

# Spotlight on GOT2 in Cancer Metabolism

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**Abstract:** GOT2 is at the nexus of several critical metabolic pathways in homeostatic cellular and dysregulated cancer metabolism. Despite this, recent work has emphasized the remarkable plasticity of cancer cells to employ compensatory pathways when GOT2 is inhibited. Here, we review the metabolic roles of GOT2, highlighting findings in both normal and cancer cells. We emphasize how cancer cells repurpose cell intrinsic metabolism and their flexibility when GOT2 is inhibited. We close by using this framework to discuss key considerations for future investigations into cancer metabolism.

**Keywords:** transaminase, mitochondria, tumor microenvironment, nucleotides, redox, pancreatic cancer

## Introduction

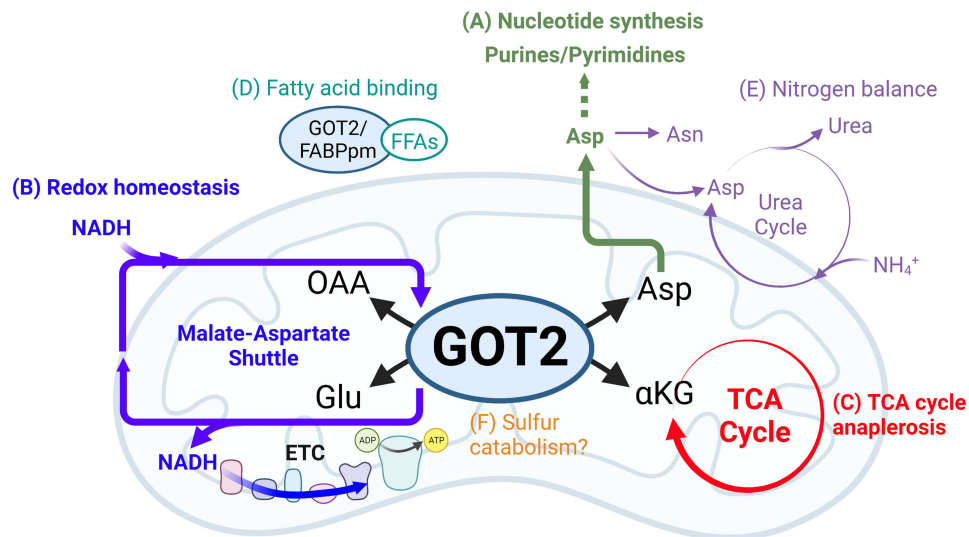
Tumors rewire cellular metabolism to sustain aberrant growth and survival.<sup>1-3</sup> Cancer cells do not create new metabolic pathways, but rather re-purpose inherent metabolic tendencies of the cells and tissues of origin.<sup>4</sup> Thus, understanding cellular metabolism under homeostatic conditions can provide insight into the metabolic underpinnings of cancer cells. Furthermore, unique aspects of cancer metabolism present vulnerabilities that can be exploited therapeutically without disrupting non-malignant cell function.<sup>5</sup>

Here, we discuss these concepts through the lens of glutamate-oxaloacetate transaminase 2 (GOT2), a mitochondrial enzyme that has recently garnered considerable interest in cancer metabolism. GOT2 is a mitochondrial aminotransferase that reversibly produces aspartate and  $\alpha$ -ketoglutarate from glutamate and oxaloacetate, while the cytosolic isoform GOT1 catalyzes the converse reaction. GOT2 is most well known as a member of the malate-aspartate shuttle (MAS), a cytosolic-mitochondrial pathway that transfers reducing equivalents into the mitochondria to support oxidative phosphorylation.<sup>6</sup> In addition to, and as a part of this function, GOT2 also participates in several critical metabolic functions: nucleotide synthesis, redox homeostasis, fueling the tricarboxylic acid (TCA) cycle, fatty acid transport, nitrogen balance, and sulfur catabolism (Figure 1). The following sections will dissect how each of these GOT2-related pathways have been investigated under both cellular homeostasis and cancer metabolism.

## Metabolic Functions of GOT2

### Aspartate Requirement for Nucleotide Synthesis

The deregulated cell growth and division in cancer cells imposes significant requirements for RNA and DNA. Perhaps, the most well-studied product of the GOT2-catalyzed reaction is the amino acid aspartate, which is a rate-limiting metabolite for cancer cells due to its contribution of both carbon and nitrogen to nucleotide synthesis<sup>7-10</sup> (Figure 1A). In vitro studies demonstrated that impaired proliferation of pancreatic cancer cells due to loss of GOT2 could be rescued by aspartate supplementation. In vivo, however, pancreatic tumors overcome loss of GOT2 by acquisition of aspartate from the tumor microenvironment using macropinocytosis.<sup>11</sup> In acute myelogenous leukemia, disruption of vitamin B6 synthesis, a co-factor in the GOT2 reaction, disrupted aspartate synthesis and impaired cancer cell growth.



**Figure 1** Cellular metabolism involving GOT2. **(A)** Asp produced by GOT2 provides carbon and nitrogen for synthesis of purine and pyrimidine nucleotide bases. **(B)** As part of the malate-aspartate shuttle, GOT2 facilitates the transfer of electrons generated from cytosolic glucose oxidation and carried by membrane-impermeable NADH into the mitochondria, where NADH is regenerated and electrons are deposited into the ETC to drive ATP production through oxidative phosphorylation. **(C)** GOT2 fuels anabolic TCA cycling through production of the intermediate  $\alpha$ KG. **(D)** Cytosolic GOT2, also known as FABPm, binds to free fatty acids with implications for FFA transport, oxidation, and signaling. **(E)** Asp from GOT2 is further utilized to synthesize the amino acid Asn and for incorporation into the urea cycle, which detoxifies cellular  $\text{NH}_4^+$  through urea excretion or recycling of nitrogen for numerous anabolic pathways. **(F)** Some evidence suggests promiscuous GOT2 activity is involved in the catabolism of sulfur-containing metabolites, though this mechanism remains to be thoroughly investigated. Created with BioRender.com.

**Abbreviations:** GOT2, glutamate-oxaloacetate transaminase 2; OAA, oxaloacetate; Glu, glutamate; Asp, aspartate;  $\alpha$ KG,  $\alpha$ -ketoglutarate; NADH, reduced nicotinamide adenine dinucleotide; ETC, electron transport chain; ADP, adenosine diphosphate; ATP, adenosine triphosphate; FFA, free fatty acid; Asn, asparagine;  $\text{NH}_4^+$ , ammonium; TCA, tricarboxylic acid.

Supplementation with aspartate was sufficient to rescue decreased GOT2 activity due to chemical and genetic inhibition of the vitamin B6 pathway.<sup>12</sup> Furthermore, GOT2 expression is increased in triple negative breast cancer and overexpression of GOT2 in these cells increased intracellular aspartate levels and promoted proliferation.<sup>13,14</sup> Interestingly, VHL-deficient clear-cell renal carcinoma tumors have decreased expression of GOT2,<sup>15,16</sup> suggesting alternative strategies to generate or acquire aspartate, like macropinocytosis, though those mechanisms remain to be identified.

Aspartate has also been reported to play important roles in metabolism in healthy cells. For example, chondrocytes depend on GOT2-derived aspartate for differentiation and to carry out bone remodeling functions.<sup>17</sup> Similarly, perturbations in hematopoietic stem cells (HSCs) that elevate intracellular aspartate levels, such as overexpression of the mitochondrial aspartate transporter or deletion of GOT1, the cytosolic isoform that consumes aspartate, boosted HSC number and activity. Deletion of both GOT1 and GOT2 was lethal for HSCs as they were unable to synthesize nucleotides or another product of aspartate metabolism, the amino acid asparagine.<sup>18</sup>

## Redox Balance

Disruption of the MAS induces “reductive stress”, characterized by increased levels of NADH that interfere with redox sensitive metabolic pathways. Patients with germline loss-of-function GOT2 variants present with neurological defects including epilepsy, and fibroblasts derived from these patients demonstrated impaired serine biosynthesis (a redox coupled pathway). Proper function could be restored through supplementation with serine or pyruvate, the latter being a metabolite that accepts electrons from NADH to resolve reductive stress.<sup>19</sup> Similarly, in vitro studies in pancreatic cancer demonstrated that loss of GOT2 induced reductive stress due to NADH accumulation, which was alleviated through NADH oxidizing mechanisms.<sup>20</sup> Furthermore, impaired mitochondrial metabolism and reduced expression of GOT2 in neurons have been identified in several neurological pathologies.<sup>21–24</sup> This suggests that GOT2 is involved in sustaining oxidative metabolism to generate energy for highly active neuronal subtypes. Dietary, chemical, or genetic

interventions that alleviate reductive stress could be effective therapies for defects in the MAS, including GOT2 inactivation.<sup>25</sup>

Maintenance of NADPH/NADP<sup>+</sup> pools is also essential to protect cells against damaging levels of reactive oxygen species (ROS). GOT2 consumes glutamate, therefore downregulation of GOT2 expression may lead to an increased availability of glutamate for production of glutathione (GSH), a tripeptide of glycine, glutamate, and cysteine and the most abundant endogenous antioxidant.<sup>26</sup> In ischemic hearts, hypoxia-inducible factor 1 $\alpha$  expression, the primary cellular factor involved in the hypoxic response, is inversely correlated with GOT2 expression and coincides with a concomitant increase in GSH.

In cancer cells, the maintenance of a redox state that supports sustained proliferation is paramount. Because the MAS sustains redox balance between the cytosol and the mitochondria (Figure 1B), its activity is modulated in various cancer types. For instance, a rewired MAS in PDAC fuels production of NADPH through malic enzyme 1 (ME1) in the cytosol to regenerate GSH and protect cells from ROS.<sup>27</sup> Several enzymes in this pathway are overexpressed in pancreatic tumors compared to normal pancreas.<sup>28</sup> Loss of GOT2 also induced senescence mediated by decreased NADPH synthesis and ROS accumulation.<sup>29</sup> Furthermore, inhibition of glutamine breakdown (or glutaminolysis) coupled with therapies that generated ROS-induced redox catastrophe in pancreatic cancer cells both *in vitro* and *in vivo*.<sup>28</sup> Conversely, in hepatocellular carcinoma, GOT2 is downregulated in response to insulin signaling,<sup>30</sup> resulting in increased glutamate availability for GSH synthesis.<sup>31</sup>

## The Dysregulated Cancer TCA Cycle

The net activity of the MAS does not consume carbon or nitrogen. However, when provided substrates in excess of MAS activity, GOT2 can generate surplus  $\alpha$ -ketoglutarate and aspartate. The aspartate can be used as a biosynthetic precursor, as described above. Production of  $\alpha$ -ketoglutarate from GOT2 provides a major carbon input that sustains anaplerotic TCA cycling in proliferating cells (Figure 1C). When functioning in this mode, the TCA cycle generates both the reducing equivalents needed to produce energy and provide biosynthetic precursors.<sup>32</sup> Pancreatic cancer cells in culture utilize glutamine-derived  $\alpha$ -ketoglutarate through GOT2 as the primary anaplerotic input to support biosynthesis and proliferation.<sup>27</sup> Similarly, GOT2 overexpression in diffuse large B cell lymphoma drives elevated glutaminolysis to support anaplerosis through  $\alpha$ -ketoglutarate production.<sup>33</sup>

The ratios of TCA cycle intermediates have also been implicated in cell fate.<sup>32</sup> For example, paragangliomas and pheochromocytomas, rare neurological tumors, are unique in that they harbor loss of function mutations in the MAS components MDH2, SLC25A11, and a gain of function mutation in GOT2.<sup>34</sup> Indeed, a whole-exome sequencing study of 11 patients revealed a patient with a germline GOT2 gain of function mutation, while two other tumors had increased GOT2 activity.<sup>35</sup> This increased GOT2 activity elevated both the succinate/fumarate ratio and the available aspartate, due to increased anaplerosis.<sup>35,36</sup> Epigenetic modifying enzymes, such as histone and DNA demethylases, consume  $\alpha$ -ketoglutarate and produce succinate. Therefore, while the TCA cycle is important for biosynthesis and energy production, it is necessary to consider how disruption of the  $\alpha$ -ketoglutarate/succinate ratio through increased GOT2 activity has broader implications on cell state and differentiation programs in these and other tumors, as well as in normal physiology.

## Emerging GOT2 Pathways in Cancer

GOT2 has been reported to have additional functions, outside of the classical roles described above, such as fatty acid binding, cysteine/sulfur breakdown, and nitrogen balance (Figure 1D–F). For example, several studies have proposed GOT2 is a plasma membrane-bound fatty acid-binding protein and have provided the alternate protein name FABPpm. In this role, GOT2/FABPpm is responsible for the import of free fatty acids under conditions of cellular stress, organ injury, or fatty acid accumulation, potentially influencing fatty acid oxidation.<sup>37–46</sup> Despite considerable literature examining the role of GOT2 as a plasma-membrane bound fatty acid transporter in several tissues, this capacity has only recently been examined in cancer. One provocative study confirmed GOT2 as a fatty acid-binding protein in pancreatic cancer<sup>47</sup> (Figure 1D). GOT2 free fatty acid-binding activity in syngeneic allografts in immune competent mice promoted anti-inflammatory PPAR $\delta$  activity, ultimately blocking cytotoxic T cell infiltration, and promoting tumor growth.<sup>47</sup> These findings indicate more remains to be investigated regarding the role of GOT2 in fatty acid metabolism in cancer.

GOT2 is also involved in nitrogen balance through production/consumption of the non-essential amino acids aspartate and glutamate. Regarding aspartate, it can enter the urea cycle, the primary mechanism by which cells either recycle or dispose of nitrogen (Figure 1E). Human patients with inborn errors of GOT2 metabolism displayed dysregulated levels of serum ammonia.<sup>19,25</sup> As such, further work is needed to identify mechanisms by which GOT2 is involved in removing toxic levels of ammonia or recovering nitrogen for incorporation into amino acids and nucleotides.

In colorectal cancer, GOT2 is essential in nitrogen balance through production of amino acids and the urea cycle via a HIF1a-SOX12-GOT2 axis that drives asparagine production from aspartate (Figure 1E). Inhibition of this pathway impairs cell growth.<sup>48</sup> Additionally, in Kaposi's sarcoma-associated herpesvirus cells, GOT2-derived nitrogen is essential for both nucleotide and amino acid production.<sup>49</sup> Cells detoxify ammonia through the urea cycle and aspartate incorporation is a critical node in fueling this cycle. Cancer cells often downregulate urea cycle enzymes to maintain aspartate pools for nucleotide production.<sup>50</sup> However, how cancer cells tune aspartate flux between the urea cycle or nucleotide synthesis remains unclear.

Promiscuous GOT2 transaminase activity has also been proposed to act on cysteine sulfinate to produce 3-sulfinylpyruvate, which is further catabolized to sulfur dioxide and pyruvate, presenting a potential link between GOT2 and taurine biosynthesis<sup>51</sup> (Figure 1F). Downregulation of GOT2 and the sulfur dioxide pathway is also implicated in myocardial injury.<sup>52</sup> Furthermore, serum levels of GOT2 correlated with increased cardiovascular disease,<sup>45</sup> and this was proposed to be due to increased sulfur dioxide production. This pathway has not yet been evaluated in cancer cells.

## GOT2 in the Tumor Microenvironment

Tumors are dynamic pseudo-organs in which malignant and non-transformed cell types work in concert to support growth and survival.<sup>53,54</sup> As such, the metabolism of immune and stromal cells can have a strong influence on cancer cells.<sup>55</sup> Aside from its myriad functions in cancer cells, GOT2 is also expressed, and potentially important, in several of these non-cancer tumor cells. For instance, in an autochthonous model of pancreatic tumorigenesis with loss of *Got2*, while immunohistochemistry (IHC) confirmed knockdown of *Got2* in transformed, epithelial lesions, the surrounding stroma retained strong expression of *Got2*.<sup>20</sup> Indeed, single-cell RNA-sequencing of mouse and human pancreatic tumors also detected significant GOT2 expression in several compartments including macrophages, fibroblasts, T cells, and endothelial cells.<sup>56</sup> Furthermore, CAR-T cells engineered to express GOT2 demonstrated enhanced cytotoxic activity against liver cancer both in vitro and in vivo.<sup>57</sup> An in-silico analysis of clear cell renal carcinoma detected GOT2 expression in both immune and stromal cell types.<sup>16</sup> In sum, these findings suggest that understanding the role of GOT2 in the tumor as whole is important when evaluating GOT2 as a potential target in cancer.

## Regulation of GOT2 in Cancer

The regulation of metabolic enzyme activity enables cells to meet the dynamic requirements imposed by both the external environment and intracellular signaling. Many cancer types induce GOT2 activity through epigenetic, post-transcriptional, or post-translational mechanisms. For example, in breast cancer, the BRCA1/ZBRK1 complex represses GOT2 promoter activity,<sup>14</sup> suggesting that loss of BRCA1 induces GOT2 expression. As mentioned earlier, VHL-deficient clear cell renal carcinoma tumors display decreased GOT2 expression.<sup>15</sup> This correlates with hypermethylation at the GOT2 promoter.<sup>16</sup> However, in CRC, hypoxic HIF1 activity induces SOX12 binding to the promoters of several metabolic enzymes, including GOT2, to promote expression.<sup>48</sup> These two studies suggest differential activity between cancer cells from different tissue types, as well as between hypoxia versus loss of VHL.

At the post-transcriptional level, some evidence in non-small cell lung cancer indicates that GOT2 transcript levels could be regulated by miRNAs.<sup>58</sup> Post-translationally, one study posited that GOT2 acetylation promoted complex formation with MDH2 to enhance activity of the MAS.<sup>59</sup> A second study found that inflammatory signaling in the liver-induced acetylation of GOT2 to promote its activity and drive the MAS.<sup>60</sup> However, the gamut of GOT2 post-translational modifications remains to be characterized. Lastly, GOT2 expression and activity can be modulated according to external stimuli. For instance, in diffuse large B cell lymphoma, STAT3 and NF- $\kappa$ B inflammatory signaling increased GOT2 expression through c-Myc.<sup>33</sup> Further work into the regulatory underpinnings of GOT2 could provide a better understanding for differential metabolic activities and dependencies between cancer cell types and environments.

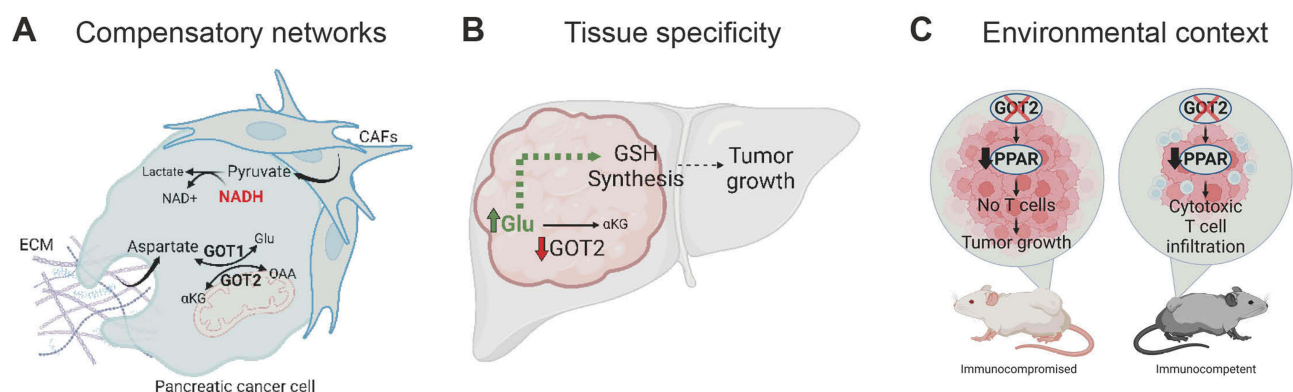
## Conclusion: Key Considerations When Interrogating Cancer Metabolism

Cumulatively, the body of work involving GOT2 highlights several key points in tumor metabolism. First, cancer cells exhibit dynamic metabolism to support malignant functions. Second, the metabolic pathways utilized by cancer cells are dictated by tissue of origin, genomic profile, environmental nutrient composition, and external signaling. Third, investigating cancer metabolism in the proper context is essential for identifying therapeutically tractable targets.

A major challenge in targeting cancer metabolism is the ability of cancer cells to rapidly adapt to metabolic pathway blockade.<sup>61</sup> As it relates to the MAS, GOT2 inhibition leads to an elevated NADH/NAD<sup>+</sup> ratio, and this reductive stress is detrimental to rapidly proliferating cells. Several essential metabolic pathways depend on this ratio, and NADH accumulation decreases flux through these pathways. Yet, while loss of GOT2 in PDAC cell lines led to reductive stress and impaired proliferation, xenograft tumors grew unimpeded in the absence of GOT2. It was discovered that pancreatic cancer-associated fibroblasts (CAFs) release sufficient pyruvate to ameliorate NADH accumulation through reduction to lactate<sup>20</sup> (Figure 2A). In addition to alleviating reductive stress, PDAC cells can acquire aspartate through macropinocytosis in the absence of GOT2 (Figure 2A). PDAC tumors are rich in extracellular matrix with a prominent stromal compartment secreting numerous proteins. These proteins can be non-specifically scavenged and broken down into their amino acid components to replenish aspartate pools in the absence of GOT2 in vivo.<sup>11</sup> Additionally, GOT1 can compensate for the loss of GOT2, and double GOT1/GOT2 knockout slowed pancreatic cancer xenograft growth<sup>11</sup> (Figure 2A). Lastly, a recent study has demonstrated how the MAS can reverse its direction to correct mitochondrial redox imbalance imposed by inhibition of the ETC.<sup>62</sup> Collectively, these studies emphasize both cell-intrinsic and -extrinsic mechanisms can compensate for loss of GOT2.

The tissue of origin plays a critical role in the tumor-promoting or -restraining function of GOT2 in cancer metabolism.<sup>4</sup> For instance, in HCC, downregulation of GOT2 is associated with more aggressive disease and worse outcomes. Indeed, overexpressing GOT2 in HCC cell lines restrains tumor growth. One potential explanation is that loss of GOT2 increases pools of the GOT2 substrate glutamate, increasing its incorporation in glutathione<sup>31</sup> (Figure 2B). Conversely, in PDAC GOT2 is part of the rewired MAS utilized to synthesize NADPH and reduce glutathione.<sup>27</sup> Perhaps, tumors arising in the liver versus the pancreas utilize distinct pathways for glutathione production, which then dictate whether GOT2 expression is detrimental or beneficial for tumor metabolism.

Lastly, the pre-clinical models utilized can influence metabolic findings, even in the same cancer type. Two studies found that loss of GOT2 in human pancreatic cancer cell lines in vitro impaired proliferation, while human xenografts in immunocompromised mice progressed unimpeded in the absence of GOT2. As discussed, cell-intrinsic rewiring and



**Figure 2** Context-specific GOT2 metabolism in cancer. **(A)** Pancreatic cancer cells compensate for loss of GOT2 via several mechanisms. CAF- and environment-derived pyruvate can be utilized to reduce pyruvate to lactate and alleviate NADH accumulation. Aspartate can either be produced by compensatory cytosolic GOT1 activity or through macropinocytosis and breakdown of ECM components. **(B)** GOT2 is tumor promoting or restraining depending on tumor type. Hepatocellular carcinoma downregulates GOT2. This increases Glu availability for GSH synthesis and sustained tumor growth. **(C)** GOT2 impairment induces different phenotypes in immune competent or compromised mouse models. A decrease in PPAR $\delta$  anti-inflammatory pathways caused by loss of GOT2 fatty acid binding activity lifts the blockade on cytotoxic T cells, leading to increased infiltration and cancer cell killing. Created with BioRender.com.

**Abbreviations:** GOT2, glutamate-oxaloacetate transaminase 2; OAA, oxaloacetate; Glu, glutamate;  $\alpha$ KG,  $\alpha$ -ketoglutarate; NADH, reduced nicotinamide adenine dinucleotide; GSH, glutathione; PPAR, peroxisome proliferator-activated receptor.

metabolic crosstalk within the TME could both contribute to this compensation.<sup>11,20,61</sup> However, intriguingly, a third study reported the opposite phenotype. Loss of GOT2 had no effect on in vitro cellular proliferation but significantly inhibited the growth of murine syngeneic allografts in immunocompetent mice. GOT2 loss induced cytotoxic T cell infiltration due to decreased fatty acid binding and PPAR $\delta$  signaling in cancer cells, which was absent in the models using human PDAC cells in immunocompromised mice<sup>47</sup> (Figure 2C). To complicate matters further, deletion of *Got2* in an autochthonous model of pancreatic tumorigenesis did not affect the number or severity of lesions.<sup>20</sup> However, these two studies compared rapidly growing, established allografts to gradually developing neoplastic lesions. Future work examining the role of GOT2 in advanced mouse models that more accurately recapitulate human disease are warranted to fully understand the role of GOT2 in pancreatic cancer. Even still, differences between mouse and human tissue will have to be considered. In sum, the model systems used to investigate metabolism can impact cancer metabolism, and careful study across several model systems is needed to accurately assess the metabolic vulnerabilities within a cancer type.

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## Disclosure

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