group ($P = 0.0053$), suggesting a directly nephroprotective role of NO. However, no assesment of right heart function and no detailed hemodynamic measurements were presented.

To determine both the cardiac and renal protective effects of NO during cardiac surgery and the potential cardio–renal interactions that might occur, we are now conducting a randomized trial in 250 cardiac surgery patients at the Massachusetts General Hospital (Boston, MA) with intraoperative echocardiography and pulmonary artery catheterization. We will measure renal biomarkers, assess pre- and postoperative redox states, and evaluate pre- and postoperative levels of plasma NO metabolomics. The primary endpoint of this ongoing study in Boston is to determine whether NO is effective in the prevention of AKI as defined by Kidney Disease Improving Global Outcomes criteria (ClinicalTrials.gov Identifier: NCT02836899). However, in contrast to our first trial in China in patients primarily with rheumatic heart disease, Americans undergoing heart surgery are generally older and are affected by endothelial dysfunction. Endothelial dysfunction is a condition characterized by impaired endothelial NO synthase. In these patients, the diseased endothelium is unable to provide appropriate vasodilation during and after ischemic events, and is unable to replenish plasma NO after consumption of NO from intravascular hemolysis (5). At present (January 2019), we are halfway through enrollment ($n = 125$). We believe that the results of this ongoing trial will be able to address the hypothesis of Dr. Coutrot and colleagues. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Chong Lei, M.D., Ph.D.
Fourth Military Medical University
Xi'an, China

Lorenzo Berra, M.D.
Harvard Medical School
Boston, Massachusetts

Lize Xiong, M.D., Ph.D.*
Fourth Military Medical University
Xi'an, China

Warren M. Zapol, M.D.
Harvard Medical School
Boston, Massachusetts

ORCID ID: 0000-0003-2702-2093 (L.B.).

*Corresponding author (e-mail: mzkxlz@126.com).

References

1. Lei C, Berra L, Rezoagli E, Yu B, Dong H, Yu S, *et al*. Nitric oxide decreases acute kidney injury and stage 3 chronic kidney disease after cardiac surgery. *Am J Respir Crit Care Med* 2018;198: 1279–1287.
2. Minneci PC, Deans KJ, Zhi H, Yuen PS, Star RA, Banks SM, *et al*. Hemolysis-associated endothelial dysfunction mediated by accelerated NO inactivation by decompartmentalized oxyhemoglobin. *J Clin Invest* 2005;115:3409–3417.
3. Frostell C, Fratacci MD, Wain JC, Jones R, Zapol WM. Inhaled nitric oxide: a selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. *Circulation* 1991;83:2038–2047.
4. Kamenshchikov KO, Mandel IA, Podoksenov YK, Svirko YS, Lomivorotov VV, Mikheev SL, *et al*. Nitric oxide exerts organoprotective effects in cardiac surgery. *Circulation* 2018;138:A11400.
5. Esper RJ, Nordaby RA, Vilariño JO, Paragano A, Cacharrón JL, Machado RA. Endothelial dysfunction: a comprehensive appraisal. *Cardiovasc Diabetol* 2006;5:4.

Enhancing the Expression of CFTR Using Antisense Molecules against MicroRNA miR-145-5p

To the Editor:

We read with great interest the article titled "MicroRNA-145 Antagonism Reverses TGF-β Inhibition of F508del CFTR Correction in Airway Epithelia" by Lutful Kabir and colleagues (1). In this paper, the authors demonstrate that miR-145 mediates TGF-β (transforming growth factor-β) inhibition of synthesis and function of CFTR (cystic fibrosis transmembrane conductance regulator) in cystic fibrosis (CF) airway epithelia. Interestingly, they found that antagonists of miR-145 were able to interrupt TGF-β signaling and restore F508del CFTR modulation. Therefore, they suggested that miR-145 targeting may provide a novel therapeutic opportunity to enhance the benefit of F508del CFTR correction in CF epithelia. In agreement with this, we have elsewhere published data supporting the use of miR-145 targeting in CF, based on an antisense peptide nucleic acid (PNA) to target miR-145-5p (PNA-a145) and enhance expression of the *CFTR* gene, which we analyzed at mRNA (qRT-PCR) and protein (Western blotting) levels (2). In support of the conclusion by Lutful Kabir and colleagues, our data suggest the use of suitably delivered antisense molecules targeting miR-145-5p to enhance the expression of CFTR (2).

With respect to a possible microRNA (miRNA)-based therapeutic option, one limitation is of course the presence of more than 300 *CFTR* gene disease-causing mutations (www.genet.sickkids.on.ca/cftr/) (3). Besides the *CFTR* mutation leading to the deletion of the phenylalanine in position 508 (F508del CFTR), accounting for 50–90% CF chromosomes, most CF-causing mutations are missense (42%), nonsense (10%), frameshift (15%), splicing (13%), in-frame deletion/insertion (2%), and promoter (0.5%) mutations, which are now operationally categorized in six classes of molecular defects of the CFTR protein (3).

In consideration of these different molecular defects, it is expected that targeting miR-145 could be useful for type IV (less function), V (less protein), and VI (less stable protein) CFTR defects. For CFTR defects such as type I (no protein), II (no traffic), and III

Supported by Fondazione Fibrosi Cistica, Project "MicroRNA Therapeutics in CF: Targeting CFTR and Inflammation Networks (MICRORNA-CF)," FFC#3/2016.

Originally Published in Press as DOI: 10.1164/rccm.201901-0019LE on February 27, 2019

(no function), miR-145 targeting may be considered only in combination with other therapeutic protocols. In conclusion, in agreement with Lutful Kabir and colleagues, and considering the increasing relevance of RNA-targeted therapies (4), combined therapy with innovative oligonucleotides or peptide–nucleotide chimeras targeting miR-145 might be considered together with CFTR correctors such as lumacaftor, tezacaftor (1, 3), and 4,6,4′-trimethylangelicin (3, 5).

In addition, using next-generation sequencing of the miRNome, we recently observed that the majority of miRNAs (81.6%) are not modulated in Calu-3 cells treated with the anti-miR145 PNA (unpublished results). On the other hand, we observed that inhibition of miR-145-5p with PNA might also be accompanied by coinhibition of other miRNAs (12.5%), among which of great interest in our opinion is miR-155-3p. Our next-generation sequencing data, validated by qRT-PCR and droplet digital RT-PCR, demonstrated that treatment of Calu-3 cells with PNA-a145 resulted in inhibition of miR-145-5p and miR-155-3p by 34–43% and 42–44%, respectively. This is of interest because miR-155-3p upregulation has been reported to be responsible for hyperactivating IL-8, thereby amplifying the deleterious effects of excessive inflammation on CF lung tissues (6). Therefore, in addition to upregulation of CFTR (1, 2), PNA-mediated targeting of miR-145-5p might be expected to lead to downregulation of IL-8, possibly combining two activities of great interest in CF therapy, i.e., the rescue and potentiation of mutant CFTR protein and the control of lung inflammation. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Alessia Finotti, Ph.D.
Jessica Gasparello, Ph.D.
Enrica Fabbri, Ph.D.
University of Ferrara
Ferrara, Italy

Anna Tamanini, Ph.D.
University Hospital of Verona
Verona, Italy

Roberto Corradini, Ph.D.
University of Parma
Parma, Italy

Maria Cristina Dechecchi, Ph.D.
University Hospital of Verona
Verona, Italy

Giulio Cabrini, M.D.
University of Ferrara
Ferrara, Italy
and
University Hospital of Verona
Verona, Italy

Roberto Gambari, Ph.D.*
University of Ferrara
Ferrara, Italy

ORCID IDs: 0000-0002-7638-515X (A.F.); 0000-0001-8557-0739 (J.G.); 0000-0001-9205-6033 (R.G.).

*Corresponding author (e-mail: gam@unife.it).

References

1. Lutful Kabir F, Ambalavanan N, Liu G, Li P, Solomon GM, Lal CV, *et al*. MicroRNA-145 antagonism reverses TGF-β inhibition of F508del CFTR correction in airway epithelia. *Am J Respir Crit Care Med* 2018; 197:632–643.
2. Fabbri E, Tamanini A, Jakova T, Gasparello J, Manicardi A, Corradini R, *et al*. A peptide nucleic acid against microRNA miR-145-5p enhances the expression of the cystic fibrosis transmembrane conductance regulator (CFTR) in Calu-3 cells. *Molecules* 2017;23:E71.
3. Dechecchi MC, Tamanini A, Cabrini G. Molecular basis of cystic fibrosis: from bench to bedside. *Ann Transl Med* 2018;6:334–347.
4. Levin AA. Treating disease at the RNA level with oligonucleotides. *N Engl J Med* 2019;380:57–70.
5. Favia M, Mancini MT, Bezzerri V, Guerra L, Laselva O, Abbattiscianni AC, *et al*. Trimethylangelicin promotes the functional rescue of mutant F508del CFTR protein in cystic fibrosis airway cells. *Am J Physiol Lung Cell Mol Physiol* 2014;307:L48–L61.
6. Bhattacharyya S, Balakathiresan NS, Dalgard C, Gutti U, Armistead D, Jozwik C, *et al*. Elevated miR-155 promotes inflammation in cystic fibrosis by driving hyperexpression of interleukin-8. *J Biol Chem* 2011; 286:11604–11615.

Reply to Finotti *et al.*

To the Editor:

Fabbri and colleagues' investigations (1) complement well the work of our group (2) and others (3–5) to emphasize the importance of the TGF-β (transforming growth factor-β)/miR-145 (microRNA 145) axis in regulating CFTR (cystic fibrosis transmembrane conductance regulator) expression and function in airway epithelia. In brief, miR-145 mediates TGF-β suppression of CFTR through miR-145 binding to the 3′-untranslated region (3′-UTR) of CFTR mRNA. TGF-β/miR-145 stimulation nullifies the benefit of currently available CFTR modulators, and miR-145 antagonism augments F508del correction. The authors outline well the therapeutic potential of miR-145 antagonism for each CFTR mutational category (e.g., class I–VI defects).

In their letter to the editor, Finotti and colleagues elucidate two important considerations in the development of oligotherapeutics: target specificity and preclinical model selection. With regard to antagonist selection, the question arises as to whether to block the microRNA (miRNA) itself (full antagonism) or the binding site on the transcript of interest (target site blockade). Pursuing the latter approach, we use an antisense oligonucleotide to block miR-145 binding to the CFTR 3′-UTR sequence and limit off-target disruption of TGF-β signaling. Finotti and colleagues describe the possible benefit of full miR-145 antagonism to suppress additional consequences of increased TGF-β stimulation. Their letter highlights

Supported by grants from the NIH (NHLBI grant K08 HL140190 and National Institute of Diabetes and Digestive and Kidney Diseases grant P30 DK072482) and the Cystic Fibrosis Foundation (HARRIS17I0).

Originally Published in Press as DOI: 10.1164/rccm.201901-0204LE on February 27, 2019