# CBI
# Hypertrophic Scar Formation in Rabbits

COMPARATIVE BIOSCIENCES, INC.
A TRANSLATIONAL APPROACH TO PRECLINICAL RESEARCH

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# COMPARATIVE BIOSCIENCES, INC.

**Premier Preclinical Contract Research Organization**

- Member of GD3 Genesis Drug Discover & Development Services
- **Over 25 years** of experience
- Conveniently **located in the heart of Silicon Valley**
- **State of the art, purpose-built facility**
- Approximately 35 employees
- Highly experienced scientific staff
- **GLP, OECD, FDA, USDA, OLAW**
- **AAALAC Accreditation**

# Hypertrophic Scar Formation Overview

- Hypertrophic scar (HS) formation is **a skin fibroproliferative disease that occurs following a cutaneous injury, leading to functional and cosmetic impairment**. To date, few therapeutic treatments exhibit satisfactory outcomes.

- Hypertrophic scar (HS) formation is a common complication of wound healing, particularly after burn injuries. HSs are raised, red, rigid, and responsible for serious functional and cosmetic problems. The underlying mechanisms of scar formation are complicated, and the process may be affected by multiple factors.

# Hypertrophic Scar Formation Services

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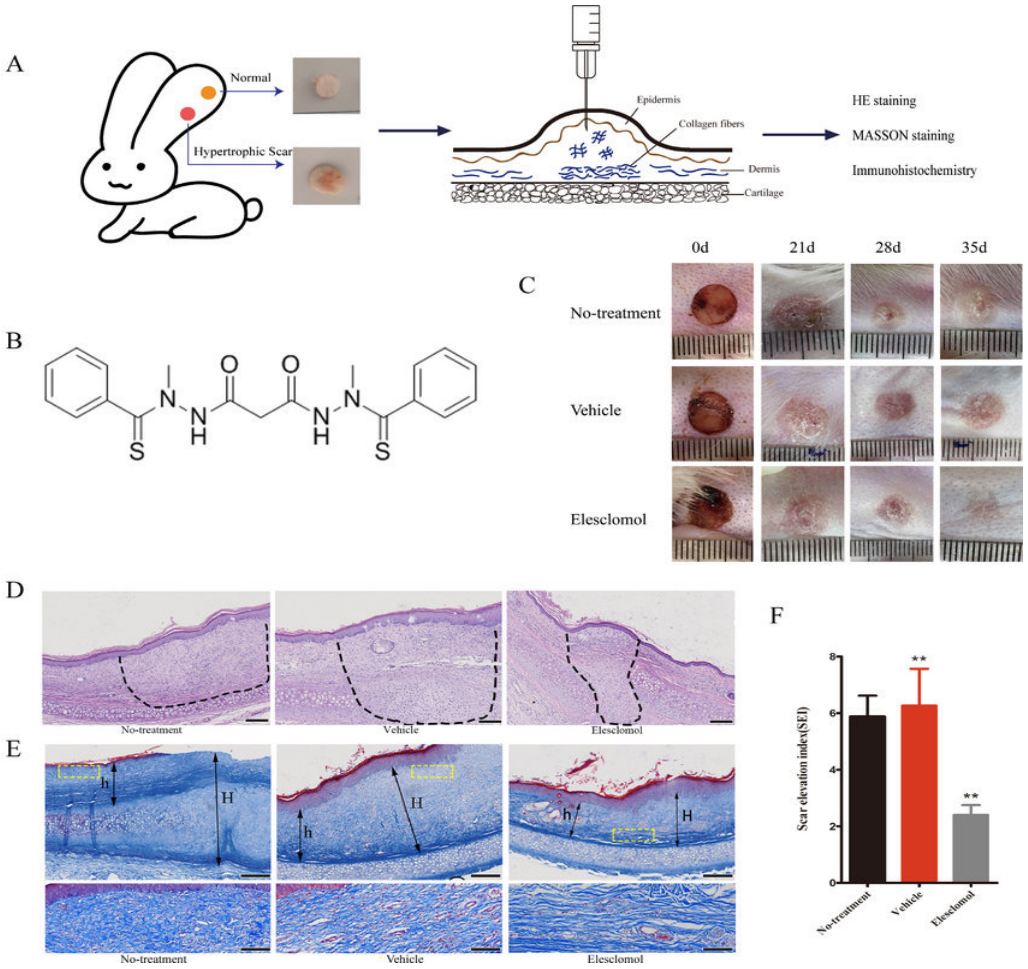
**Creation of a circular lesion with removal of the perichondrium elicits a proliferative fibrosis resulting in scar formation on the rabbit ear.**

- This lesion can be measured and effects of test article determined
- Typical study setup:
  - 6 weeks with dosing at day of wound formation
  - After formation of scar (~3 weeks) with 3-4 weeks treatment
  - With 2-3x weekly assessments and followed by histopathology for each
- Test article may be applied topically or intralesion injection
- Vehicle and test article applied to lesions

# Hypertrophic Scar Studies



# Hypertrophic scar formation in Rabbits


Appearance immediately post surgically


Untreated lesions:
There is clear scar formation present


Triamcinolone treated:
There is more healing and less scar formation

Four hypertrophic scars on the ventral side of a rabbit's ear 4 weeks' post-wound.

Hypertrophic scars are benign and not harmful to a person's general health. They do not develop into skin cancer. **A hypertrophic scar will often regress completely between 6 months and 3 years after it first appears**.

# Screening Study- Proposed Design

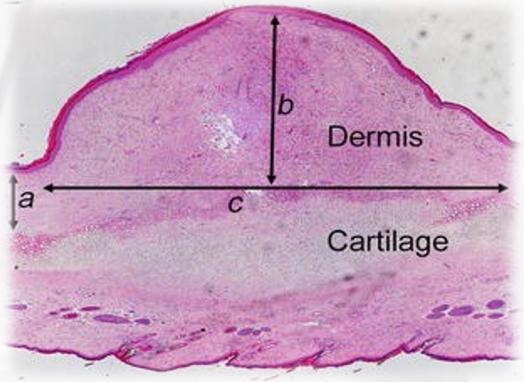
Sham would be **the one that had the cirurgy but not received the vehicle** (just to have the lesion/stress of the cirurgy).

The vehicle group would have the lesion/cirurgy and the vehicle used to dilute the drug/substance.

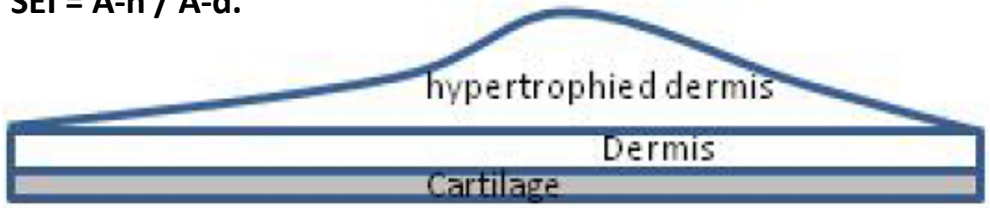
| Group | No. Rabbits | In life observations | Necropsy |
|---|---|---|---|
| Vehicle and sham | 5 | Photos of lesions 1x week for 6 weeks | • Histopathology (including IHC) and quantitative assessment of scar formation<br>• qPCR of Scar Samples |
| TA low dose | 5 | | |
| TA mid dose | 5 | | |
| TA high dose | 5 | | |

# Hypertrophic Scar Area in the Rabbit Ear:

- Quantitative Histology Assessment
  - Scar Elevation Index (SEI)
    - Morris et al. (1997)



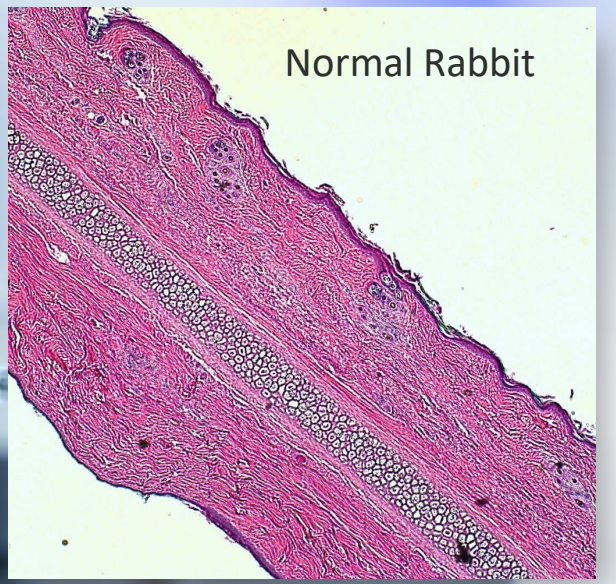

**SEI = A-h / A-d.**




In brief, the SEI measures the ratio of total scar connective tissue area to the area of underlying dermis. **SEI = A-h / A-d.**

The scar area was measured in 1 or 2 sections per scar. Measurements were made digitally. A computerized visual imaging system was used to photograph the areas of interest using an Olympus (Tokyo, Japan) camera, with the image digitized using MicroSuite Basic Edition Software (Tokyo, Japan). For a cross-section of each scar, the external perimeters of the underlying dermis (P-d) and the newly formed hypertrophied dermis including the underlying dermis (P-h) were collected. The software automatically calculates the area bounded by each perimeter; A-h and A-d respectively.

# Hypertrophic scar formation in Rabbits

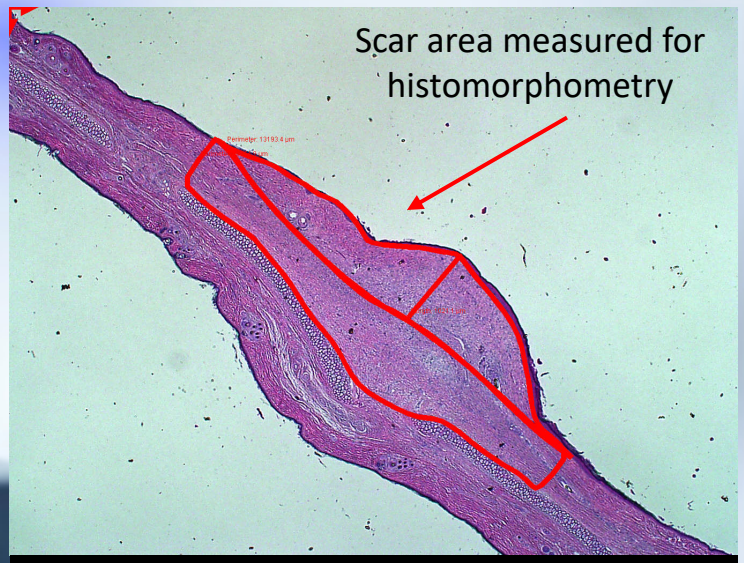




Hypertrophic scarring is a common proliferative disorder of dermal fibroblasts characterized by collagen overexpression and excessive extracellular matrix (ECM) deposition in healing wounds elicited by deep burns, inflammatory reactions, and trauma. This condition has been a major concern for patients and a challenge to surgeons for centuries; therefore, effective strategies to inhibit hypertrophic scar formation are highly important.

# Hypertrophic scar formation in Rabbits

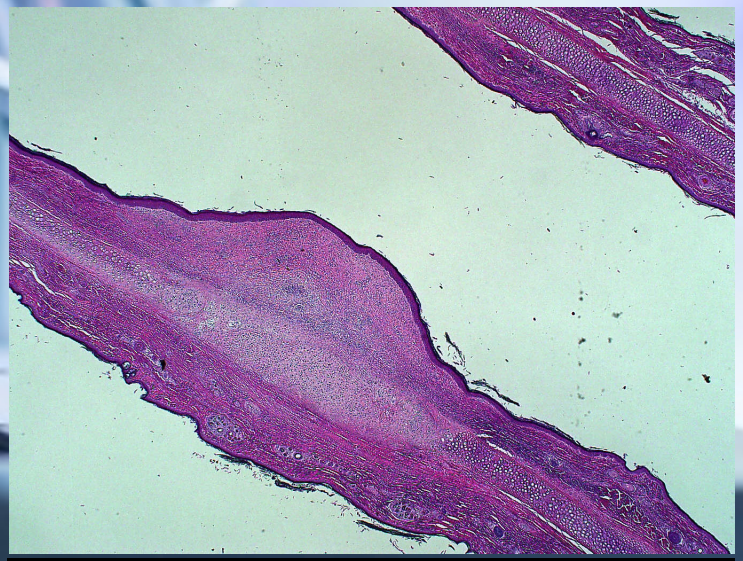
Three weeks, scar induction, no treatment




Three weeks, scar induction, no treatment, demonstrating area measured for histomorphometry

Scars form **when the dermis (deep, thick layer of skin) is damaged**. The body forms new collagen fibers (a naturally occurring protein in the body) to mend the damage, resulting in a scar tissue.

# Hypertrophic scar formation in Rabbits



Three weeks, scar induction,
no treatment

Three weeks, scar induction,
treatment with Test Article

**Replacement** refers to a type of healing in which severely damaged or non-regenerable tissues are repaired by the laying down of connective tissue, a process commonly referred to as scarring.

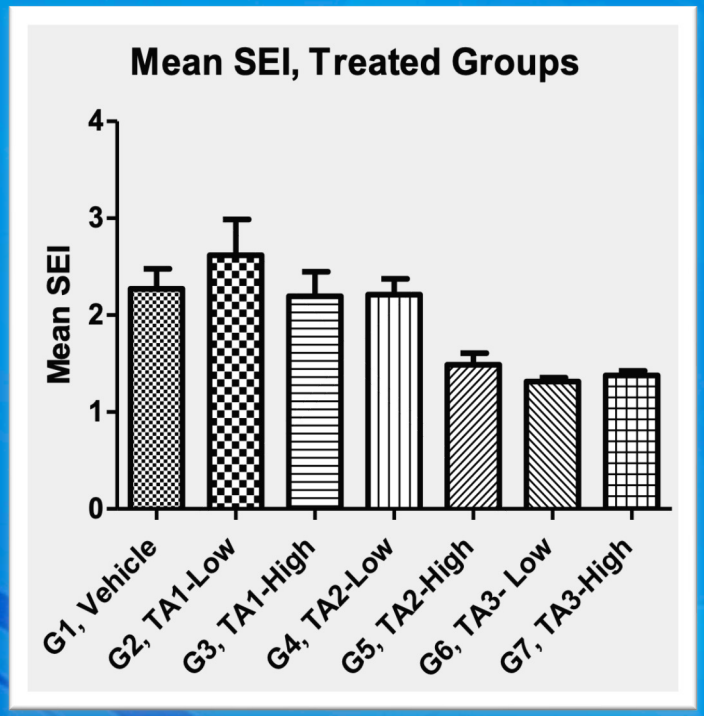
# Hypertrophic Scar Area in the Rabbit Ear:

SEI reflects the scars' hyperplasia. SEI: dermal thickness after wound healing/dermal thickness of adjacent normal tissues. SEI > 1.5 indicates hypertrophic scars.

**Study Summary, SEI of Wounds Treated**

Please note

- Decreased SEI compared to vehicle shows efficacy
- TA 1 shows no efficacy at both doses
- TA 2 shows efficacy only in high dose
- TA 3 shows efficacy in both doses

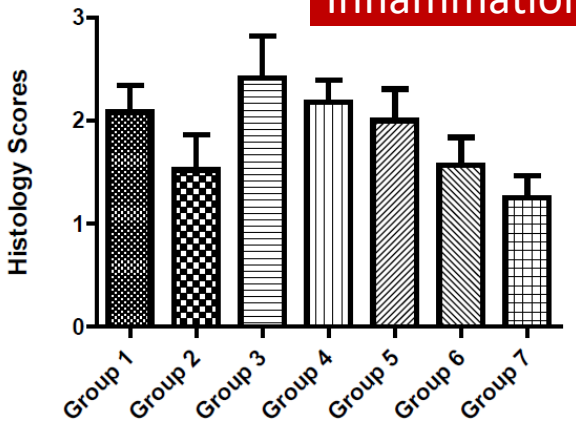



# Hypertrophic scar formation in Rabbits

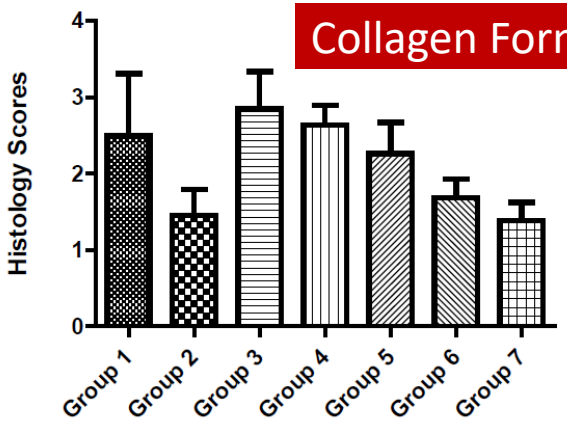
## Histopathology Scores

Histopathologic scoring is **a tool by which semi-quantitative data can be obtained from tissues**. Initially, a thorough understanding of the experimental design, study objectives and methods are required to allow the pathologist to appropriately examine tissues and develop lesion scoring approaches.

Inflammation



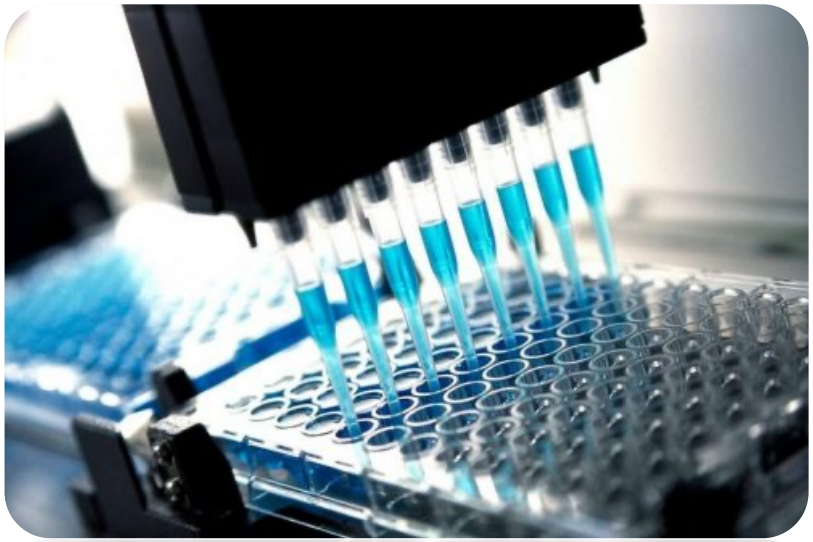

Collagen Formation



# Hypertrophic scar formation in Rabbits

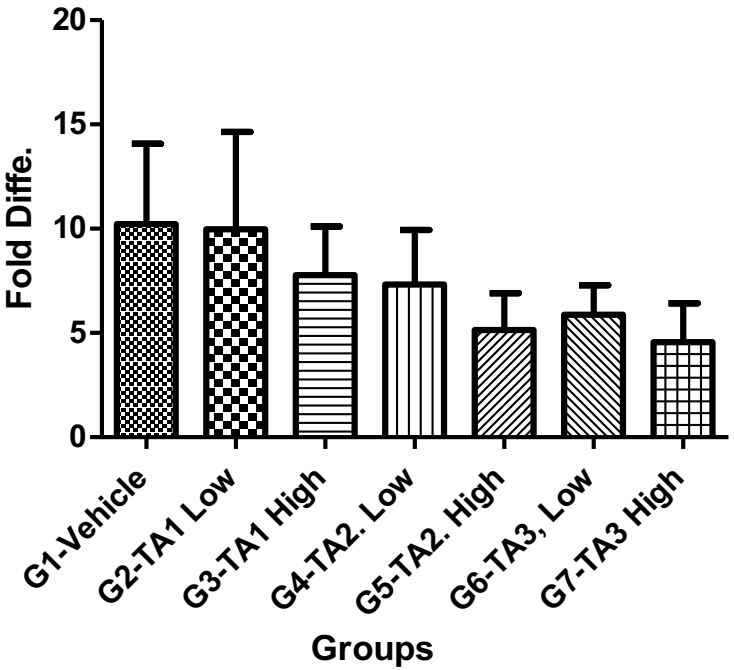
**qPCR – gene of interest expression**

Real-time quantitative PCR (qPCR) is an efficient, simple, and low-cost technique frequently used by molecular biologists to quantify gene expression. **The calculation of the relative expression of a target-gene by qPCR is based on the use of reference gene(s) as endogenous control(s)**.



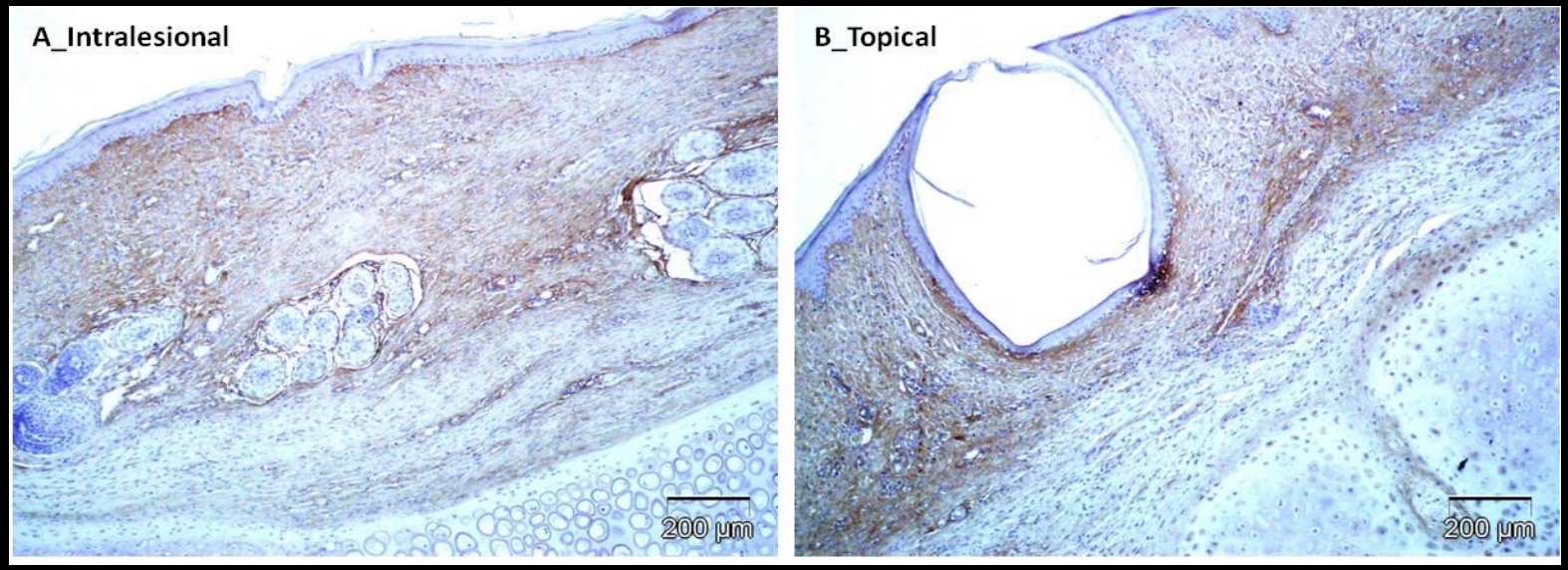
- High gene expression in vehicle & TA 1 correlates with lack of efficacy.
- Low gene expression in TA 3 correlates with efficacy seen.

qPCR – gene of interest expression



# Hypertrophic scar formation in Rabbits

Immunohistochemical detection




Immunohistochemical detection of Periostin in Hypertrophic Scar. The testarticle was applied intralesional (A) or topical (B). Periosotin leads to altered regenerations through TGF-Beta Signage.

Immunohistochemistry (IHC) and immunofluorescence (IF) are **molecular assays that involve the use of antibodies to detect specific proteins within tissues** on microscope slides.

# Service and Quality

- ***Thoroughness in planning and execution is key to a successful study***. All protocols are vetted and approved by multiple personnel. Our QAU has a rigorous training program. All non-GLP studies are conducted in the spirit of GLP.
- ***We believe in sound science.*** Our ratio of scientists to non-scientists is one of the highest in the industry. Every study director is a PhD-level scientist.
- ***We believe in communication.*** Timely responses to your inquiries and frequent updates on your study are mandatory.
- ***We welcome visitors.*** You are always welcome at CBI to meet the staff, tour the laboratory and discuss the progress and results of your study.