What to do with PKM2

By Tracey Baas, Senior Editor

Novartis AG researchers have shown that the absence of pyruvate kinase M2 isozyme has no effect on cancer cell proliferation in mice and suggest that inhibiting the enzyme alone might not be an effective strategy to stop tumor growth.¹ The finding is surprising, given that knocking down the target is known to impair proliferation of cultured cancer cells. Biotechs working on modulators of the enzyme think it is too early to write off the target.

Pyruvate kinase occurs as two main isoforms—pyruvate kinase M1 isozyme (PKM1) is expressed in most normal cells as a constitutively active tetramer, whereas PKM2 is expressed in cancer cells either as a high-activity tetramer or a low-activity dimer. PKM2's oligomerization and activity is allosterically regulated by glycolysis intermediates.

The PKM1 tetramer and each form of PKM2 can catalyze the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP, producing ATP and pyruvate. The PKM2 dimer does so at a much slower rate than either tetramer, leading to a bottleneck in the metabolic pathway.

This slowdown effectively redirects the use of glucose from energy production to biomass synthesis, allowing tumor cells to rapidly proliferate.

There are two opposing schools of thought on controlling PKM2 activity: inhibition or activation (see Figure 1, "Metabolic pathways regulated by pyruvate kinase").

Both strategies have yielded positive results. Research from groups working with PKM2 inhibitors have shown that the kinase was required for initial growth and for establishing tumors in mice.^{2,3} In contrast, a **Massachusetts Institute of Technology** team working with a PKM2 activator showed that the high-activity enzyme increased the tumor latency period and decreased tumor size compared with vehicle during initial tumor growth.⁴

Novartis asked a slightly different question: whether PKM2 is required for the maintenance and growth of established tumors.

In cultured colon carcinoma or adenocarcinoma cells, small hairpin RNA-mediated knockdown of both PKM1 and PKM2 increased levels of PEP and decreased pyruvate kinase activity and cellular proliferation compared with no knockdown. Knocking down either PKM1 or PKM2 alone had little effect.

Metabolic labeling studies showed that knockdown of PKM2 reduced the conversion of glucose to pyruvate and diminished, but did not block, lactate production. However, biosynthesis of serine and glycine was ramped up, suggesting that alternative pathways were being turned on to feed cancer cells' need for biomass to proliferate.

In mice with established colon carcinoma or adenocarcinoma, inhibiting Pkm1 and Pkm2—either separately or in combination—increased PEP levels and decreased pyruvate kinase activity but did not decrease tumor growth.

Together, the results suggest that inhibiting pyruvate kinase activity is not enough to stop the growth of established tumors and instead may cause cancer cells to meet their need for energy and biomass by turning to alternative metabolic pathways.

Data were published in the *Proceedings of the National Academy of Sciences*. Novartis declined requests for an interview.

PKM2 ups and downs

Biotechs contacted by SciBX said it is too early to give up on targeting PKM2 because there is so much metabolic variation from tumor to tumor.

"Targeting metabolic pathways to treat cancer is a difficult business," said Neil Thompson, SVP of biology at **Astex Pharmaceuticals Inc.** "Astex is taking a very unbiased approach to screening molecules that target PKM2 and has identified both activators and inhibitors, some that bind to novel sites on PKM2."

He continued: "We're going to have to take the time to find specific features of a tumor that make it more sensitive to metabolic pathway disruptions—perhaps an abnormality that would make the tumor more dependent on ATP. You need to find the right patients with specific tumor biomarkers that will respond to your targeted small molecule. If a PKM2 therapeutic causes the tumor to rewire its metabolism, then the next step is to understand that shift and find a complementary control point and companion

drug to block tumor metabolism and proliferation."

At least three companies—Astex, **Dynamix Pharmaceuticals Ltd.** and **Tolero Pharmaceuticals Inc.**—are trying to block tumor growth by turning the low-activity dimeric form of PKM2 into the high-activity tetramer form.

"We have demonstrated that Dynamix's PKM2 activators indeed inhibit tumor growth *in vitro*, and we have also demonstrated robust, statistically significant inhibition of tumor growth *in vivo*," said Oren Becker, president and CEO of Dynamix. "The inherent selectivity of these activators, which are allosteric in nature and affect only PKM2 and not PKM1, suggest that they will have favorable safety as well."

The company's DNX-03047 PKM2 activator is in preclinical development to treat cancer.

Tolero's PKM2 activator, TP-1454, is in preclinical development to treat cancer. The company licensed the molecule from Astex.

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**—Neil Thompson,
Astex Pharmaceuticals Inc.**

Figure 1. Metabolic pathways regulated by pyruvate kinase.

To block the cancer metabolism pathway driven by the pyruvate kinase M2 isozyme (PKM2) dimer, researchers are working to develop small molecules that either inhibit the enzyme or keep the enzyme in its high-activity tetramer state. However, results from Cortés-Cros *et al.* suggest that blocking PKM2 may not be enough to prevent tumor proliferation.

PKM1 is expressed in most normal cells as a constitutively active tetramer, whereas PKM2 is expressed in cancer cells either as a high-activity tetramer or a low-activity dimer.

(I) In normal cells with the PKM1 tetramer or cancer cells with the PKM2 tetramer, the kinase catalyzes the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP, generating ATP and pyruvate. Pyruvate then is shuttled into the tricarboxylic acid cycle to produce acetyl-CoA and citrate. The process is balanced to allow the production of both ATP and the biosynthesis of cellular macromolecules to sustain growing cells.

(II) In cancer cells with the PKM2 dimer, the low-activity kinase also catalyzes the transfer of phosphate from PEP to ADP, generating ATP and pyruvate. The PKM2 dimer does so at a much slower rate than the PKM2 tetramer or PKM1 tetramer, leading to a bottleneck in the metabolic pathway. The buildup of intermediate metabolites of glycolysis feed other biosynthetic pathways to produce large quantities of cellular macromolecules. In addition, the decreased generation of pyruvate leads to lactate production.

A fourth company, **Agios Pharmaceuticals Inc.**, has PKM2 modulators in preclinical development to treat cancer. Agios declined to discuss its program.

The patent and licensing status of Novartis' findings are undisclosed.

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COMPANIES AND INSTITUTIONS MENTIONED

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