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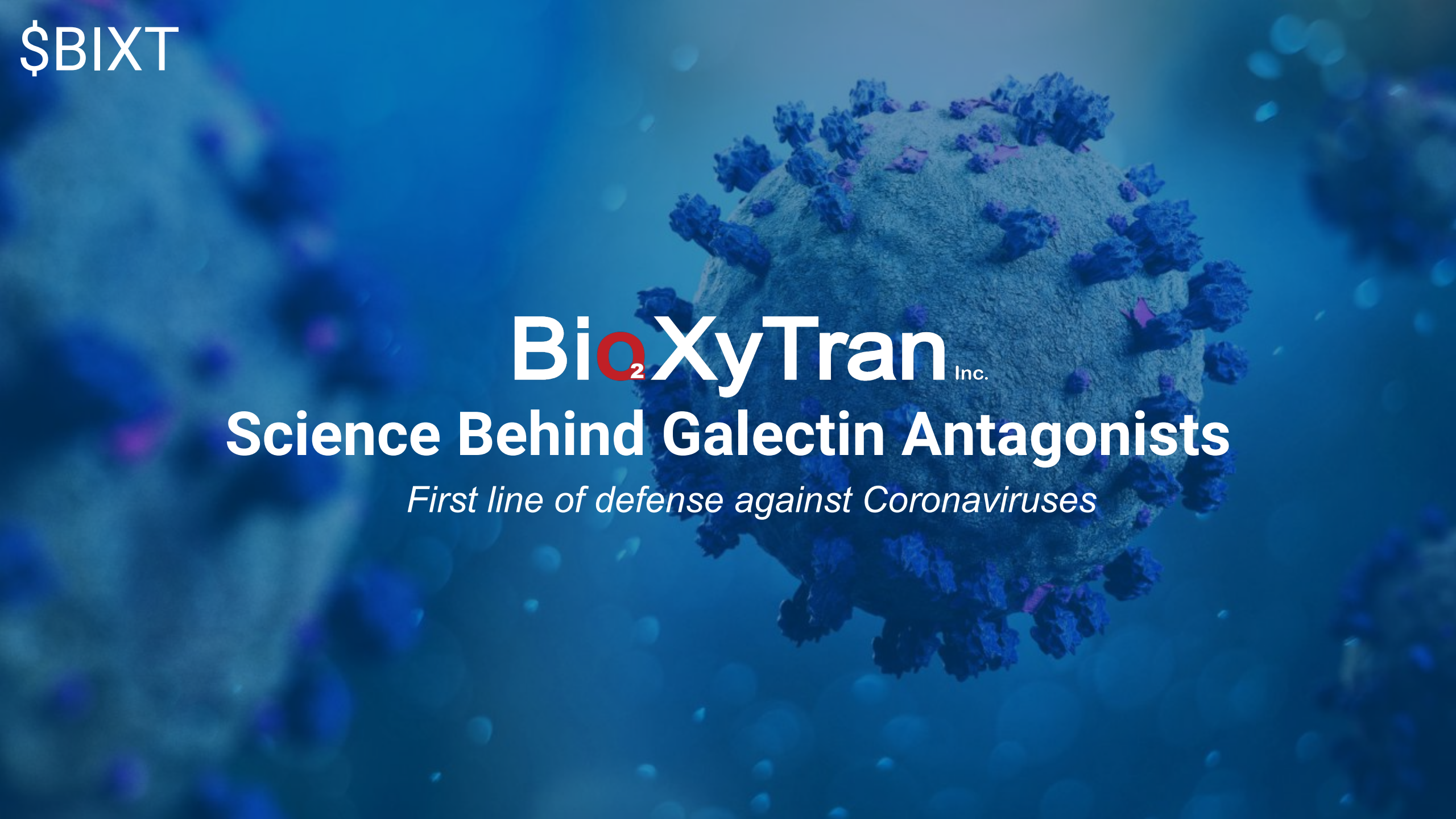
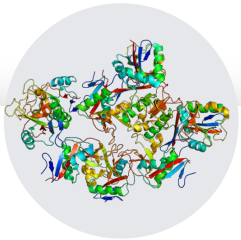


BioXyTran Inc.

Science Behind Galectin Antagonists

First line of defense against Coronaviruses

Inspiration For Glycovirology



Adhesion Drug

- Most common means of viral adhesion are surface lectins combining with carbohydrates
- Carbohydrates block surface lectins.
- Galectins are adhesion molecules (extracellular matrix)
- Galectins thought to aid in viral docking
- Galectins strongly implicated in viral diseases

Entry Inhibitor

- Galectin Fold discovered on spike protein in a conserved region
- Interfere with spike protein activation
- Creation of a physical barrier

Galectins Explained

A Galectin is a protein that recognizes carbohydrates and modulates intra cellular and extracellular interactions primarily related to the immune system. In some cases Galectins act as a glue bringing molecules together. The major focus of research is on extracellular interactions.

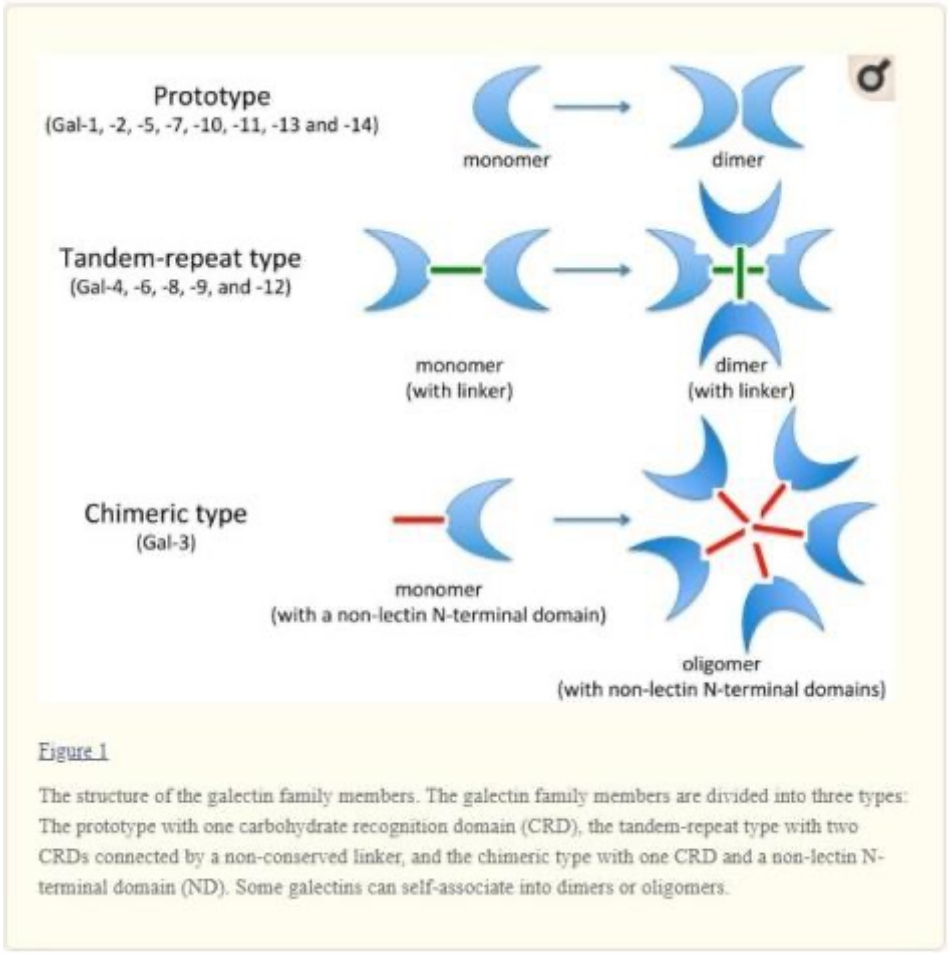


Figure 1

The structure of the galectin family members. The galectin family members are divided into three types: The prototype with one carbohydrate recognition domain (CRD), the tandem-repeat type with two CRDs connected by a non-conserved linker, and the chimeric type with one CRD and a non-lectin N-terminal domain (ND). Some galectins can self-associate into dimers or oligomers.

https://www.pharma-iq.com/pre-clinical-discovery-and-development/articles/why-galectin-3-has-emerged-as-a-focus-for-drug-research-and-development-1

Galectins Linked to Chronic Disease

30 years of research

Over 4000 Journal articles on Galectins

Galectins are a key biomarker of chronic disease

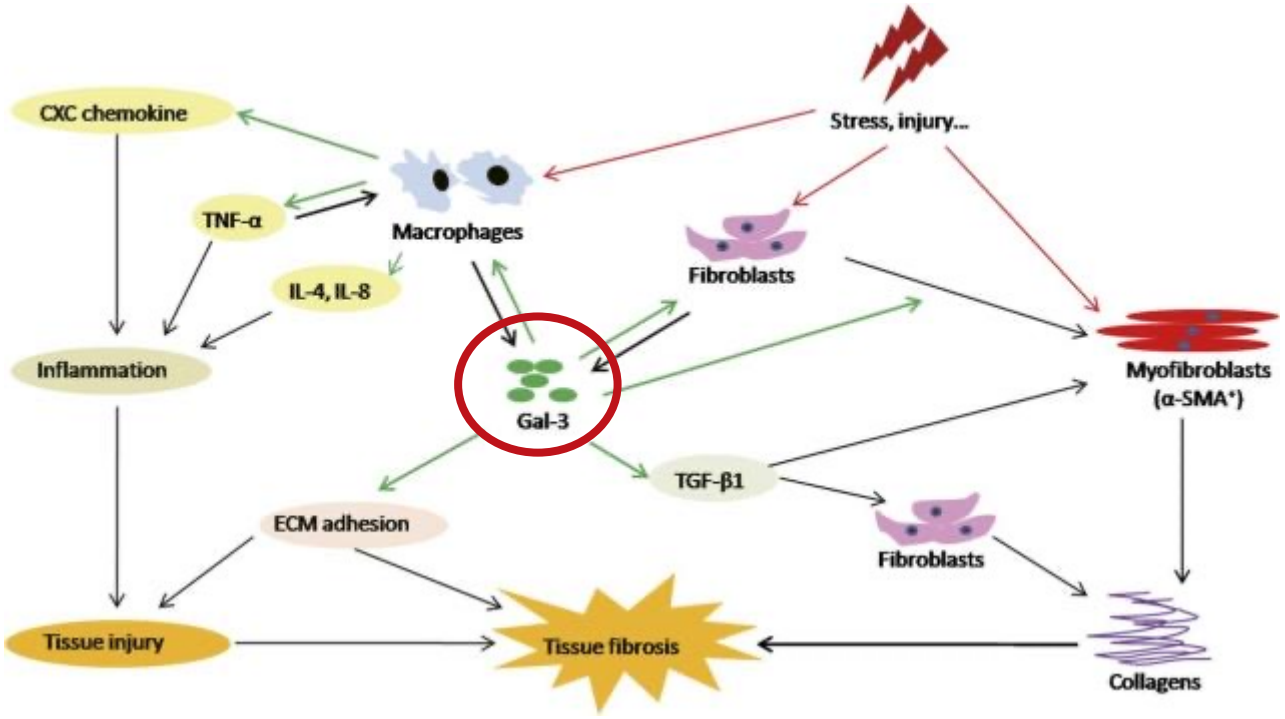
No approved Galectin inhibitor - YET

Disease Indication	Journals	Areas of Focus
Cancer	1500	Cervical, Breast, Endometrial, Pancreatic, Thyroid, CRC, **Biomarker**
Cardiovascular Disease	622	**Biomarker** for heart failure, stroke, other cardiovascular disease
Brain	350	Predictive **Biomarker** stroke, TBI, Postpartum Depression
Kidney	211	Fibrosis, **Biomarker** in chronic kidney disease
Lung	200	Cancer, Fibrosis, **Biomarker**
Liver	185	NASH, NAFLD, Fibrosis, **Biomarker**
Skin	127	Wound Healing, infection, Lupus, Psoriasis, Cancer, **Biomarker**
Digestive System	109	Gastric & Colorectal Cancer, Metastasis, Inflammatory, **Biomarker**

https://www.pharma-iq.com/pre-clinical-discovery-and-development/articles/why-galectin-3-has-emerged-as-a-focus-for-drug-research-and-development-1

Galectin Trouble-Maker

The Center of Inflammatory Feedback Loops



Gal-3 is a Pro-inflammatory Molecule

Inhibiting it blocks cycle of inflammation

Galectin is the KEY modulator of inflammatory molecules

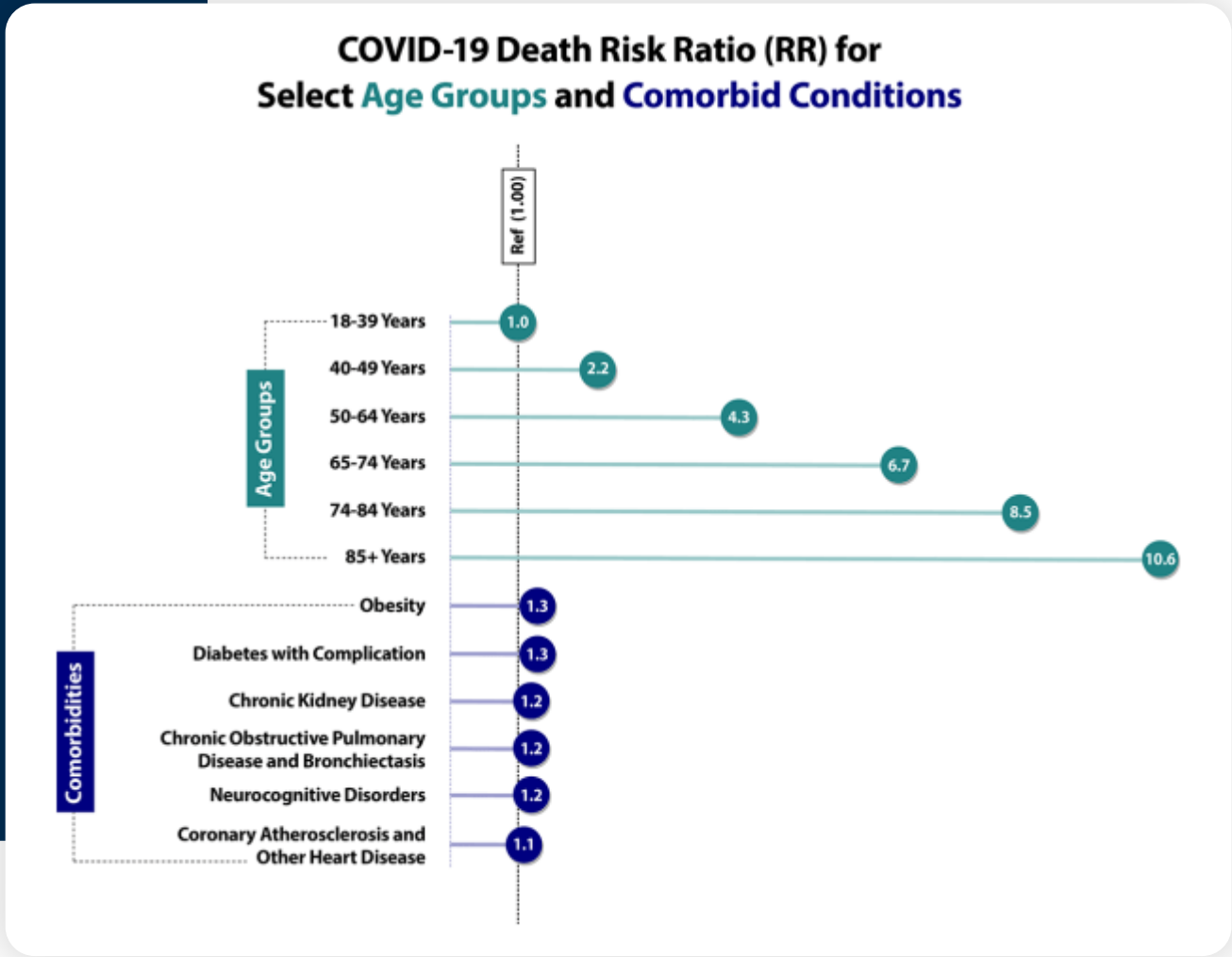
http://jpet.aspetjournals.org/content/351/2/336
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5752178/

There is little downside to blocking galectin-3 entirely from the body potentially ameliorating many chronic and deadly diseases

Galectin Linkage to COVID-19

"People With Underlying Medical Conditions" aka tendency for high galectin serum levels

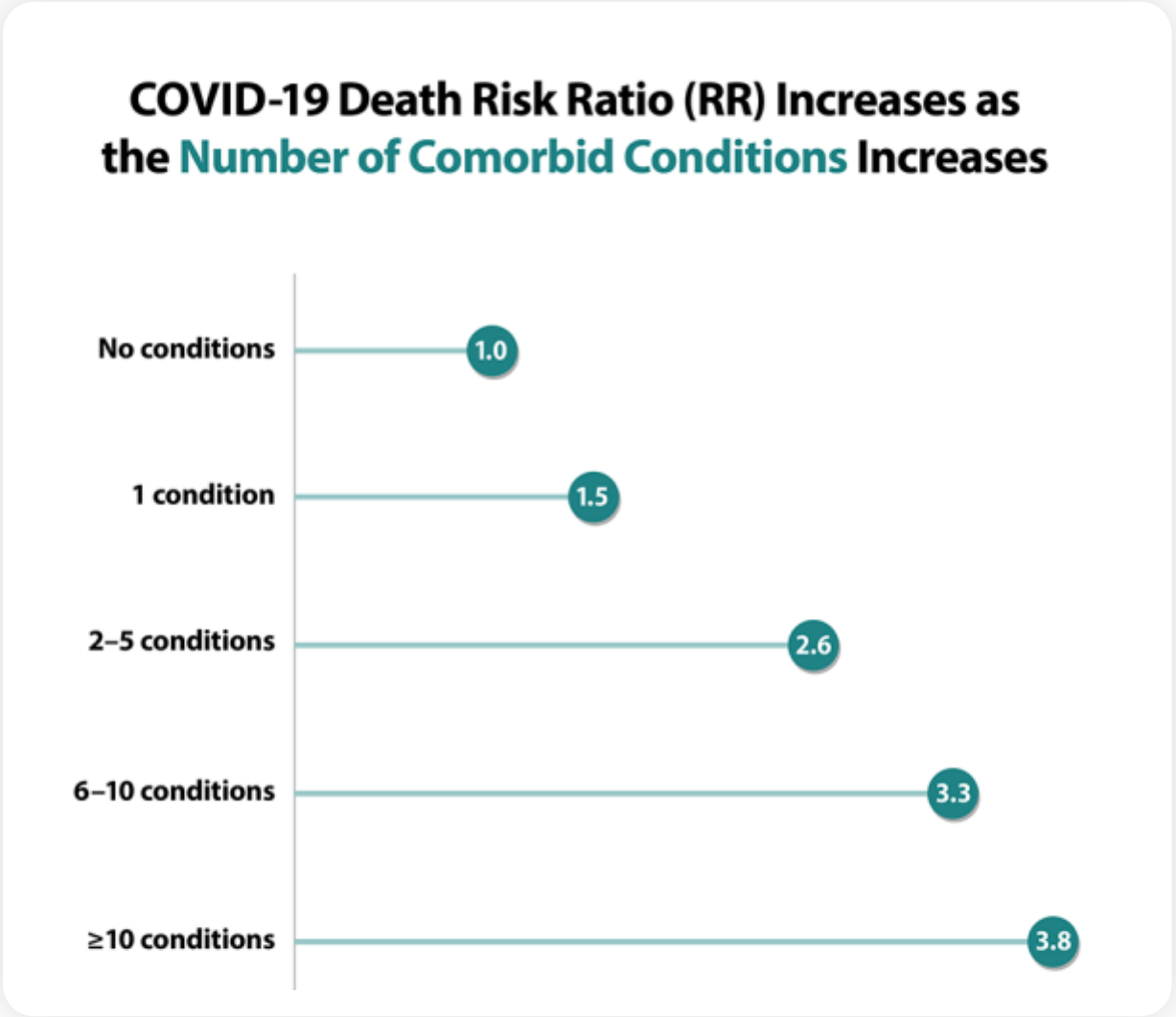
- Cancer
- Kidney Disease
- Liver Disease
- Lung Disease (COPD, Asthma, Cystic Fibrosis)
- Dementia or Alzheimer's Disease
- Diabetes
- Downs Syndrome
- Heart Disease
- HIV
- Immunocompromised
- Mental Health Conditions
- Obesity
- Sickle Cell Anemia
- Smoker
- Organ Transplant
- Stroke
- Substance Abuse



https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html

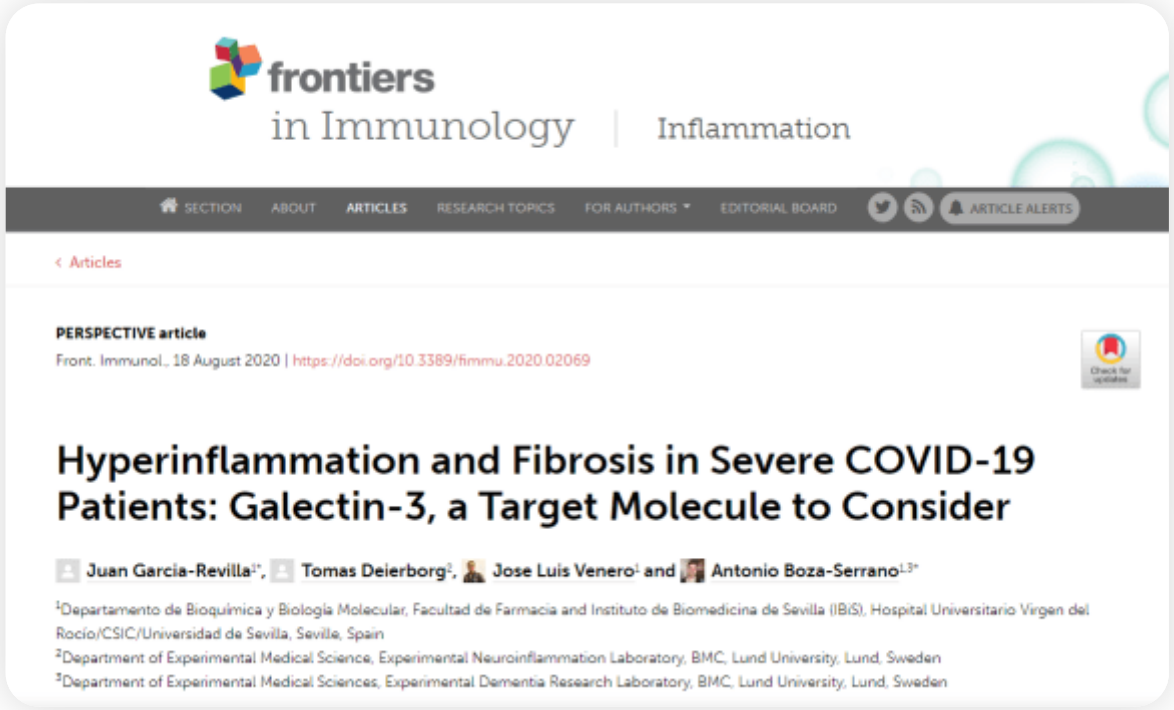
“Underlying Conditions” Increase Galectin Serum Markers

Galectin-3 & Galectin-9 Serum Levels Rise with the Number of Underlying Conditions



https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/underlyingconditions.html

Galectins Predict Mortality



frontiers in Immunology | Inflammation

SECTION ABOUT ARTICLES RESEARCH TOPICS FOR AUTHORS EDITORIAL BOARD ARTICLE ALERTS

‹ Articles

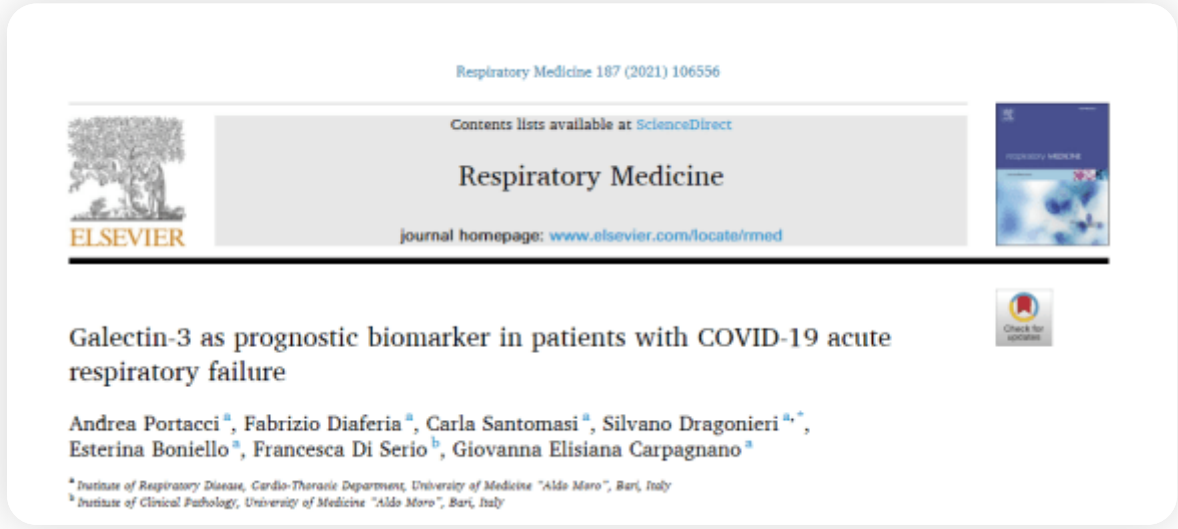
PERSPECTIVE article

Front. Immunol., 18 August 2020 | https://doi.org/10.3389/fimmu.2020.02069

Hyperinflammation and Fibrosis in Severe COVID-19 Patients: Galectin-3, a Target Molecule to Consider

Juan Garcia-Revilla[1*], Tomas Deierborg[2], Jose Luis Venero[1] and Antonio Boza-Serrano[1,3*]

[1]Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia and Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain
[2]Department of Experimental Medical Science, Experimental Neuroinflammation Laboratory, BMC, Lund University, Lund, Sweden
[3]Department of Experimental Medical Sciences, Experimental Dementia Research Laboratory, BMC, Lund University, Lund, Sweden



Respiratory Medicine 187 (2021) 106556

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

ELSEVIER

Galectin-3 as prognostic biomarker in patients with COVID-19 acute respiratory failure

Andrea Portacci[a], Fabrizio Diaferia[a], Carla Santomasi[a], Silvano Dragonieri[a,*], Esterina Boniello[a], Francesca Di Serio[b], Giovanna Elisiana Carpagnano[a]

[a] Institute of Respiratory Disease, Cardio-Thoracic Department, University of Medicine "Aldo Moro", Bari, Italy
[b] Institute of Clinical Pathology, University of Medicine "Aldo Moro", Bari, Italy

This was one of many journal articles that served as a rationale for a Galectin-3 Prognostic Test.

Highlights

- Assess the utility of Galectin-3 for prognosis prediction
- Increased Galectin-3 serum levels are associated with higher risk of death, ICU, and Severe ARDS development
- Galectin-3 can provide important prognostic information in patients with COVID-19 acute respiratory failure

https://www.frontiersin.org/articles/10.3389/fimmu.2020.02069/full
https://www.sciencedirect.com/science/article/pii/S0954611121002626

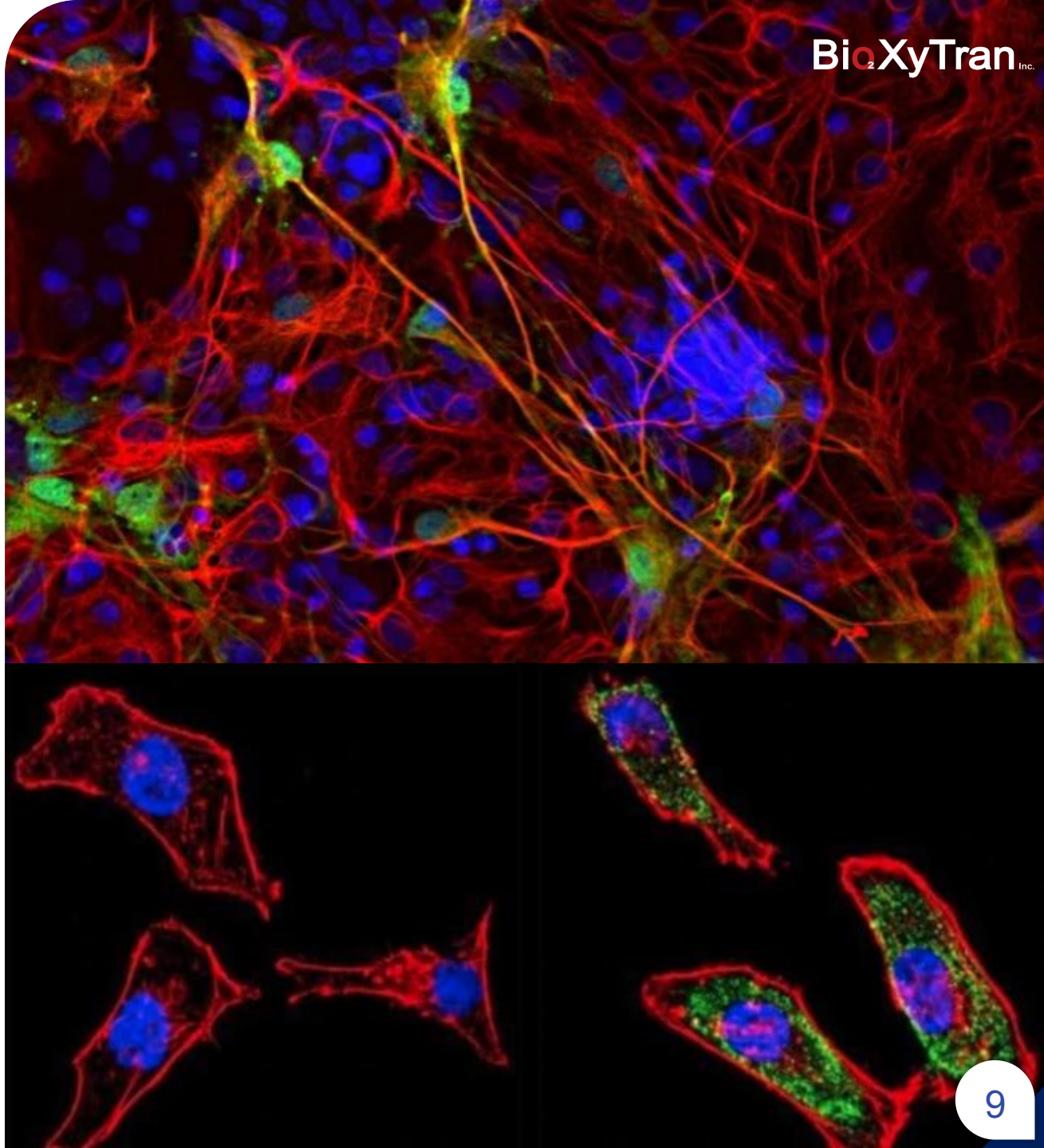
Galectin Serum Test Data Summary

Results

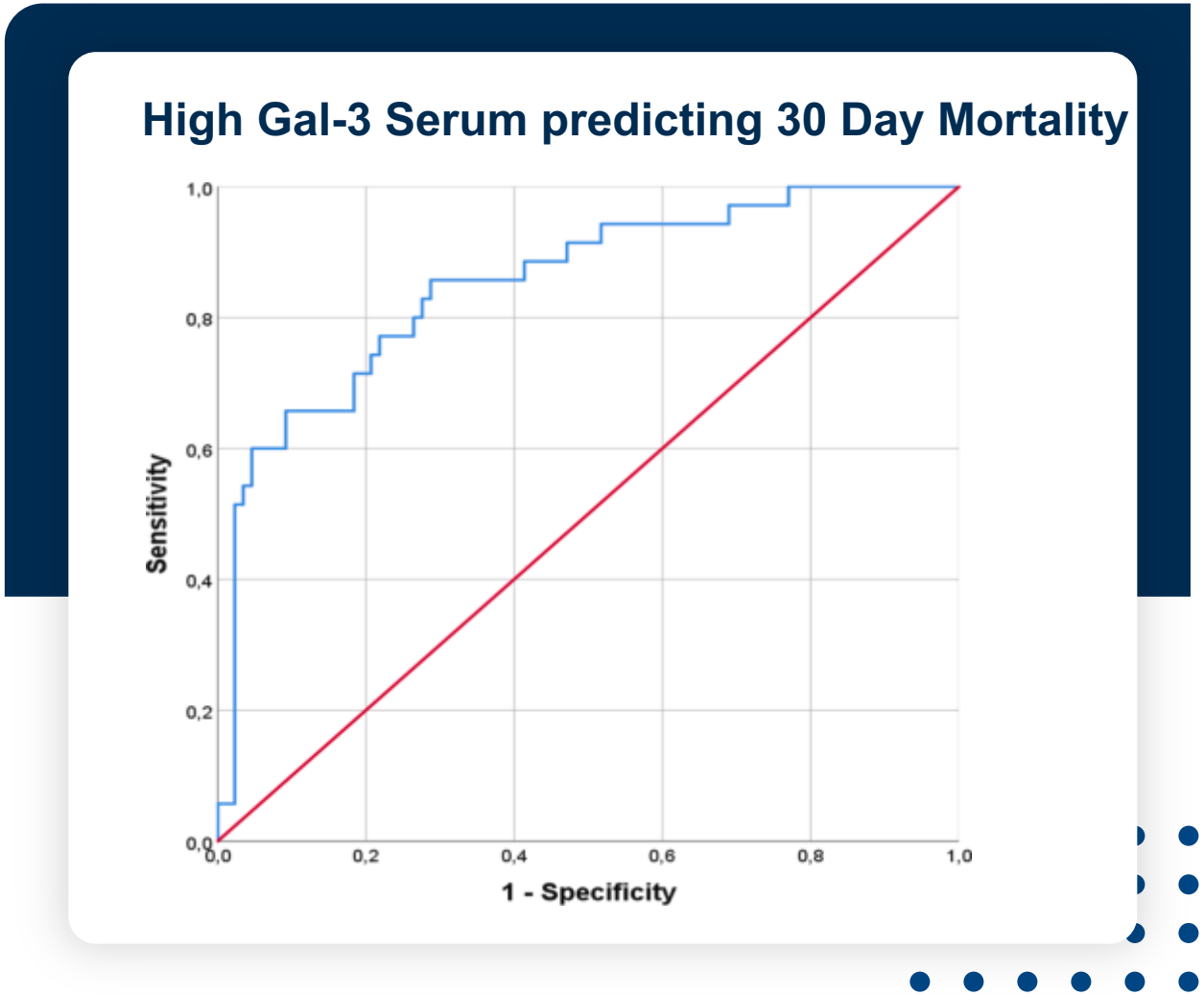
Galectin-3 correlated with many other prognostic predictors tested in our analysis. Moreover, patients with serum levels of Galectin-3 above 35.3 ng/ml had increased risk for mortality, Intensive Care Unit admission and severe Acute Respiratory Distress Syndrome.

- 27.9% non-survivors
- 35.3 ng/ml Galectin Serum Level cutoff

Galectin-3 predicts mortality, ICU Stays, and can stratify patients all with one quick cheap and easy to take test.



ROC Curve – Galectin -3



Receiver Operating Characteristic Analysis

n=39
Gal-3 Serum >= 35.3 ng/ml

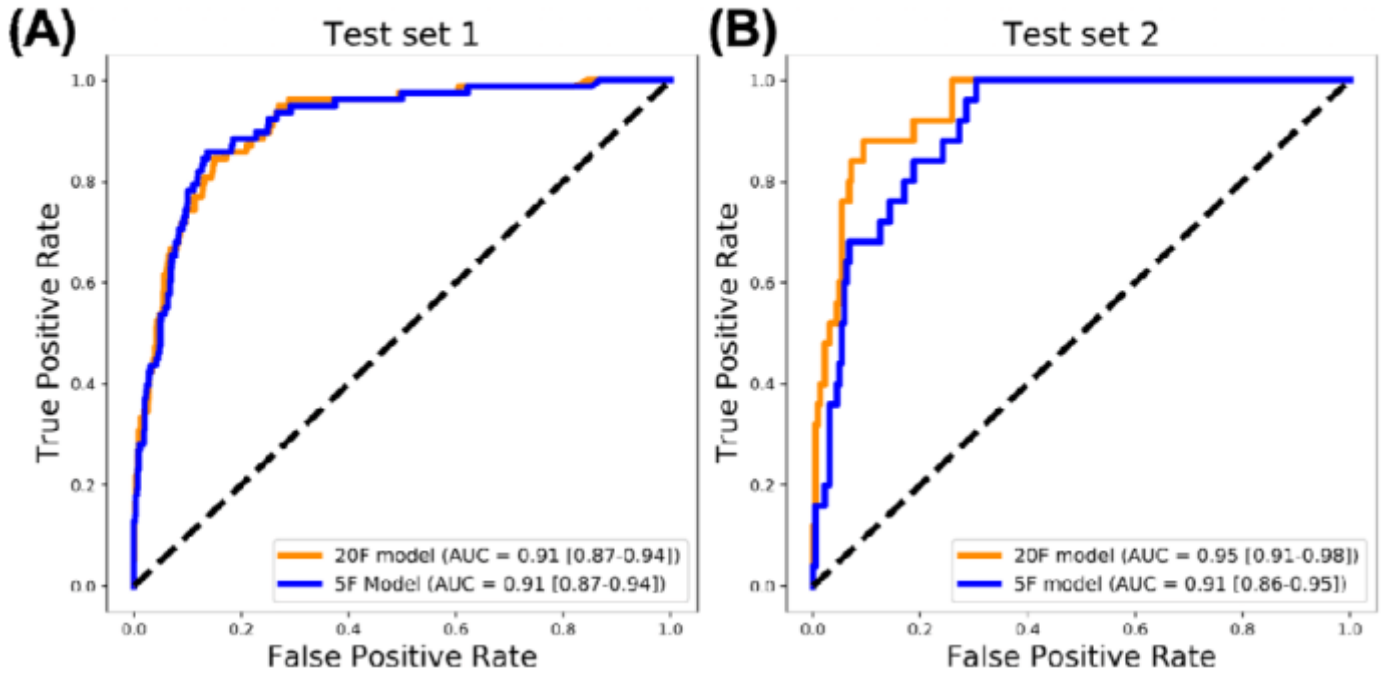
80% sensitivity and 92.1% specificity
for mortality prediction.

AUC = .906 (95% CI .85-.96, p,.0001)

ROC Curve – WHO Comparison

5 predictive factors vs 20 factors

Figure 3



5 Clinical Features

- Age
- Min O2 saturation
- Type of Patient
- Hydroxycloroquine use
- Max Body Temp

NYC – Mount Sinai Health

Development Cohort
N=3,841

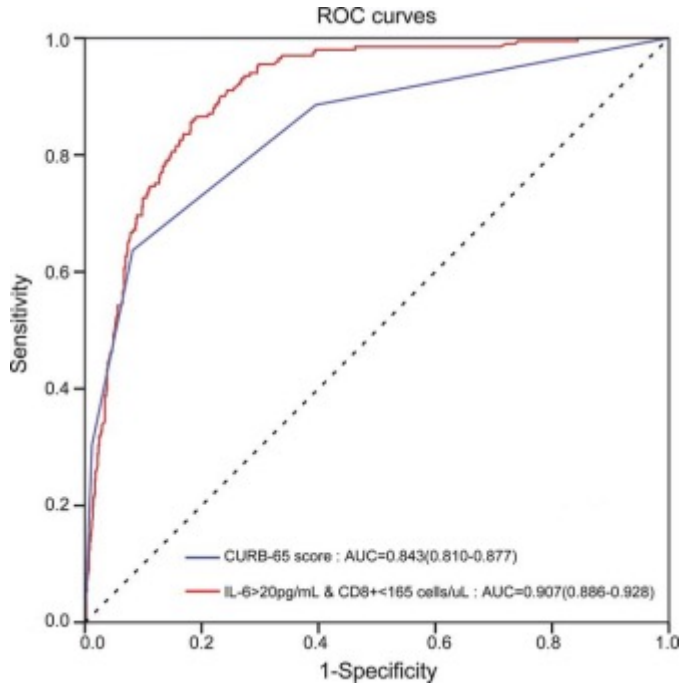
Retrospective Analysis
N=961

AUC = .91

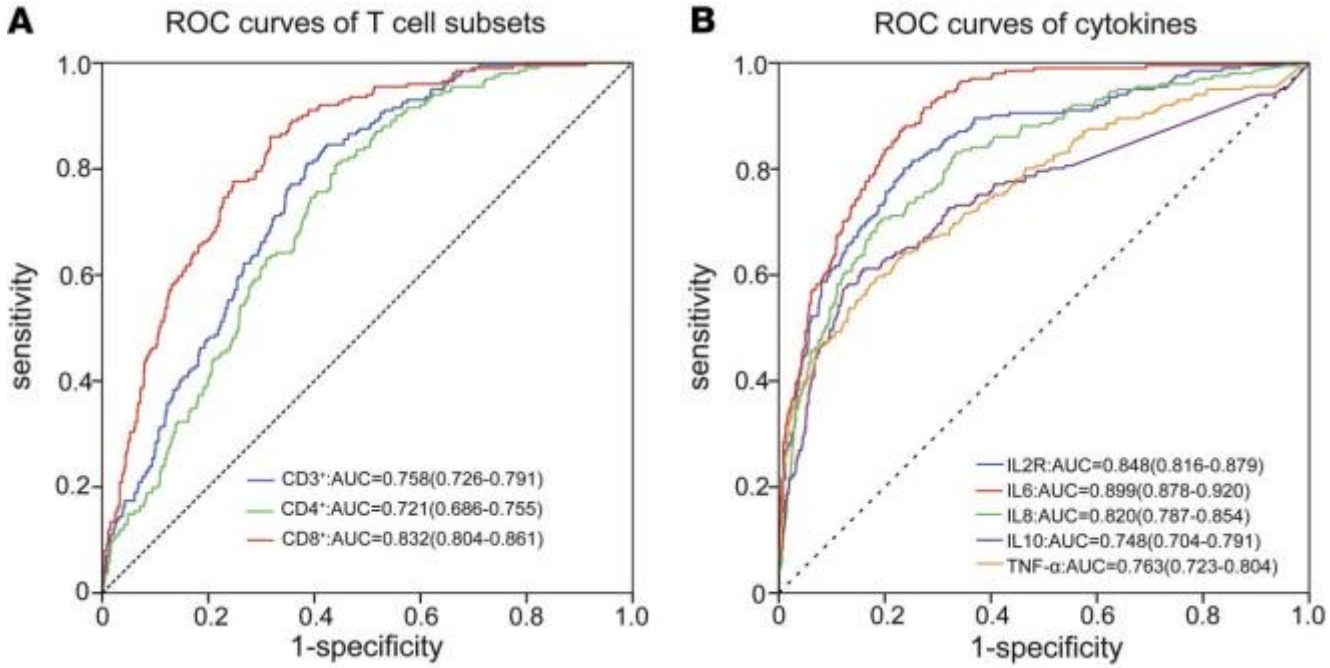
WHO Recommended Clinical Predictor of COVID-19 Mortality

ROC Curve – Other Biomarkers

IL-6 and CD8 counts Combined



ROC curves derived from the model of combined elevated IL-6 and reduced $CD8^+$ T cell counts and CURB-65 scores in our cohort. The ROC curves of this predictive model showed a better performance than that of the CURB-65 score ($P < 0.001$).

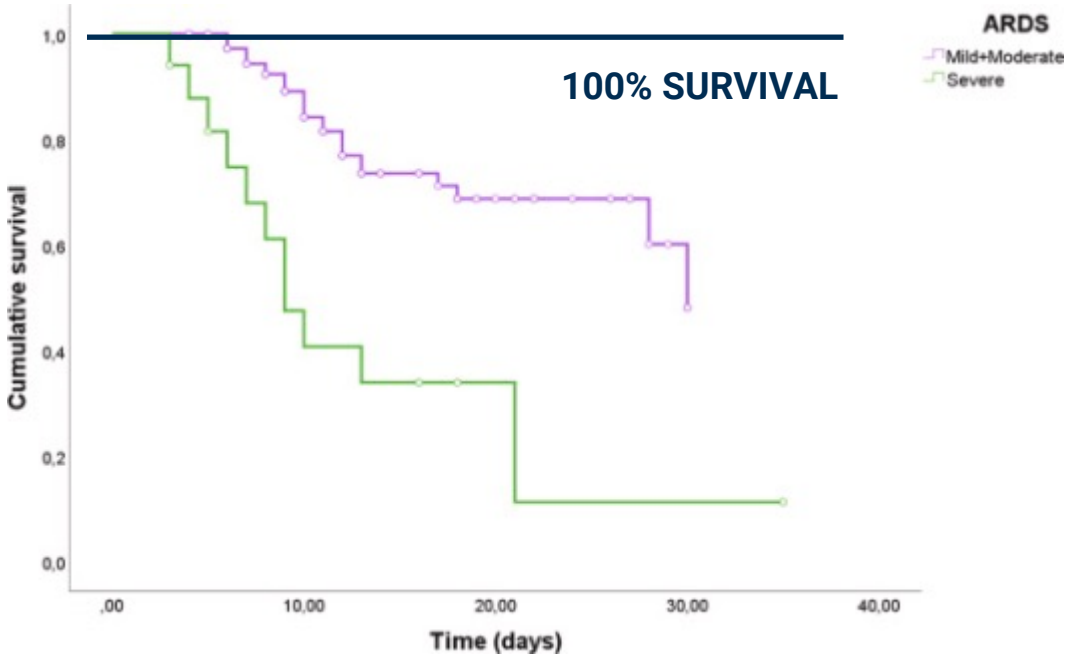


(A) ROC curves of each category of T lymphocyte subgroup. (B) ROC curves for each category of serum cytokines. AUC, area under the ROC curve; ROC, receiver operating characteristic.

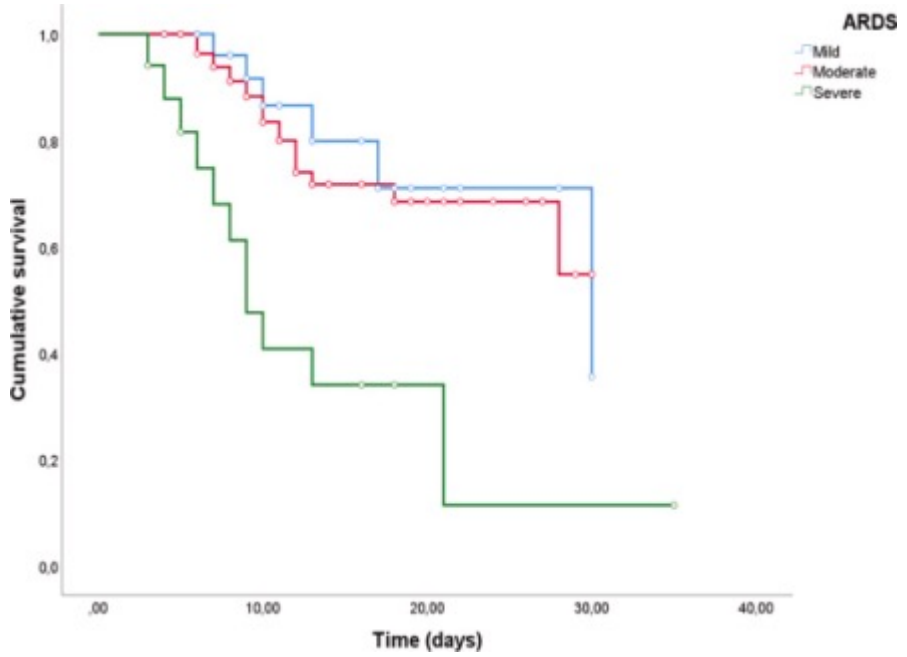
IL-6 and $CD8^+$ T cell counts combined are an early predictor of in-hospital mortality of patients with COVID-19

Kaplan Meir Curve – COVID-19

Gal-3 Serum Levels



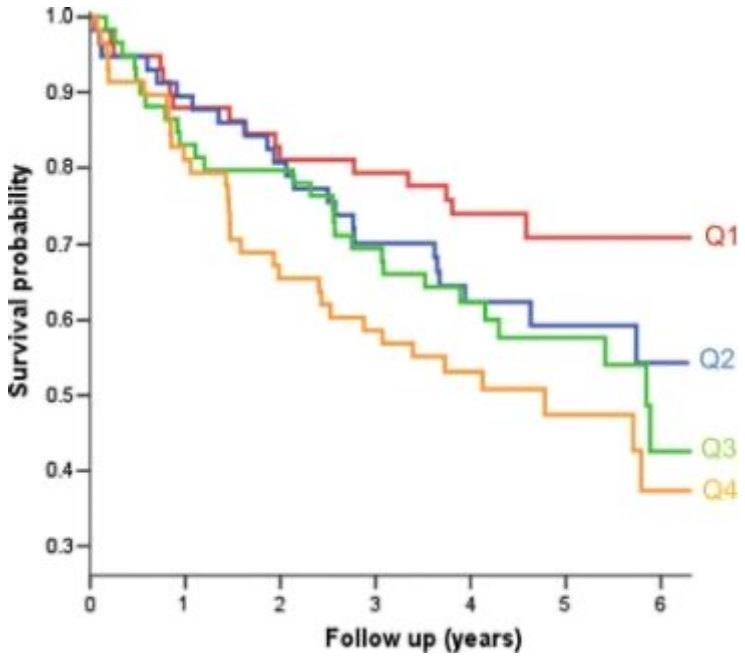
Galectin-3 has shown good diagnostic power for severe ARDS (AUC 0.75, p =0.001). In this case, using the fixed cut-off of 35.3 ng/ml, we found a sensitivity of 70.6% and a specificity of 78% for the outcome.



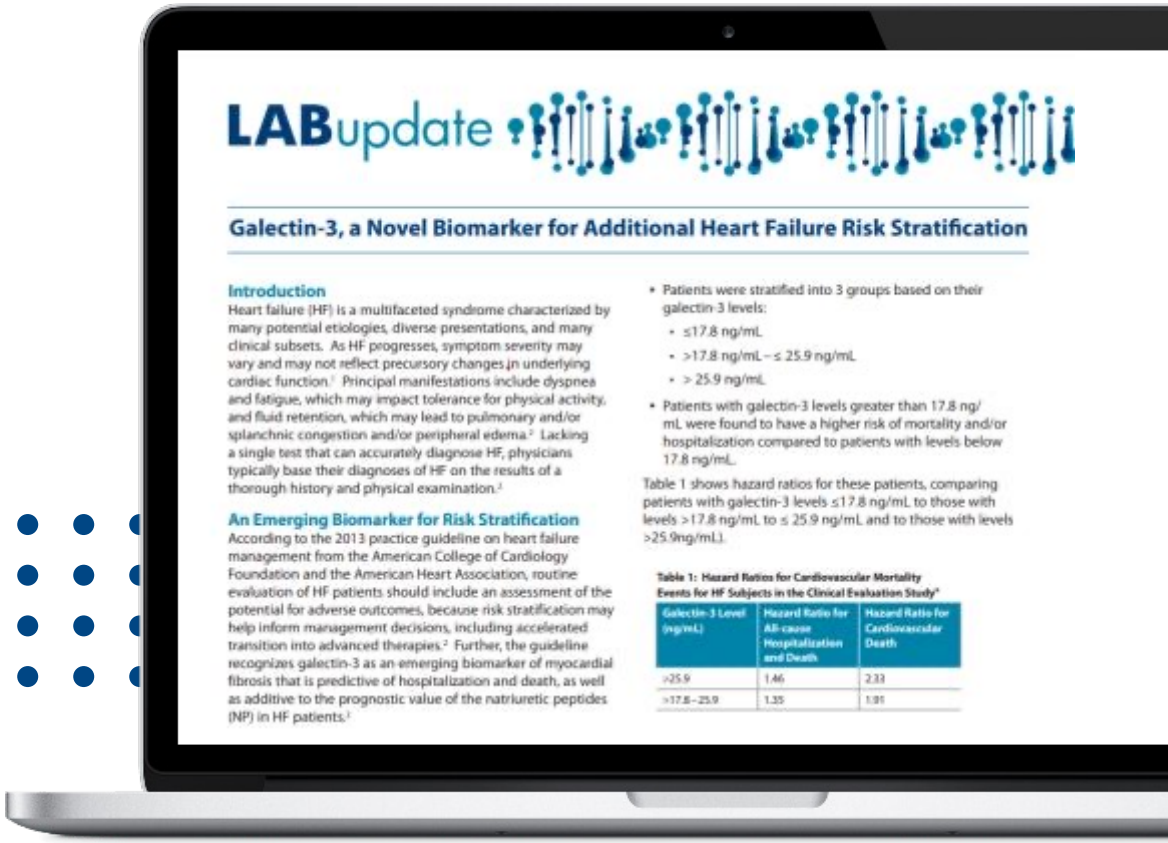
Low to moderate levels of Gal-3 serum were not good prognosticators of ARDS.

Kaplan Meir Curve – Chronic Heart Failure

Gal-3 Serum Levels Heart Failure



Kaplan–Meier curves according to quartiles of baseline galectin-3 values. Log-rank P = 0.048. Q1 galectin-3 values <13.63 ng/mL, Q2 13.63–17.63 ng/mL, Q3 17.64–21.62 ng/mL, Q4 >21.62 ng/mL

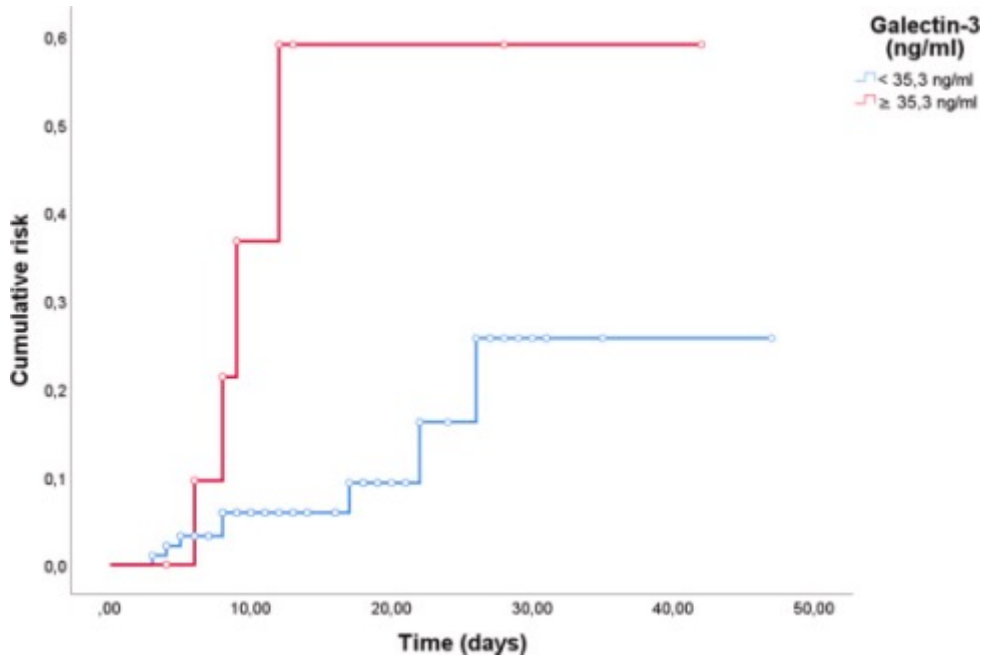


FDA Approved test shows patient stratification Heart Disease using Gal-3 serum levels a prognostic for disease severity. Similar stratification in COVID-19

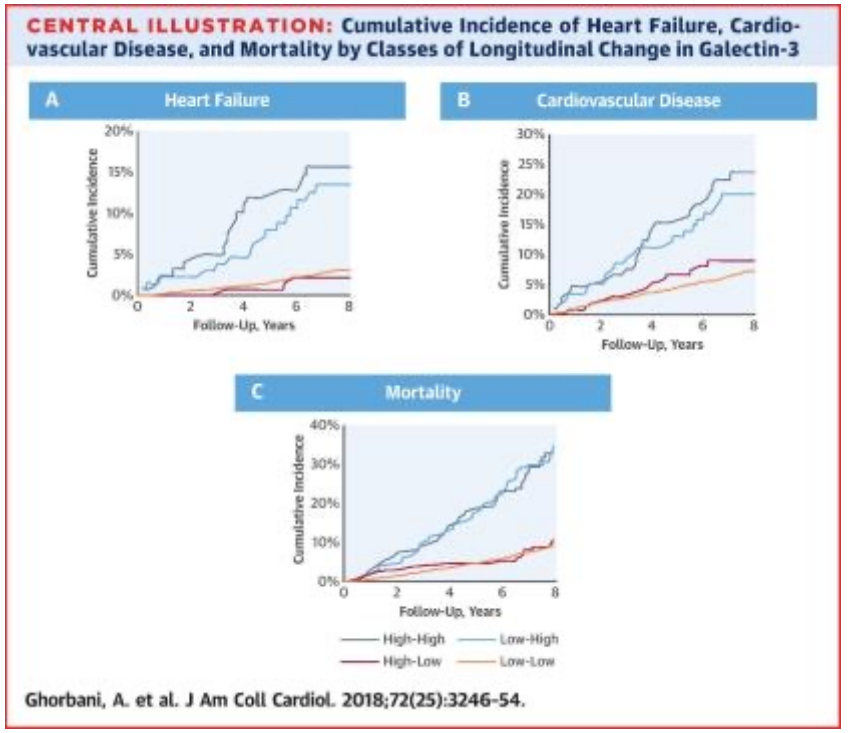
Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure

Mortality Risk – Galectin -3 Serum Levels

Gal-3 Serum Levels COVID-19



Day 12 there is a 60% mortality with high Gal-3 levels upon admission.

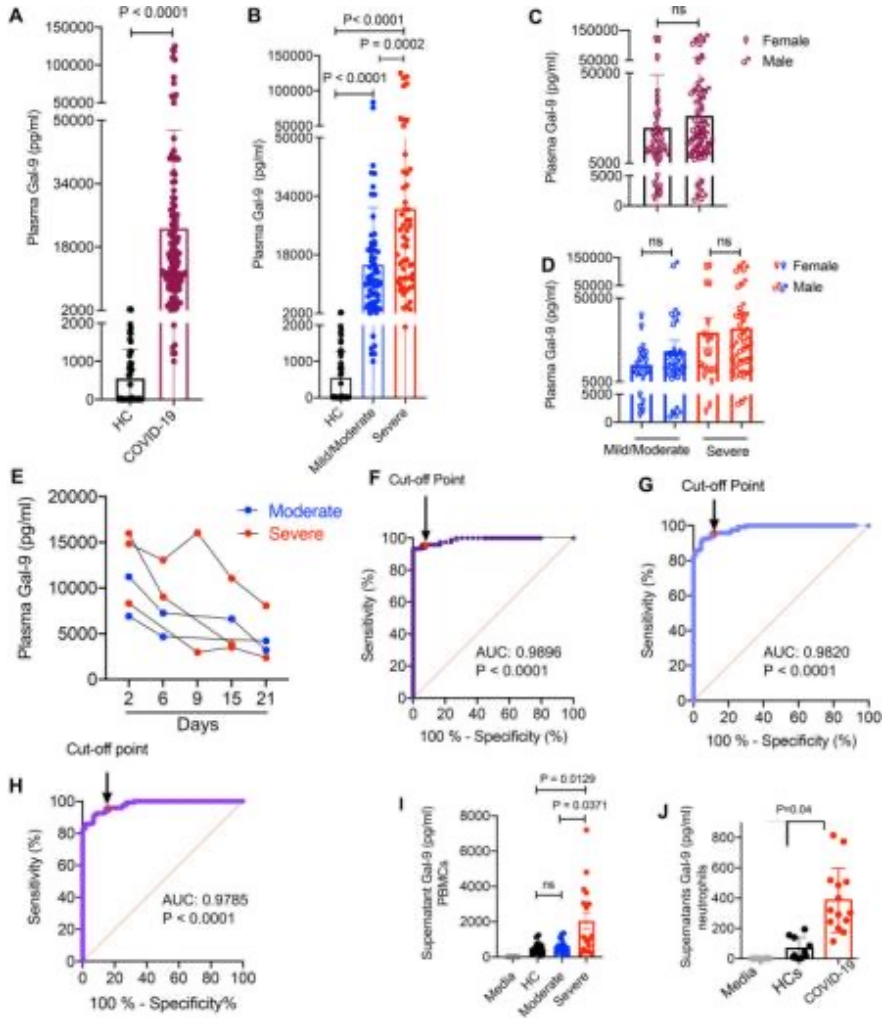


Similar stratification in Heart Disease based on serum levels.

Galectin-9 Prognosticates Severe COVID-19

Patients with high levels of Galectin-9 serum are extremely likely to die

https://pubmed.ncbi.nlm.nih.gov/33947753/

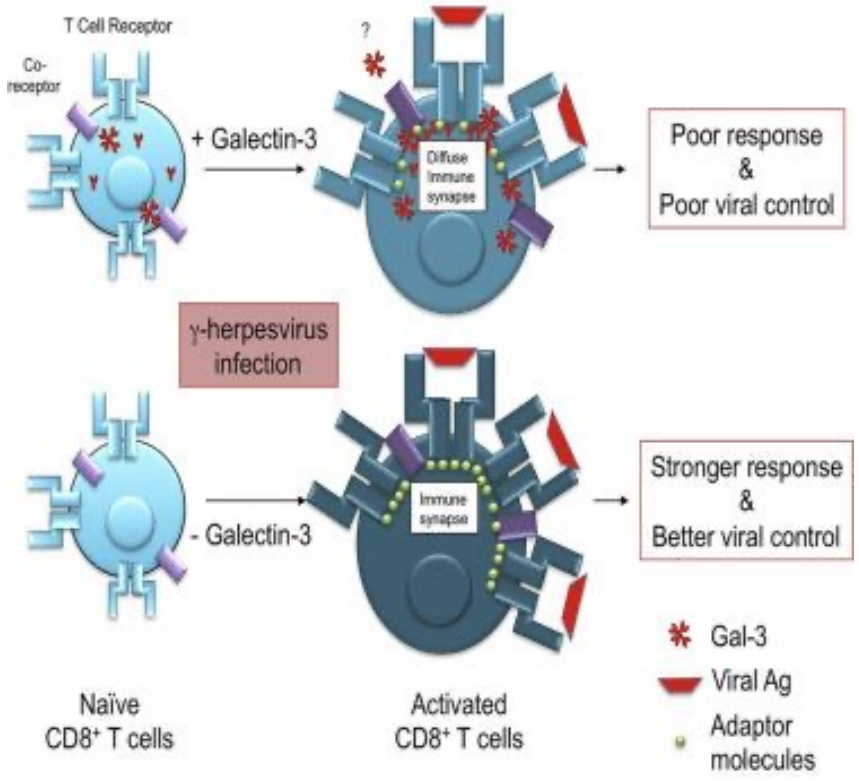


Galectin-3 Upregulated

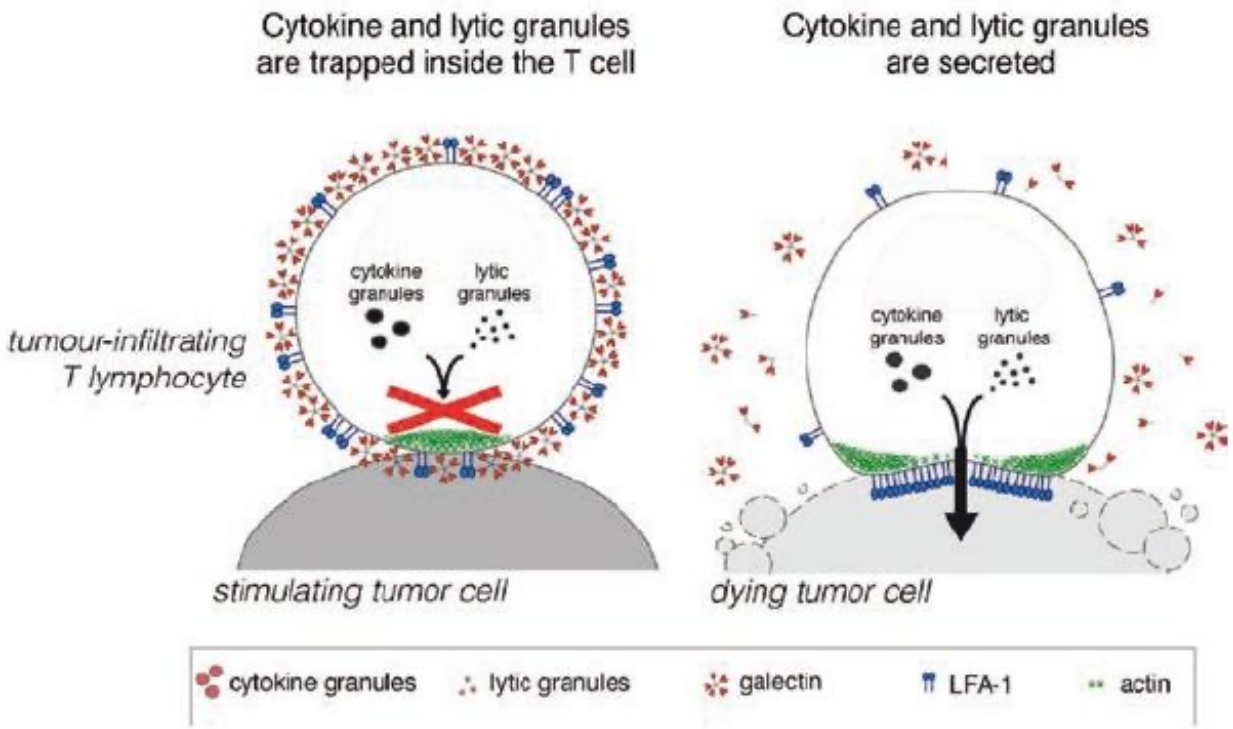
Virally infected cells upregulate Gal-3 which is used in the budding process of virion. When cells burst after the nuclear material is used up it goes into the inflammatory environment.

Effect of Elevated - Galectin-3

Galectin-3 plaque creates CD8+ T-Cell Anergy. Gal-3 also promotes the trafficking of inflammatory macrophages via adhesion that allows invasion and extravasation into the vasculature. Gal-3 is also responsible for all types of organ fibrosis (brain, heart, lungs, kidney, GI tract).



Galectin Effect (T-Cell Anergy)



Cytokines and lytic enzymes are produced normally by human tumor-infiltrating T lymphocytes but remain trapped inside the cells.

Galectins are responsible for T-Cell anergy and prevent the LFA-1 lectins (depicted in blue) from coalescing at the target cell and developing good adhesion in order to destroy it with cytotoxins.

SAME MOA IN VIRUSES

https://www.deduveinstitute.be/sites/default/files/upload/%20Annual%20Report%20DDUV%202017.pdf
https://seekingalpha.com/instablog/47560554-vision-and-value/5210387-gr-mdminus-02-keytruda-s-partner

Functions of Galectin-3 in Viral Infections

Figure 2. Gal-3 may amplify the cytokine storm syndrome associated with severe COVID-19.

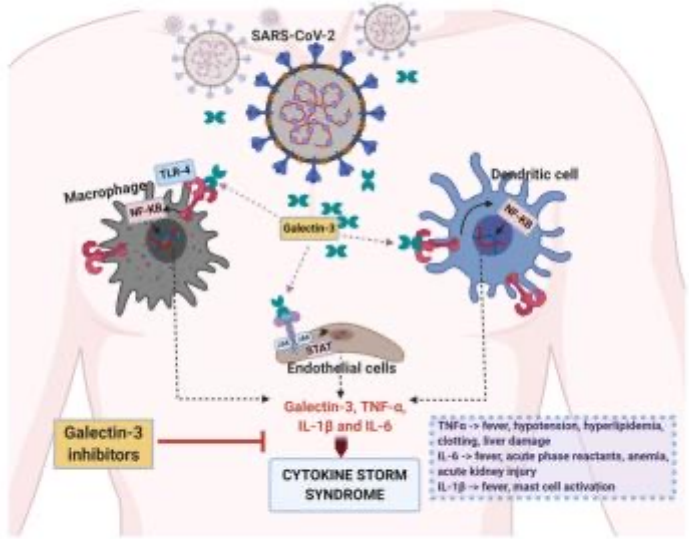
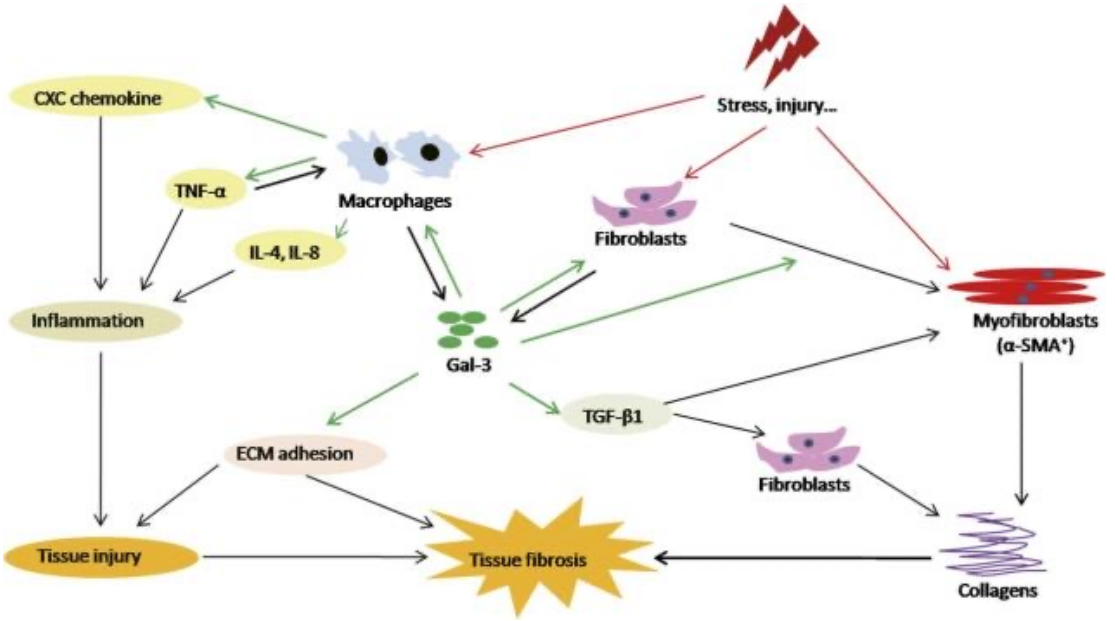


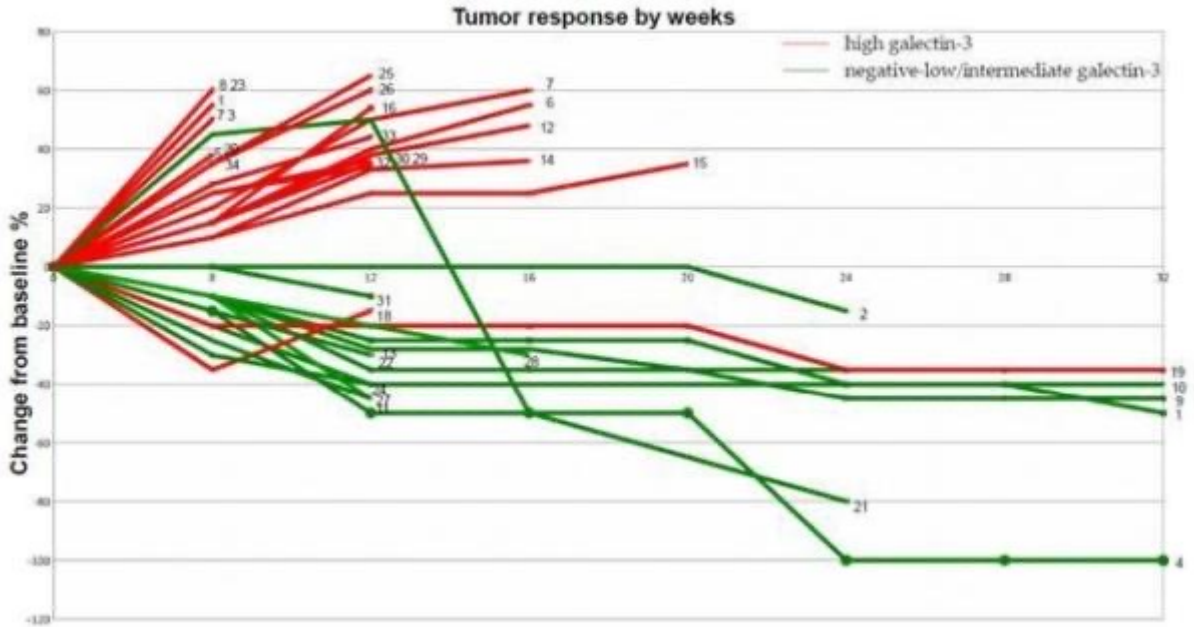
Figure 2. Gal-3 may amplify the cytokine storm syndrome associated with severe COVID-19.
During severe SARS-CoV2 infection, increased plasma concentrations of Gal-3 are observed in circulating macrophages, monocytes, and dendritic cells. When secreted, Gal-3 can then agonize TLR4 receptors on their surfaces and induce the release of inflammatory cytokines such as IL-1, IL-6, and TNF-α. This process also results in the secretion of further Gal-3, resulting in a positive feedback loop that may contribute to the development of CSS.



Drivers of the Cytokine Storm

- Innate Immune System – helping the trafficking of cells
- Dampening of adaptive immune system (the CD8+ response)
- Feeds the inflammatory cycle of fibrosis.

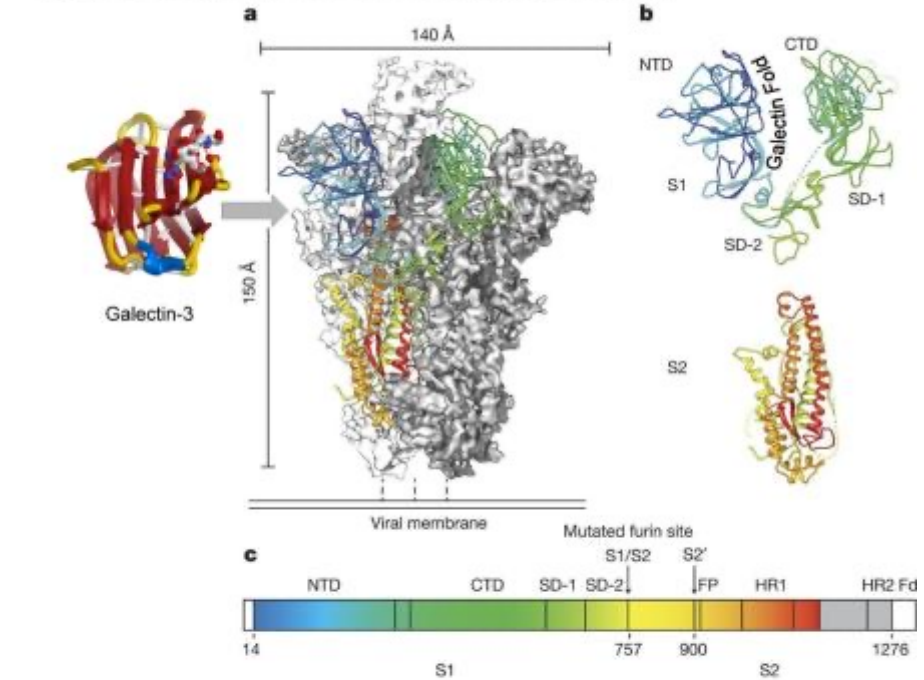
Galectin Linkage to Cancer



Binding to the spike protein and acting as entry inhibitor

Open Label Clinical Trial demonstrated viral elimination in 2-5 days
Zero safety signals. Increased IgG levels on par with vaccinated

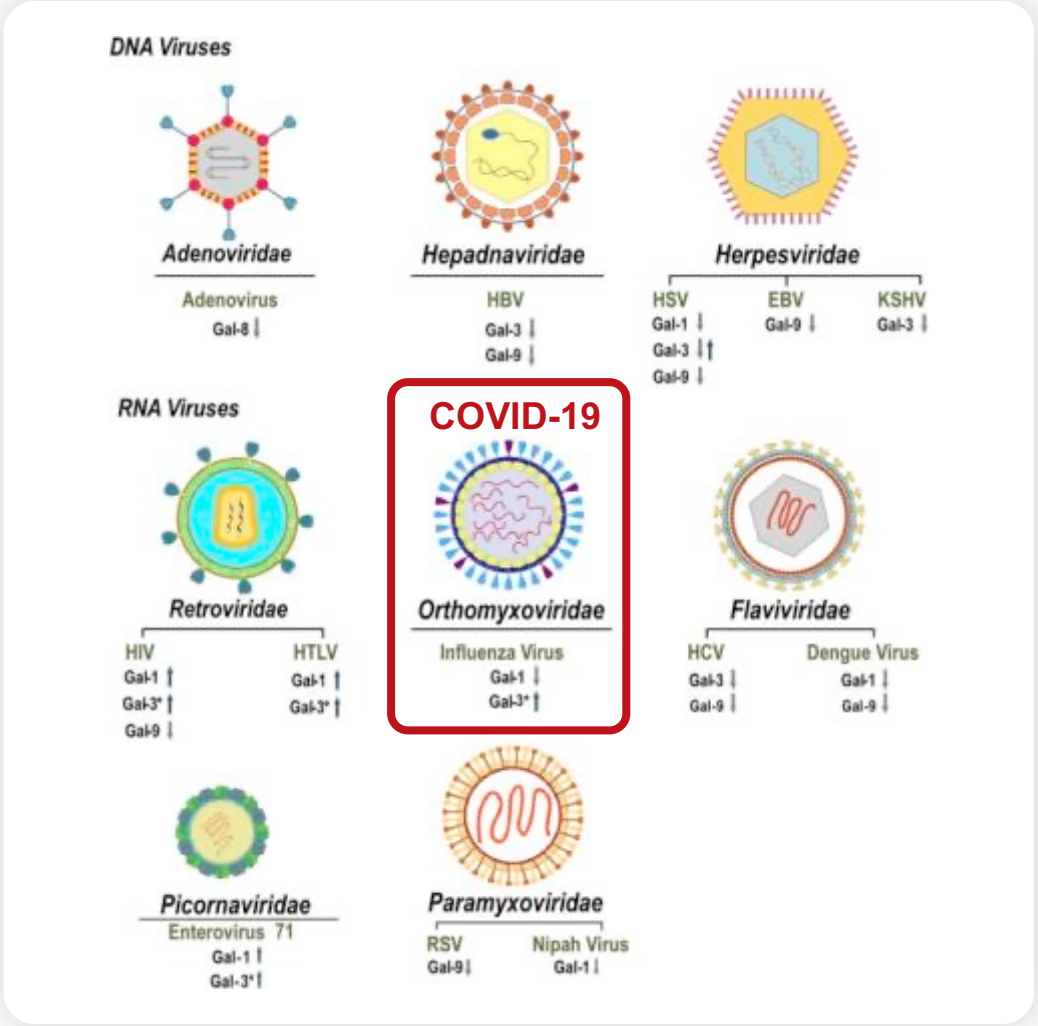
Makes Keytruda 100% effective if excluded for high Galectin-3



a, A single protomer of the trimeric S protein is shown in cartoon representation coloured as a rainbow from the N to C terminus (blue to red) with the reconstructed EM density of remaining protomers shown in white and grey. b, The S1 subunit is composed of the NTD and CTD as well as two sub-domains (SD-1 and SD-2). The S2 subunit contains the coronavirus fusion machinery and is primarily α-helical. c, Domain architecture of the HKU1 S protein coloured as in a.

Kirchdoerfer, R., Cottrell, C., Wang, N. et al. Pre-fusion structure of a human coronavirus spike protein. Nature 531, 118–121 (2016)

Galectins Modulate Viral Infection



https://www.sciencedirect.com/science/article/pii/S1684118219301495

Galectin-1,3, and 9 are modulators of viral infection

Gal-1 has been reported to participate in the regulation of influenza A virus infection. An *in vitro* study showed that Gal-1 expression inhibited the Influenza A (H1N1) virus infection on BEAS-2B (human bronchial epithelial cells) and induced an arrest of the cell cycle, largely at the G0/G1 phase.[20] Another study showed an association between the levels of Gal-1 and viral loads during the acute phase of influenza A/WSN/33H1N1 infection, using an intranasal treatment of hrGal-1 enhancing mice survival that were challenged with A/WSN/33 (H1N1) influenza virus, suggesting Gal-1 as an inhibitor to ameliorate influenza A virus infection.[21]

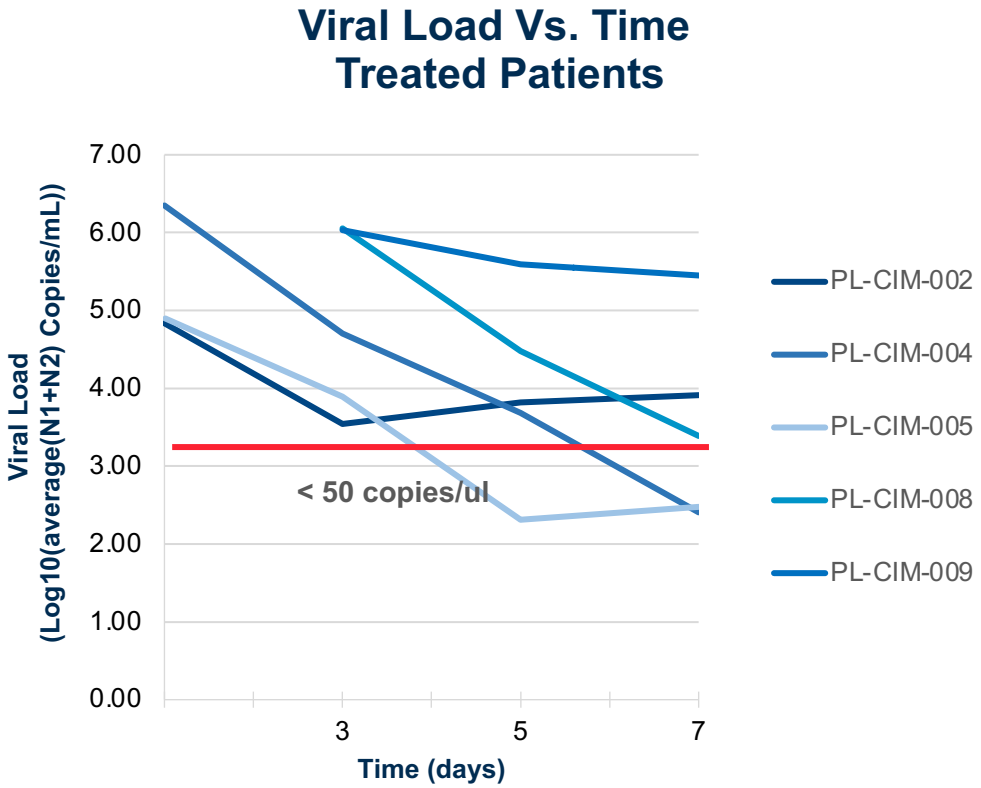
High Galectin Expression Leads to Bad Outcomes

Galectins are the Smoking Gun of COVID-19

- Extremely accurate predictors of mortality
- Galectin serum levels are more accurate than composite scores
- Entire disease pathogenesis explained by their MOA
- Responsible for the cytokine storm
- Creates T-Cell Anergy

What if Galectins Were Blocked?

Patients Treated With Galectin Antagonist Experienced Reductions In Viral Load



N1+N2 Copies/mL

Day	1	3	5	7
PL-CIM-002	137080	6970	13180	16340
PL-CIM-004	4468590	101170	9660	510
PL-CIM-005	159730	15630	410	600
PL-CIM-008	N/A	2268630	60180	4890
PL-CIM-009	N/A	2154530	783750	563430

Patient 9 appeared to be an anomaly. Additionally, Patient 8 had no PCR measurement of viral load on day 1, so day 1's PCR measurement was assumed to equal day 3's measurement.

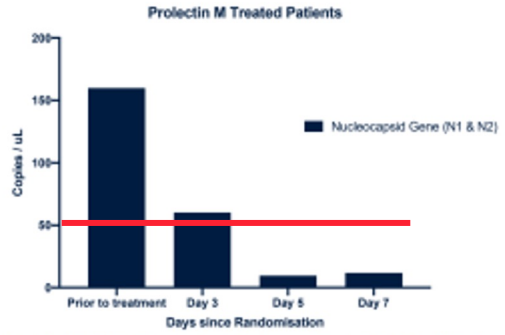
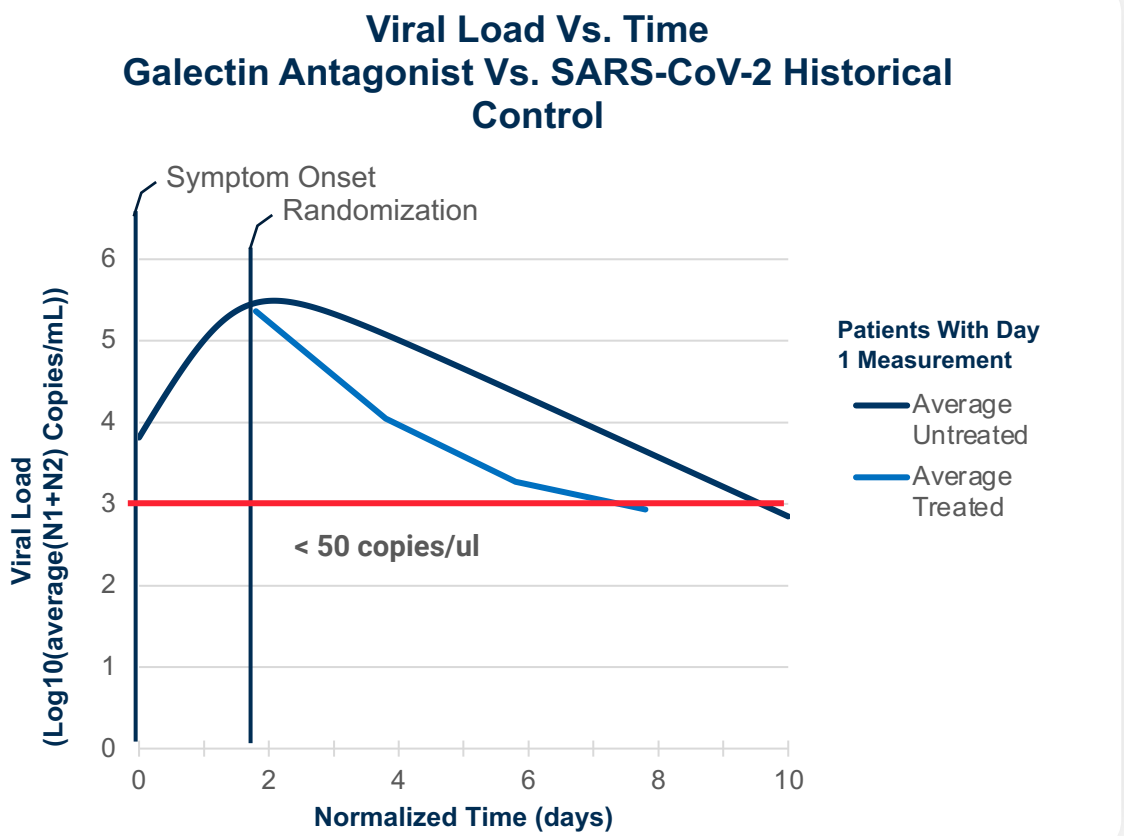


Figure 3 – drop in absolute copy numbers of nucleocapsid gene over time – Treated group

[1] Galectin Antagonist use in Mild Cases of SARS-CoV-2; Pilot Feasibility Randomised, Open Label, Controlled Trial (longdom.org)

Viral Curve Comparison



Historical control is taken from a mathematical model using longitudinal data across four different studies of symptomatic, untreated cases[1]

Assumed symptom onset at a viral load of 6500 copies/mL (i.e. log10(3.81))[1]

Patients treated within 2 days of symptom onset (average 1.80 days)[2]

Upper and lower bounds of the model are 95% confidence interval[1]

[1] A quantitative model used to compare within-host SARS-CoV-2, MERS-CoV, and SARS-CoV dynamics provides insights into the pathogenesis and treatment of SARS-CoV-2 (plos.org)
[2] Galectin Antagonist use in Mild Cases of SARS-CoV-2; Pilot Feasibility Randomised, Open Label, Controlled Trial (longdom.org)

Galectin Antagonist Treatment Results in SARS-CoV-2 Spike Protein Specific Antibody Immunity

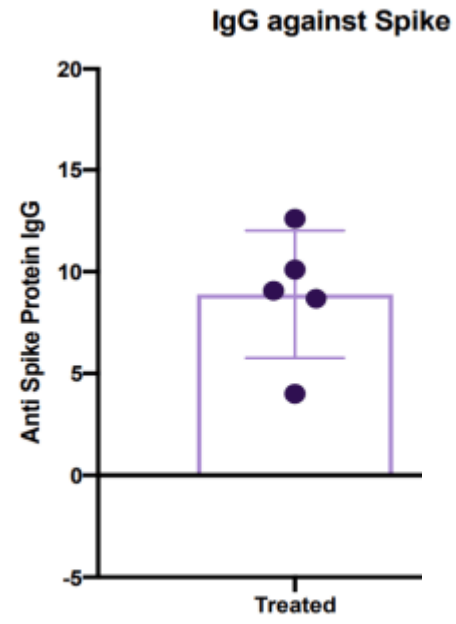


Figure 4 – difference in IgG on day 28

Introducing Post Infection Immunization

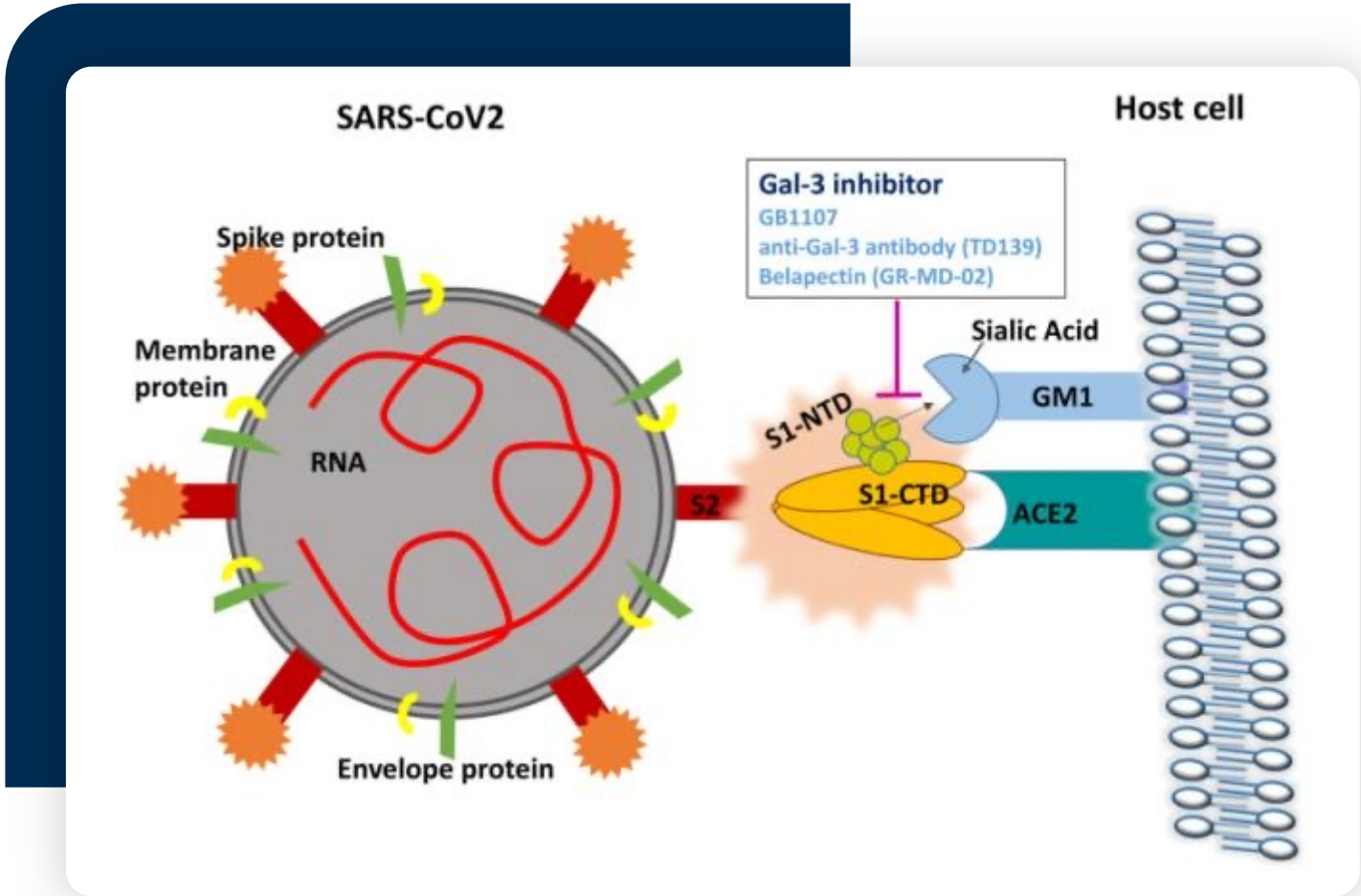
Galectin antagonists clear the blood of viral load thereby reducing the strain on the Innate immune system so the Adaptive immune system can build a robust response toward future infection.

[1] Galectin Antagonist use in Mild Cases of SARS-CoV-2; Pilot Feasibility Randomised, Open Label, Controlled Trial (longdom.org)

Clinical Trial Result Summary

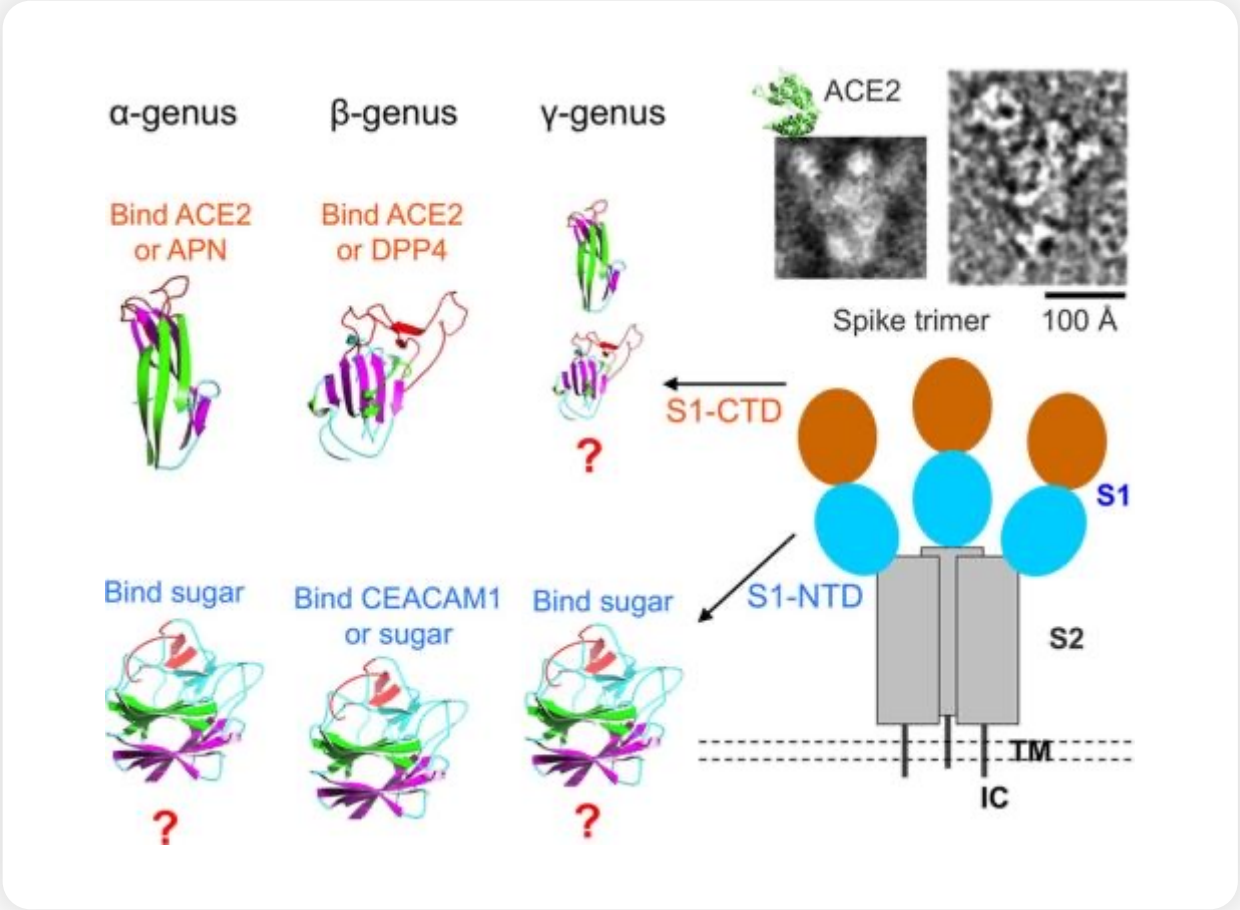
- Compete elimination of the viral load within 5 days
- Reduction of infectivity
- Quieting the cytokine storm
- Robust antibody response (Post Infection Immunization)

Galectin Inhibition MOA



- Binding to the spike protein in Blood
- Liver removes carbohydrates drug and virus.
- Restoration of Adaptive immune system – peel off galectin plaque
- Adaptive immune system creates long term immunity

Galectin-3 Ideal Target



Coronavirus Receptor Recognition Pattern

- Spikes use one or more S1 domains as receptor binding domains (RBD)
- Galectin receptors appear to have no effect on viral entry

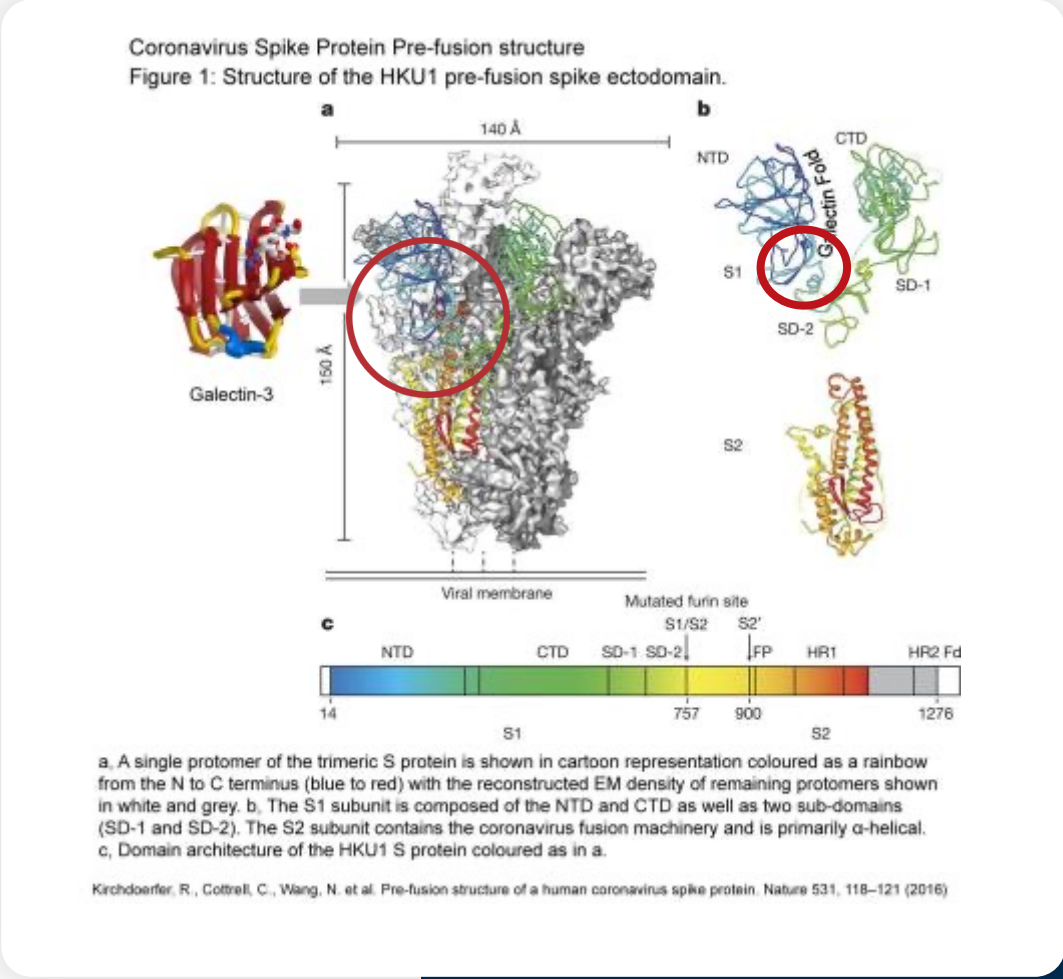
In the viral evolutionary path it appears that the CoV stole a host galectin gene and inserted it at the end of their spike gene. The S1-NTD CoV has a galectin fold that is likely shared amongst different CoV genus but they are programmed to recognize different sugar receptors. Theory suspects viral lectins originated from host galectins but evolved the galectin fold to evade the immune system.

Source: Fang, Li. Receptor Recognition Mechanisms of Coronaviruses: a Decade of Structural Studies, *Feb, 2015 89 (4), pgs. 1954 - 1964*

Universal Target – Galectin Fold

Attaching to the Virus

- S1 NTD Region across all Coronaviruses is the ideal Binding site for a Galectin-3 Inhibitor
- Monoclonal Antibodies(Mab) cannot take advantage of this spot because the Mab's are too large.
- This includes INFLUENZA



Finding the Virus

The Immune System

Virus in the blood

Spreads through blood - Innate Immune System

Virus in tissue

Removing Infected Tissue – Adaptive Immune

Clearing the Virus

The Immune System

Virus in the blood

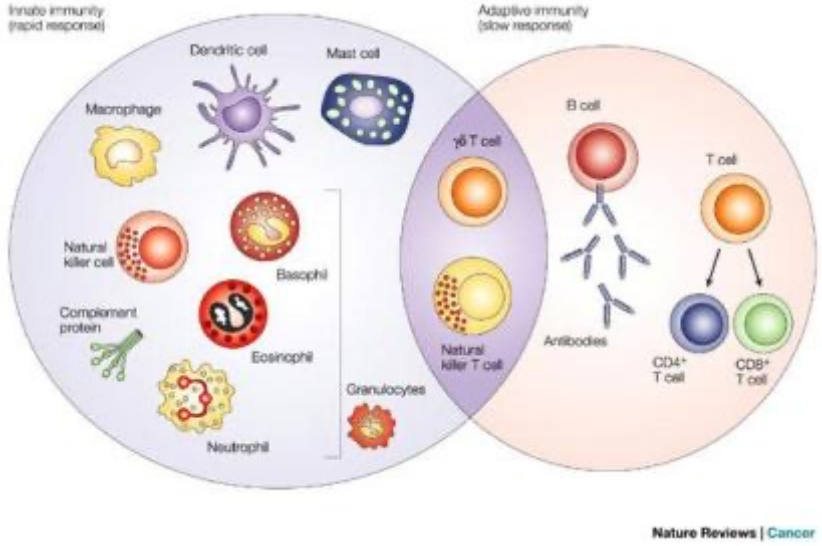
Innate Defense

- Dendritic Cell
- Mast Cell
- Macrophage
- Natural Killer Cells
- Neutrophil
- Eosinophil
- Basophil
- Compliment Protein

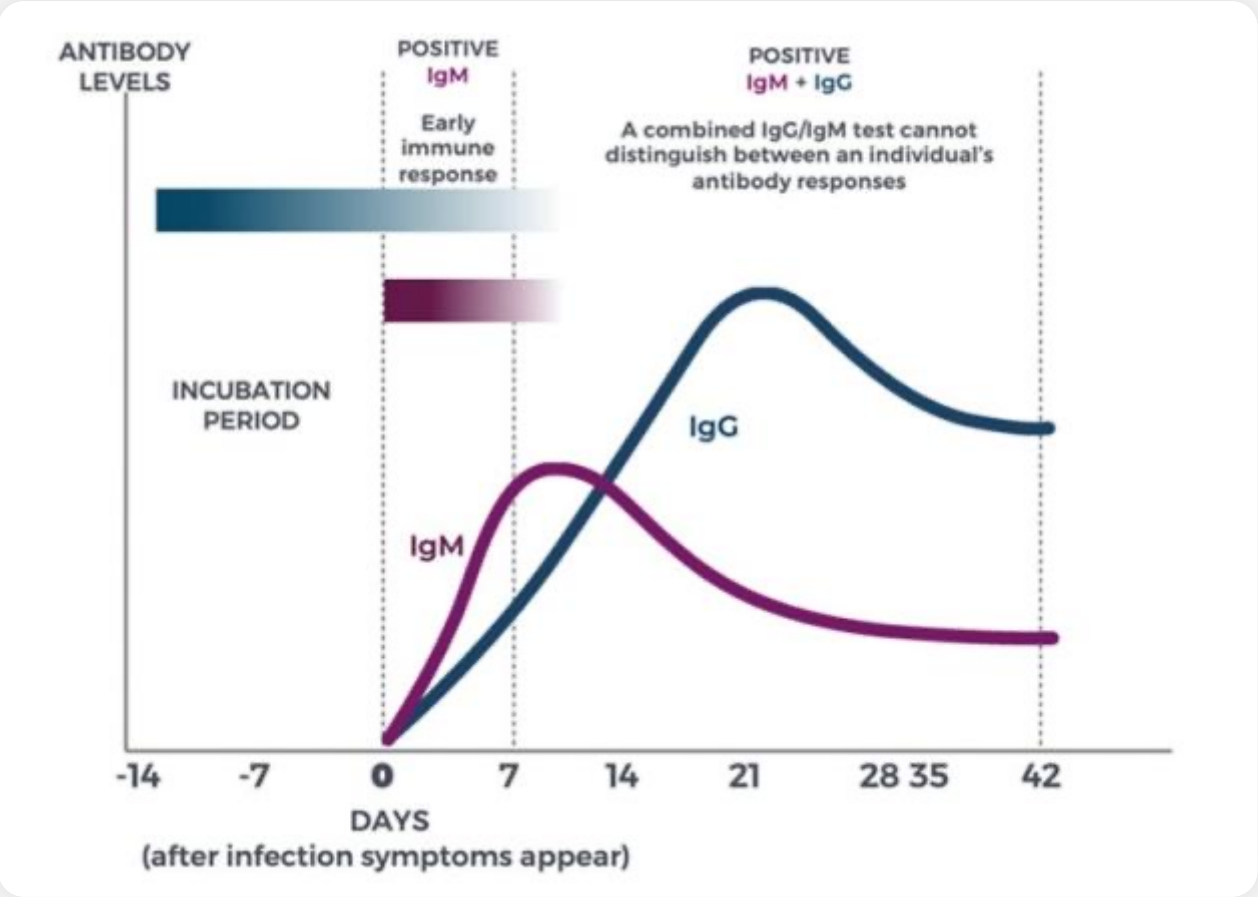
Virus in diseased tissue

Adaptive Defense

- T-Cell (CD4+ CD8+)
- B Cell
- Antibodies



Targeting the Blood

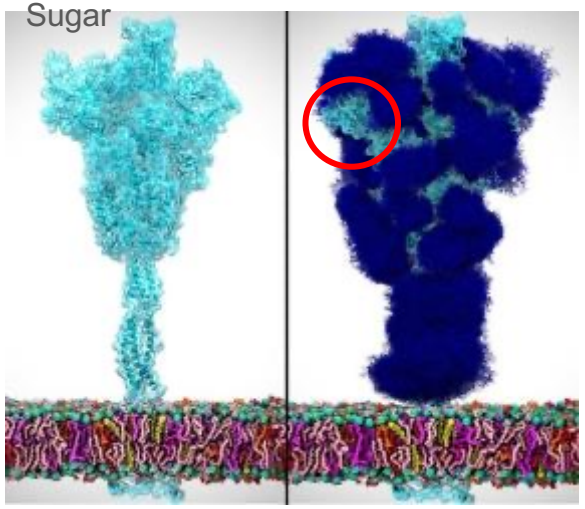


Galectin Inhibitors eliminates the virus in the blood & build long term immunity

- Eliminating the viral load in the blood **prevents activation of innate immune response** (it does the heavy lifting)
- Halting the spread of the disease to other organs and thereby reduces clinical symptoms and contagiousness
- Overactive Innate response can lead to the cytokine storm and complications
- Adaptive immune system works in the background to remove virally infected cells and tissue while boosting IgG antibody production

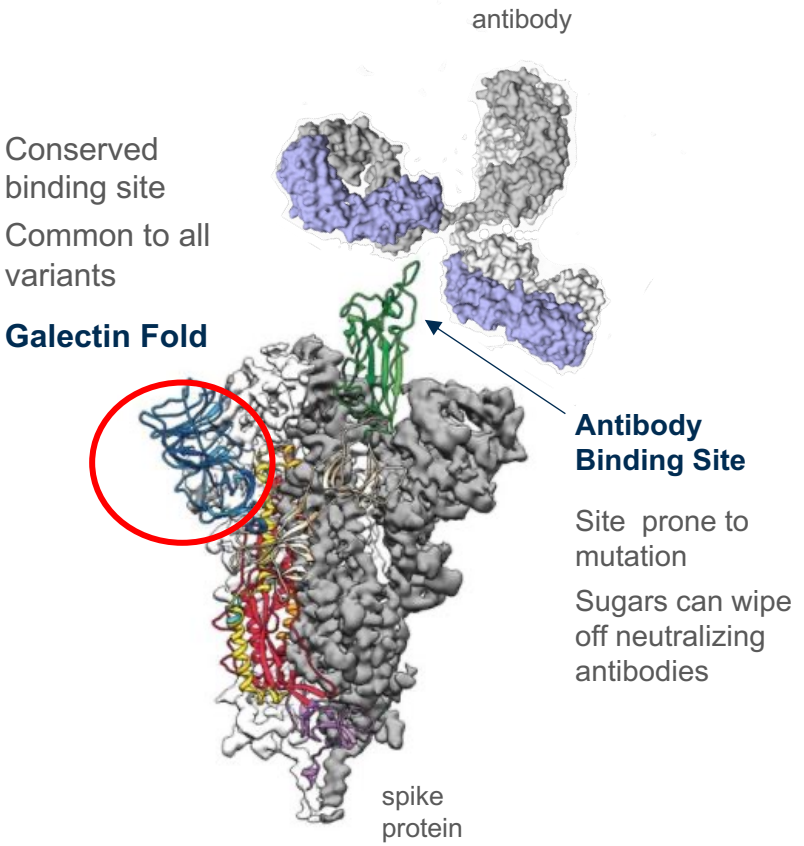
Neutralizing the Spike Protein

How it Works



Part of the Problem

Antibodies need a place to attach. The sugar shield is not static, but rather a dynamic shape shifting like coating with windshield wipers on the surface that limit areas of attachment.



Galectin Fold Ideal Binding Site

Binding to the spike protein prevents viral entry

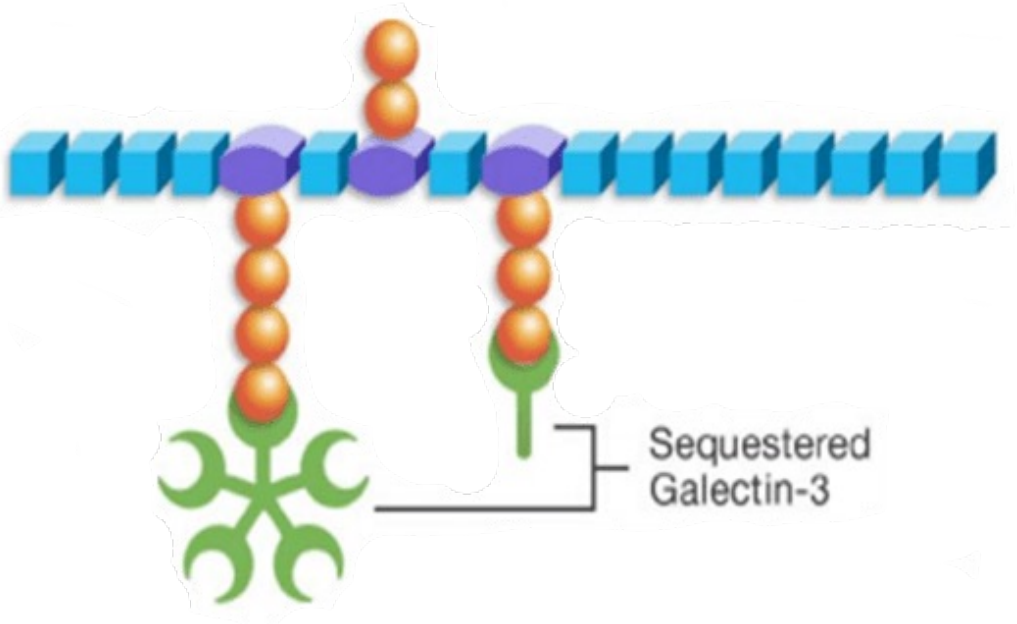
Immensely tighter bond to galectin fold vs tip (Prolectin-RX 99% binding affinity)

Antibodies take time to be produced – slower response to infection

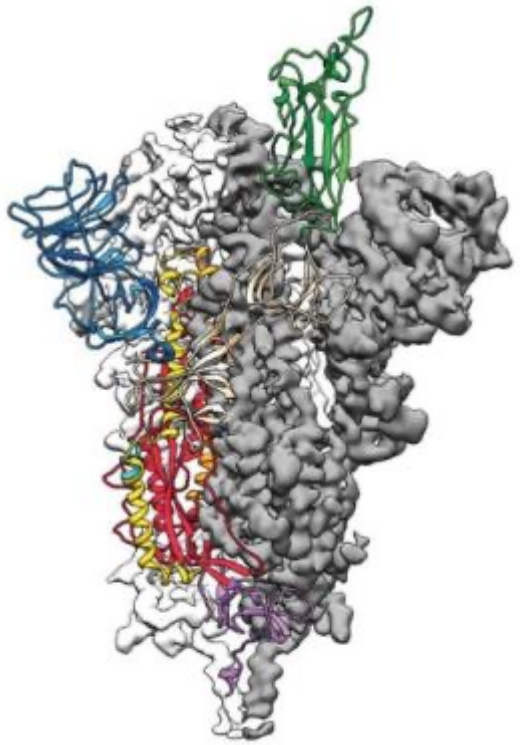
Galectin Inhibitors are Superior to Antibodies

Key and Lock Mechanism

Galectin Inhibitor Acts as Key and the Galectin Fold is the Lock



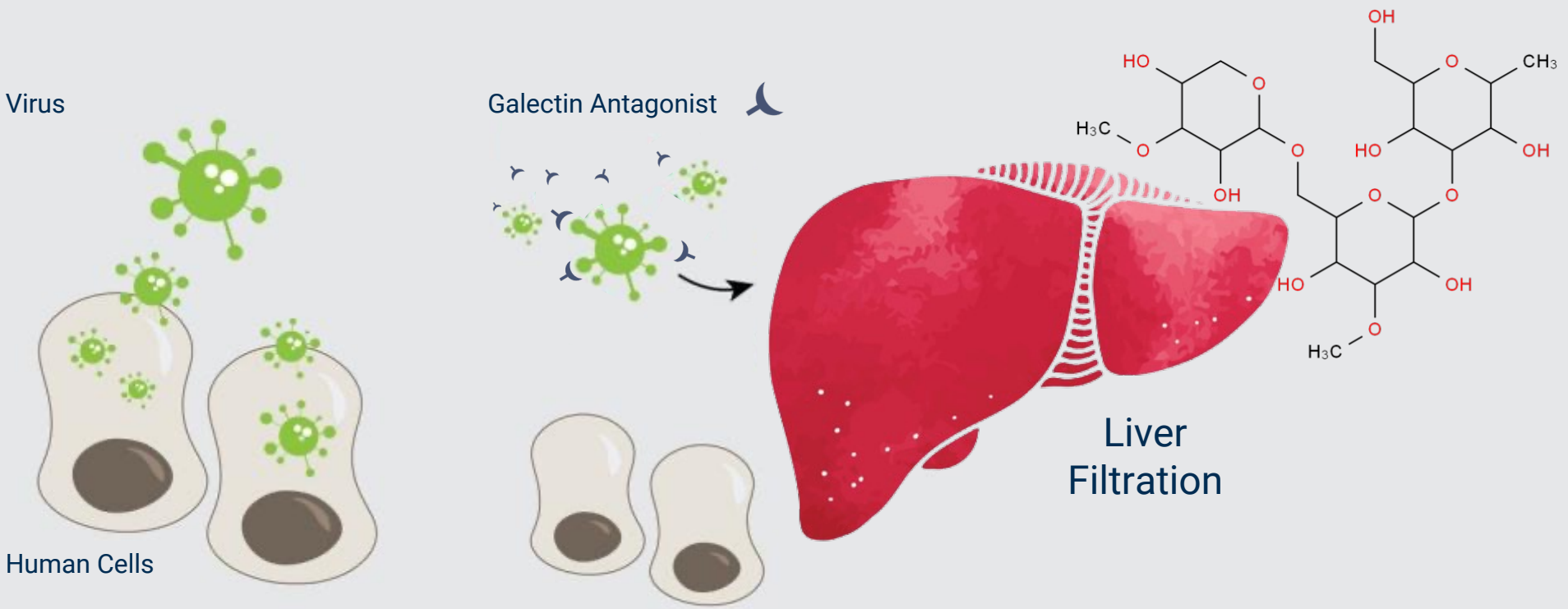
Galectin Inhibitor Key



The tight binding affinity allows the virion stick to the galectin inhibitor like a fly to fly-paper

Galectin Antagonist Tags Virus For Elimination

Theoretical Mechanism of Action



Approach used for the 1st Time in History of Drug Development

Clinical Research

Galectin Antagonist use in Mild Cases of SARS-CoV-2: Pilot Feasibility Randomised, Open Label, Controlled Trial

Alben Sigamani*, Mathu Ruthra, Sudhishma, Samarth Shetty, Madhavi, Anup Chugani, Hana Chen-Walden, David Platt and Thomas Kutty

Importance: Novel SARS-CoV-2 virus has infected nearly 100 million people across the world and is highly contagious. There is a need for a novel mechanism to block viral entry and stop its replication.

Background: Spike protein N Terminal Domain (NTD) of the novel SARS-CoV-2 is essential for viral entry and replication in human cell. Thus the S1 NTD of human coronavirus family, which is similar to a galectin binding site-human galactose binding lectins, is a potential novel target for early treatment in COVID-19.

Objectives: To study the feasibility of performing a definitive trial of using galectin antagonist–Prolectin-M as treatment for mild, symptomatic, rRT-PCR positive, COVID-19.

Main outcomes and measures: Cycle threshold (Ct) value is number of cycles needed to express fluorescence, on real time reverse transcriptase polymerase chain reaction. Ct values expressed for RNA polymerase (Rd/RP) gene+Nucleocapsid gene and the small envelope (E) genes determine infectivity of the individual. A digital droplet PCR based estimation of the Nucleocapid genes (N1+N2) in absolute copies/μL determines active viral replication.

https://www.longdom.org/abstract/galectin-antagonist-use-in-mild-cases-of-sarscov2-pilot-feasibility-randomised-open-label-controlled-trial-61087.html

Product

Benefits

- Eliminates virus and stops the spread
- Promotes immunity
- Easy to transport and administer
- No adverse effects
- Conserved binding region resistant to mutations
- Universally compatible with other therapies

Science Behind Galectin Antagonists

Clinical Research

- Proven Safety Profile in Drug Class
- Peer-reviewed clinical trial in COVID-19
- Galectin Inhibitors in phase 2 & 3 trials for IPF, NASH, Cancer, Atopic Dermatitis, Psoriasis, Covid-19

4000+

Journal Articles on Target Receptors

medRxiv

THE PREPRINT SERVER FOR HEALTH SCIENCES

Comment on this paper

Galectin antagonist use in mild cases of SARS-CoV-2 cases; pilot feasibility randomised, open label, controlled trial

ALBEN SIGAMANI, ALBEN SIGAMANI, MADHAVI KADAMBI, MATHU RUTHRA, SUDHISHMA SHIVAPRASAD, ANUP CHUGANI, HANA CHEN-WALDEN, THOMASKUTTY ALUMPARAMBILL, DAVID PLATT

doi: https://doi.org/10.1101/2020.12.03.20238840

This article is a preprint and has not been certified by peer review [what does this mean?]. It reports new medical research that has yet to be evaluated and so should *not* be used to guide clinical practice.

Abstract | Info/History | Metrics

Abstract

Importance Novel SARS-CoV-2 virus has infected n
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Journal of Vaccines & Vaccination

Research Article

Galectin Antagonist use in Mild Cases of SARS-CoV-2: Pilot Feasibility Randomised, Open Label, Controlled Trial

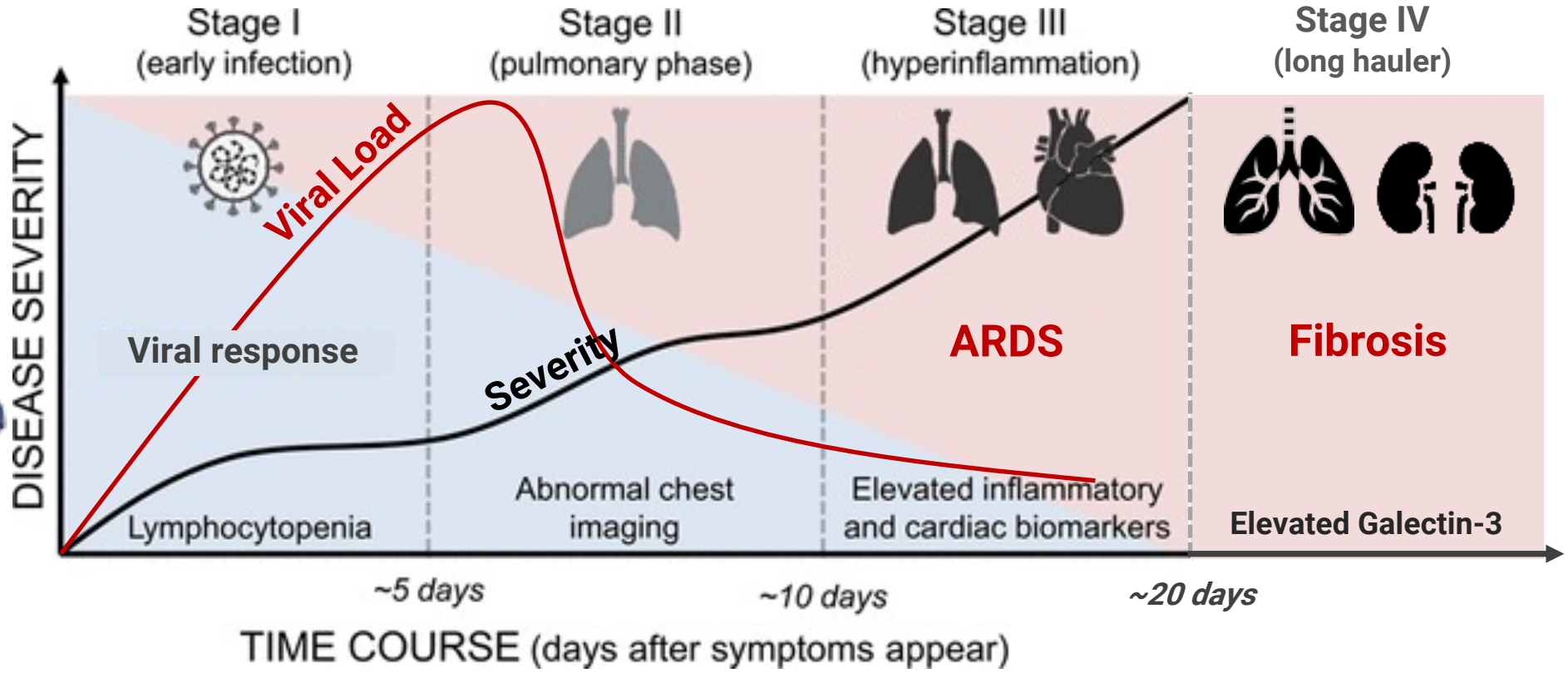
ABSTRACT

30+ years

of research in Galectins, carbohydrate-binding proteins

End-to-End Solution

Treatment	ProLectin-M Oral	ProLectin-I Intravenous	ProLectin-A Intravenous	ProLectin-F Intravenous
Combination			MDX-Viewer	



Version 2.01.01

Technology Comparison

	Stops the spread	Easy to administer	Safety	Resistant to mutations	Storage Condition	Promotes Immunity
Galectin Antagonists	+	+	+	+	+	+
Vaccines	-	-	+	-	-	+
Anti-replication drugs	+	+	-	-	+	-
Immunomodulatory drugs	-	-	+	+	+	-
Regenerative medicine	-	-	-	+	-	-

Business Model

Galectin Antagonist Scalability

Chewable tablet formulation

United States and Worldwide Pharmacopeia Supply of API (Active Pharmaceutical Ingredients)

Direct cost:

10-12 cents

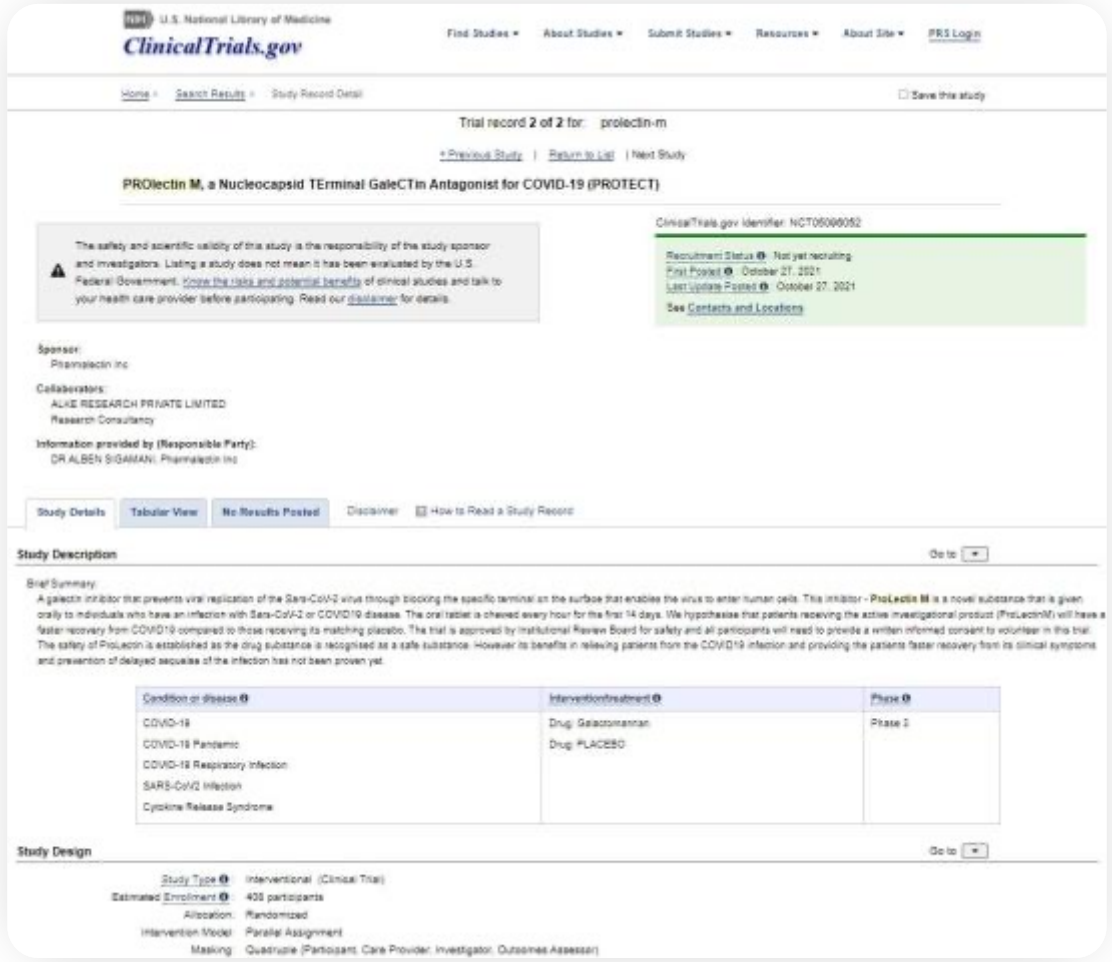
per tablet

Treatment:

45 tablets

per bottle, 9 tablets a day

Proposed Clinical Trial Design



Clinical Trial

- 408 participants
- Double Blind Randomized Controlled Trial (DBRCT)
- Change in seropositivity at day 14
- Broad inclusion criteria (Vaccination status irrelevant)

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