 100 Hz).
Scientific Publications	- Triviño A, Ramírez JM, Andrés MV, Salazar JJ, Ramírez AI, García-Sánchez J. **Comparison between chorioretinal effects of the single-pulse versus micropulsed diode laser in pigmented rabbits**. Laser and Light 1997; 8: 31-38. - Gómez-Ulla F, Marín F, Ramírez JM, Triviño A. **La mácula senil**. Barcelona; Edika-Med S.A. 1993. 250 pages.
Institution	Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, University Complutense of Madrid, Madrid

Experimental Model 70	**Organotypic retinal cultures**
Target Disease	Retinal diseases
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	Organotypic cell cultures provide a convenient tool for studying the development of the CNS. They permit the manipulation of fluid and cell environments and are therefore an ideal complement to studies in vivo. Moreover, organotypic cultures have the advantage of retaining their cytoarchitecture, a condition that cannot be fulfilled by dissociated or reaggregate cell cultures. In addition, they are an useful tool to perform preclinical drug testing. Neonatal murine animals are quickly decapitated and the eyes are immediately enucleated under sterile conditions. Eyeballs are maintained in sterile medium enriched with glucose and after enzymatic digestion, the retina and the attached pigment epithelium are dissected. Following dissection, four radial cuts are made to flatten the tissue. The retina with the pigment epithelium are placed with the ganglion cell layer facing up into the upper compartment of a culture chamber containing sterile enriched medium in the lower compartment. The explants are maintained in an incubator with an atmosphere of 5% CO2, balanced air and 100% humidity at 37°C. The medium is changed the first day in culture and every second day thereafter. After days in vitro the cultures could be fixed for analysis under light microscopy or electron microscopy.
Scientific Publications	Ongoing
Institution	Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, University Complutense of Madrid, Madrid

Experimental Model 71	**Experimental glaucoma**
Target Disease	Glaucoma
Species	Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)
Short Description	The induction of unilateral ocular hypertension is achieved in anesthetized rats or mice by treating the animals in a single session with a series of diode laser burns. The laser beam is directly delivered without any lenses, aimed at the limbal and episcleral veins. The spot size, duration, and power are 50-100 μm, 0.5 s and 0.3 W, respectively. Each eye received between 55-76 burns. In rats, the IOP increases between 34 and 125% over the baseline within the first 72 h after lasering and peak at 12 h. At 1 and 2 weeks the IOP is approximately 35 and 39% over baseline. By three weeks the IOP starts to decline, acquiring close to normal levels at 12 weeks. In mice, a substantial increase of the IOP is evident 24h after lasering, this continuing for 4 days and then gradually returning to the basal value after the fifth day, so that by one week after lasering, the IOP values in the treated animals are comparable for both eyes.
Scientific Publications	- Ramírez AI, Salazar JJ, de Hoz R, Rojas B, Gallego BI, Salinas-Navarro M, Alarcón-Martínez L, Ortín-Martínez A, Avilés-Trigueros M, Vidal-Sanz M, Triviño A, Ramírez JM. **Quantification of the effect of different levels of IOP in the astroglia of the rat retina ipsilateral and contralateral to experimental glaucoma**. Invest Ophthalmol Vis Sci. 2010; 5:5690-5696. - Gallego BI, Salazar JJ, de Hoz R, Rojas B, Ramírez AI, Salinas-Navarro M, Ortín-Martínez A, Valiente-Soriano FJ, Avilés-Trigueros M, Villegas-Pérez MP, Vidal-Sanz M, Triviño A, Ramirez JM. **IOP induces upregulation of GFAP and MHC-II and microglia reactivity in mice retina contralateral to experimental glaucoma**. J Neuroinflammation. 2012; 9(1):92.
Institution	Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, University Complutense of Madrid, Madrid

Experimental Model 72	**Chronic Glaucoma induction by a series of intracamerular injections of hyaluronic acid (Wistar rat)**
Target Disease	Glaucoma
Species	Rat (*Ratus Norvergicus)*: wistar
Short Description	Wistar rats (aged 6 months) were weekly injected during 10 consecutive weeks in the anterior chamber with either high density hyaluronic acid (left eyes) or balanced salt solution (right eyes). Other rats were used as the controls. Intraocular pressure and electrorretinograms were performed. After euthanized, rat aqueous humor (AH) was collected in criotubes, while the retina and optic nerve were processed to morphologic, morphometric and immunohistochemic assays. Protons available in metabolites can be managed by high resolution nuclear magnetic resonance (1H-NMR) spectroscopy, to simultaneously quantify metabolites within biological fluids, according to their chemical shift. Metabolomic profile of the AH was assessed in this chronic glaucoma rat model to better understand biochemical mechanisms related to the AH homeostasis in normal and pathologic conditions. Microscopic data mimics glaucomatous retinal-optic nerve changes, validating our rat chronic glaucoma model. Furthermore, a database of 1H-NMR from various AH metabolites were obtained. Compounds identified as alanine, valine, lysine, leucine, tyrosine, fenilalanine, N-acetil glutamate, acetoacetate, glutamine, taurine, oxo-glutarate, glucose, VLDL cholesterol, and lactate correlated well with data from conventional techniques, but significantly different relative intensity values were found in the AH spectrums from the glaucoma eyes, as compared to both the sham-operated and control rats. With this rat chronic glaucoma model, a different metabolomic profile was detected in the aqueous humor.
Scientific Publications	- Mayordomo A, Lopez-Murcia M, Morales JM, Diaz-Llopis M, Pinazo-Durán MD, **Metabolomics of the aqueous humor in glaucomatous rats.** Ophthalmic Res 2013.
Institution	Ophthalmic Research Unit “Santiago Grisolía”, Faculty of Medicine, University of Valencia, Valencia

Experimental Model 73	**Proliferation, apoptosis and angiogenesis in ophthalmic diseases (C57BL/6 mouse)**
Target Disease	Retinopathies
Species	Mice (*Mus Musculus*): C57BL/6
Short Description	Adult C57BL/6 mice were genetically manipulated to obtain transgenic mice with two extra copies of p53 gene (super p53; sp53-tg/tg) by Prof. Manuel Serrano (National Center for Oncology Research; CNIO, Madrid). This model has been extensively used for ophthalmic studies by our research group. The p53 gene encodes for proteins that regulates cell cycle. Our main goal is to analyse the role of p53 gene in the molecular events involving retinal angiogenesis and/or apoptosis. Increased p53 gene dosage suggest a set of actions in mice retina mainly by regulating the sensitvitity to endogenous oxidative stress and by controlling the expression of molecules involved in cell proliferation, survival and death.
Scientific publications	- Gallego-Pinazo R, Zanón-Moreno V, Sanz S, Andrés V, Serrano M, García-Cao I, Pinazo-Durán MD. **Biochemical characterization of the optic nerve in mice overexpressing the P53 gen**. Oxidative stress assays. Arch Soc Esp Oftalmol. 2008;83:105-11. - Gallego-Pinazo R, Pinazo-Durán MD, Serrano M. **The cell cycle and gene p53. An approach to molecular ophthalmology**. Arch Soc Esp Oftalmol. 2010; 85:229-231. - Pinazo-Duran MD. **Genetics and something else**. Arch Soc Esp Oftalmol. 2012;87:35-37 - Marco-Ramirez C, Gallego-Pinazo R, Zanon-Moreno V, Garcia-Medina JJ, Galbis-Estrada C, Ramirez-Sebastian AI. **P53 gene protects the retina by regulating endogenous oxidative stress**. Ophthalmic Res. 2013 - Salazar JJ, Gallego-Pinazo R, De Hoz R, Rojas B, Serrano M, Pinazo-Duran MD, Ramirez-Sebastian JM. **Retinal astroglia display morphologic changes in the super p53 mice model**. Plos One 2013.
Institution	Ophthalmic Research Unit “Santiago Grisolía”, Faculty of Medicine, University of Valencia, Valencia

Experimental Model 74	**Neuroprotection and apolipoprotein E (apoE$^{-/-}$ mouse)**
Target Disease	Retinopathies and Neuropathies
Species	Mice (*Mus Musculus*): apoE$^{-/-}$
Short Description	To study the retinal astroglia by their intermediate filament protein in the apoE knock out (apoE-/-) mice. The apoE -/- mice and wild type counterparts eyes were processed to immunohistochemical and biochemical methods. Retinal wholemounts were immunostained with antibodies against GFAP. Retinal homogenates were processed to GFAP expression. Statistical processing was done by the SPSS 15.0 program. In comparison with naïve, astrocytes in apoE-/- mice showed several changes as follows: there was a significantly reduced astrocyte profiles density in the whole retinas. Moreover, the GFAP expression was significantly decreased; as shown by the immunohistochemical and immunoblotting assays. We are trying to address morphologic and molecular facts that help managing vitreo retinal disorders closely related to visual impairment. We speculate that apoE could enhance the protective effects of astrocytes on eye neurons and neurodegeneration disorders.
Scientific publications	- Lopez-Sanchez E, Frances-Muñoz E, Diez-Juan A, Andres V, Menezo JL, Pinazo-Duran MD. **Optic nerve alterations in apolipoprotein E deficient mice**. Eur J Ophthalmol. 2003; 13(6):560-5.
Institution	Ophthalmic Research Unit “Santiago Grisolía”, Faculty of Medicine, University of Valencia, Valencia

Experimental Model 75	**Rat model of glaucoma by intracamerular injections of sodium hyaluronate**
Target Disease	Glaucoma
Species	Rat (*Ratus Norvergicus)*: wistar
Short Description	Glaucoma models are helpful to study disease characteristics and to design new therapeutic options. Juvenile male Wistar rats experimental and controls are used for our glaucoma model, as follows: experimental rats receive weekly an intracamerular injection of 25 µl of sodium hyaluronate in the left eye and sterile saline solution in the right eye (sham operated), consecutively for ten weeks. Control rats were followed in parallel without manipulation. All rats were subjected to slitlamp anterior/posterior eye segment examinations, intraocular pressure (IOP), and flash electroretinograms (ERG). The aqueous humor is also collected at endpoints to be analyzed by Nuclear Magnetic Resonance. After euthanasia, eyeballs and the attached optic nerves were enucleated from all rats, and processed to histochemical and immunocytochemical analyses. Elevated IOP and significant reduction of a, b waves and amplitude of oscillatory potential was observed in the left eyes compared to control eyes. Histochemistry demonstrates retinal and optic nerve changes closely related to glaucoma descriptions. The aqueous humor metabolomic profile from control and experimental eyes were compared and concentrations of metabolites (amino acids, lipids and carbohydrates) significantly changed after the sodium hyaluronate injections series, compared to the sham-operated eyes. Metabolic changes in the hypertensive eyes correlated with the impaired retinal function. We have set up this experimental model of glaucoma in rats for further research.
Scientific publications	- Mayordomo-Febrer A, López-Murcia M, Morales-Tatay JM, Monleón-Salvado D, Pinazo-Durán MD. **Metabolomics of the aqueous humor in the rat glaucoma model induced by a series of intracamerular sodium hyaluronate injection**. Exp Eye Res. 2015;131:84-92.
Institution	Ophthalmic Research Unit “Santiago Grisolía”, Faculty of Medicine, University of Valencia, Valencia

Experimental Model 76	**Inflammation and immune response in retinal pigment epithelial cell cultures [immortalized human retinal pigment epithelial cell line (ARPE19)]: Immune aggression in vitro model**
Target Disease	Retinopathies
Species	Human: cell line (ARPE19)
Short Description	Discrimination of potential pathogens with the toll-like receptors (TLRs) is main objective of the innate immune system. This recognition leads to expression of genes such as inflammatory cytokines and co-stimulatory molecules. We deal with analyzing the pro-inflammatory capacity of cultured human retinal pigment epithelium (RPE) in response to TLRs challenge. A human RPE cell line (ARPE19) was established by trypsinization of a primary RPE culture resulting in a uniform cell population (displaying strong growth potential). We assessed pro-inflammatory TLRs-induced activation in these cells, by western-blot/immunoblotting, confocal microscopy and flow cytometry of conditioned media. PI cytokines expression-secretion is induced in RPE cultures. Unravelling the regulated activities of TLRs in RPE cells and its plasticity in immune response, new therapeutic avenues to control retinal inflammation may be revealed.
Scientific publications	- Puchades-Lanza L, Prieto-Chinchilla P, Pinazo-Durán MD, Jiménez-Santana P, Gallego-Pinazo R, Boscá-Gomar L. **Pro-inflammatory capacity of ARPE19 cells in response to toll like receptors challenge**. Ophthalmic Res 2013.
Institution	Ophthalmic Research Unit “Santiago Grisolía”, Faculty of Medicine, University of Valencia, Valencia

Experimental Model 77	**Development of the visual system**
Target Disease	Ocular Malformations
Species	Rat (*Ratus Norvergicus*)
Short Description	Rat model of Chronic Ethanol Intoxication. The foetal alcohol syndrome. Rat model of Chronic Pre- and Postnatal Exposure to drugs. We have been studying the ocular development in normal and pathologic conditions. Our goal was to analyse the retinal and optic nerve development by morphologic, morphometric and molecular approaches. We have used control rats to study by scanning, light and electron transmission microscopy the characteristics of the pre- and postnatal eye and optic nerve development at key stages (prenatal day 19 and 21; postnatal days 5, 7, 10 14. 21. 28). The retinal cells and layering were examined and several developmental parameters were analysed (retinal thickness, each retinal layer thickness, number of cell profiles, neuronal axonal and glial cell markers (Neurofilament antibody, Glial fibrillary acidic protein antibody, myelin basic protein antibody).

Scientific publications	- Pons-Vázquez S, Gallego-Pinazo R, Galbis-Estrada C, Zanon-Moreno V, Garcia-Medina JJ, Vila-Bou V, Sanz-Solana P, Pinazo-Durán MD. **Combined pre- and postnatal ethanol exposure in rats disturbs the myelination of optic axons**. Alcohol Alcohol. 2011;46(5):514-22. - Melo P, Zanon-Moreno V, Alves CJ, Magalhães A, Tavares MA, Pinazo-Duran MD, Moradas-Ferreira P. **Oxidative stress response in the adult rat retina and plasma after repeated administration of methamphetamine**. Neurochem Int. 2010;56(3):431-6. - Melo P, Pinazo-Durán MD, Salgado-Borges J, Tavares MA. **Correlation of axon size and myelin occupancy in rats prenatally exposed to methamphetamine**. Brain Res. 2008;1222:61-8. - Pons-Vázquez S, Vila-Bou V, Zanon-Moreno V, Iborra FJ, Gallego-Pinazo R, Melo PM, García-Medina JJ, Pinazo-Durán MD. **Study of apoptosis and mitosis mechanisms in the eye. Experimental model of gestational toxic syndrome**. Arch Soc Esp Oftalmol. 2008;83(1):37-44. - Pons S, Zanón-Moreno V, Melo P, Vila V, Gallego-Pinazo R, Pinazo-Durán MD. **Optic neuropathy induced by prenatal drug or alcohol exposure**. Arch Soc Esp Oftalmol. 2007;82(1):21-6. - Pinazo-Durán MD. **State-of-the-art in ocular malformations. Human and animal model studies**. Arch Soc Esp Oftalmol. 2002 Sep;77(9):473-6. - Melo P, Rodrigues LG, Pinazo-Durán MD, Tavares MA. **Methamphetamine and lipid peroxidation in the rat retina**. Birth Defects Res A Clin Mol Teratol. 2005;73(6):455-60. - Pinazo-Durán MD, Cervera R, Pons S, Zanón-Moreno VC, Gallego-Pinazo R, Guerri C. **Mechanisms of protein expression in the rat optic nerve. Modifications by alcohol exposure**. Arch Soc Esp Oftalmol. 2005;80(2): 99-104 - Melo P, Moreno VZ, Vázquez SP, Pinazo-Durán MD, Tavares MA. **Myelination changes in the rat optic nerve after prenatal exposure to methamphetamine**. Brain Res. 20061106(1):21-9. - Pons S, Zanón-Moreno V, Melo P, Vila V, Gallego-Pinazo R, Pinazo-Durán MD. **Optic neuropathy induced by prenatal drug or alcohol exposure**. Arch Soc Esp Oftalmol. 2007;82(1):21-6. - Strömland K, Pinazo-Durán MD. **Ophthalmic involvement in the fetal alcohol syndrome: clinical and animal model studies**. Alcohol Alcohol. 2002;37(1):2-8. - Gamborino MJ, Sevilla-Romero E, Muñoz A, Hernández-Yago J, Renau-Piqueras J, Pinazo-Durán MD. **Role of thyroid hormone in craniofacial and eye development using a rat model**. Ophthalmic Res. 2001;33(5):283-91 - Pinazo-Duran MD, Renau-Piqueras J, Guerri C, Strömland K. **Optic nerve hypoplasia in fetal alcohol syndrome: an update**. Eur J Ophthalmol. 1997;7(3):262-70 - Pinazo-Duran MD, Valles S, Guerri C, Renau-Piqueras J. **Gestational ethanol-induced changes in protein expression during the morphogenesis of the rat optic nerve**. Int J Dev Biol. 1996;Suppl 1:141S-142S. - Strömland K, Pinazo-Durán MD. **Optic nerve hypoplasia: comparative effects in children and rats exposed to alcohol during pregnancy**. Teratology. 1994;50(2):100-11. - Pinazo-Duran MD, Renau-Piqueras J, Guerri C. **Developmental changes in the optic nerve related to ethanol consumption in pregnant rats: analysis of the ethanol-exposed optic nerve**. Teratology. 1993;48(4): 305-22.
Institution	Ophthalmic Research Unit “Santiago Grisolía”, Faculty of Medicine, University of Valencia, Valencia; and Institute of Cytological Research/Principe Felipe Research Center, Valencia

Experimental Model 78	**Development of the visual system**
Target Disease	Ocular malformations and retinal diseases.
Species	Rat (*Ratus Norvergicus*)
Short Description	Rat model of Thyroid hormone deprivation. The congenital-neonatal hypothyroidism. Hormonal neurodevelopment. We have been studying the ocular development in normal and pathologic conditions. Our goal was to analyse the retinal and optic nerve development by morphologic, morphometric and molecular approaches. We have used control rats and pre- and postnatally thyroid hormone deprived animas to study by scanning, light and electron transmission microscopy the characteristics of the pre- and postnatal eye and optic nerve development at key stages (prenatal day 19 and 21; postnatal days 5, 7, 10 14. 21. 28). The retinal cells and layering were examined and several developmental parameters were analysed (retinal thickness, each retinal layer thickness, number of cell profiles, neuronal axonal and glial cell markers (Neurofilament antibody, Glial fibrillary acidic protein antibody, myelin basic protein antibody).
Scientific publications	- Pinazo-Durán MD, Pons-Vázquez S, Gallego-Pinazo R, Galbis Estrada C, Zanón-Moreno V, Vila Bou V, Sanz Solana P. **Thyroid hormone deficiency disrupts rat eye neurodevelopment**. Brain Res. 2011;1392:16-26. - Pinazo-Durán MD, Iborra FJ, Pons S, Sevilla-Romero E, Gallego-Pinazo R, Muñoz A. **Postnatal thyroid hormone supplementation rescues developmental abnormalities induced by congenital-neonatal hypothyroidism in the rat retina**. Ophthalmic Res. 2005;37(4):225-34. - Sevilla-Romero E, Muñoz A, Pinazo-Durán MD. **Low thyroid hormone levels impair the perinatal development of the rat retina**. Ophthalmic Res. 2002;34(4):181-91. - Bort AR, Sevilla-Romero E, Gamborino MJ, Muñoz A, Pinazo-Durán MD. **A study on developmental characteristics of optic nerve in a rat model of materno-foetal hypothyroidism**. Arch Soc Esp Oftalmol. 2002;77(2):73-80 - Pinazo-Durán MD. **State-of-the-art in ocular malformations**. Human and animal model studies. Arch Soc Esp Oftalmol. 2002;77(9):473-6. - Bort AR, Sevilla-Romero E, Gamborino MJ, Muñoz A, Pinazo-Durán MD. **A study on developmental characteristics of optic nerve in a rat model of materno-foetal hypothyroidism**. Arch Soc Esp Oftalmol. 2002;77(2):73-80. - Gamborino MJ, Sevilla-Romero E, Muñoz A, Hernández-Yago J, Renau-Piqueras J, Pinazo-Durán MD. **Role of thyroid hormone in craniofacial and eye development using a rat model**. Ophthalmic Res. 2001;33(5): 283-91. - Pinazo-Duran MD, Renau-Piqueras J, Guerri C. **Developmental changes in the optic nerve related to ethanol consumption in pregnant rats: analysis of the ethanol-exposed optic nerve**. Teratology. 1993;48(4): 305-22.
Institution	Ophthalmic Research Unit “Santiago Grisolía”, Faculty of Medicine, University of Valencia, Valencia; and * Institute of Biomedical Research Alberto Sols, CSIC, Madrid

Experimental Model 79	**Preclinical safety of intravitreal docosahexaenoic acid in a rabbit model**
Target Disease	Retinopathies
Species	Rabbit (*Oryctolagus cuniculus*)
Short Description	We try to evaluate the retinal toxicity of a single dose of intravitreal pure docosahexaenoic acid (DHA) in rabbit eyes over a short-term period. We set up an experimental model of pre-clinical toxicity in New Zealand albino rabbits. Six concentrations of DHA were used and injected intravitreally in the right eyes. Vehicle solution was injected in the contralateral eyes. Retinal safety was studied by slit-lamp examination, electroretinography and morphologic/morphometric histological examination. Aqueous humor samples were taken to quantify the concentration of omega-3 acids by gas chromatography. Our results indicate that administration of intravitreal DHA is safe in the albino rabbit model up to the maximum tolerated dose of 25µg/50µl. Further studies should be performed in order to evaluate the effect of intravitreal injection of DHA as a treatment, alone or in combination, of different retinal diseases.
Scientific Publications	
Institution	Universitary and Polytechnic Hospital La Fe, Valencia

Experimental Model 80	**Genetic glaucoma (lrp2 associated)**
Target Disease	Glaucoma
Species	Zebrafish (*Danio Rerio*): strain AB (mw1)
Short Description	Bugeye zebrafish was identified through a mutational screen for adult ocular defects in zebrafish. Its phenotype included glaucoma with enlarged eyes, myopia, elevated IOP, and damage to retinal ganglion cells. Linkage analysis allowed to identify nonsense mutations in low density lipoprotein receptor-related protein 2 (lrp2) a large transmembrane protein of the LDL-receptor related protein (Lrp) family. In humans, mutations in LRP2 result in Donnai-Barrow syndrome, a rare disease characterized by a spectrum of phenotypes including agenesis of the corpus callosum, diaphragmatic hernia, sensonurial deafness, hypertelorism, buphthalmia (enlarged eye globes) and high myopia.
Scientific Publications	- Veth KN, Willer JR, Collery RF, Gray MP, Willer GB, Wagner DS, Mullins MC, Udvadia AJ, Smith RS, John SW, Gregg RG, Link BA. **Mutations in Zebrafish lrp2 Result in Adult-Onset Ocular Pathogenesis That Models Myopia and Other Risk Factors for Glaucoma**. PLoS Genet. 201;7(2):e1001310
Institution	Human Molecular Genetics Laboratory, School of Medicine, University of Castilla-La Mancha; Albacete