Glucokinase alternative

By Chris Cain, Senior Writer

Over the last decade, numerous compounds that activate glucokinase have advanced into the clinic to treat diabetes, but the approach has faced safety challenges because of, amongst other issues, high rates of adverse hypoglycemic events. Now, researchers from **Amgen Inc.** have identified compounds that instead target glucokinase regulator, opening the door to a potentially safer strategy for modulating hepatic glucokinase activity.¹

Glucokinase (GCK; GK) catalyzes the conversion of glucose into glucose-6-phosphate, the first step of glycolysis, and is a key regulatory component for maintaining homeostatic levels of glucose in humans. Most of the enzyme is concentrated in the liver, but it also plays a key role in glucose sensing in the pancreas.

Since **Roche** scientists described the first allosteric GK activator a decade ago,² diverse classes of compounds have been synthesized and advanced into the clinic as therapies to reduce blood glucose levels in diabetes.³

Although activating GK has been clearly shown to reduce blood glucose levels in patients with diabetes, controlling the effect so as not to tip the balance and cause adverse hypoglycemic events has turned out to be a tall order.⁴

To address this, companies have designed compounds that only partially agonize GK or have increased selectivity for the liver. A liver-selective GK activator was shown to reduce the occurrence of hypoglycemia in animal models.⁵ At least eight GK activators remain in the clinic (see Table 1, “Glucokinase activators in clinical development”).

Now, Amgen has taken a different tack to address the problem by targeting not GK but glucokinase regulator (GCKR; GKR), a GK regulatory subunit present in the liver.

GKR inhibits GK in hepatocytes by sequestering the protein in an inactive complex in the nucleus—disrupting the GKR-GK interaction relieves this sequestration. This process is naturally regulated by sugars such as fructose-6-phosphate and fructose-1-

phosphate, which bind GKR and strengthen or weaken its interaction with GK, respectively.

To tap into this regulatory mechanism, Amgen scientists performed an *in vitro* screen to identify compounds that could disrupt the GK-GKR interaction and then eliminated compounds that bound GK directly. This led to the identification of a thiophene sulphanomidopiperazine, AMG-1694, which was further optimized to bind GKR and promote GK-GKR dissociation at a concentration of 7 nM.

Crystal structures of AMG-1694 in complex with GKR and sorbitol-6-phosphate, which stabilizes the complex, showed that it bound to a site on the protein distinct from the GK-GKR interface or its sugar-binding site.

David Lloyd, a principal scientist at Amgen and a corresponding author on the paper, told *SciBX* that identifying this compound was a challenge. “It’s important to note that the hit rate for the screen was extremely low,” he said. “In fact, we only obtained two weak hits in the related screen. Careful biochemical follow-up determined they bound to GKR, and this was indeed a surprise. It was at this point we realized we had something different.” Amgen principal scientist Clarence Hale was also a corresponding author on the paper.

In liver sections taken from normal or diabetic rats dosed with AMG-1694, GK was dose-dependently translocated out of the nucleus and blood glucose levels were reduced only in the diabetic rats, whereas both normal and diabetic rats dosed with a direct activator of GK had reductions in blood glucose.

To test the effect of GKR disruption in mouse models, a related compound with improved pharmacokinetics in mice, AMG-3969, was synthesized. In three mouse models of obesity, the compound dose-dependently reduced blood glucose concentration, but it had no effect in normal mice. It also did not affect levels of triglycerides, which was a concern because human genetic studies have suggested that inhibiting GKR may raise triglyceride levels.

Results were published in *Nature*.

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—David Lloyd, Amgen Inc.

Table 1. Glucokinase activators in clinical development. Despite the discontinuation of some programs from large pharmas, including **Merck & Co. Inc.** (NYSE:MRK), **Takeda Pharmaceutical Co. Ltd.** (Tokyo:4502), **AstraZeneca plc** (LSE:AZN; NYSE:AZN) and **Roche** (SIX:ROG; OTCQX:RHHBY), at least eight glucokinase activators remain in clinical development.

Source: BCIQ: BioCentury Online Intelligence

Company	Product	Development
Advinus Therapeutics Ltd.	GKM-001	Phase I/II
Array BioPharma Inc. (NASDAQ:ARRY)	ARRY-403 (formerly AMG151) ^A	Phase II
Daiichi Sankyo Co. Ltd. (Tokyo:4568)	DS-7309	Phase I
Pfizer Inc. (NYSE:PFE)	PF-04937319	Phase II
Roche	HMS5552 (formerly RO5305552)	Phase I
Teijin Pharma Ltd.	TMG-123	Phase I
TransTech Pharma Inc.	GKI-399 (TTP399)	Phase I/II
Zydus Cadila Group (NSE:CADILAH; BSE:532321)	ZYGK1	Phase I

^AThis year, **Amgen Inc.** (NASDAQ:AMGN) returned all rights to AMG151 to Array BioPharma.

Leveraging the liver

Although a thorough mechanistic explanation for how the GKRPs binding compounds lower blood glucose remains to be elucidated, their liver-specific, high blood glucose concentration-dependent activity sets them apart from GK activators.

Brian Miller, an associate professor of chemistry at **Florida State**

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**—Brian Miller,
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University, told *SciBX*, “In principle, targeting GKRPs is advantageous because to the best of our knowledge GKRPs-mediated regulation of GK is exclusive to the liver. As such, a GKRPs therapeutic would solve the liver specificity issues that are currently haunting the remaining GK activator programs.”

He added, “Therapeutic intervention in GK activity by targeting GKRPs is not expected to impact the cooperativity of GK kinetics, which is quite important in whole-body glucose homeostasis. All the direct GK activators reduce or eliminate this important functional characteristic of GK.”

Amgen’s Lloyd emphasized that targeting GKRPs enhances GK activity only at high glucose concentrations and not when glucose levels are normal. “A crucial difference between a GKRPs disruptor and a liver-specific GK [activator] is that the GKRPs [disruptor] promotes the actions of GK in the physiological range of glucose actions,” he said.

Earlier this year, Miller published the crystal structure of the mammalian GK-GKRPs complex.⁶ Also this year, **Boehringer Ingelheim GmbH** published the structure of inactive GKRPs bound to fructose-6-phosphate.⁷ **Boehringer Ingelheim** has not disclosed any programs targeting GKRPs.

Matthew Vander Heiden, an assistant professor of biology at the Koch Institute for Integrative Cancer Research at the **Massachusetts Institute of Technology**, told *SciBX* that he was impressed that the researchers identified a compound that potentially disrupts a protein-protein interaction. “Everyone says, ‘Well, it’s a protein-protein

interaction, so it’s not druggable’, but this is an example that shows that is not always the case.”

He added that he wanted to see more detail of how the compound disrupts GK-GKRPs interaction. “If a small molecule can do this, it is suggestive that an endogenous molecule could also engage this mechanism.”

Lloyd said that working to understand this mechanism is among his team’s next steps. “We are very interested in how the GKRPs disruptors have altered or restored the metabolic physiology of the liver. Along these lines, we wish to investigate the outcome of hepatic glucose with these compounds. We are also interested in the structural release of GK.”

Amgen spokesperson Kristen Davis said that this is an early research-stage program and did not disclose further development plans. The company has filed a patent covering GKRPs-binding compounds and did not disclose their availability for partnering.

In August, Amgen discontinued development of its clinical-stage GK activator, ARRY-403 (formerly AMG151) and returned rights to partner **Array BioPharma Inc.**

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COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Array BioPharma Inc. (NASDAQ:ARRY), Boulder, Colo.
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