SARS-CoV-2 Cell Entry Mechanisms in Intact Human Heart Published in JACC: Basic to Translational Science by University of Colorado Anschutz Medical Campus and ARCA biopharma Investigators

- Integrin A5 may be a new target for intervening in the cell infectious process
- Findings may lead to the development of precision therapeutic approaches to prevent SARS-CoV-2-cell entry while preserving the functional activity of ACE2

Westminster and Aurora, CO, July 7, 2020 – The University of Colorado Anschutz Medical Campus and ARCA biopharma, Inc. (Nasdaq: ABIO) today announced that the paper entitled "Dynamic Regulation of SARS-CoV-2 Binding and Cell Entry Mechanisms in Remodeled Human Ventricular Myocardium" (Bristow MR, et al. *https://doi.org/10.1016/j.jacbts.2020.06.007* was published in JACC: Basic to Translational Science (JBTS), a member of the Journal of the American College of Cardiology (JACC) family of journals. The paper provides new information on mechanisms involved in host cell binding and entry of the SARS-CoV-2 in the human heart. Investigators affiliated with the Bristow Laboratory at CU Anschutz Medical Campus, Cardiovascular Institute at the University of Colorado School of Medicine and ARCA biopharma determined how known and potential mechanisms responsible for COVID-19 infection in the intact heart are altered by prior heart muscle disease, and to what extent they are changed when damaged heart muscle improves through a process called reverse remodeling.

COVID-19 infection occurs when SARS-CoV-2 infects host cells by binding to receptor sites on cell surface membranes, then merging its viral membrane with host cell surface and intracellular membranes to facilitate internalization, and finally taking control over host cell RNA synthesis to replicate virus. When heart muscle is damaged, enlarges and weakens (a process termed "remodeling"), various myocardial cell constituents change their expression, including some that may participate in CoV-2 host cell binding and internalization. Foremost of those that have been previously identified is angiotensin converting enzyme-2 (ACE2), which based on work performed in the Bristow Laboratory, was first reported to be increased ("upregulated") at the protein and enzyme activity levels in explanted remodeled human hearts in 2003 (Zisman LS et al, Circulation 108:1709-12). Shortly thereafter, ACE2 was reported by others to be the receptor for SARS-CoV binding to host cells (Li W et al, Nature 426:450-4, 2003), and recently, ACE2 was identified as the receptor for SARS-CoV-2 cell binding (Hoffman et al, Cell 181:1-10, 2020). However, it was not known if ACE2 upregulation in remodeled explanted hearts was due to heart failure medications that can affect ACE2 expression or was only found in late stage heart failure and remodeling present in hearts obtained from cardiac transplant recipients. Moreover, mechanisms or constituents other than ACE2 that could participate in CoV2 host cell binding and internalization had not been previously investigated in remodeled human left ventricles (LVs).

In the JBTS reported study, 46 patients with mild-moderate heart failure and remodeling from nonischemic dilated cardiomyopathy and nonfailing, non-remodeled controls had RNA extracted from interventricular septum endomyocardial biopsies. From the extracted RNA, genes known to participate in CoV-2 host cell binding and cell entry or who were possible candidates for these processes had mRNA expression measured by two independent platforms. The 46 dilated cardiomyopathy patients were then treated for 12 months with beta-blocking agents to produce reverse remodeling, measured by improvement in left ventricular ejection fraction (LVEF), which occurred in 65 percent of the patients. Gene expression in patients with reverse remodeling was then compared to the 35 percent of patients whose LVEFs/remodeling did not change. Importantly, the dilated cardiomyopathy patients were being treated with inhibitors of the renin-angiotensin system prior to baseline measurements and throughout the study, eliminating the possibility that such therapy could have affected ACE2 expression.

At baseline, ACE2 myocardial mRNA expression was markedly upregulated in the dilated cardiomyopathy patients, by nearly two-fold. With reverse remodeling, ACE2 gene expression normalized, and was unchanged in those with no remodeling improvement. The behavior of ACE2 as well as the degree of baseline expression was highly correlated with that of the natriuretic peptide B gene, whose processed protein product BNP is considered the gold standard biomarker for heart failure and remodeling. These data indicate that the increased expression of ACE2 begins much earlier than in end stage heart failure, that it is directly related to the remodeling process and not to the administration of heart failure therapy, and that by virtue of its potential for increasing the amount of virus internalization into host cells including cardiac myocytes, it is a likely a contributor to the increased adverse outcomes of patients with underlying heart disease who have COVID-19 infection.

ACE2 is a "protective" or counter-regulatory enzyme that catalyzes the conversion of angiotensin-II to angiotensin-(1-7), a peptide that mitigates abnormal cell growth, blood vessel constriction and thrombosis (blood clots) mediated by angiotensin-II, which is known to be elevated in COVID-19 patients with clinically significant disease. However, the increased expression of ACE2 in the remodeled heart means that its beneficial effects may also predispose to increased cell infection by CoV-2. Based on these observations, the paper concludes that an optimal ACE2 targeted treatment for COVID-19 would consist of an agent that blocks CoV-2 binding without diminishing or perhaps even increasing ACE2 enzyme activity.

The study also examined the expression of multiple proteases that facilitate cell entry through virus-host cell membrane fusion, and found that none previously shown to be involved in CoV or CoV-2 membrane coalescence were altered, and that 11 additional expressed proteases were not upregulated in remodeling. In contrast, the study found that integrin A5 subunit (ITGA5), which binds to ACE2 and can mediate host cell internalization of viruses, was upregulated in remodeled heart and normalized its expression on reverse remodeling similar to ACE2. The study concluded that the ITGA5 protein gene product or the $\alpha 5 \beta 1$ heterodimer is a candidate for facilitating CoV-2 binding and entry in myocardial cells. Thus, in remodeled, intact human hearts one (ACE2) and possibly two (ITGA5) myocardial cell components are altered to favor enhanced infection by CoV-2, which may explain COVID-19 increased adverse outcomes in patients with underlying heart muscle disorders.

Michael Bristow MD, PhD, Director of Pharmacogenomics at the Cardiovascular Institute, and ARCA's President and Chief Executive Officer commented, "These findings add to the evidence that increased ACE2 expression contributes to the increased adverse outcomes risk observed for COVID-19 in patients with underlying myocardial disease. In addition, we detected a possible additional route of CoV-2 binding and host cell internalization that is upregulated in remodeled human ventricles, involving integrin A5. This information sets the stage for the development of diagnostic approaches to and therapeutic manipulation of ACE2 for preventing CoV-2-host cell internalization while preserving functional activity, and may identify a new target for intervening in the cell infectious process."

About the University of Colorado School of Medicine Cardiovascular Institute
The University of Colorado School of Medicine Cardiovascular Institute (CU-CVI) was co-founded in 1998 by Dr. Bristow and Leslie Leinwand, PhD with the mission of integrating cardiovascular research, treatment, and discovery through a collaboration of the Anschutz Medical and Boulder campuses. The CU-CVI is now Co-directed by Dr. Leinwand and Peter Buttrick, MD, Division Head of Cardiology and Senior Associate Dean for Academic Affairs at the CU School of Medicine, who is a co-author on the JBTS paper.

The scientific goals of the Institute are to understand the genetic basis and specific molecular mechanisms responsible for heart muscle disease and heart failure and to produce new diagnostic techniques and treatments for patients. By integrating the effort of those committed to curing heart muscle disease and heart failure, the collaborative nature of the Institute encourages the sharing of findings and data, which ultimately translate into improved treatments and therapies of patients. www.ucdenver.edu/academics/colleges/medicalschool/institutes/CardiovascularInstitute.

In work sponsored by the American Heart Association the research team that worked on the current study is now investigating the effects of CoV-2 on host cell entry and internalization mechanisms in the intact heart of patients with COVID-19 cardiac involvement.

About the University of Colorado Anschutz Medical Campus
The University of Colorado Anschutz Medical Campus is a world-class medical destination at the forefront of transformative science, medicine, education, and patient care. The campus encompasses the University of Colorado health professional schools, more than 60 centers and institutes, and two nationally ranked independent hospitals that treat more than two million adult and pediatric patients each year. Innovative, interconnected and highly collaborative, together we deliver life-changing treatments, patient care, professional training, and conduct world-renowned research. For more information, visit www.cuanschutz.edu.

About ARCA biopharma
ARCA biopharma is dedicated to developing genetically targeted and other precision therapies for cardiovascular diseases through a precision medicine approach to drug development. ARCA is developing AB201 (rNAPc2) as a potential treatment for diseases caused by RNA viruses, initially focusing on COVID-19. ARCA is also developing Gencaro™ (bucindolol hydrochloride), an investigational, pharmacologically unique beta-blocker and mild vasodilator, as a potential treatment for atrial fibrillation in heart failure patients. ARCA has identified common genetic variations that it believes predict individual patient response to Gencaro, giving it the potential to

be the first genetically targeted AF prevention treatment. The U.S. FDA has granted the Gencaro development program Fast Track designation and a Special Protocol Assessment (SPA) agreement. For more information, please visit www.arcabio.com or follow the Company on LinkedIn.

Safe Harbor Statement

This press release contains "forward-looking statements" for purposes of the safe harbor provided by the Private Securities Litigation Reform Act of 1995. These statements include, but are not limited to, statements regarding the ability of ARCA's financial resources to support its operations through the end of the third quarter of 2020, potential future development plans for AB201 and Gencaro, the expected features and characteristics of AB201 or Gencaro, including the potential for AB201 to treat COVID-19/CAC, the potential for genetic variations to predict individual patient response to Gencaro, Gencaro's potential to treat AF, future vaccines and/or treatment options for patients with COVID-19, future treatment options for patients with AF, and the potential for Gencaro to be the first genetically targeted AF prevention treatment. Such statements are based on management's current expectations and involve risks and uncertainties. Actual results and performance could differ materially from those projected in the forward-looking statements as a result of many factors, including, without limitation, the risks and uncertainties associated with: ARCA's financial resources and whether they will be sufficient to meet its business objectives and operational requirements; ARCA may not be able to raise sufficient capital on acceptable terms, or at all, to continue development of AB201 or Gencaro or to otherwise continue operations in the future; results of earlier clinical trials may not be confirmed in future trials; the protection and market exclusivity provided by ARCA's intellectual property; risks related to the drug discovery and the regulatory approval process; and, the impact of competitive products and technological changes. These and other factors are identified and described in more detail in ARCA's filings with the Securities and Exchange Commission, including without limitation ARCA's annual report on Form 10-K for the year ended December 31, 2019, and subsequent filings. ARCA disclaims any intent or obligation to update these forward-looking statements.

CU Anschutz and School of Medicine Media Contacts:
Julia Milzer (CU Anschutz), **E:** julia.milzer@cuanschutz.edu **C:** 303.725.0733
Chanthy Na (School of Medicine), **E:** chanthy.na@cuanschutz.edu

ARCA biopharma Investor & Media Contact:
Derek Cole
720.940.2163
derek.cole@arcabio.com

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