

# Myocardial Virus and Gene Expression in SARS-CoV-2 Positive Patients with Clinically Important Myocardial Dysfunction

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# Disclosures

- President & CEO of a biotech company (ARCA biopharma) developing a drug for COVID-19 Coagulopathy (CAC)
  - Drug (rNAPc2) and indication have no direct relationship to this presentation or research program



# Angiotensin II Formation in the Intact Human Heart

## Predominance of the Angiotensin-converting Enzyme Pathway

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Larry Zisman

# Angiotensin-(1-7) Formation in the Intact Human Heart

## In Vivo Dependence on Angiotensin II as Substrate

Lawrence S. Zisman, MD; Glenn E. Meixell, PhD; Michael R. Bristow, MD, PhD; Charles C. Canver, MD

*Circulation.* 2003;108:1679-1681.  
October 7, 2003

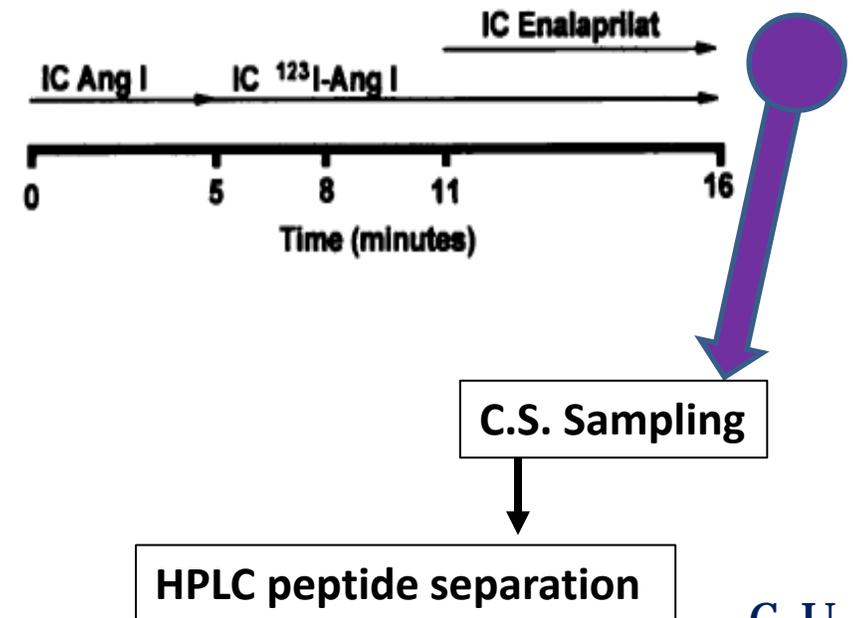
# Increased Angiotensin-(1-7)-Forming Activity in Failing Human Heart Ventricles

## Evidence for Upregulation of the Angiotensin-Converting Enzyme Homologue ACE2

Lawrence S. Zisman, MD; Rebecca S. Keller, PhD; Barbara Weaver, MS; Qishan Lin, PhD; Robert Speth, PhD; Michael R. Bristow, MD, PhD; Charles C. Canver, MD

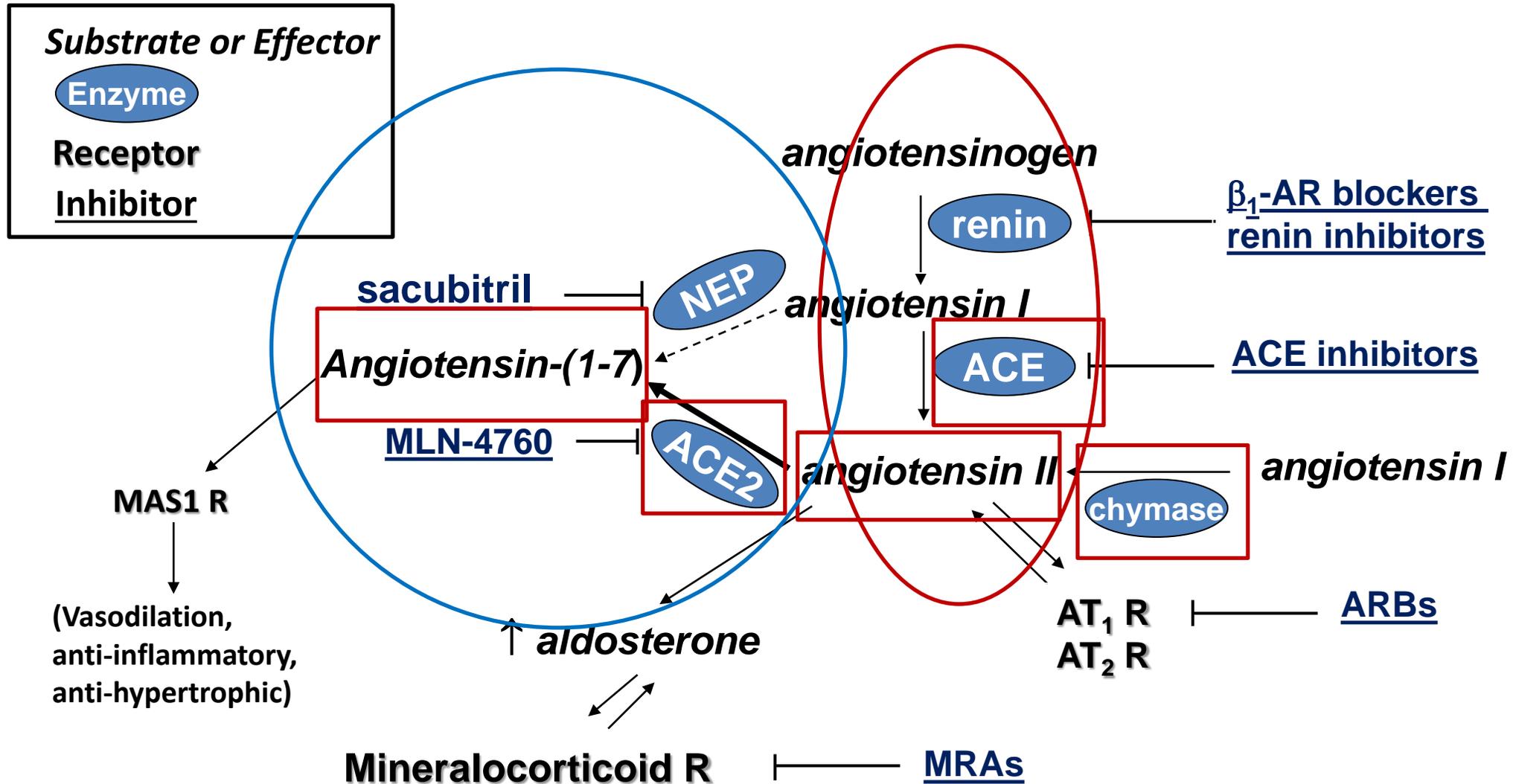
*Circulation.* 2003;108:1707-1712.  
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published in abstract form (*Circulation.* 1999;100(suppl I):I-625).



C U  
V I

# The Renin-Angiotensin-Aldosterone System



## Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus

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## Article

## A pneumonia outbreak associated with a new coronavirus of probable bat origin

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Since the outbreak of severe acute respiratory syndrome (SARS) 18 years ago, a large number of SARS-related coronaviruses (SARSr-CoVs) have been discovered in their natural reservoir host, bats<sup>1–4</sup>. Previous studies have shown that some bat SARSr-CoVs have the potential to infect humans<sup>5–7</sup>. Here we report the identification and characterization of a new coronavirus (2019-nCoV), which caused an epidemic of acute respiratory syndrome in humans in Wuhan, China. The epidemic, which started on 12 December 2019, had caused 2,794 laboratory-confirmed infections including 80 deaths by 26 January 2020. Full-length genome sequences were obtained from five patients at an early stage of the outbreak. The sequences are almost identical and share 79.6% sequence identity to SARS-CoV. Furthermore, we show that 2019-nCoV is 96% identical at the whole-genome level to a bat coronavirus. Pairwise protein sequence analysis of seven conserved non-structural proteins domains show that this virus belongs to the species of SARSr-CoV. In addition, 2019-nCoV virus isolated from the bronchoalveolar lavage fluid of a critically ill patient could be neutralized by sera from several patients. Notably, we confirmed that 2019-nCoV uses the same cell entry receptor—angiotensin converting enzyme II (ACE2)—as SARS-CoV.

# Myocardial Involvement in COVID-19: *Published Information*

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## As of 4/6/20 (AHA grant submission):

- Myocardial injury & dysfunction minimal reports, mechanism uncertain
  - Myopericarditis by CMR (1 report, no tissue)
  - 1 heart autopsy biopsy, SCD in severe lung Dz, “no obvious histologic changes” in heart
- Myocardial injury evidence by ↑ hs-cTn, associated with adverse outcomes
- ACE2 is CoV-2 receptor for cell entry, ↑ in failing/remodeled human LVs and in animal models Rxd with ARBs

# Myocardial Virus and Gene Expression in SARS-CoV-2 Positive Patients with Clinically Important Myocardial Dysfunction: *Aims*

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## **Aim 1. Detection of CoV-2 in cardiac myocytes.**

- N = 10, EMBx
- Histopathology including EM, patients with evidence of CoV-2 myocardial involvement
- RT-PCR for viral genome

## **Aim 2. Determine the degree of inflammatory reaction vs. direct myocardial injury.**

- Histopathology
- Cytokine gene expression, circulating levels

## **Aim 3. Measure mRNA expression of the binding target (ACE2), proteases and integrins** that have been shown to be key to cellular entry in non-cardiac cells.

- mRNA abundance by RNA-Seq and microarray
- ACE2, ACE, NPPB,  $\alpha 5$  ITG, TF, mRNA abundance by RT-PCR rapid turnaround; circulating ACE2, ACE, ANG II, TF

## **Aim 4. Measure mRNA expression of candidate and global genes**, and compare results to nonfailing controls and reduced LVEF nonischemic dilated cardiomyopathy (NDC) patients

- mRNA abundance by RNA-Seq and microarray, n = 10 patients with CoV-2 myocardial involvement
- 12 NF, 12 F/NDC septal biopsies from explanted hearts; previous EmBx data (4 NF, 46 F/NDC)

# Myocardial Virus and Gene Expression in SARS-CoV-2 Positive Patients with Clinically Important Myocardial Dysfunction: *Revised Entry Criteria (10/1/20)*

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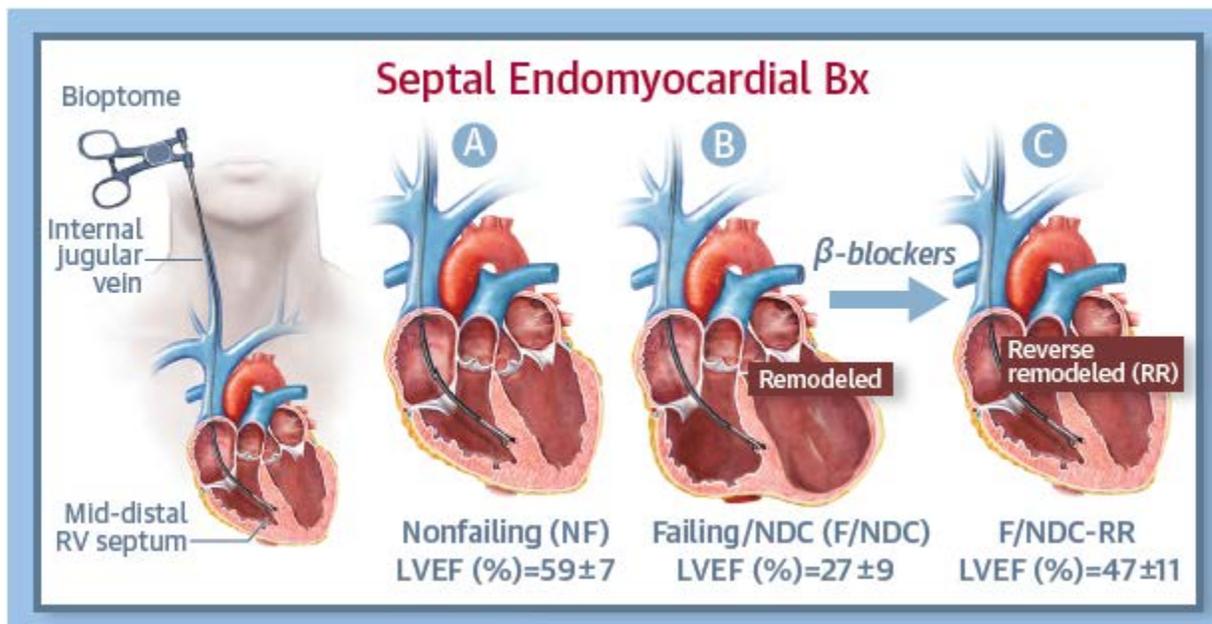
## Inclusion, Hospitalized Patients

- In or recently in ICU, PCR + for CoV-2, Age  $\geq 18$ , COVID-19 myocardial involvement in the DDx, stable enough for cardiac catheterization
- LVEF  $< 50\%$  OR  
TnI  $\geq 0.05$  ng/ml OR  
global longitudinal strain  $> -16$  OR  
ST-T changes suggesting STEMI, NSTEMI or myopericarditis with patent coronary arteries OR  
new onset sustained VT or VF
- Patient or authorized representative able to give informed consent

## Inclusion, Outpatients

- In ICU in the past 3 mos, PCR + for CoV-2, Age  $\geq 18$ , COVID-19 myocardial involvement in the DDx, stable enough for cardiac catheterization
- LVEF, TnI, GLS, ST-T and VT/VF criteria same as for hospitalized patients
- Patient able to give informed consent

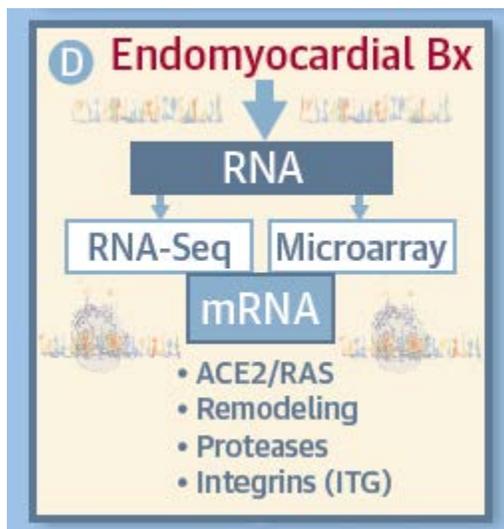
# Dynamic Regulation of SARS-CoV-2 Binding and Cell Entry Mechanisms in Remodeled Human Ventricular Myocardium



## Highlights:

1. Cellular receptor for CoV-2 (ACE2) and 5 proteases previously implicated in membrane fusion are expressed.
2. ACE2 upregulated  $\approx 2$  fold in remodeled LV, proteases NSC.
3. ACE2 normalizes on reverse remodeling independent of ACEIs or ARBs.
4. ITGA5, which encodes an integrin ( $\alpha 5$  ITG) that binds to ACE2 and to a motif (RGD) in the CoV-2 spike protein receptor binding domain, is upregulated in remodeled LV and normalizes on reverse remodeling, and is a candidate for facilitating or mediating CoV-2 cell binding and entry.

**Thus upregulated CoV-2 cell binding mechanisms may explain heightened risk of COVID-19 in patients with underlying heart muscle disease.**



**E Myocardial mRNA expression**

	NF	F/NDC	F/NDC-RR
ACE2	→	↑	→
Proteases	→	→	→
ITGA5	→	↑	→

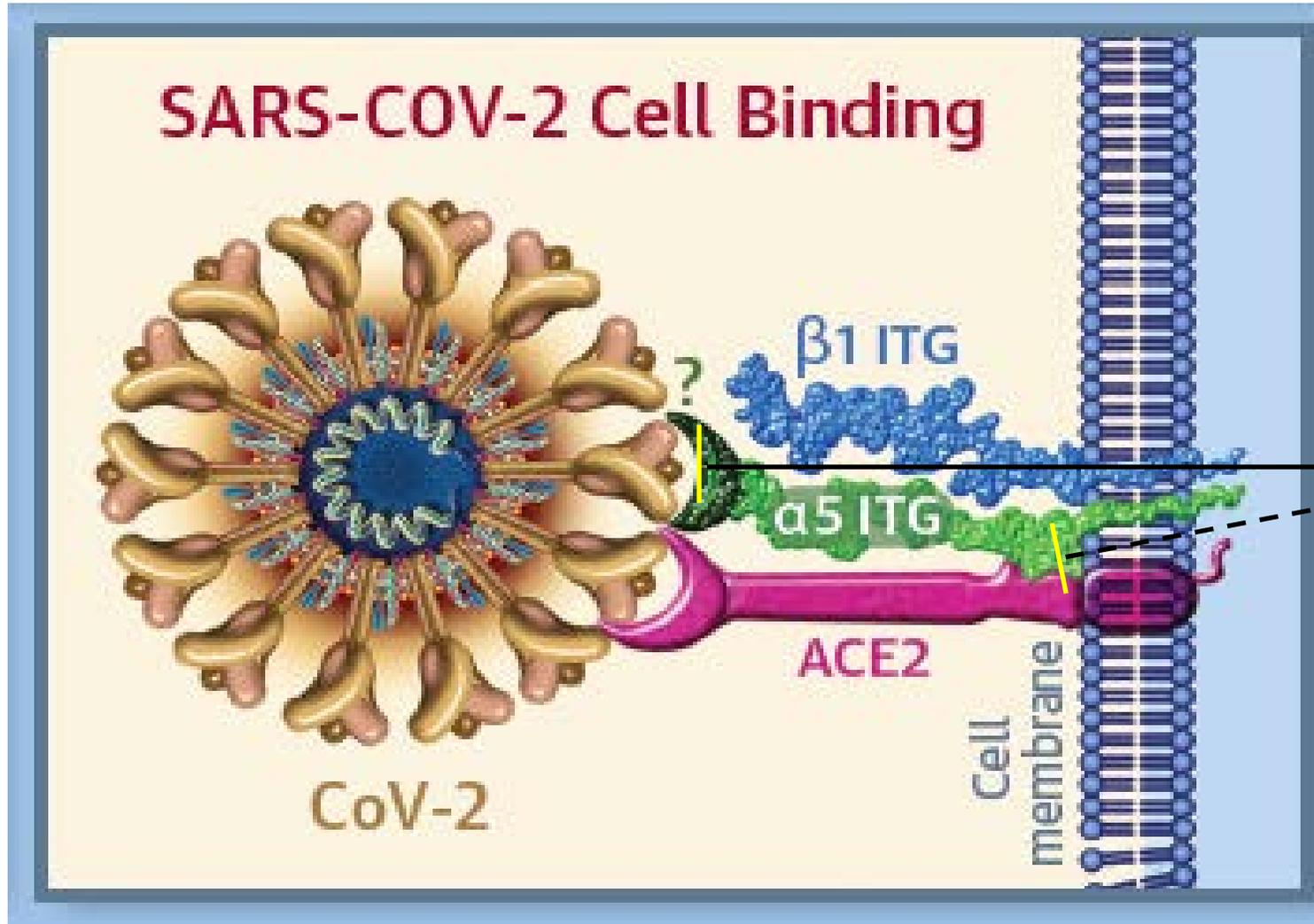
# Integrin $\alpha 5 \beta 1$ facilitates CoV-2 binding and cell entry

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Research Center, Tulane



**The Integrin Binding Peptide, ATN-161,  
as a Novel Therapy for SARS-CoV-2  
Infection (in review, Greg Bix Laboratory  
& BSL3 Core, Tulane SoM).**



**ATN-161**

(non RGD peptide derived from fibronectin, binds to  $\alpha 5 \beta 1$  at an  $\alpha 5$  ITG binding site). In VeroE6 cells:

- CoV-2 binds to  $\alpha 5 \beta 1$
- ATN-161 prevents CoV-2 binding to  $\alpha 5 \beta 1$ , (nM affinity), and  $\alpha 5$  binding to ACE2 ( $\mu\text{M}$ )
- **ATN-161 prevents CoV-2 cell infection (3.16  $\mu\text{M}$  IC<sub>50</sub>)**

<https://doi.org/10.1101/2020.06.15.153387>doi:  
bioRxiv preprint

Bristow MR et al, JBTS Sept 2020. DOI: 10.1016/j.jacbts.2020.06.007  
Published online June 25, 2020

# Myocardial Involvement in COVID-19: *Summary so far*

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- Clinically significant **myocardial involvement** in COVID-19 patients occurs with **uncertain but not uncommon** incidence, and is important to detect and monitor following the acute infection
- Myocardial injury, most commonly detected by an elevation in hs-cTn, may be of **several types**
  - **Inflammation** (myocarditis); probably over Dx'd based on uncontrolled CMR studies
  - **Cytopathic effects** in cardiac myocytes including myofibril disruption and loss, with no or little evidence of inflammation
  - **Vascular involvement**, including **microthrombi**
- **ACE2 is upregulated in ventricular remodeling** similar to NPPB, doesn't appear to be modifiable by RASi therapy and may be a major reason for worse outcomes in some patients
- **Integrin  $\alpha 5$  or its  $\alpha 5\beta 1$  dimer is a co-receptor for CoV-2**, and is a potential therapeutic target in COVID-19