Destabilizing data in CF

By Benjamin Boettner, Associate Editor

Two independent labs have produced *in vitro* evidence that combining Kalydeco ivacaftor with lumacaftor destabilizes the compounds' cystic fibrosis transmembrane conductance regulator target^{1,2}—contradicting the positive data from **Vertex Pharmaceuticals Inc.**'s recent Phase III trials in cystic fibrosis.

Although the preclinical evidence will not trump clinical data, the researchers believe the findings could prompt the design of next-generation, more synergistic compounds. Vertex declined an interview request but noted in an e-mail response that it has been aware of these preclinical data since last year. The company is planning a 2014 FDA submission of the Kalydeco and lumacaftor combination.

The **McGill University** and **The University of North Carolina at Chapel Hill** groups behind the two studies are now screening other compound classes for molecules that promote cystic fibrosis transmembrane conductance regulator (CFTR) stability and rescue channel function.

Vertex's TRAFFIC and TRANSPORT studies in 1,100 patients with CF found that combinations of Kalydeco and lumacaftor produced modest but statistically significant improvements in lung function versus placebo.³

Kalydeco is highly effective as a single agent in patients with the G551D mutation in the *CFTR* gene. The drug—a CFTR potentiator—increases channel opening and thus corrects the gating deficiency caused by G551D. However, G551D is only one of many *CFTR* mutations that cause CF, affecting only 5% of patients.

Lumacaftor—a CFTR corrector—targets a folding deficiency caused by *CFTR* DF508, a mutation found in about 90% of patients, and tags the mutant proteins for intracellular destruction. By fixing the folding, lumacaftor allows *CFTR* DF508 channels to be trafficked to the plasma membrane.

However, the DF508 mutation also causes a secondary gating defect, and channels that do reach the plasma membrane do not open like wild-type channels. Because lumacaftor improves trafficking but not gating activity, the compound does not provide enough improvement for clinical benefit when given as a single therapy.

The goal of CF therapy is to enable enough functional CFTR channels to reach the plasma membrane to control ion balance and fluid homeostasis in airway epithelia and prevent the mucous buildup, secondary infections and inflammation that characterize the disease. Thus, Vertex thought combining the potentiator and corrector

properties of Kalydeco and lumacaftor would have an additive effect and produce enough active CFTR on the cell surface to restore lung function.

The clinical data suggest the combination works. But the mechanism proposed has now been called into question by results from Gergely Lukacs' lab at McGill and Martina Gentzsch's lab at UNC Chapel Hill that suggest the combination destabilizes rather than promotes channel function.

According to Lukacs, the combination treatment hitting two distinct deficiencies of the mutant is an elegant approach. The explanation for the suboptimal efficacy in the clinic in relation to predictions based on previous *in vitro* studies lies in the dichotomy of Kalydeco's mechanisms of action; although the drug can open up the small number of mutant channels that do reach the plasma membrane in the presence or absence of lumacaftor, it also reduces the number of channels at the cell surface. "Despite Kalydeco's destabilizing effects on CFTR protein during transport to the plasma membrane, small amounts of resilient channel proteins could reach the plasma membrane and be functionally stimulated by the drug," he said.

The mutant channel density was reduced at the cell surface by approximately 15%–40% upon prolonged exposure to Kalydeco in the presence of correctors versus exposure only to corrector.

Thus, although the clinical results were substantial, the improvement in lung function was "modest" according to Lukacs, and the evidence from his and Gentzsch's groups suggests that greater clinical efficacy might be obtained by developing optimized potentiators for combination therapy.

Lukacs is a professor of physiology at McGill. Gentzsch is an assistant professor in the Department of Cell Biology and Physiology at **The University of North Carolina at Chapel Hill School of Medicine**.

Channel breakdown

Both teams used human primary bronchial epithelial cells expressing *CFTR* DF508 to study combinations of the two compounds. When cells treated with lumacaftor were given acute exposure to Kalydeco, CFTR-mediated ion currents increased within minutes. By contrast, adding Kalydeco for 24–48 hours decreased channel conductance.

In the UNC study, Kalydeco decreased the half-life of *CFTR* DF508 in the presence of lumacaftor and reduced the steady-state levels of mature protein. Similarly, the McGill study showed that when combined with lumacaftor, Kalydeco accelerated lysosomal degradation of *CFTR* DF508 proteins that reached the plasma membrane. Thus, both groups demonstrated that Kalydeco destabilizes *CFTR* DF508 when lumacaftor is present.

The UNC group also showed that Kalydeco destabilizes wild-type CFTR—even in the absence of lumacaftor—but the McGill team found it did not. Gentzsch and Lukacs both told *SciBX* the discrepancy was likely due to differences in dosing. Whereas Gentzsch's group used Kalydeco at concentrations above 5 μM , the Lukacs lab worked in a range under 1 μM . Lukacs told *SciBX* that based on the clinical efficacy of Kalydeco in patients with G551D CF and its EC_{50} value for *CFTR* G551D activation, the concentration of the drug seen in bronchial epithelial cells in the lung is likely between 0.1 and 1 μM .

Next, the McGill team tested a panel of 10 investigational potentiators in combination with lumacaftor to find out whether

CFTR destabilization was a class effect. The compounds, eight of which enhanced CFTR DF508 activity in acute treatment, were provided by the **Cystic Fibrosis Foundation**.

Most of the potentiators mimicked Kalydeco and destabilized CFTR DF508 when combined with lumacaftor. However, one of the Cystic Fibrosis Foundation compounds—dubbed P5—persistently caused a sevenfold increase in channel activity and did not decrease the channel density at the plasma membrane.

Although Lukacs told *SciBX* that P5 is unlikely to become a lead compound because of its relatively low potency, the result suggested that compound optimization could yield a potentiator that does not have a negative drug-drug interaction with lumacaftor.

Lukacs said he is working with Alan Verkman's group at the **University of California, San Francisco School of Medicine** to use P5 as a scaffold for designing better potentiators. Verkman is a professor of medicine and physiology at the UCSF School of Medicine and has a cell-based, high throughput screening technology for CFTR potentiators.

The UNC and McGill studies were published in *Science Translational Medicine*.

Potentiator potential

The next step for designing better potentiators will be to understand how Kalydeco destabilizes the mutant CFTR and how both Kalydeco and lumacaftor interact with the channel protein.

"The simplest solution would be to conduct potentiator screens followed by secondary screens to determine the surface density of DF508 CFTR upon drug exposure," Lukacs said. Surface density studies would provide information about the amount of functional, stabilized CFTR DF508 present on the plasma membrane.

But screening is only one approach. For rational drug design, understanding the molecular interactions will be key.

"It is clear that some form of drug-drug interaction is leading to a loss of efficacy *in vitro*. Elucidating this mechanism may provide an opportunity for further improving the combination's activity in patients," said Philip Thomas. He is a professor of physiology at **The University of Texas Southwestern Medical Center** and a cofounder of **Reata Pharmaceuticals Inc.** Reata has a protein folding-based drug discovery program.

"What I would like to see ideally are binding studies for P5. These could help identify a site and mechanism that may allow for more effective potentiation of DF508 CFTR," David Thomas told *SciBX*. He is chair in molecular genetics and a professor of biochemistry at McGill.

He added, "Nobody knows whether the two drugs bind to proximal sites nor whether Kalydeco is still bound to the channel at the plasma membrane. Modeling these interactions is extremely difficult, and expressing individual domains or smaller parts of CFTR and determining drug binding through NMR and Biacore studies likely will be the way forward."

Biacore is a platform for studying real-time interactions between compounds and their targets.

Clinical clues

To reconcile the clinical and preclinical data, the researchers will need to gather data from patients that can give clues about the mechanism.

According to Gentzsch, measurements of chloride levels in sweat, the nasal potential difference and ion transport in rectal biopsy tissue—coupled with biochemical analysis of CFTR—can provide data on how the drug combination affects CFTR correction and function in patients.

Organoids also provide an option for studying the mechanism in an *ex vivo* model that mimics the CF tissue environment better than cell-based systems, Jeffrey Beekman told *SciBX*.⁴ He added that patient-specific intestinal organoids could be used to assess the impact of genetic variability and the breadth of responses to combinations of potentiators and correctors.

"We are starting clinical studies to evaluate the value of organoid models for predicting pharmacodynamics of CFTR-targeting drugs, eventually also including Kalydeco and lumacaftor. Combined with patient-specific pharmacokinetic drug measurements in serum and improved genotyping, this could lead to more effective precision medicine," he said.

Beekman is an associate professor at **University Medical Center Utrecht**. He is working on CFTR assays in primary cystic fibrosis intestinal organoids with Hans Clevers, a professor at UMC Utrecht and group leader at the **Hubrecht Institute**.

The IP covering the Clevers group's intestinal organoid-based CF assay system is owned by **The HUB Foundation for Organoid Technology** and UMC Utrecht and is available for licensing. HUB is generating biobanks of personal organoids.

The findings from the *Science Translational Medicine* studies have not been patented.

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REFERENCES

1. Veit, G. *et al. Sci. Transl. Med.*; published online July 23, 2014; doi:10.1126/scitranslmed.3008889
Contact: Gergely L. Lukacs, McGill University, Montreal, Quebec, Canada
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2. Cholon, D.M. *et al. Sci. Transl. Med.*; published online July 23, 2014; doi:10.1126/scitranslmed.3008680
Contact: Martina Gentzsch, The University of North Carolina at Chapel Hill, Chapel Hill, N.C.
e-mail: gentzsch@med.unc.edu
3. McCallister, E. *BioCentury* Week of June 30, A7–A9 (2014)
4. Dekkers, J.F. *et al. Nat. Med.* **19**, 939–945 (2013)

COMPANIES AND INSTITUTIONS MENTIONED

Cystic Fibrosis Foundation, Bethesda, Md.
Food and Drug Administration, Silver Spring, Md.
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