Immunomodulation / immunotherapy

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Immunotherapy

- Ocular Surface Disease
  - Keratoconjunctivitis sicca
- Intraocular disease
  - Uveitis
- Posterior segment disease
  - Neovascularization
Future / Emerging Therapies

• Ocular surface disease
  – Dry eye*
  – Allergy*
  – Inhibition of corneal transplant rejection
  – Anti-fibrosis

2013
Future / Emerging Therapies

- Intraocular disease
  - Uveitis*
  - Post-surgical inflammation*
  - Anti-neovascular drugs
    - Wet Age Related Macular Degeneration
    - Diabetic retinopathy
New Drugs / Treatments for Immunologic Diseases

• Ocular Surface Disease
  – Keratoconjunctivitis sicca

• Intraocular disease
  – Uveitis

• Posterior segment disease
  – Neovascularization
Ocular Surface Disease

• Keratoconjunctivitis sicca
  – Huge market
  – Lots of new products and devices
• Allergic conjunctivitis
Keratoconjunctivitis sicca (dry eye)

- Lifitegrast
  - (Xiidra™)
- Nanomicellar CsA
  - (Seciera™)
- Secretagogues
  - (mucin; sodium channel blockers) (β-ENAC inhib)
- Forsight Vision5
  - (CsA rings)
- Gene Therapy
  - (HLA-G)
- Cell therapy

https://www.allergan.com/
Keratoconjunctivitis sicca (dry eye)

- Lifitegrast 5%
  - (Xiidra™) (Shire pharma)
  - LFA-1/ICAM inhibitor

Murphy et al. The pharmacologic assessment of a novel lymphocyte function-associated antigen-1 antagonist (SAR 1118) for the treatment of keratoconjunctivitis sicca in dogs. IOVS 52.6 (2011): 3174-3180.
Keratoconjunctivitis sicca (dry eye)

- Nanomicellar CsA 0.09%
  - (Seciera™)
  - SUN pharma
Keratoconjunctivitis sicca (dry eye)

- Forsight Vision5
  - (CsA rings)
  - 6 month release
  - Current use for delivery of bimatoprost

https://www.allergan.com/
Keratoconjunctivitis sicca (dry eye)

- Forsight Rings
  - Current clinical trial ongoing with canine KCS (NC State)
    - Well tolerated
    - Great retention
    - Results are pending

https://www.allergan.com/
Keratoconjunctivitis sicca (dry eye)

• Gene Therapy
  – Gene addition therapy
  – Add DNA to transcribe and release immunosuppressive proteins
    • AAV-HLA-G
    • AAV-IL-10
New Drugs / Treatments for Immunologic Diseases

- Ocular Surface Disease
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- Intraocular disease
  - Uveitis
- Posterior segment disease
  - Neovascularization
Anti-inflammatory / immunosuppressants

- Uveitis
- Post-operative inflammation
Anti-inflammatory / immunosuppressants

• Intraocular implants
• Suprachoroidal injection
  – Steroid
• Biologics
  – Antibodies and fusion proteins
• Gene and cell therapy
Anti-inflammatory / immunosuppressants

- Intraocular implants
  - OZURDEX®
    - (0.7 mg dexamethasone intravitreal implant)
    - 6 month drug release
    - Posterior uveitis
    - Diabetic macular edema
    - Retinal vein occlusion

www.ozurdex.com
Anti-inflammatory / immunosuppressants

- Suprachoroidal injection
  - Steroid

*Drug contained in posterior segment of eye
No anterior chamber involvement

https://doi.org/10.1016/j.drudis.2014.10.010
Anti-inflammatory / immunosuppressants

- Suprachoroidal injection
  - Steroid
  - Pig model
    - Acute uveitis
    - Triamcinolone (TA)

SCS injection in horses with ERU?

- Intravitreal Triamcinolone Acetonide
  - Intravitreal up to 20 mg “safe” in horses\(^1\)
  - Clinically – development of keratitis and fungal disease
  - Would SCS TA be effective and safe in horses with active ERU?
  - What is the feasibility?

Methods for TA SCS injection - Horses

- Horses with active ERU poorly responsive to medications
- 5 mg of TA injected into SCS
  - 1100um length 30G microneedle (Clearside Bio, Alpharetta, GA)
  - TA = Triesence® (Alcon laboratories, Fort Worth, TX)
  - Surgical preparation (5% betadine)
  - Topical proparacaine HCL
  - 8-10 mm posterior to dorso-temporal limbus
SCS TA in Horses with ERU

- Injected 12 horses to date
  - No difficulties in making the injection
  - No vitreous drug visible
  - Within 24 hours, substantial improvement in active inflammation
  - One of 12 horses developed corneal ulcer 24 hours after injection – reepithelialized without developing fungal keratitis
  - Two of 12 horses had CsA implantation
New Drugs / Treatments for Immunologic Diseases

• Ocular Surface Disease
  – Keratoconjunctivitis sicca

• Intraocular disease
  – Uveitis

• Posterior segment disease
  – Neovascularization
Anti-inflammatory / Immunosuppressants

- **Biologics**
  - Antibodies and fusion proteins
    - Anti-TNF therapy – uveitis
      - Adalimumab; infliximab
    - Daclizumab (Zymbrytra™)- anti-IL-2 receptor (MS)
    - Gevokizumab - anti IL1
    - Tocilizumab – anti IL-6
    - Abatacept (Orenica™)
      - fusion protein composed of the Fc region of the immunoglobulin IgG1 fused to the extracellular domain of **CTLA-4**
<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Route of administration</th>
<th>Cost of typical therapy ($)</th>
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<tr>
<td><strong>TNF inhibitors</strong></td>
<td></td>
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<tr>
<td>Infliximab</td>
<td>TNF alpha</td>
<td>Intravenous</td>
<td>105,000</td>
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<td>Adalimumab</td>
<td>TNF alpha</td>
<td>Subcutaneous</td>
<td>20,000</td>
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<tr>
<td>Etanercept</td>
<td>TNF alpha, beta</td>
<td>Subcutaneous</td>
<td>12,000</td>
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<tr>
<td>Golimumab</td>
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<td>12,000</td>
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<tr>
<td><strong>Specific receptor antagonists</strong></td>
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<tr>
<td>Canakinumab</td>
<td>IL-1 beta</td>
<td>IV or subcutaneous</td>
<td>155,000</td>
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<td>Tocilizumab</td>
<td>IL-6</td>
<td>Intravenous</td>
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<td>Anakinra</td>
<td>IL-1 receptor</td>
<td>Subcutaneous</td>
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<td>Gevokizumab</td>
<td>IL-1 beta</td>
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<td><strong>Lymphocyte inhibitors</strong></td>
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<td>Rituximab</td>
<td>B cells via CD20</td>
<td>Intravenous</td>
<td>5,000</td>
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</table>

Anti-inflammatory / Immunosuppressants

- Gene and stem cell therapy
- Immunomodulatory
Stem cell therapy for ocular disease

- Stem cell therapy
  - Retinal regeneration
  - Corneal surface healing
  - Glaucoma
  - (lots of other potentially)
Stem cell therapy for ocular disease

- Stem cell therapy
- Immunomodulatory
  - Ocular surface immune
  - Uveitis

https://doi.org/10.1016/j.jocit.2014.12.001
Mesenchymal stem cells

• Self-renewing multipotent cells
  • Nonhematopoietic
  • Derived from adult bone marrow
• Differentiate into multiple connective tissue cell types
• Soluble factors of mesenchymal stem cells (MSCs) alter the tissue microenvironment
Bone marrow-derived mesenchymal stem cells

- Regenerative medicine therapeutic for equine musculoskeletal injury
- Shown to ameliorate tissue damage from injury
  - Able to migrate to site of tissue injury

Schnabel et al. 2013
Mesenchymal stem cells for ocular therapy

- MSCs undergo epithelial cell differentiation and acceleration of corneal healing in rabbits (Gu et al. 2009, Almaliotis et al. 2015)

- Reduce alkali-induced oxidative stress (Cejkova et al. 2013)

- Corneal reconstruction in rats (Ma et al. 2006, Jiang et al. 2010)
  - Decrease inflammation and neovascularization

- Intravenous injection of MSC targets corneal injury and promotes regeneration (Lan et al. 2012)
Equine corneal primary cultures treated with equine Msc

- Scratch made through center of each well using 200uL pipette tip
- Evaluated for 72 hours

Sherman et al. Stem Cell Research & Therapy (2017) 8:120. DOI 10.1186/s13287-017-0577-3
Equine corneal primary cultures treated with equine MSC

Media only

Stem cell supernatant

MSC
Teddy

A 12-year-old Warmblood gelding was diagnosed with superficial IMMK in the left eye (OS) in 2011 and had periodic flare-ups of cloudiness that responded well to topical flurbiprofen and oral previcox.

After developing mild corneal degeneration, topical cyclosporine ointment two to four times daily was initiated.
Mesenchymal stem cells - Case report

- Autologous BM-MSCs therapy
  - Immuno-modulatory
  - Decrease frequency of topical medications

- BM aspirated and MSCs were cultured
  - Three weeks later, two separate 0.25 ml bulbar subconjunctival injections
  - Approximately 5x10^6 MSCs (injected) suspended in the horse’s own serum.

- No immediate adverse effect
Mesenchymal stem cells - Case report

• Topical medications were decreased and on recheck 1 month later the cornea was clearer and the degeneration was resolving.

• No additional flare-ups developed over the 6 month follow-up period.

• Currently on no medications
Mesenchymal stem cells - Case report

Appearance over time

Appearance at injection

1 month after injection

6 months after injection
Gene Therapy

https://www.yourgenome.org
Gene Therapy Strategies

- Gene replacement
- Gene augmentation /Addition
- Gene correction (Chimeraplasty)
- Gene inhibition
- Gene editing (CRISPR)
**Gene Therapy**

- **DNA**: Represents the structure of DNA.
- **Mutant gene**: Shows a red segment indicating a mutated gene.
- **Normal gene**: Shows a green segment indicating a normal gene.
- **Replacement of mutant gene**: Illustrates the process of replacing a mutant gene with a normal gene.
- **Addition of normal gene**: Demonstrates the process of adding a normal gene to the DNA sequence.

**Gene Editing**

- **Normal**: Shows the sequence `ATTGCGATC`.
- **Diseased**: Shows the sequence `ATTGAGATC`.
- **A to C Editing**: Highlights the change from `ATTGAGATC` to `ATTGCGATC` indicating a mutation correction.
Ocular Gene therapy

• Gene replacement
  – Inherited retinopathies
  – *RPE65*, Leber's congenital amaurosis 2 (LCA2)
  – Briard Dog
    (2001 – Dr. Gus Aguirre)
  – University of Pennsylvania
Human leukocyte antigen G

- Molecule is used to prevent maternal-mediated fetal rejection by forming an “immune barrier” at the maternal/fetal interface
- Induces immune tolerance directly via inhibition of NK and APCs, T and B cells lysis, and anti-neovascularization effects
HLA-G Gene Therapy

- Gene therapy is delivered via adeno-associated virus (AAV) vectors
- Reduce vascularization in order to prevent graft rejection
- Blocks the action of T-cells and antigen presenting cells
Corneal Transplants

- Most common form of tissue transplant
- Full Thickness Corneal Transplant
  - All layers of cornea are replaced with donor tissue
- Corneal Button
  - Corneal donor tissue

http://news.bbc.co.uk
Corneal AAV-HLA-G prevents corneal vascularization and inflammation

Hirsh, et al. AAV-HLA-G gene therapy to re-establish corneal immune tolerance. Sci Report 2017
Corneal AAV-HLA-G prevents corneal transplant rejection

Gilger, et al. AAV-HLA-G gene therapy to prevent corneal transplant rejection. In prep
Additional uses of immunosuppressive protein – gene addition strategies

• HLA-G
  – Dry eye
  – Immune-mediated keratitis / pannus
  – Uveitis
  – Retinal inflammation
  – Wet macular degeneration (humans)

• Other
  – IL-10
  – TGFβ
  – LFA-1 (integrin)
Combination of gene and cell therapy

1. Cells are removed from patient.
2. In the laboratory, a virus is altered so that it cannot reproduce.
3. A gene is inserted into the virus.
4. The altered virus is mixed with cells from the patient.
5. The cells from the patient become genetically altered.
6. The altered cells are injected into the patient.
7. The genetically altered cells produce the desired protein or hormone.
Research-in-progress and future treatments for immune-mediated ocular disease

• New devices
  – Ozurdex (dexamethasone)
  – Forsight (Cyclosporine)
  – Microneedle
    • Suprachoroidal injection

• New Medications
  – Lifitegrast (Xiidra™)
  – Nanomicellar CsA
    • (Seciera™)
Research-in-progress and future treatments for immune-mediated ocular disease

• Biologics
  – Geared toward human diseases
  – Expensive

• Gene and stem cell therapy
  – Key for future
Questions?