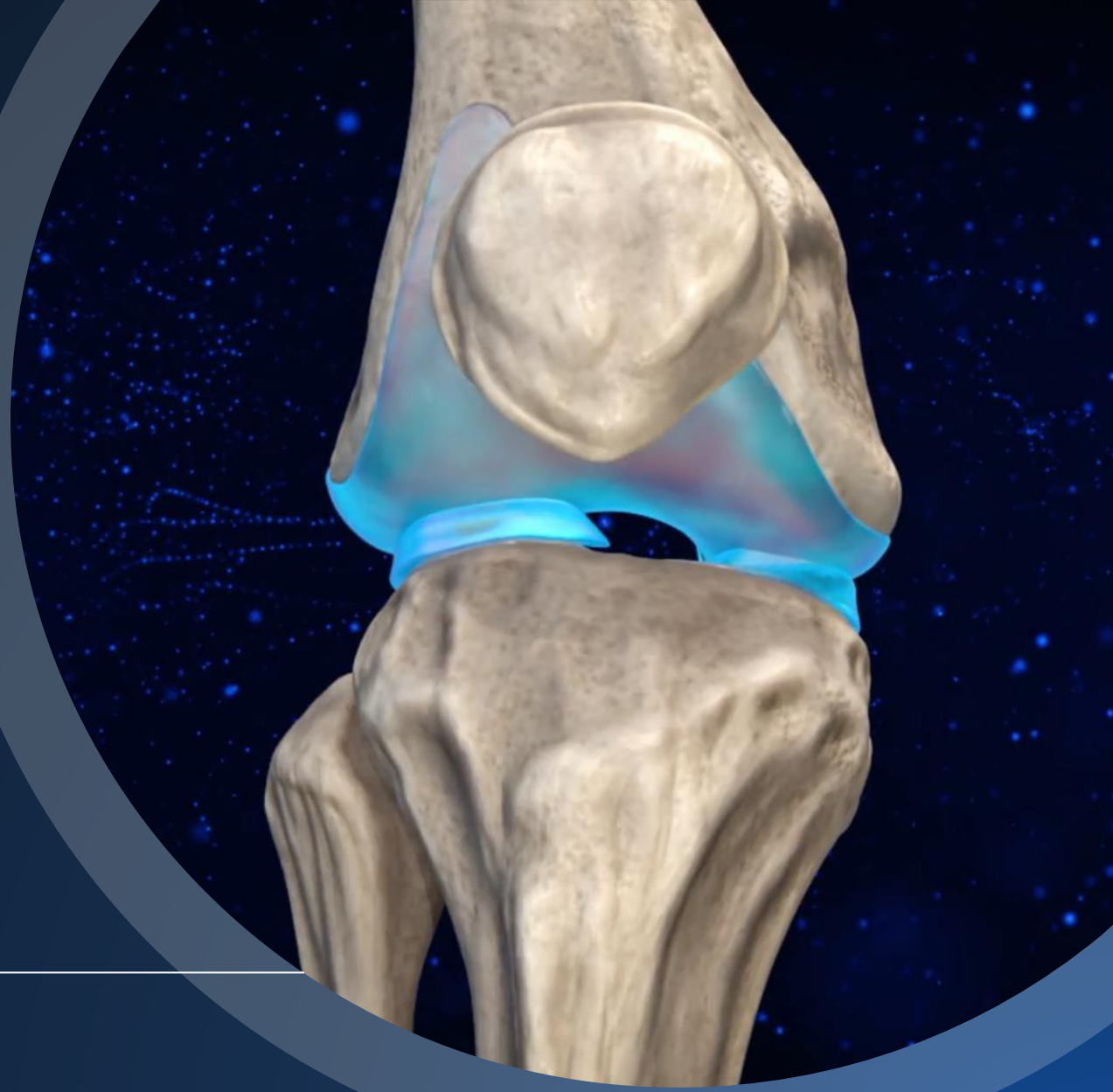
CYTONICS

Engineering the Future of Joint Health with Molecular Precision



Join us in a *grassroots* effort to reclaim biotech innovation from Big Pharma and Wall Street

Reg A+ Disclaimer

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CYTONICS

"He who has a Why can endure any How"

~ Friedrich Nietzsche

The What

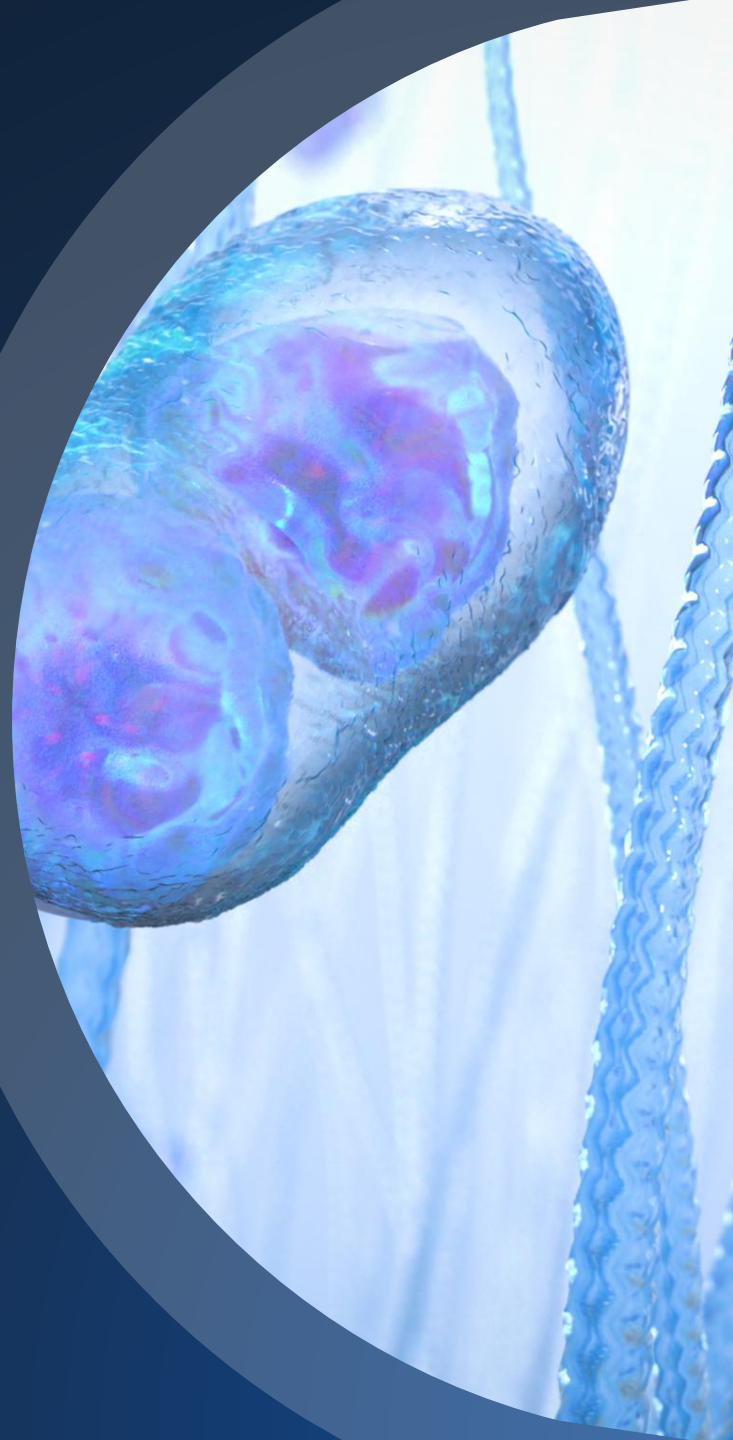
We are on a mission to develop the *first* **disease-modifying** therapy for OA, solving a problem Big Pharma has not been able to crack for decades

The Why

Osteoarthritis is currently a life sentence of pain and debilitated quality of life due to Big Pharma's failure. This is simply not acceptable.

The How

We are positioning ourselves to succeed where Beat Big Pharma has failed by addressing their *myopic* approach to drug development. In doing so, we will reclaim biotech innovation from Silicon Valley and Wall Street's stranglehold



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The Osteoarthritis Epidemic

Osteoarthritis (OA) is a progressive joint disease where cartilage breaks down, leading to pain, stiffness, and reduced mobility. Unlike a single-cause illness, OA is driven by multiple enzymes that degrade cartilage, leaving joints stiff and painful.

currently suffering from OA in the **USA***

This figure is projected to reach **25%** of the adult population by **2030.**

Global prevalence**

Agnostic to wealth or standard of living

Post-traumatic or chronic condition

Affecting both athletes and the elderly

$1.1T burden to the **global economy*****

*https://oaaction.unc.edu/oa-module/oa-prevalence-and-burden
** https://www.who.int/news-room/fact-sheets/detail/osteoarthritis
*** https://www.sciencedirect.com/science/article/pii/S106345842100738X

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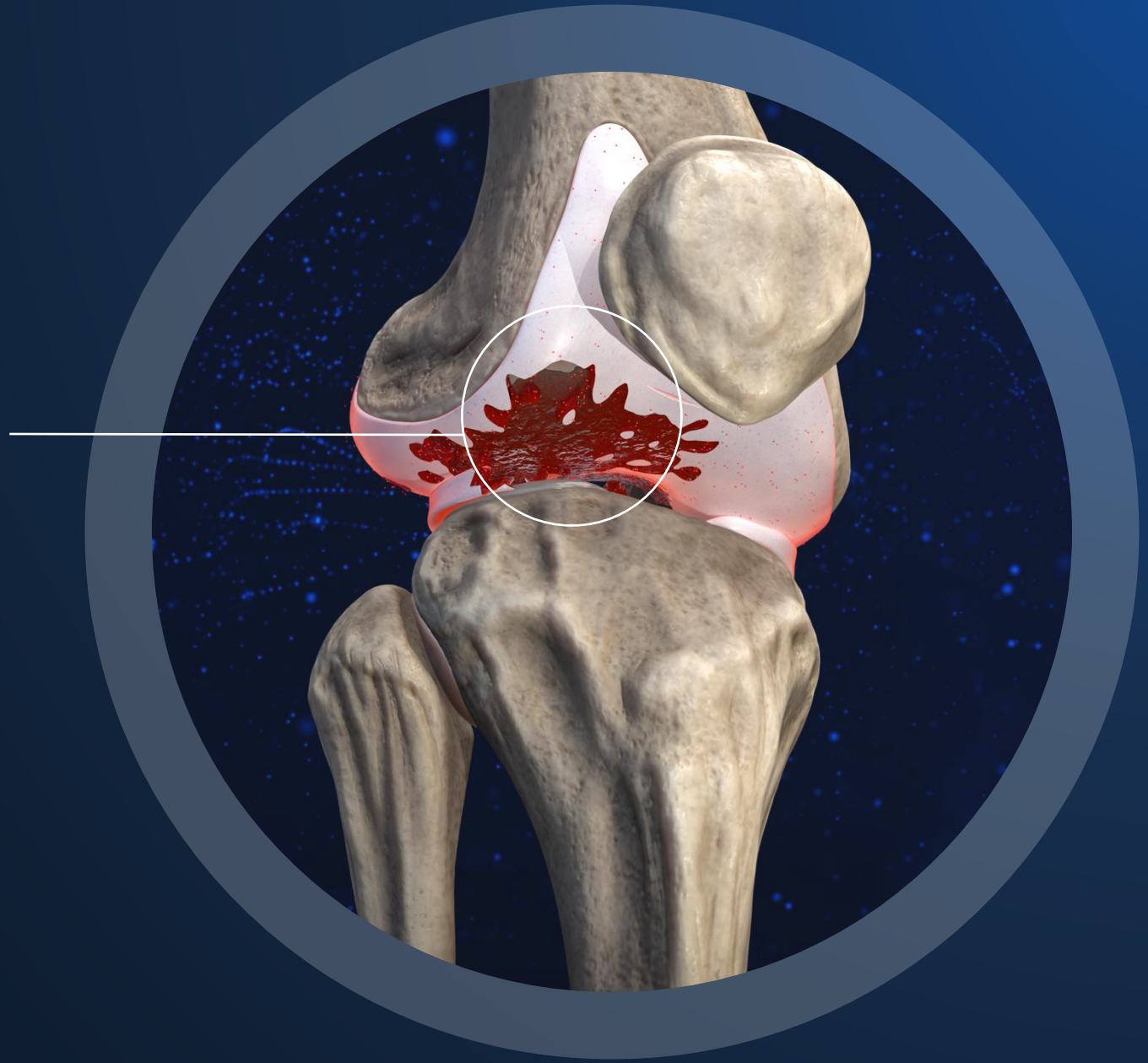
The Root Cause of OA

OA is caused by **hyperactive proteases**, a class of catabolic enzymes that degrade cartilage tissue.



A successful **disease-modifying OA drug** (DMOAD) must address target these protease enzymes. It restore joint health by encouraging tissue repair, reduce pain, and ultimately improve long-term quality of life.

Current OA Treatments are Failing

Current OA treatments relieve symptoms but don't address the root cause.

Conventional therapies are palliative, masking joint pain and inflammation temporarily, but fail to address the root molecular cause of the disease.

Non-steroidal Anti-inflammatory Drugs

(e.g., Advil)

▼

Temporary inflammation and pain reduction

Hyaluronic Acid

(viscous supplementation)

▼

Temporary artificial cushioning

Corticosteroids

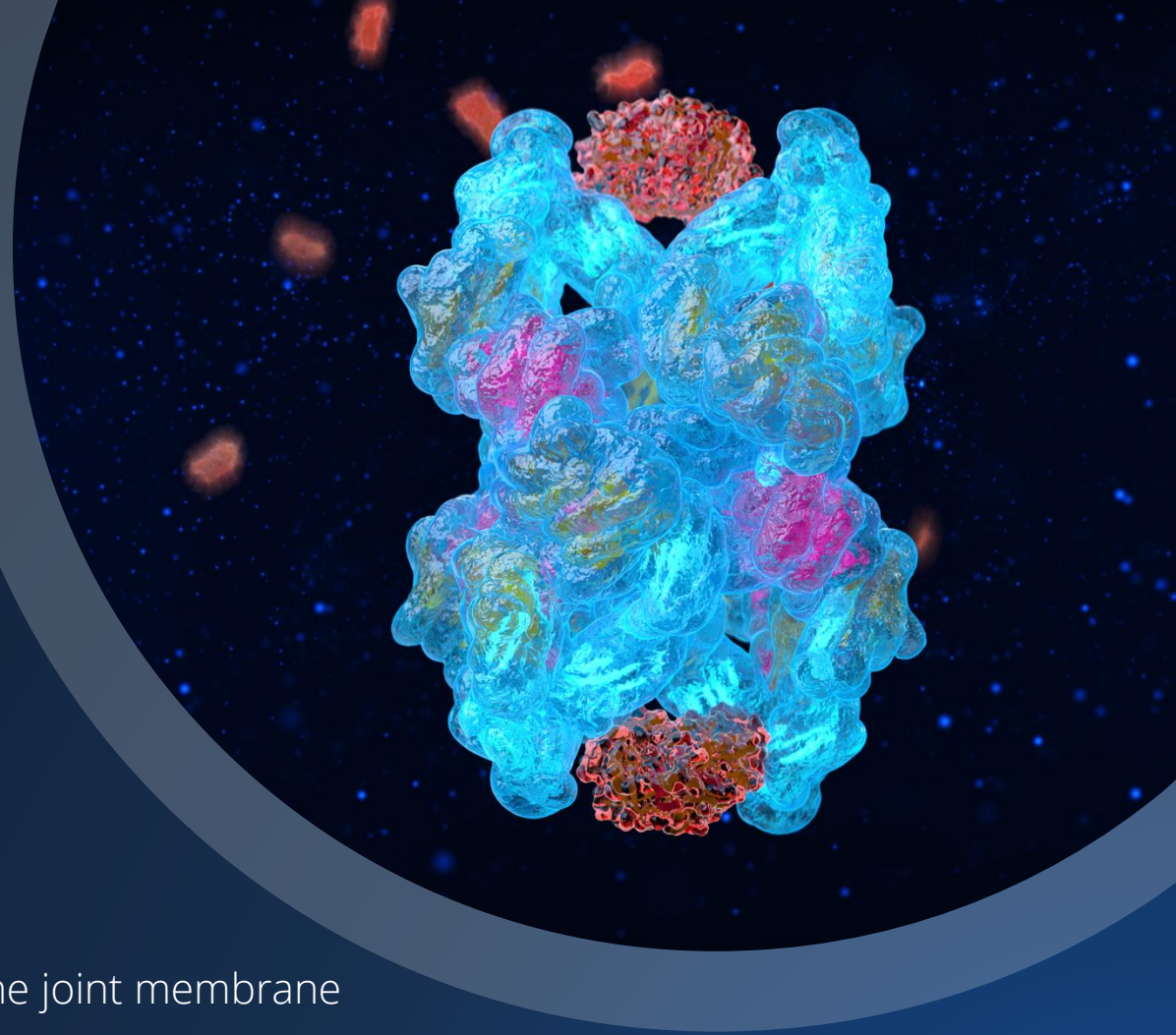
(e.g., Prednisone)

▼

Temporary inflammation reduction, many side effects of chronic use

Our Solution:

CYT-108: The First Disease-Modifying Therapy for Osteoarthritis



A Precision-Engineered "Super" Alpha-2-Macroglobulin (A2M) that Targets OA at its Root Cause

- Protease inhibitor with potent inhibitory activity against the proteases that are upregulated in OA
- Targets OA at its root cause to protect cartilage damage and the joint membrane
- Highly concentrated for targeted delivery directly into joint space
- Preclinical studies demonstrate significant reduction in the progression of OA
- Capable of manufacturing at scale and global distribution as a shelf-stable compound

CYTONICS®

"Nature's Blueprint, Perfected."

How CYT-108 Works

A2M is a naturally-occurring protease inhibitor that protects against cartilage degradation. Though levels are too low in joints to be an effective treatment…

CYT-108 is a genetically engineered **"super" A2M"** protein that is **200%** more potent than the naturally-occurring A2M.

Intra-articular (targeted) delivery for maximum concentration and minimum off-target effects

Sequesters cytokines and neutralizes destructive proteases, cutting off the inflammatory feedback loop

Designed to quickly reduce joint pain and inflammation while halting progressive cartilage degradation

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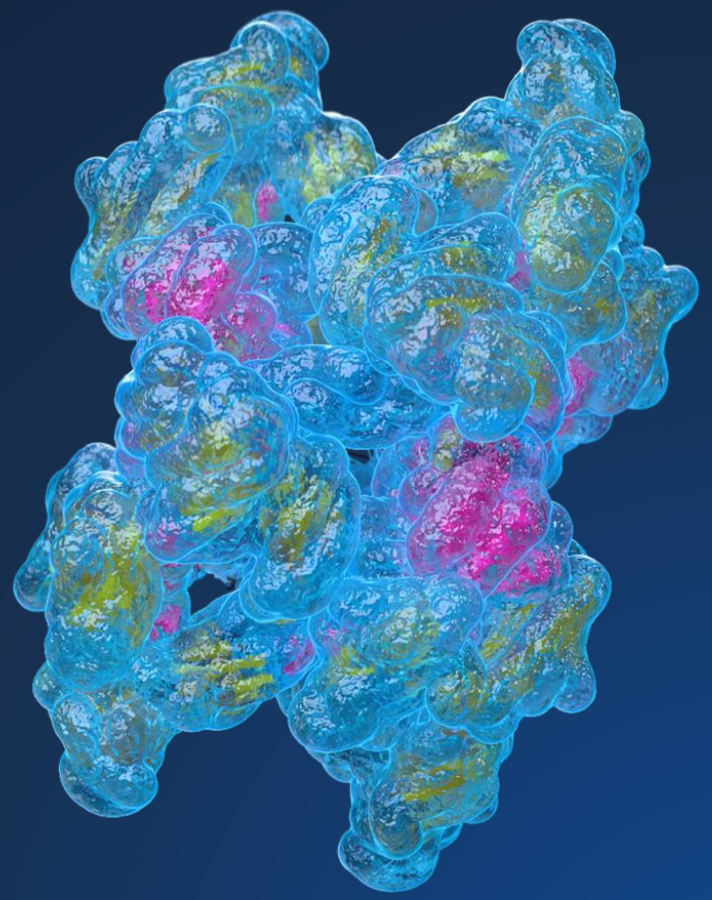
Leveraging Nature's Beautiful Design

the power of Alpha-2-Macroglobulin (A2M)

- **Alpha-2-Macroglobulin (A2M)** is a blood serum protein that plays a role in the clotting cascade.
- A2M is a *very* well characterized, broad-spectrum **protease inhibitor** that has demonstrated potent inhibitory activity against the proteases that are upregulated in OA.
- A2M's two "*bait regions*" act as a **Venus Flytrap** for proteases, encapsulating and rendering them inactive.
- Unfortunately, the naturally occurring levels of A2M are **too low** to lend any therapeutic benefit to damaged joints.
- We theorized that delivering **high concentrations of A2M** directly **into the joint spac**e could bind to and inhibit the proteases, slowing and eventually **halting the progression of OA.**



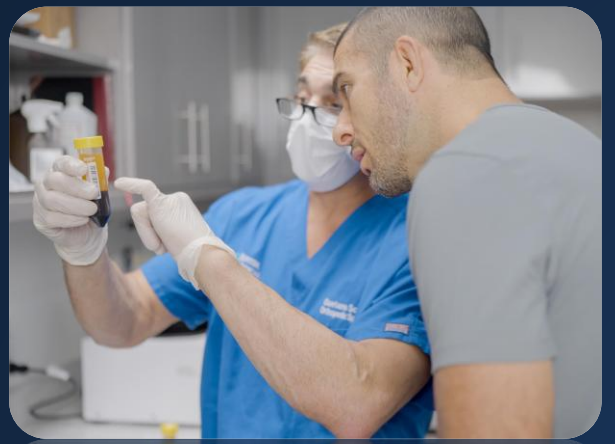
Now, we just had to prove it.

See Appendix for supporting A2M efficacy data and related scientific publications

Harnessing the Power of Alpha-2-Macroglobulin (A2M)

Our first-generation technology, the **A**utologous **P**rotease **I**nhibitor **C**oncentrate (**APIC™**) therapy put A2M on the map.

- The **APIC™** therapy selectively concentrates A2M from a patient's *own blood* to treat OA.
- **Successfully treated over 10,000 patients nationwide**, providing clinical and commercial proof of the therapeutic potential of the A2M protein. *See testimonials in Appendix.*
- Limitations: patient-to-patient variability in A2M levels, requires equipment and training, A2M extraction ~2hrs, insurance coverage



What if we don't have to rely on natural A2M…?

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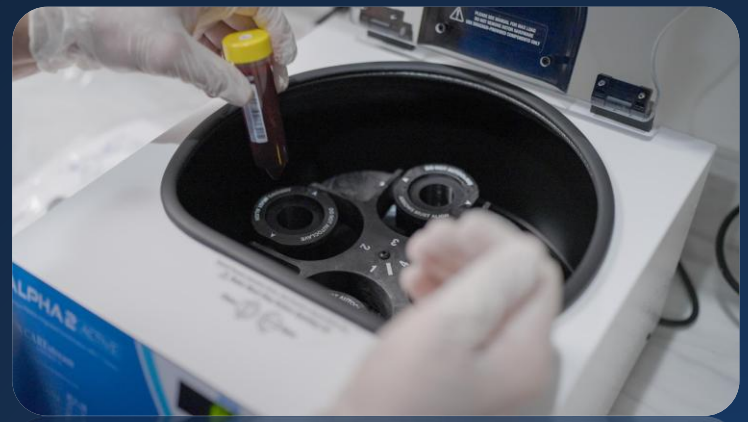
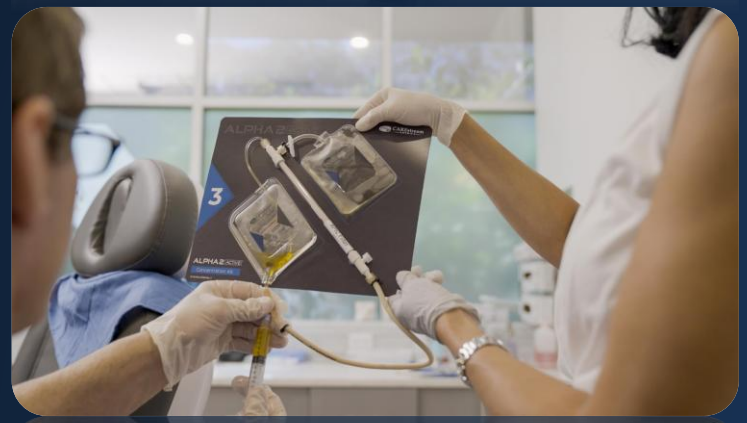
APIC™ Blood Processing & A2M Extraction





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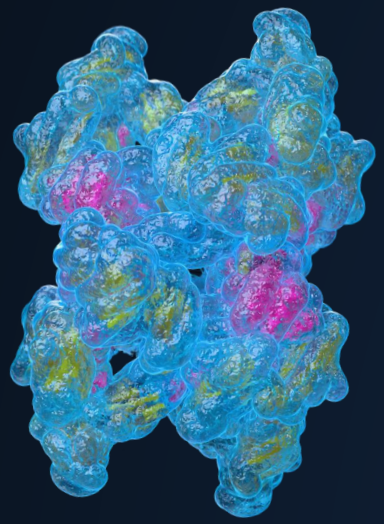
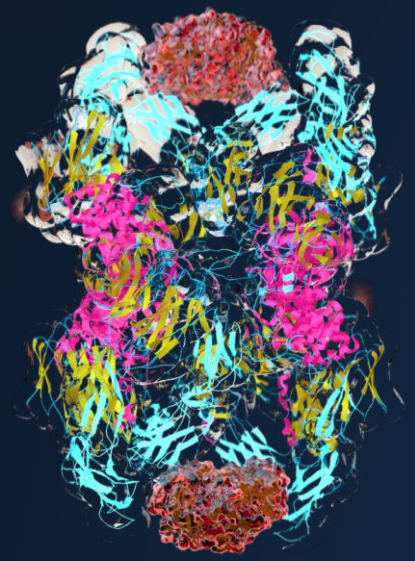
From APIC™ to CYT-108
The next generation of A2M therapy

Cytonics has genetically engineered a **"super A2M"** designed to specifically target and efficiently eliminate **ALL major classes proteases** involved in OA.



Natural A2M: APIC purified from patient blood



CYT-108: genetically-engineered (recombinant) A2M

	APIC™	CYT-108
Source	Patient's own blood	Genetically-engineered (recombinant A2M)
Efficacy	Patient-to-patient variability	Precisely dosed, 200% more potent & optimized against MMP-13 and ADAMTS-5
Scalability	Limited by blood processing	Laboratory production
Insurance Coverage	Uncertain	High potential

CYTONICS

Nature's Blueprint, Perfected

APIC™
validated A2M's effectiveness

▼

CYT-108
is *Nature's Blueprint, Perfected*

APIC™
is limited by patient-derived A2M

▼

CYT-108
is scalable, shelf-stable, and mass-producible for global distribution

APIC™
was a great first step

▼

CYT-108
is the future of OA treatment, high-probability of becoming 1st line therapy

CYTONICS

CYT-108 is Non-Toxic and Safe to Administer

Preclinical Safety Results

ORGAN PATHOLOGY

Does administration of CYT-108 affect the health of major organs?

"...pathology revealed findings consistent with those commonly observed in laboratory subjects, and not in laboratory subjects, and ***[no organ damage was] attributed to treatment with CYT-108."***

NO ORGAN DAMAGE

IMMUNOGENICITY

Does administration of CYT-108 **induce antibody creation?**

Anti-CYT-108 antibodies are created within 1 week of administration, and rapidly clear out "excess" CYT-108 that leaks into the bloodstream. These antibodies remain active over time. No additional CYT-108 was detected in the bloodstream upon subsequent injection**, indicating a rapid and robust clearance without adverse effect on the animal.**

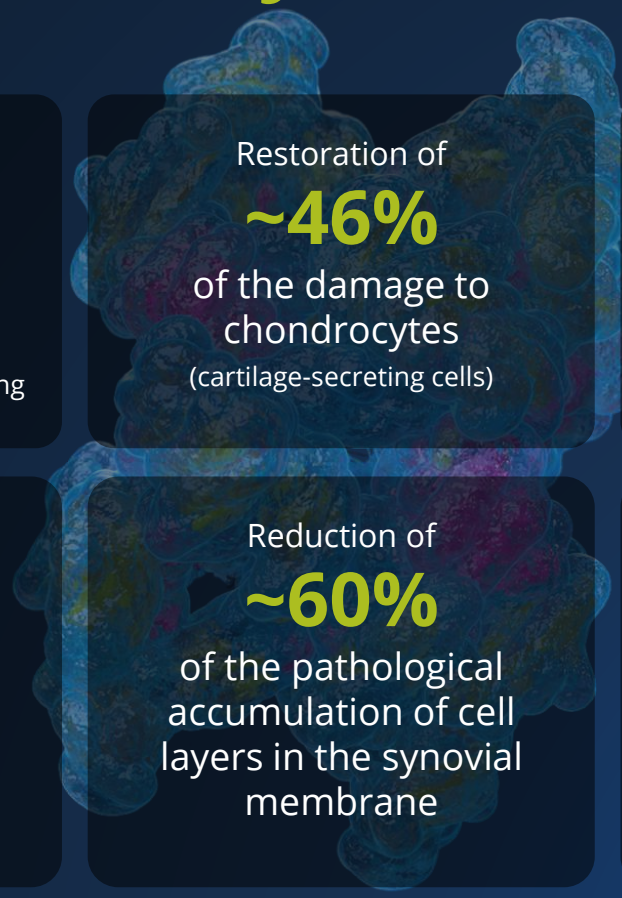
IMMUNE CLEARANCE

Note: The data represented in this slide is from a preclinical experiment conducted by Sinclair Research Center LLC ("Sinclair"), a Contract Research Organization located in Auxvasse, MO. Sinclair was contracted by Cytonics to perform the preclinical studies measuring the effect of CYT-108 on cartilage integrity and other joint tissues. Data was analyzed by an independent pathologist.

CYT-108 Protects Cartilage and Joint Tissue

Preclinical Efficacy Results

CYT-108 has therapeutic effect in **protecting** articular cartilage, bone, and synovial membrane physiology when administered to subjects suffering from post-traumatic osteoarthritis, and substantially **restores** the cartilage matrix, underlying subchondral bone, and the synovial membrane back to normal, healthy anatomy and physiology.



Recovery of
~57%
of the damage to the cartilage structure
as measured by Safrinin-O staining

Restoration of
~46%
of the damage to chondrocytes
(cartilage-secreting cells)

Enhancement of
~105
proteoglycan content
(key component of cartilage)

Reduction of
~77%
of the subchondral bone plate thickness
back to normal levels

Reduction of
~60%
of the pathological accumulation of cell layers in the synovial membrane

Reduction of
~31%
of the synovial tissue hyperplasia back to normal levels
(pathological membrane thickening)

See Appendix for supplemental CYT-108 efficacy data

Note: The data represented in this slide is from a preclinical experiment conducted by Sinclair Research Center LLC ("Sinclair"), a Contract Research Organization located in Auxvasse, MO. Sinclair was contracted by Cytonics to perform the preclinical studies measuring the effect of CYT-108 on cartilage integrity and other joint tissues. Data was analyzed by an independent pathologist.

CYTONICS

CYT-108 Phase 1 Clinical Study

As a true "disease-modifying" treatment, CYT-108 will catalyze a **new era of quality of life**, enabling everyone to enjoy an active, pain-free existence into their golden years.

If approved by the FDA, CYT-108 will likely be the first and only biopharmaceutical capable of targeting OA's root cause, providing *both* **symptomatic relief** and **restoring the joint** to a healthy state.

JUNE 2024
First-in-human trial for CYT-108 initiated; first patient first dose

MAR 2025
Last patient last visit

Q3 2025
Clinical Study Report

Savings up to
$27B
annually*

Attractive to Insurance Payers

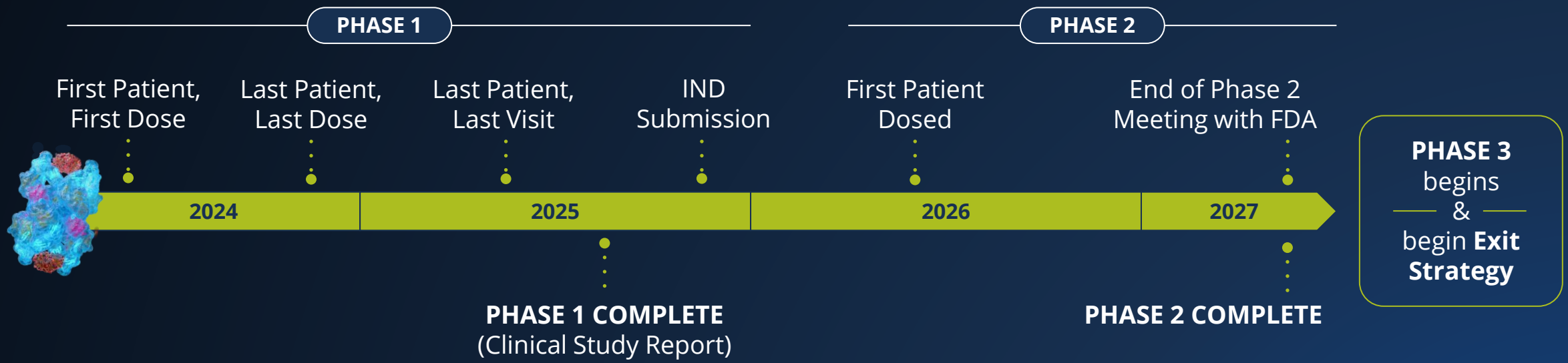
If approved, CYT-108 will be an **attractive first-line therapy** due to its ability to **obviate joint replacement surgeries**, as a biopharmaceutical that has demonstrable efficacy in randomized clinical trials, can be synthesized on an industrial scale and distributed globally.

Avg cost of U.S. Total Knee Replacement:
$33,000

*https://www.healthpartners.com/blog/cost-of-a-knee-replacement-surgery | 790,000 TKR surgeries conducted annually (https://anmed.org/wellness/blog/total-knee-hip-replacement-surgeries-help-millions

CYTONICS

The Path to CYT-108 Approval



PHASE 3 SCENARIO:

- Initiate Phase 3 **3Q '27**
- Phase 3 concludes **4Q '28**
- Biologic License Application submitted to FDA for possible approval **1Q '29**

POTENTIAL EXIT SCENARIOS:

IPO

- **Raise capital** for PHASE 3
- Uplist to **NASDAQ**
- Stock becomes **liquid** (buy/sell)

ACQUISITION

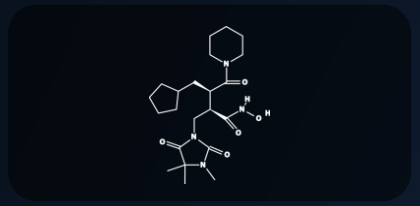
- Acquirer buys PHASE 2 asset
- All shareholders bought out in **private deal**

STRATEGIC PARTNERSHIP

- **"BioBucks":** upfront cash, milestones, and royalties
- Decision: IPO *or* remain private use cash to fund new R&D

CYTONICS

Succeeding where Big Pharma has failed



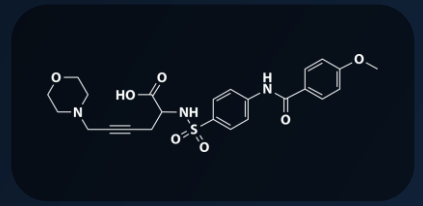
Cipemastat (Trocade)

Manufacturer:
Roche

Date of Failure:
~2001

Reason:
Lack of efficacy; musculoskeletal side effects due to off-target toxicity

CYT-108 broadly inhibits multiple protease classes simultaneously and closely mimics a natural human protein, ensuring safety and tolerability even at supraphysiological levels due to its endogenous origin



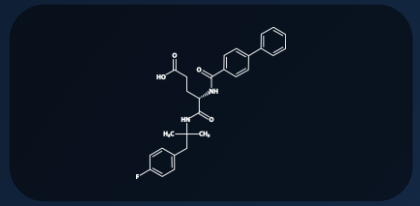
PG-116800

Manufacturer:
Proctor & Gamble

Date of Failure:
2004

Reason:
No efficacy; severe musculoskeletal toxicity (shoulder pain, tendon fibrosis)

CYT-108 has proven safety in preclinical studies at doses up to 10x intra-articular concentrations, indicating significantly reduced risk of musculoskeletal adverse events compared to traditional synthetic inhibitors



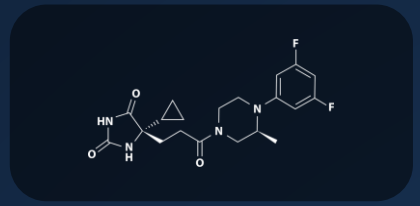
AGG-523

Manufacturer:
Wyeth (Pfizer)

Date of Failure:
~2009

Reason:
Terminated early due to strategic reprioritization; no established efficacy signal

CYT-108's development is substantially de-risked because its effectiveness is supported by both robust *in vivo* animal data and commercial success of natural A2M in APIC™ therapy



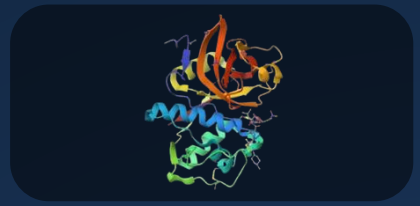
GLPG1972 (S201086)

Manufacturer:
Galapagos NV/Servier

Date of Failure:
2020

Reason:
Lack of structural and symptomatic efficacy; failed primary endpoint of cartilage preservation.

CYT-108 has been shown to restore joint tissues (cartilage, synovial membrane, bone) back to healthy states *in vivo*. It also positively influenced anabolic metabolism markers, providing evidence of potential clinical symptomatic relief and disease-modifying effects



MIV-711

Manufacturer:
Medivir AB

Date of Failure:
2019

Reason:
Structural efficacy without symptomatic improvement

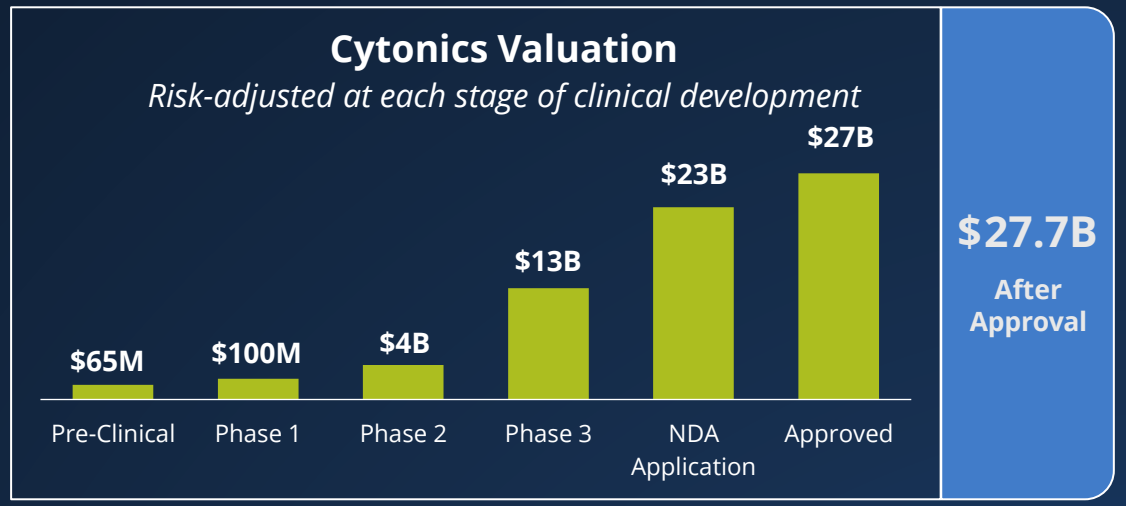
CYT-108 has demonstrated *both* cartilage preservation and significant anabolic activity, suggesting likely disease-modifying improvements as well as symptomatic relief. It addresses patient pain and mobility which are clinically meaningful endpoints that MIV-711 failed to meet

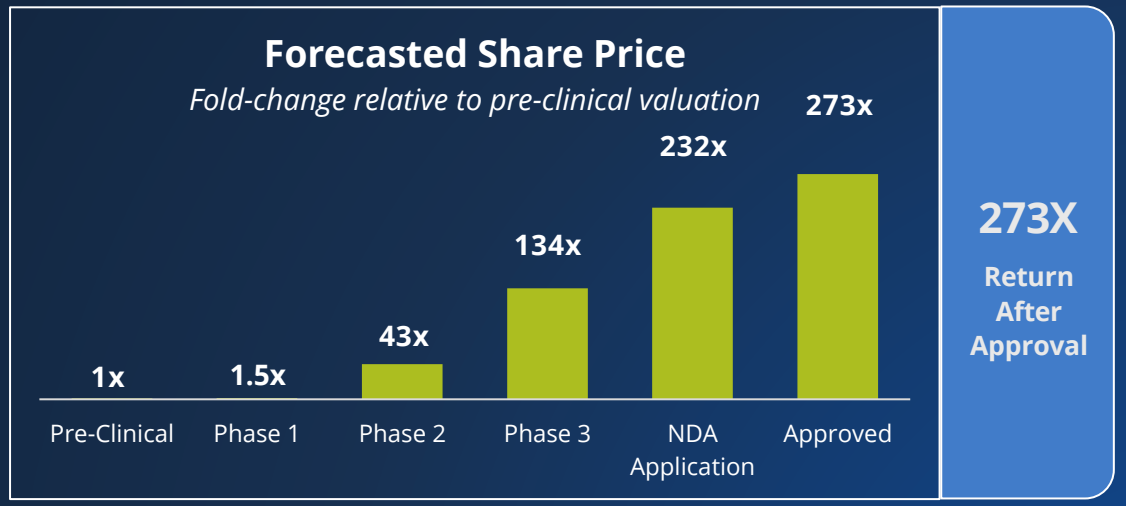
CYTONICS

Let's talk Growth
increasing shareholder value

Risk-Adjusted Discounted Cash Flow Assumptions:

- **Discount rate of 50%**
  (this is appropriate for pre-clinical stage biotech)*
- **20% market capture**
  (conservative estimate) in the US human orthopedic market *only*
  (does not include expansion into other markets)
- **$2,000 per treatment, avg. 2 treatments per year**
  (Hyaluronic injections cost >$2,000 each)
- Cytonics assumes full development cost of bringing CYT-108 to market and producing and selling the drug upon FDA approval
  - COGS = 15% of revenue (According to a meta-analysis compiled in Biotech Forecasting & Valuation (2016)**. Data was retrieved from company10-k filings.)
  - SG&A = 34% (According to an analysis of 35 small- and mid-cap drug companies in the NASDAQ Biotechnology Index in 2015, reported in Biotech Forecasting & Valuation (2016)**
- Upon patent expiry, Cytonics loses 20% of CYT-108 sales per year (Terminal Value in perpetuity)





These figures are derived from a rDCF financial forecast and not guaranteed.

*https://www.linkedin.com/pulse/valuation-methodologies-life-science-companies-crean-ph-d-mba/

**David, Frank S, et al. "The Pharmagellan Guide to Biotech Forecasting and Valuation,, Pharmagellan LLC, Pharmagellan, www.pharmagellan.com/book.

***Note: Increase in shareholder value calculated as the relative change in share price compared to the preclinical valuation, and accounts for future anticipated dilution to fund the Phase 2 and Phase 3 clinical trials.

CYTONICS

A Half-Trillion Market Waiting for a Real Solution.

This debilitating disease must be stopped.

Over 500M people with OA have no access to an effective treatment that targets the disease at its source

OA affects 13X more people than Rheumatoid Arthritis (RA)

Global RA Sales:
(TNF-α inhibitors)
$43 billion

13x more people have **OA** than RA

Potential Global
OA Therapeutic Market:
$560+ billion

OA prevalence: https://www.who.int/news-room/fact-sheets/detail/osteoarthritis
RA prevalence: https://pubmed.ncbi.nlm.nih.gov/30285183/
RA market size: https://www.researchandmarkets.com/reports/5733874/tnf-alpha-inhibitors-global-market-report

CYTONICS

A Grassroots Effort: *For the People, By the People*

Biopharma
almost exclusively capitalized by Venture Capital.

Cytonics' CYT-108
program is exclusively funded by "everyday" investors, who have equipped us to take on Big Pharma and the Wall Street behemoths with the single cast of a stone.

Invest like a Venture Capitalist

- Private deal, pre-IPO / acquisition / strategic partnership
- High growth sector, biotech exit multiples

Challenge Big Pharma's stranglehold on innovation

- Big Pharma has failed to develop a disease-modifying therapy for OA, leaving patients with only palliative treatments
- Estimated ~ $1B spent on failed clinical trials
- We know *why* they failed, and *what* to do about it

Subvert Wall Street's grip on the biotech sector

- Crowdfunding is *democratizing* biopharma investing
- Cytonics is backed by over 6,000 retail investors
- Disrupting the traditional VC funding model (and they are taking notice)

CYTONICS

The Core Team

MANAGEMENT TEAM

BOARD OF DIRECTORS

Gaetano Scuderi, MD

Founder and Chairman of the Board

Joey Bose, MS

President & CEO

Lewis Hanna, PhD

Chief Scientific Officer

Gordon Ramseier, MBA

Independent Director

Tracy Goeken, MD

Independent Director

Stanford University

UC San Diego

University of Virginia

Johns Hopkins University

Alexion

Bristol Myers Squibb

The R.W. Johnson Pharmaceutical Research Institute

ONCOtherapeutics

dura Pharmaceuticals

BCI

Pfizer

Clinical Development Partner Linical

Nuron Biotech

somahlution

Pharm-Olam Helping Create A Healthier World

CYTONICS

The OA Dream Team

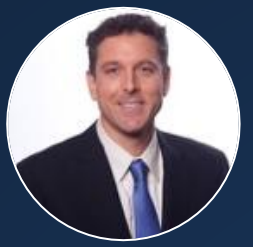
MEDICAL ADVISORY BOARD

Ketan Desai, MD, PhD

Chief Medical Officer, Levolta Pharmaceuticals

Mark Schweitzer, MD

Vice President for Health Affairs, Wayne State University



Geoffrey D. Abrams, MD

Associate Professor of Orthopedic Surgery, Stanford University

Iain Lachlan (Lachy) McLean, MD, PhD

Chief Medical Officer, Genascence Corporation

Mukesh Ahuja, MD

Global Clinical Head of Osteoarthritis, Paradigm Biopharmaceutical

Michael Wyand, DVM

CEO of Oxeia Biopharma

OUR COLLABORATORS

BROWN

The Scripps Research Institute

Stanford MEDICINE

Jefferson University and Hospitals

Beaumont HEALTH SYSTEM

bme University of Michigan Biomedical Engineering

FAU Florida Atlantic University

CYTONICS

Patent Status Summary

APIC™, FACT™, and Recombinant A2M Variant (CYT-108) Claims

COMPOSITION OF MATTER

AUTOLOGOUS "APIC™"

Liquid A2M composition from blood

- GB 2501611B
- AU 2013222414
- U.S. 10,265,388
- CA 3,095,010
- U.S. 18/777,657
- CA 2865170
- U.S.11,040,092
- EP 2827882
- GB 2827882
- FR 2827882
- DE 2827882

Non-immunogenic Liquid A2M composition

- AU 2013222414

A2M Enriched by +1.1x (10%+)

- GB 2501611B
- AU 2013222414
- U.S. 10,265,388

RECOMBINANT "CYT-108"

CYT-108 engineered bait region

- GB 2503131B
- U.S. 10,400,028
- U.S. 10,940,189
- U.S. 12,195,521
- U.S. 18/947,045
- U.S. 18/777,657
- AU 2015349782
- JP 6861152
- U.S. 12,967,973

CYT-108 bait region comprises protease recognition sites

- GB 2503131B
- U.S. 10,400,028
- U.S. 10,940,189
- AU 2015349782
- CA 2,967,973
- U.S. 11,634,475

CYT-108 protease Inhibition

- GB2 503131B
- U.S. 10,889,631
- EP 3221341
- DE 3221341
- FR 3221341
- GB 3221341

METHODS OF USE / TREATMENT

Method of treating chronic wounds with autologous A2M

- U.S. Pat. No. 9,352,021

Method of treating chronic wounds with autologous A2M at 1.1x higher tha sample

- U.S. Pat. No. 9,352,021

Method of treating chronic wounds with autologous A2M + non-A2M proteins

- U.S. Pat. No. 9,352,021

Method of treating chronic wounds with recombinant A2M

- U.S. Pat. No. 9,498,514

Autologous composition of enriched A2M to treat degenerative joint diseases

- EP 13751112.7
- CA 2865170
- JP 6861152
- U.S. 10,889,631
- CA 2,967,973
- CA 3,095,010

DEVICES

APIC flow filtration module + centrifuge

- GB2522561B

DIAGNOTICS

Detection of FAC biomarker

- U.S. 10,889,631

METHODS OF DEVELOPMENT

Engineering recombinant A2M polypeptides

- U.S. 10,889,631

Issued Pending

CYTONICS

Self-Evident Success

Navigating FDA's complex regulatory process by commercializing APIC ™ (510k)

Proven tech with over 10,000 patients treated with APIC™ (first gen A2M therapy)

Awarded $1.8M in NIH grants to pursue innovative treatments for osteoarthritis

$25M in funding raised from HNWI, private equity, and equity crowdfunding (Reg A+/CF)

Over 6,000 equity crowdfunding shareholders invested over $15M

De-risked development CYT-108 has demonstrated over 200% more potency than the naturally occurring A2M purified by APIC™

Initiated Phase 1 clinical study of CYT-108 as a treatment for osteoarthritis of the knee

A robust and multi-layered IP fortress of 25 patent spanning key global markets (USA, EU, AU, JP, CA)

CYTONICS

Join the Movement: Why Invest?

- Succeeding where Big Pharma has failed
- CYT-108 poised to be the *first* DMOAD *ever* created
- APIC™ uniquely de-risks development of CYT-108
- Track record of navigating FDA's complexity
- $500B+ market potential for DMOAD drug
- Broad patent coverage in key geographies
- OA Dream Team: 6 MDs > 80 OA clinical trials
- No Corporate Backers, Just People on a Mission
- Uniting Individuals to Disrupt Big Pharma's Stranglehold

THE OFFERING

$20M Reg A & Reg D

USE OF FUNDS

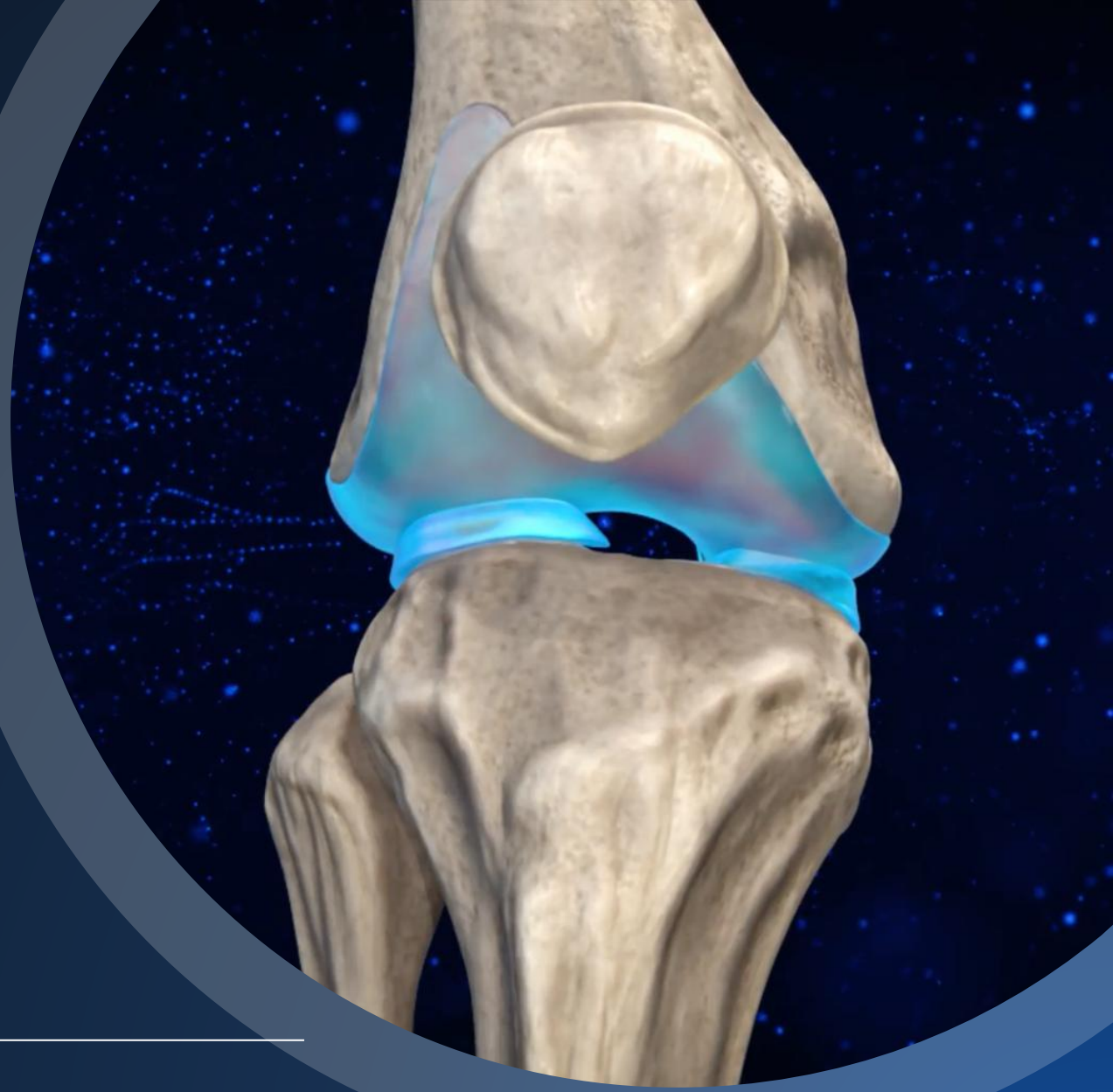
CYT-108 Phase 2 Clinical Trial

EXIT STRATEGY

- Uplist to the NASDAQ
- Strategic Partnership
- Acquisition

CYTONICS

Engineering the Future of Joint Health with Molecular Precision



invest.cytonics.com

Join us in a *grassroots* effort to reclaim biotech innovation from Big Pharma and Wall Street

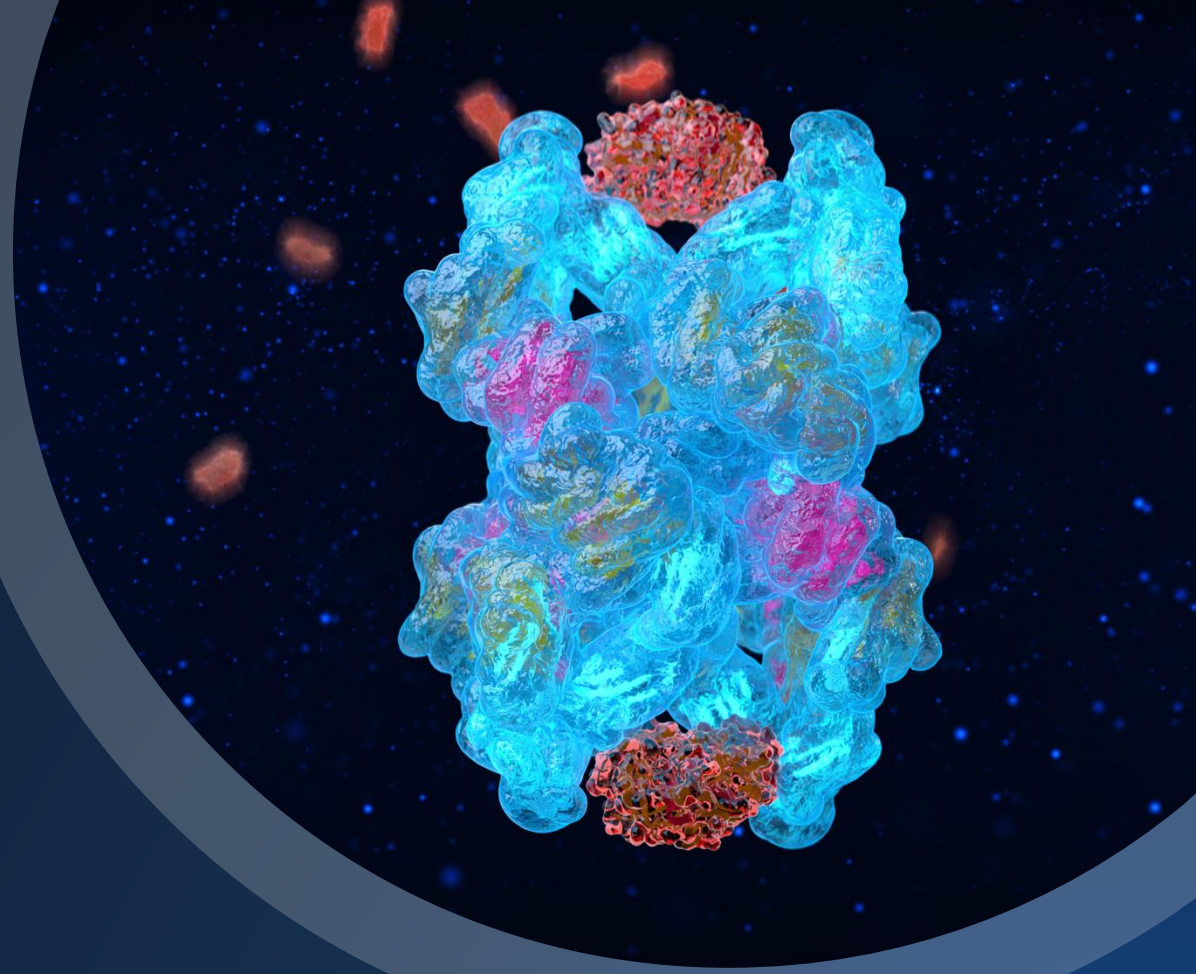
Appendix

Testimonials

Scientific Publications

Supporting A2M Efficacy Data

Supplemental CYT-108 Efficacy Data



CYTONICS

“Nature’s Blueprint, Perfected.”

APIC™ is Patient Tested, Physician Approved

A2M has been instrumental in treating my patients with cartilage injuries that are **otherwise not amenable to cartilage restoration procedures**... I have returned the majority of my patients who received an A2M injection to participation in sports successfully **without the use of a scalpel**.

—David E. Dominguez, MD, President/Owner: 3D Sports Medicine and Orthopedic Center

I have been using Cytonics' APIC kits to treat various joint pains (mostly in the knee). This is part of my regenerative medicine practice. I've seen **remarkable results** such that I have suggested that **my wife and my son** undergo treatments as well as patients.

—Laurence Rosenfield, MD, orthopedist, advocate of APIC treatment for patients and family

I had a **single treatment** of Cytonics' APIC therapy, APIC. **Within 2 days the swelling and stiffness was gone** and hasn't returned 6 months later. I was so impressed with these results that I have been evangelizing for APIC treatment to my doctors and friends ever since.

—Gabe, patient and APIC advocate

After almost eight months of therapy and various treatments, Richard Grossman, MD told me about Cytonics and the available APIC treatment. I received my first injection in April of 2018 and **within weeks the large nodule in my Achilles had shrunk** significantly. While I was feeling much better and able to **start playing basketball and tennis again**.

—Daryle Bobb, patient

These testimonials may not be representative of the experience of other customers and are not a guarantee of future performance or success.

Scientific Publications

Zhang, Yang, et al. " *Targeted Designed Variants of Alpha 2 Macroglobulin (A2MJ Attenuate artilage Degeneration in a Rat Model of Osteoarthritis Induced by Anterior ruciate ligament ransection*." Arthritis Research & Therapy, vol. 19, no. 1, 2017, doi:10.1186/s13075-017-1363-4.

Abrams, Geoffrey D., et al. *"Fibronectin–Aggrecan Complex as a Marker for Cartilage Degradation in Non-Arthritic Hips."* Knee Surgery, Sports Traumatology, Arthroscopy, vol. 22, no. 4, 2014, pp. 768—773., doi:10.1007/s0167-014-2863-2.

Bedi, Asheesh, et al. *"The Effect of Matrix Metalloproteinase Inhibition on Tendon-to-Bone Healing in a Rotator Cuff Repair Model."* Journal of Shoulder and Elbow Surgery, vol. 19, no. 3, 2010, pp. 384—391., doi:10.1016/j.jse.2009.07.010.

Browning, Shawn R, et al. *"Platelet-Rich Plasma Increases Matrix Metalloproteinases in Cultures of Human Synovial Fibroblasts."* The Journal of Bone and Joint Surgery-American Volume, vol. 94, no. 23, 2012, doi:10.2106/jbjs.k.01501.

Cuellar, Jason M. *"Intradiscal Injection of an Autologous Alpha-2-Macroglobulin (A2MJ Concentrate Alleviates Back Pain in FAC-Positive Patients."* Orthopedics and Rheumatology Open Access Journal, vol. 4, no. 2, Mar. 2017, doi:10.19080/oroaj.2017.04.555634.

Demirag, Burak, et al. *"The Effect of Alpha-2 Macroglobulin on the Healing of Ruptured Anterior Cruciate Ligament in Rabbits."* Connective Tissue Research, vol. 45, no. 1, 2004, pp. 23—27., doi:10.1080/03008200490278115.

Scientific Publications

Demirag, Burak. *"Enhancement of Tendon-Bone Healing of Anterior Cruciate Ligament Grafts by Blockage of Matrix Metalloproteinases."* The Journal of Bone and Joint Surgery (American), vol. 87, no. 11, Jan. 2005, p. 2401., doi:10.2106/jbjs.d.01952.

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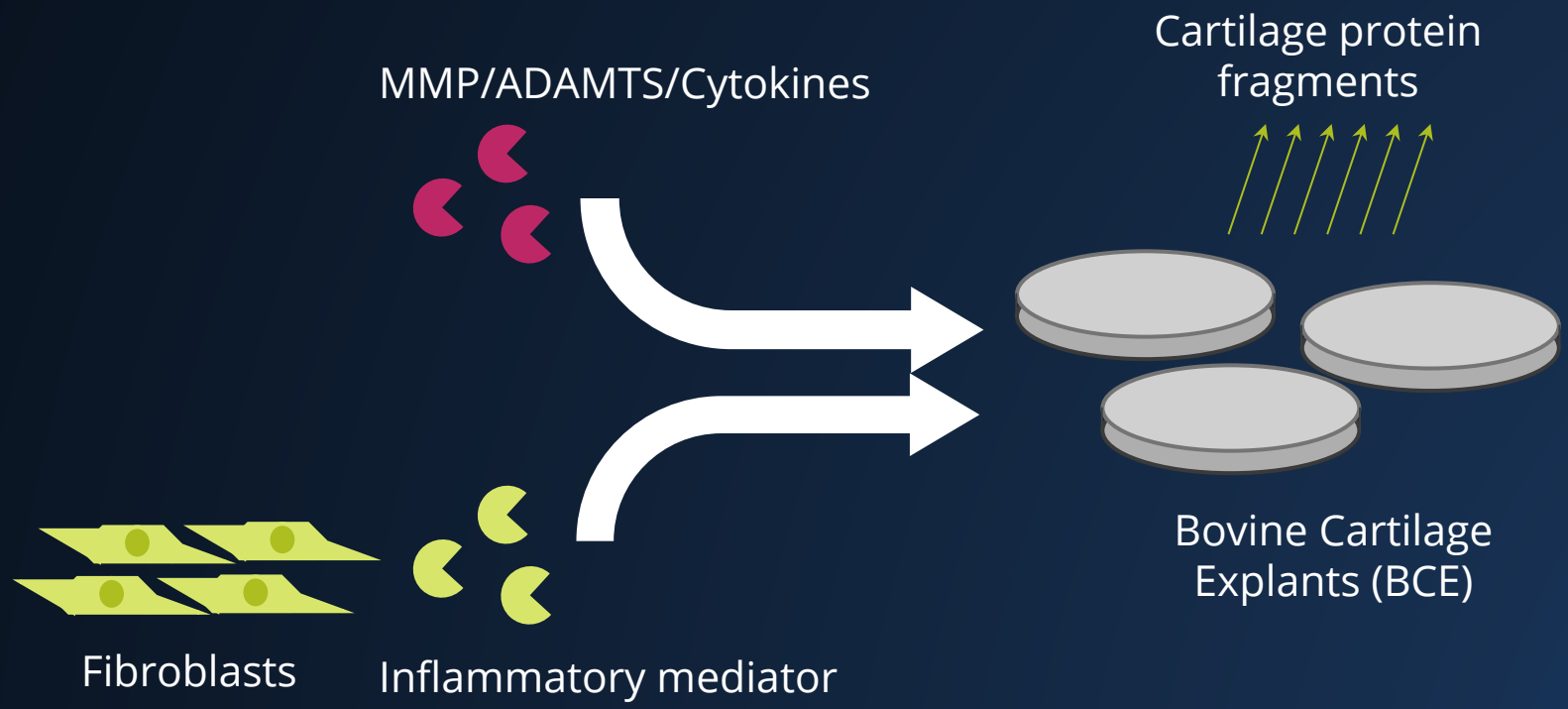
Luan, Y., et al. *"Inhibition of ADAMTS-7 and ADAMTS-12 Degradation of Cartilage Oligomeric Matrix Protein by Alpha-2-Macroglobulin."* Osteoarthritis and Cartilage, vol. 16, no. 11, 2008, pp. 1413—1420., doi:10.1016/j.joca.2008.03.017.

Marynen, P., et al. *"A Genetic Polymorphism in a Functional Domain of Human Pregnancy Zone Protein: the Bait Region."* FEBS Letters, vol. 262, no. 2, 1990, pp. 349—352., doi:10.1016/0014-5793(90)80226-9.

Tortorella, Micky D., et al. *"a2-Macroglobulin Is a Novel Substrate for ADAMTS-4 and ADAMTS-5 and Represents an Endogenous Inhibitor of These Enzymes."* Journal Biological Chemistry, vol. 279, no. 17, July 2004, of pp. 17554—17561., doi:10.1074/jbc.m313041200.

CYTONICS

Bovine Cartilage Explants (BCE)
a quantitative *ex vivo* assay to measure cartilage degradation

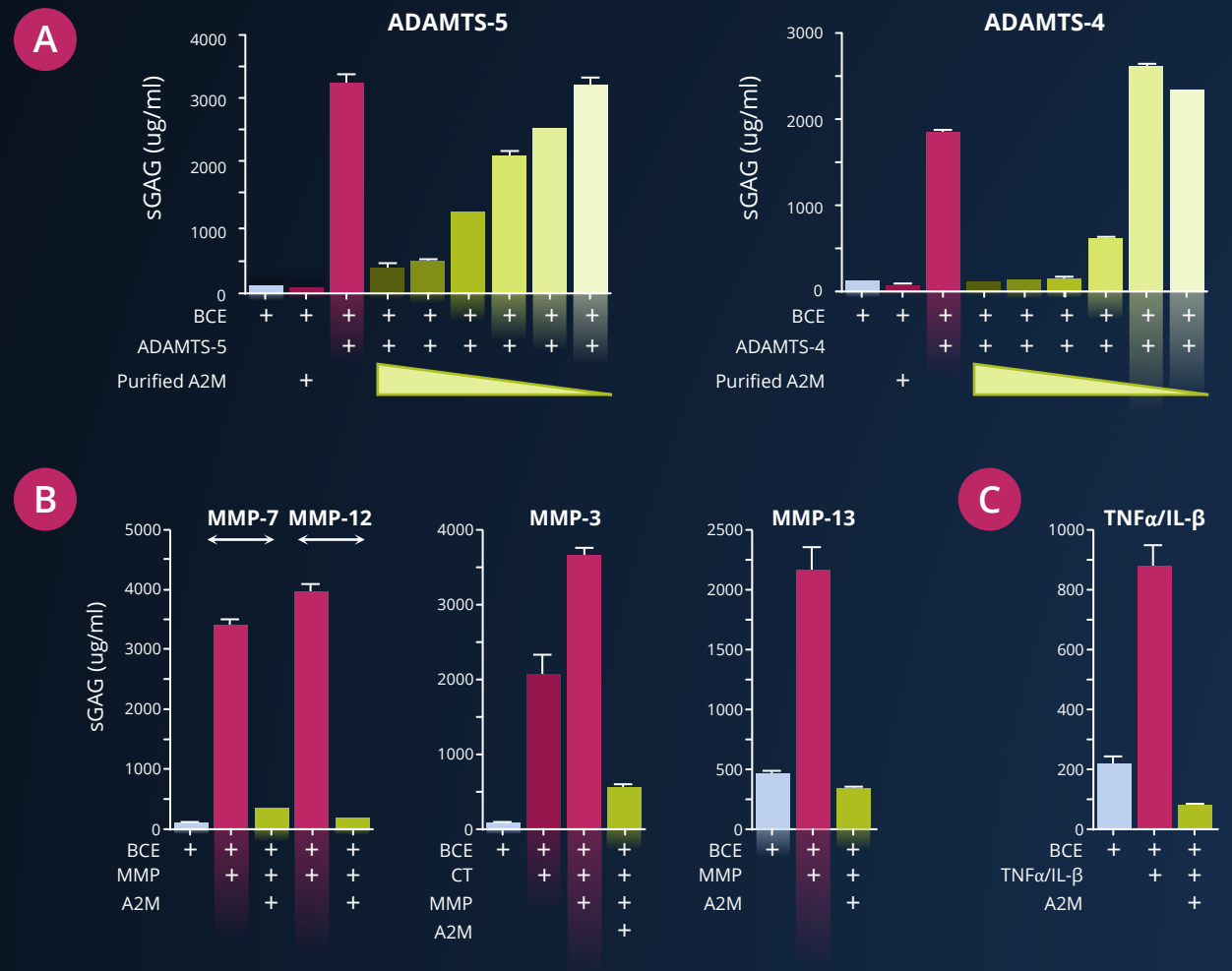


Cartilage digestion products and protein aggregates can be detected in supernatant: *sulfated proteoglycans and glycosaminoglycans (**sGAG**)*

Cartilage explants can be **macroscopically graded** to assess tissue damage using *OARSI or Mankin scoring systems.*

CYTONICS

Human purified A2M reduces protease-induced cartilage degradation in a dose-dependent manner



Following published BCE methods, cartilage degradation was quantified by sulfated glycosaminoglycan release (sGAG) at baseline (blue bars) and after the addition of the known catabolic enzymes, 500 ng/ml ADAMTS-4 and ADAMTS-5 (**A**), 5 ug/ml MMP-7, MMP-12, MMP-3, or MMP-13 (**B**), or the combined treatment of 80 ng/ml TNF-α and 8 ng/ml IL-1β (**C**); all caused significant release of sGAG into the culture media (red bars). MMP-3 requires activation with chymotrypsin (CT) 30 minutes prior to treatment of BCE, which also led to digestion of cartilage and release of sGAG (purple bar). The co-administration of **purified wt-A2M (commercially available blood protein purified from plasma)** (3.1 - 100µg/ml) significantly reduced the augmented sGAG release by ADAMTS-5 or ADAMTS-4 in a dose-dependent manner (**A**, green bars) and addition of 100 ug/ml or 5 mg/ml purified wt-A2M was sufficient to inhibit cartilage catabolism by MMPs and proinflammatory cytokines, respectively. Values mean +/- SEM; n=5.

A2M may be the protease inhibitor responsible for the chondroprotective activity of purified blood serum

CYTONICS

"High-IQ" Screening of A2M Variants

Relative protease inhibitory activity (IC50) of the recombinant A2M variants compared to wt-A2M using FITC-conjugated protease substrates.

		Cyt-98 Avg ± SD	Cyt-102 Avg ± SD	Cyt-105 Avg ± SD	Cyt-108 Avg ± SD
Aggrecanases	ADAMTS-4	208	290	176	201
	ADAMTS-5 (Casein)	170 ± 2	175 ± 10	173 ± 2	161 ± 9
	ADAMTS-5 (Aggrecan)	440	194	200	246
Collagenases	MMP-1	108 ± 2	91 ± 6	133 ± 2	119 ± 20
	MMP-8	54 ± 1	81 ± 3	132 ± 5	64 ± 2
	MMP-13	72 ± 10	76 ± 11	133 ± 16	100 ± 15
Gelatinases	MMP-2	16 ± 6	65 ± 6	80 ± 0	37 ± 6
	MMP-9	0 ± 0	71 ± 4	71 ± 3	41 ± 3
Inflam. Proteases	Elastase Cathepsin-G	117 ± 7	97 ± 8	147 ± 5	124 ± 12
		105 ± 5	54 ± 10	145 ± 9	117 ± 11

Table 3: A2M variants' efficacy at inhibiting 9 proteases implicated in OA. Results are expressed as a percent inhibition by each variant compared to wt-A2M.

CYTONICS

Ex vivo efficacy of A2M variants: CYT-98 CYT-108

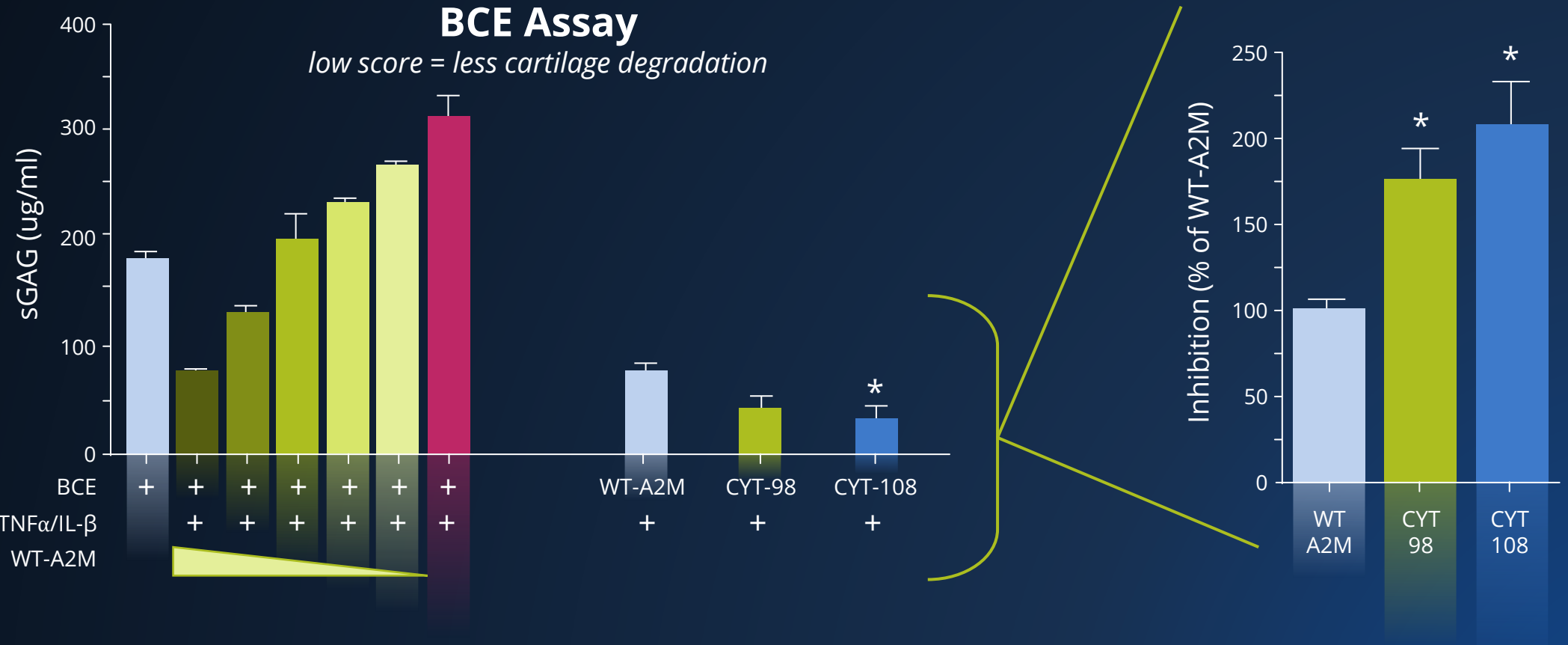


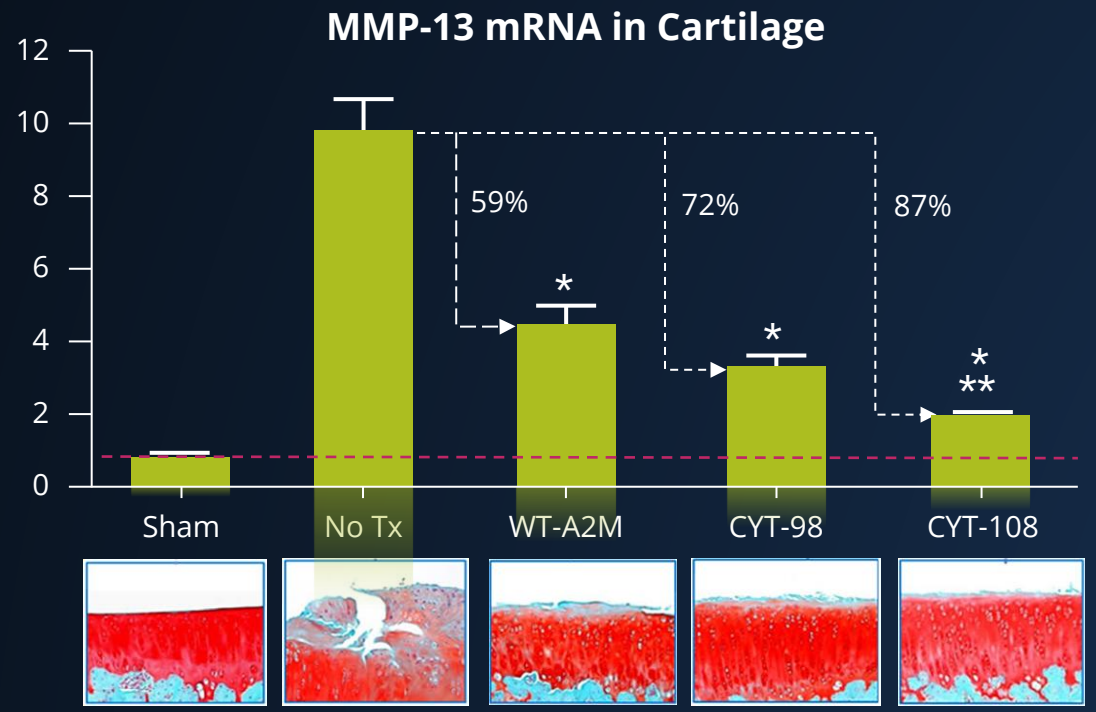
Fig. 1 Wild-type alpha-2-macroglobulin *(WT-A2M) (left)* and A2M variants CYT-98 and CYT-108 *(right)* inhibit cartilage catabolism induced by TNFα and IL-1β. *Compared with wt-A2M, $P<0.05$. BCE, bovine articular cartilage explants, SGAG sulfated glycosaminoglycan

Zhang, Y., Wei, X., Browning, S. et al. Targeted designed variants of alpha-2-macroglobulin (A2M) attenuate cartilage degeneration in a rat model of osteoarthritis induced by anterior cruciate ligament transection. Arthritis Res Ther 19, 175 (2017). https://doi.org/10.1186/s13075-017-1363-4

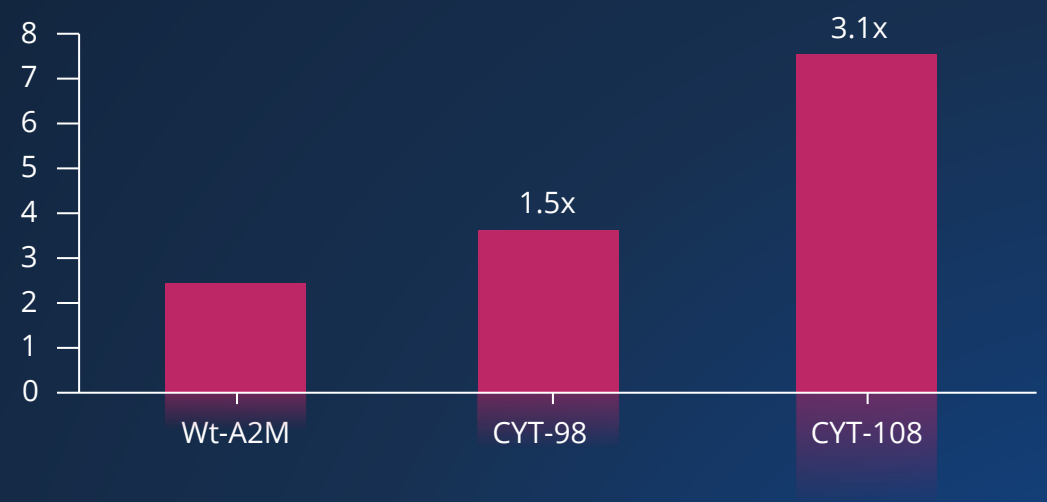
CYTONICS

Cartilage damage assessed using OARSI histological scoring

Do our A2M variants confer greater chondroprotection compared to wt-A2M in vivo?



Treatment with wt-A2M, CYT-98, and CYT-108 (0.153mg/ml for all) reduces cartilage damage by 59%, 72% and 87%, respectively (Sham subtracted). Values are the mean ± SE; n=11 for each group; *****compared with No Treatment (PBS), $P < 0.05$; ****** compared with wt-A2M, $P < 0.05$.



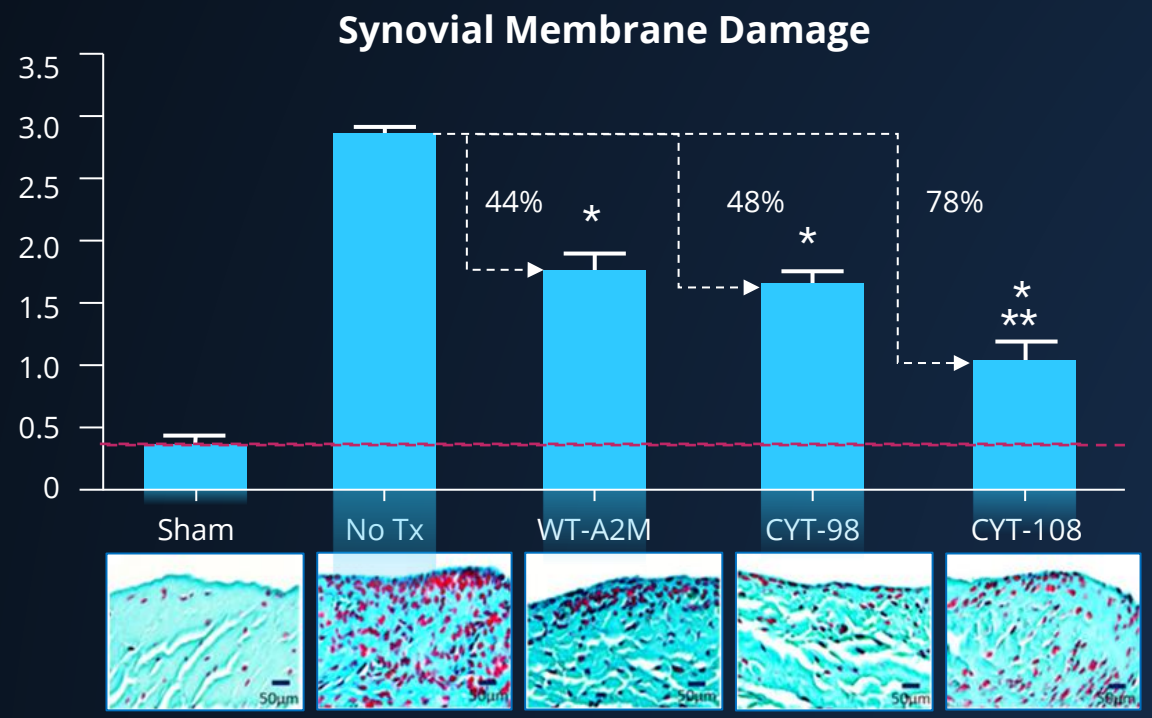
CYT-98 and CYT-108 provide 1.5-fold and 3.1-fold greater cartilage protection than wt-A2M, respectively.

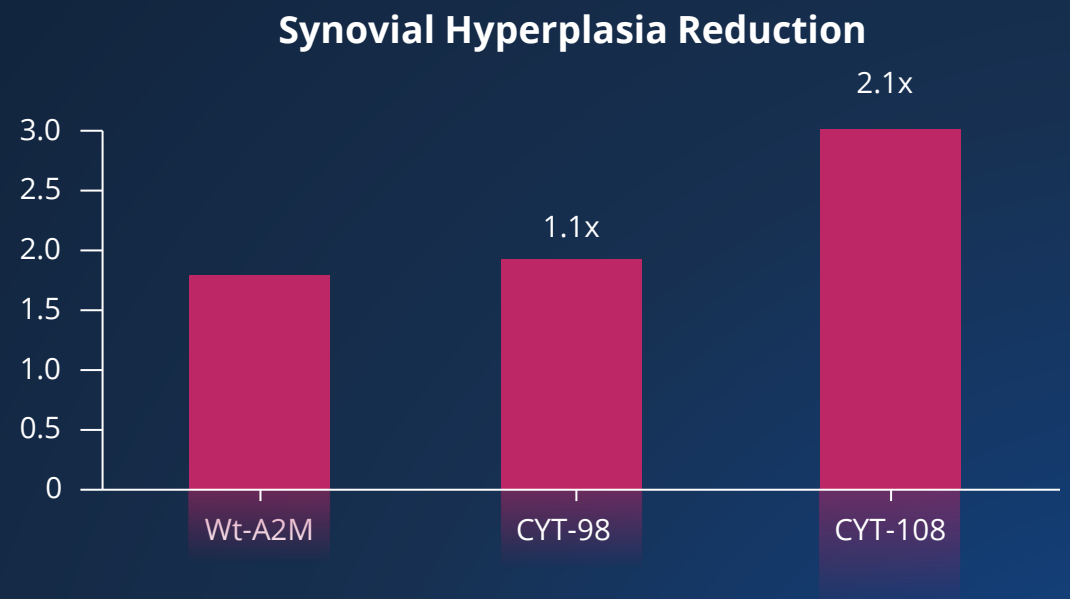
Zhang, Y., Wei, X., Browning, S. et al. Targeted designed variants of alpha-2-macroglobulin (A2M) attenuate cartilage degeneration in a rat model of osteoarthritis induced by anterior cruciate ligament transection. Arthritis Res Ther 19, 175 (2017). https://doi.org/10.1186/s13075-017-1363-4

CYTONICS

Synovial membrane inflammation by histopathology

Do our A2M variants reduce **synovial hyperplasia** compared to wt-A2M in vivo?





CYT-98 and CYT-108 reduce synovial hyperplasia by 1.1-fold and 2.1-fold more than wt-A2M, respectively.

Treatment with wt-A2M, CYT-98 and CYT-108 (0.153mg/ml for all) reduced synovial hyperplasia by 44%, 48% and 78%, respectively (Sham subtracted) . Values are the mean ± SE; n=11 for each group; *compared with No Treatment (PBS), $P < 0.05$; ** compared with wt-A2M, $P < 0.05$.

Zhang, Y., Wei, X., Browning, S. et al. Targeted designed variants of alpha-2-macroglobulin (A2M) attenuate cartilage degeneration in a rat model of osteoarthritis induced by anterior cruciate ligament transection. Arthritis Res Ther 19, 175 (2017). https://doi.org/10.1186/s13075-017-1363-4

CYTONICS

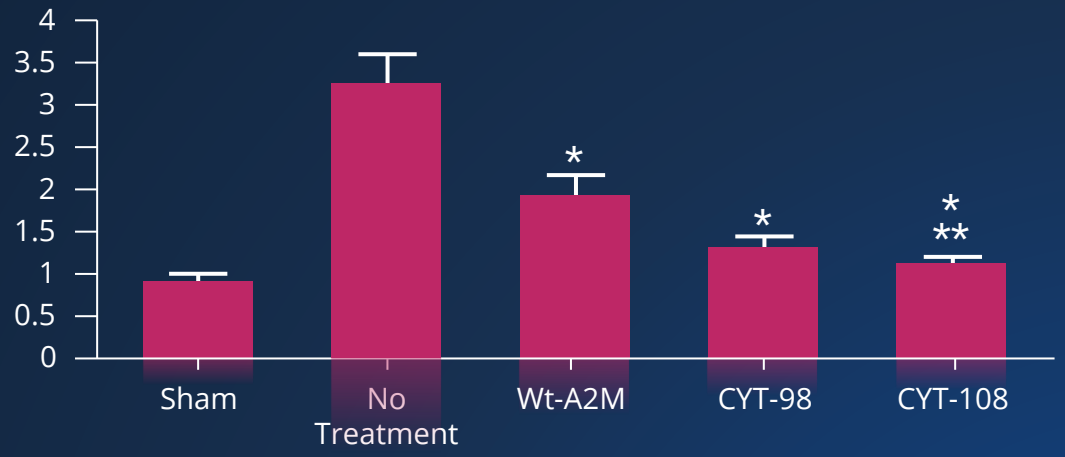
MMP-13 in synovial fluid and cartilage

How do our A2M variants affect **MMP-13 expression** compared to (natural) wt-A2M?

MMP-13 mRNA in Cartilage

Rt-PCR provides further evidence that treatment with our A2M variants reduces MMP-13 transcription >3-fold compared to wt-A2M in rat cartilage tissue. The disease modifying effects of our A2M variants may not limited to modulating protease activity and may act on transcriptional and translational pathways. Values are the mean ± SE. *Compared with No Treatment (ACLT + PBS), P < 0.05; **Compared with wt-A2M, P < 0.05;

MMP-13 Levels in Synovial Fluid (ELISA)

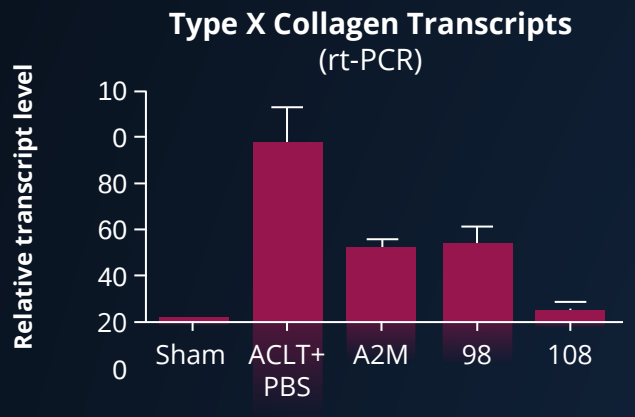


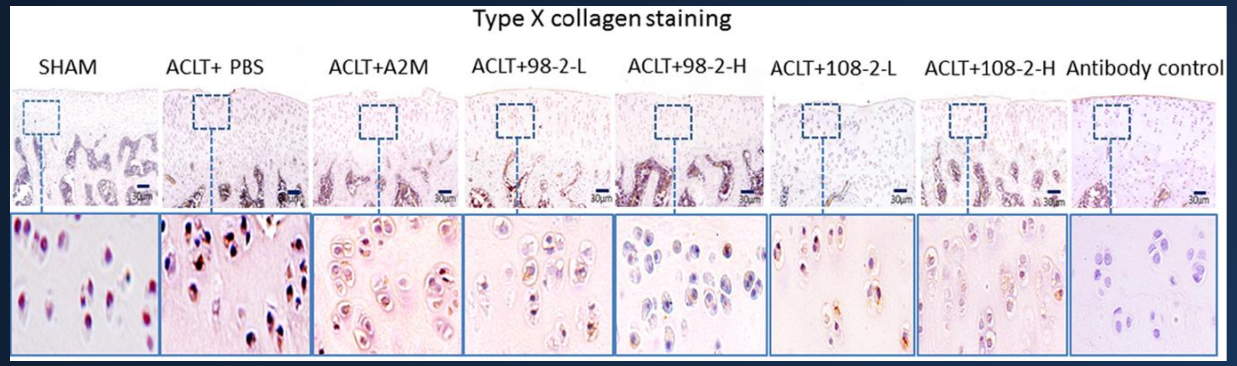
MMP-13 protein levels are significantly reduced in the synovial fluid of rats treated with wt-A2M, CYT-98, and CYT-108. Values are the mean ± SE. *Compared with No Treatment, P < 0.05; **Compared with wt-A2M, P < 0.05;

Zhang, Y., Wei, X., Browning, S. et al. Targeted designed variants of alpha-2-macroglobulin (A2M) attenuate cartilage degeneration in a rat model of osteoarthritis induced by anterior cruciate ligament transection. Arthritis Res Ther 19, 175 (2017). https://doi.org/10.1186/s13075-017-1363-4

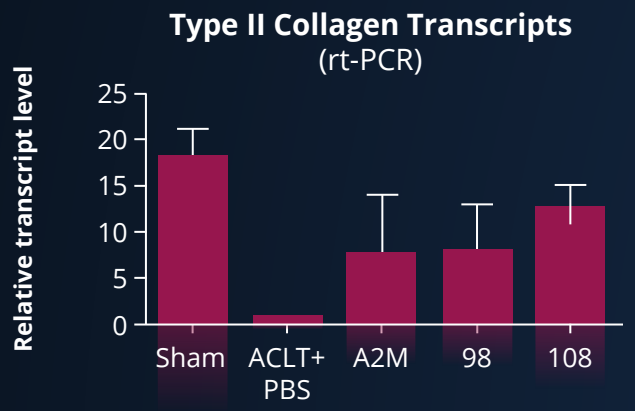
Collagen in cartilage quantified by rt-PCR and IHC

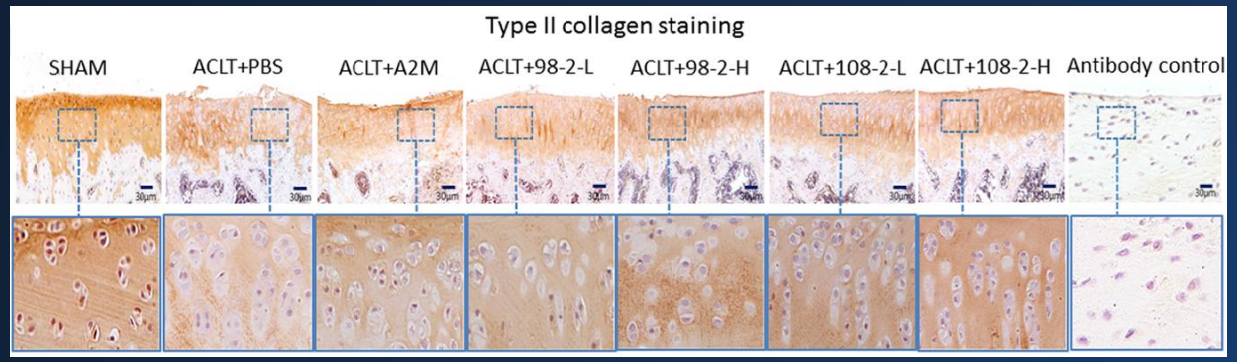
How do our A2M variants affect the **protein composition** of cartilage tissue compared to wt-A2M?





Type-X Collagen produced due to chondrocyte hypertrophy, associated with cartilage degradation and inflammation
Lighter = Good



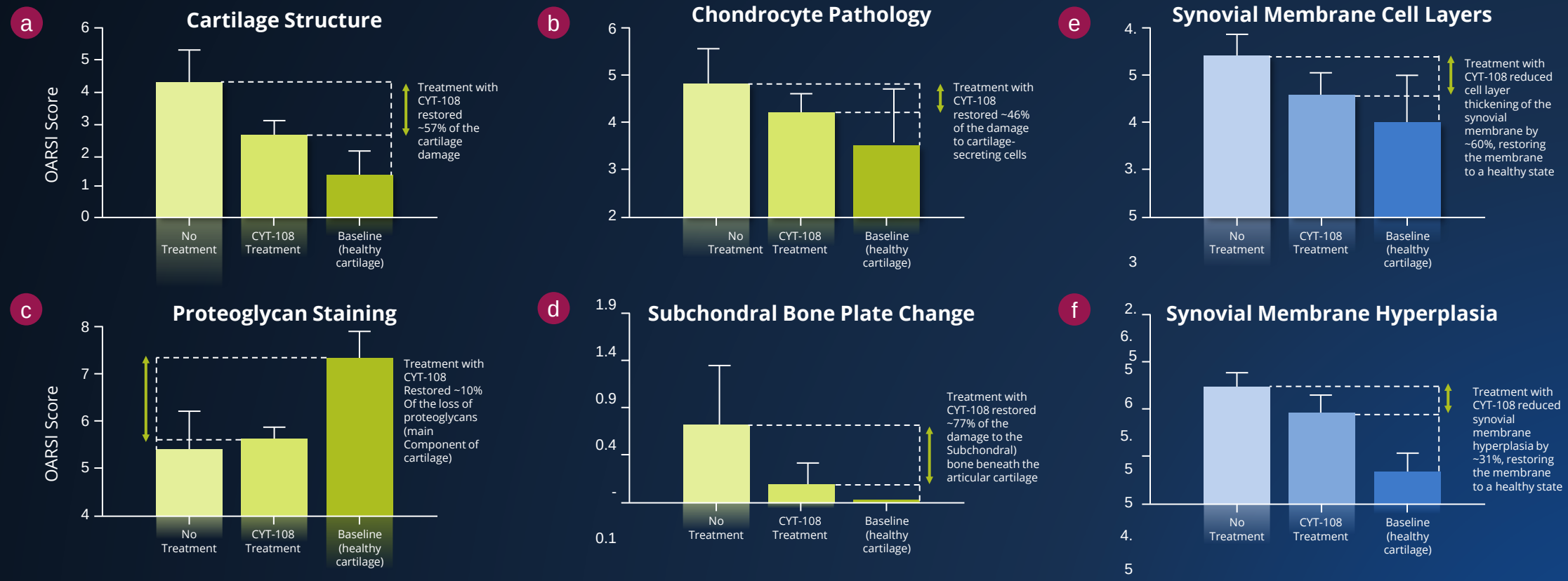


Type II Collagen associated with normal chondrocyte physiology and healthy cartilage
Darker = Good

Zhang, Y., Wei, X., Browning, S. et al. Targeted designed variants of alpha-2-macroglobulin (A2M) attenuate cartilage degeneration in a rat model of osteoarthritis induced by anterior cruciate ligament transection. Arthritis Res Ther 19, 175 (2017). https://doi.org/10.1186/s13075-017-1363-4

CYT-108 Protects Cartilage and Joint Tissue

Confirmatory preclinical efficacy experiments



Intra-articular injection of our recombinant A2M variant, CYT-108, results in articular cartilage preservation, improved remodeling of the cartilate tissue, recovery of cartilage structure and subchondral bone integrity, and preservation of synovial membrane characteristics in large animal subjects suffering from post-traumatic osteoarthritis. Histopathological grading (modified AORSI scoring system) of the articular cartilage and subchondral bone plate reveals that treatment with CYT-108 results in (a) recovery of ~57% of the damage to the cartilage structure as measured by Safrinin-O staining, (b) restoration of ~46% of the damage to chondrocytes (cartilage-secreting cells, (c) enhancement of ~10% proteoglycan content (key component of cartilage), and (d) reduction of ~77% of the subchondral bone place (bone underneath the cartlage) thinkness back to normal levels. Histopathological grading (modified OARSI scoring system) of the synovial membranes of subjects in Groups 1-3 reveals that treatment with CYT-108 results in (e) reduction of ~60% of the pathological accumulation of cell layers in the synovial membrane and (f) reduction of 31% of synovial tissue hyperplasia (pathological membrane thickening) back to normal levels. Taken together, this data indicates that CYT-108 has therapeutic effect in preserving normal articular cartilage, bone, and synovial membrane physiology when administered to animals suffering from post-traumatic osteoarthritis, and substantially restores the cartilage matrix, underlying subchondral bone, and the synovial membrane back to normal, helathy anatomy and physiology. *Error bars+/- SEM.*