General Considerations of Using Non-Human Primate in Non-Clinical Ocular Drug Research

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1. Ocular Anatomy of NHP
2. Ocular Examinations in NHP
3. Ocular Toxicity Studies in NHP
4. Summary
Ocular Anatomy of NHP

- More similar to Human than other species
  - Vision and vision field
  - Eyeball size and volume
    - Anterior chamber: human(0.17ml), cynomolgus(0.101ml), dog(0.77ml), rabbit(0.28ml), rat(0.015ml)
    - Vitreous: Human(4ml), cynomolgus(2ml), rabbit(1.5 ml), rat(0.02ml)
  - Cornea
  - Shape of lens
  - Fundus
    - Only NHP present fovea, while others present visual streak (pig, rabbit)
    - Retinal blood supply in NHP similar to human, while rabbit present of blood vessels in a limited part of retina
Ocular Examinations in NHP

*In vivo Ocular Endpoints in NHP ocular studies*

- Clinical signs
- Slit-lamp biomicroscopy
- Indirect fundus biomicroscopy / funduscoppy / ophthalmoscopy
- Tonometry
- Electroretinography (ERG)
- Other measurements
  - Fundus photo, fundus fluorescein angiography (FFA), fundus autofluorescence (FAF)
  - Optical coherence tomography (OCT), OCT angiography (OCTA)
  - Specular microscope
Evaluate the conditions of the anterior segment of the eye

- Conjunctiva, cornea, anterior chamber, iris, lens and anterior vitreous
- Several scoring systems have been published with limitations, especially for pigmented larger animals.
Slit-lamp Examination in NHP

Scoring systems
- Scoring schemes for aqueous and vitreous cells were infrequently described.
- Semiquantitative preclinical ocular toxicology scoring (SPOTS) system are more and more accepted in non-clinical ocular drug research
  - Anterior segment
  - Posterior segment
  - Characterization of intravitreal test articles
- Lens Opacities Classification System III (LOCS III) can be applied in NHP cataract scoring

Funduscopic Examination in NHP

Evaluate the conditions of the posterior segment of the eye

- Posterior vitreous, retina (optical nerve head, retinal vessels, macular fovea)
- NHP present similar funds feature to human
- Abnormalities observed should be noted
- Refer to FP, FFA, OCT or OCTA for further information if needed
Tonometry Examination in NHP

Evaluate the intraocular pressure (IOP)
  - Animals under same anesthesia condition
  - Training for conscious IOP measurement may be needed in pharmacology studies
Electroretinography Examination in NHP

Evaluate the functional status of the retina
- Full-field electroretinogram (ff ERG), provide non-invasive objective quantitative measures of the electrical activity in the retina
- Suitable for the differentiation of the functions of the rod and cone systems
- International Society for Clinical Electrophysiology of Vision (ISCEV) guidelines

- **a-wave:** the initial negative deflection corresponding to the early hyperpolarization of the rod and cone photoreceptors. This wave-component reflects outer retinal function.
- **b-wave:** the positive deflection following the a-wave that originates from the depolarization of inner retinal Muller and bipolar cells. This wave-component reflects phototransduction activity.
- **Oscillatory potentials (OPs):** high-frequency rhythmic wavelets seen on the rising slope of the b-wave. OPs are visible at greater signal intensities and reflect the electrical activity of inner retinal feedback synaptic circuits, namely amacrine cells, as well as some vascular function.

ERG Examination in NHP

ISCEV Standard protocol

- DA 0.01 cd*s/m² ERG - rod system response
  a-wave: rods
  b-wave: bipolar cells

- DA 3.0 cd*s/m² ERG - rod and cone system response
  a-wave: rods and cones
  b-wave: bipolar cells

- DA oscillatory potentials (OPs) - amacrine cell response
  b-wave: Predominantly inner retinal amacrine cells as well as some vascular function

- LA 3.0 cd*s/m² ERG - cone system response
  a-wave: cones
  b-wave: bipolar cells

- LA 30 Hz flicker ERG - cone system response
  a-wave: cones

ERG Examination in NHP

- **Factors affecting the ff-ERG**
  - Size of retinal area illuminated (amplitude can be reduced if the stimulus is not full-field because the animal is positioned too far from the stimulus source)
  - Size of pupil
  - Clarity of ocular media (cornea, lens and vitreous condition)
  - Development of retina
  - Age
  - ERG amplitude can be reduced in high myopia (trends towards myopia in NHP of mature age)
  - Anesthesia condition
Other Examinations in NHP

- **Fundus Fluorescein Angiography (FFA)**
  - Assess the anatomy, physiology and pathology of retinal and choroidal circulation
  - NHP present similar retinal and choroidal circulation to human
  - Abnormalities observed should be noted, different phase of the circulation
  - Provide useful information in addition to the fundoscopy and fundus photo

- **Fundus Autofluorescence (FAF)**
  - Assess the fluorophores (naturally, pathologically or delivered) in the retina
  - In NHP studies, FAF usually served as a method to evaluate the distribution pattern of the derived fluorophores labeled test article in the retina
Other Examinations in NHP

- **Optical Coherence Tomography (OCT)**
  - Provide non invasive *in vivo* cross section imaging of vitreous, retina and choroid
  - Well accepted examination in ocular toxicology studies, especially for subretinal route delivery
  - NHP present macular fovea and retinal layers similar to human
  - Nomenclature for human OCT can be applied in NHP

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**Figure 1.** Nomenclature for normal anatomic landmarks seen on spectral domain optical coherence tomography (OCT) images proposed and adopted by the International Nomenclature for Optical Coherence Tomography Panel. Healthy retina imaged using Heidelberg Spectralis. RPE = retinal pigment epithelium.
Other Examinations in NHP

- **Optical Coherence Tomography Angiography (OCTA)**
  - Non invasive technique for imaging the microvasculature of the retina and the choroid
  - Provide quantitative analysis of the retinal vessels
  - NHP present similar OCTA parameters with human
  - More and more preclinical NHP studies employ OCTA to elucidate the vascular changes
Other Examinations in NHP

Specular Microscope
- Non-invasive diagnostic modality to image the corneal endothelium
- *In vivo* analyze the morphology of the corneal endothelium
- Number of cells counted (NUM) in analysis
- Cell density (CD): the number of endothelial cells per mm²
- Average cell area (AVG)
- SD: standard deviation of mean cell area within the analysis
- Coefficient of variation (CV): the amount of variation in cell size
- HEX: the percentage of cells that have hexagonal shape
- Central corneal thickness (CCT)

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Ocular Toxicity Studies in NHP

- For ocular biologics, usually NHP is one of the pharmacologically relevant species
- With the anatomy similarity to human, pharmacology models well developed in NHP to mimic the clinical condition of the ocular diseases
  - Laser induced choroidal neovascularization (CNV) model
  - Laser induced ocular hypertension model
  - Aged monkeys with spontaneous ocular diseases (cataract, glaucoma, AMD)
- Clinical use drug delivery instruments can be used in NHP directly
- Rabbits tend to be more immunogenic, may not be adequate for repeat-dose studies
- NHPs are widely used in ocular toxicity studies for ocular biologics and gene therapy
Ocular Toxicity Study – Case 1

- Bispecific antibody, clinical indication - wet AMD

- **Pharmacology study**
  - laser induced CNV model in cynomolgus monkey

- **Ocular toxicity study in NHP**
  - Four groups: vehicle control, low dose, middle dose, high dose, 5M+5F/group
  - Intravitreal injection, 4 repeat dose on Day1, Day30, Day60 and Day90
  - Ocular Examinations
    - Slit-lamp
    - Fundoscopy
    - FP & FFA
    - OCT
    - ERG
Ocular Toxicity Study – Case 1

- **Ocular Findings**: severe ocular inflammation in some animals in the middle and high dose group, before 2nd dose and continue in the whole study period. No ocular inflammation observed in vehicle control and low dose group.
  - **Slit-lamp**: corneal edema, keratic precipitates (KP), anterior chamber cell infiltrates, vitreous cells
  - **Fundoscopy**: vitreous opacities, perivascular sheathing
  - **FFA**: hyperfluorescent leakage in the retina
  - **OCT**: retinal edema with increased retinal thickness, increased retinal vessel size
  - **ERG**: decreased ERG response

- **Question**
  - Are these findings related to the injection procedure, or direct effect of the test article, or immunogenicity?
Injection procedure related ocular inflammation often occurs within 3 days post dosing.

Immunogenicity related ocular inflammation
- Many biologics intended for humans are immunogenic in animals
- Immunogenicity usually present at 2 weeks post dose
- Anti-drug antibody (ADA) level in the vitreous and serum
  - Animals with ocular inflammation present high ADA titer in the vitreous and serum
  - Animals without ocular inflammation present low ADA titer in the vitreous
- Ocular inflammation occurs after ADA present, starts from anterior chamber, then vitreous
- Loss of test article exposure in TK samples

Ocular inflammation primarily related to the formation of ADA
Ocular Toxicity Study – Case 1

- To further support the ocular inflammation was related to the ADA formation, immunohistochemistry evaluation in the ocular tissue was conducted in high dose group
  - Granular deposits containing test article (humanized IgG), monkey IgG, IgM were observed in the retina

- All of these data support ocular inflammation findings were primarily related to the formation of ADA, and not related to the injection procedure or direct pro-inflammatory effect of the test article.
Ocular Toxicity Study – Case 2

- Adeno-associated virus (AAV) gene therapy, clinical indication - retinitis pigmentosa
- **Ocular toxicity study in NHP**
  - Four groups: vehicle, low dose, middle dose, high dose, 5M+5F/group
  - Subretinal injection, 75 μL/eye, one dose on Day 1
  - Ocular Examinations
    - Slit-lamp
    - Fundoscopy
    - FP/FFA
    - OCT
    - ERG
Ocular Toxicity Study – Case 2

- **Ocular Findings**
  - **Slit-lamp:** pigment particles in anterior vitreous in the middle and high dose group
  - **Fundoscopy and FFA:**
    - pigment deposition at the boundary of drug solution coverage area (blocked fluorescence of choroidal in FFA), local depigmentation in the center (window defect hyper-fluorescence in FFA) – in all groups, more severe in middle and high dose group
    - fluorescence leakage in drug solution coverage area – in test article injected groups
  - **OCT:** thinning and discontinuity of EZ, disorganization of RPE/IZ, thinning of ONL
  - **ERG:** decreased ERG response
Ocular Toxicity Study – Case 2

Question
- Are these findings related to the injection procedure, or direct effect of the test article, or immunogenicity?

Subretinal injection procedure could cause pigment changes in the retina
- Related to the injection pressure
  - Injection pressure control using viscous fluid control system
- Use the pressure as low as possible to minimize the retinal damage
- Subretinal depigmentation in the middle region of the bleb area, while a band of hyper-pigmentation inferiorly

100μL
70μL
50μL
Ocular Toxicity Study – Case 2

- Anti-drug antibodies
  - Anti-AAV antibody: low tier in serum and aqueous humor
  - Anti-expression product antibody: low tier in the serum and not detected in aqueous humor

- Overall findings support these ocular abnormalities related to the injection procedure, while test articles could exacerbate some abnormalities.
Ocular Toxicity Study – Case 3

- Monoclonal antibody, clinical indication - wet AMD
- **Pharmacology study**
  - laser induced CNV model in cynomolgus monkey
- **Ocular toxicity study in NHP**
  - Study design
    - Four groups: vehicle control, low dose, middle dose, high dose, 5M+5F/group
    - Intravitreal injection, 4 repeat dose on Day1, Day30, Day60 and Day90
  - Ocular Examinations
    - Slit-lamp
    - Fundoscopy
    - FP & FFA
    - OCT
    - ERG
Ocular Toxicity Study – Case 3

- **Ocular Findings:** severe ocular inflammation in all injected eyes in *low dose* and *middle dose* group, on the 2\textsuperscript{nd} day after first dosing. No ocular inflammation observed in vehicle control and high dose group
  - **Slit-lamp:** keratic precipitates, anterior chamber cell infiltrates and exudate, vitreous cells
  - **Fundoscopy:** vitreous opacities, blurry or invisible fundus
  - **FP/FFA and OCT:** can not be performed

- **Question**
  - Are these findings related to the injection procedure, or direct effect of the test article?
Ocular Toxicity Study – Case 3

- Ocular inflammation occurs within 3 days post dosing, in all the eyes injected with test articles in low dose and middle dose group, not in control and high dose group
- Test articles were checked for contamination
- Low and middle concentrations of the test articles were prepared and diluted from high concentration test article in an uncertified lab, with possibility of contamination
- The study was stopped
- The test article should be prepared ready for dosing in a certified lab. To minimize the risk of contamination, endotoxin free syringes are recommended.
Summary

- NHP present similar ocular anatomy to human
- *In vivo* ocular examinations are widely used and provide useful information in NHP preclinical studies
- In NHP ocular toxicity studies, ocular findings should be evaluated and assessed as thoroughly as possible
- Closely work with toxicology and pathology team
Questions?