Pancreatic ductal adenocarcinoma is on pace to become the second leading cause of cancer-related death. The high mortality rate results from a lack of methods for early detection and the inability to successfully treat patients once diagnosed. Pancreatic cancer cells have extensively reprogrammed metabolism, which is driven by oncogene-mediated cell-autonomous pathways, the unique physiology of the tumor microenvironment, and interactions with non-cancer cells. In this review, we discuss how recent efforts delineating rewired metabolic networks in pancreatic cancer have revealed new in-roads to develop detection and treatment strategies for this dreadful disease.

Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer and the most deadly major cancer, with a 5-year survival rate of 8% (Siegel et al., 2016). The reasons for this high mortality rate can be ascribed largely to late presentation of disease when patients are no longer candidates for surgical resection (Hidalgo, 2010). Driving this challenge is the anatomically inaccessible location of the pancreas, which prevents routine examination, and the lack of clinically informative early diagnostic symptoms and biomarkers (Chan et al., 2013). Furthermore, pancreatic tumorigenesis and progression occurs undetected in a process thought to take upward of two decades (Yachida et al., 2010), while PDAC cells disseminate readily, resulting in early metastasis (Rhim et al., 2012). As a consequence of these collective characteristics, patients are often unaware of their disease until very late in its course.

Paralleling the diagnostic shortcomings, efficacious therapeutic options are limited. While some progress in developing combination therapies has been achieved in the recent past (Conroy et al., 2010; Ryan et al., 2014; Von Hoff et al., 2013), these are based on standard cytotoxic chemotherapy backbones that can be difficult to tolerate, while only modestly extending survival. Moreover, targeted therapies, which can minimize harmful side effects while providing meaningful and durable responses, have not made a real impact on this disease (Moore et al., 2007). Given this information, it is clear that new strategies are required to develop effective treatment options.

Cancer cells are defined by their ability to survive and proliferate in non-native settings under nutrient and oxygen deprivation and immune cell attack (Hanahan and Weinberg, 2011). Metabolic rewiring is central to these processes, and treating cancer by targeting the unique ways malignant cells take in and use nutrients has emerged as a promising therapeutic approach (Vander Heiden, 2011). Such a strategy has considerable potential in PDAC, as pancreatic tumors are under significant physical, oxidative, and inflammatory stress while the nutrients needed to combat these stressors are sparse. Accordingly, major headway has been made in recent years to understand metabolic adaptations in pancreatic cancer cells that facilitate survival under these conditions (Chini et al., 2014; Commissio et al., 2013; Guillammond et al., 2013, 2015; Perera et al., 2015; Son et al., 2013; Ying et al., 2012), and efforts are underway to utilize this information to design metabolism-targeted diagnostic tools and therapies. These studies have revealed a high level of metabolic heterogeneity in pancreatic cancer cells (Baek et al., 2014; Boudreau et al., 2016; Daemen et al., 2015; Sancho et al., 2015; Viale et al., 2014), mirroring the more widely described genetic heterogeneity in pancreatic cancer (Bailey et al., 2016; Colisson et al., 2011; Moffitt et al., 2015).

In this review, we provide a detailed overview of how the unique physiology of pancreatic tumors creates a hostile and nutrient-poor microenvironment, and the biochemical and metabolic adaptations that occur within and among heterogeneous cell types of a pancreatic tumor to facilitate growth. We discuss how these features enable therapeutic resistance and how a detailed understanding of metabolic rearrangements will provide new actionable drug targets, and potentially improve imaging methods to detect and monitor disease.

**A New “Addiction” in Metabolism**

Oncogene addiction defines the phenomenon by which cancer cells become dependent on the activity of an oncogene for survival and proliferation (Weinstein and Joe, 2008). Inhibition of the addicting oncogene directly, or through downstream mediators, is lethal to the addicted cell. Implicit in this definition is that normal cells can tolerate inhibition of oncogenic activity without obvious consequence. This principle has led to development of several kinase-targeted cancer therapies (Gross et al., 2015). In addition, it has also given rise to the notion that discrete processes can be addicting (Luo et al., 2009), such as dependence on heat shock factor 1 under proteotoxic stress (Dai et al., 2012) or poly(ADP-ribose) polymerase activity in the context of BRCA deficiency (Bryant et al., 2005; Farmer et al., 2005). In this same vein, certain cancer cells may become dependent on defined metabolic pathways, activities, or processes: a type of non-oncogene addiction defined as a “metabolic addiction.” Importantly, these metabolic addictions can be tissue specific...
in context, due to both genetic and environmental factors (Mayers et al., 2016; Yuneva et al., 2012). Such addictions can present therapeutic vulnerabilities, and several recent studies in pancreatic cancer have provided concrete evidence of these addictions and their therapeutic utility.

**Pancreatic Tumor Physiology**

The pancreatic tumor microenvironment is a source of both intense physical and oxidative stress. Interstitial pressures in pancreatic tumors can exceed ten times that observed in a normal pancreas (Provenzano et al., 2012). These tumors have low carcinoma cellularity, and the tumor is largely composed of a dense fibrotic stroma, populated mainly by fibroblasts and immune cells (Chu et al., 2007). When activated, fibroblasts deposit extensive extracellular matrix proteins including those containing a high concentration of the fluid-rich glycosaminoglycan hyaluronan (Jacobetz et al., 2013; Provenzano et al., 2012), a major contributor to the elevated interstitial pressure (Provenzano et al., 2012). This intense pressure results in vascular collapse and tumor hypoperfusion, which limits oxygen and nutrient availability (Kamphorst et al., 2015; Koong et al., 2000) and hinders drug delivery to cancer cells (Olive et al., 2009).

Importantly, the lack of oxygen and nutrients impose major challenges for cancer cells to maintain redox and metabolic homeostasis, as well as support macromolecular biosynthesis. To sustain tumor viability under these circumstances, stromal components create a metabolically supportive niche for the cancer cells; however, the dense fibrotic stroma has also been demonstrated to restrain cancer progression (Ozdemir et al., 2014; Rhim et al., 2014). The mechanisms of stromal interaction can be grouped into three categories: physical changes, immune suppression, and crosstalk mechanisms. These concepts are described in detail in Figure 1.

**Cell-Autonomous Reprogramming of Intermediary Metabolism**

Nutrients in the form of carbohydrates, amino acids, and lipids are used by cells to maintain energy balance, assist in detoxification, and support biosynthesis. The pathways that perform this function are collectively referred to as intermediary metabolism. Pancreatic cancer cells rewire intermediary metabolism to support different energetic and biosynthetic demands compared with normal cells. Much of what has been described for this reprogramming is driven by mutations in the oncogene KRAS, which is nearly universally mutated in PDAC (Biankin et al., 2012). Recent studies have begun to unravel how these pathways are reprogrammed and the functional relevance for cancer cells.

**Glucose Metabolism**

Glucose is a principle metabolic and biosynthetic nutrient. When used as a fuel by normal cells, it is completely oxidized to carbon dioxide in the mitochondria to produce ATP. Proliferating cells such as cancer cells also use glucose to make ATP, but proportionally more glucose carbon is used for biosynthesis of ribose, glycosylation precursors, amino acids, and lipids (Lunt and Vander Heiden, 2011) (Figure 2). The hypovascular nature of the pancreatic tumor niche imposes a bottleneck on this increased demand for glucose carbon. To compensate, oncogenic Kras signaling promotes extracellular glucose avidity and capture...
via upregulation of the glucose transporter GLUT1 and hexoki-

nase (HK), respectively (Ying et al., 2012). This glucose is utilized in glycolysis and non-mitochondrial biosynthetic reactions.

Downstream of HK, mutant Kras also activates the expression of several additional enzymes in glycolysis (Gaglio et al., 2011; Ying et al., 2012). Furthermore, hypoxia and other mechanisms also activate glycolytic gene expression (Baek et al., 2014; Chaika et al., 2012; Cui et al., 2014; Shi et al., 2014) and coordinate with mutant Kras to maintain cytosolic ATP. In contrast, mitochondrial metabolism and ATP generation is predominantly fueled by glutamine (Gln) carbon in cultured PDAC cells (Son et al., 2013; Ying et al., 2012). It is important to note that recent evidence from lung cancer studies have demonstrated that the source of carbon used to fuel mitochondrial metabolism is context dependent. In vitro, Gln is the predominant carbon source for mitochondrial metabolism, whereas, in vivo, glucose carbon contributes to a greater degree (Davidson et al., 2016; Hensley et al., 2016; Schug et al., 2016).

Glucose carbon also plays important roles in anabolic pathways. Oncogenic Kras diverts glucose flux into the hexosamine biosynthetic pathway (HBP) (Ying et al., 2012) to enhance the generation of precursor moieties required for protein glycosylation (Ma et al., 2013; Ying et al., 2012) (Figure 2). This is accomplished both through increased glycolytic flux and transcriptional upregulation of the rate limiting enzyme in the HBP, glutamine fructose-6-phosphate transamidase 1 (GFPT1) (Ying et al., 2012). HBP flux can also be increased through hypoxia-mediated induction of the GFPT2 isoform (Guillaumond et al., 2013).

Oncogenic Kras activity also leads to enhanced entry of glucose carbon into the pentose phosphate pathway (PPP) (Figure 2). The PPP is the predominant pathway by which proliferating cells make ribose-5-phosphate (R5P) for DNA and RNA biosynthesis. This pathway is traditionally subdivided into two branches: oxidative and non-oxidative. Kras-transformed pancreatic cancer cells activate and become dependent on non-oxidative PPP. Knock down of Kras-regulated enzymes that govern non-oxidative PPP flux in this context is strongly growth inhibitory (Ying et al., 2012). Since most normal cells generate R5P through the oxidative arm of the PPP, this differential dependence on the non-oxidative PPP represents a potential metabolic vulnerability in PDAC (Boros et al., 1997).

**Glutamine Metabolism**

Gln is a non-essential amino acid (NEAA) and the most abundant amino acid in circulation (Hensley et al., 2013). In addition to its role in protein biosynthesis, Gln is a major source of carbon and nitrogen for proliferating cells. Accordingly, Gln avidity is a feature of many cancer types (DeBerardinis and Cheng, 2010; Wise and Thompson, 2010). We recently demonstrated that PDAC cells grown in culture are strictly dependent on Gln for proliferation, and that Gln is used to maintain redox balance (Lysiotis et al., 2013; Son et al., 2013). Gln serves two functions in this regard. First Gln-derived glutamate (Glu) is used for glutathione (GSH) biosynthesis (Figure 2), a principle component in cellular redox balance (DeBerardinis et al., 2007). Secondly, Gln facilitates generation of reducing equivalents in the form of NADPH, a high-energy molecule involved in biosynthesis and redox balance. This latter pathway is driven by oncopgenic...
Pancreatic tumors are known to have elevated basal macroautophagy (Yang et al., 2011), also known more generally as autophagy. In healthy cells, autophagy is engaged by cellular stress to clear damaged structures (e.g., protein aggregates or dysfunctional organelles) or by starvation, which is regulated by signaling of the mechanistic target of rapamycin (Neufeld et al., 2010; White, 2012; Yang and Klionsky, 2010). Once catabolized by lysosomes, these digested biomolecules become available to the cell as nutrients (Figure 3).

During PDAC progression, autophagy plays important but opposing roles (Kimmelman, 2011). In tumor initiation, autophagy is antitumorigenic owing to its role in cellular quality control. In established tumors, autophagy can support survival and energy balance in hypoxic, nutrient-poor regions of the tumor. Indeed, constitutive engagement of autophagy in pancreatic cancers is nearly ubiquitous (Yang et al., 2011), even in cells grown in culture under nutrient-replete conditions, illustrating that this process is, at least in part, cell autonomous. More importantly, inhibition of autophagy causes proliferative defects across numerous pancreatic cancer models. The role of the tumor suppressor p53 in this response has been debated and likely depends on contextual factors (Rosenfeldt et al., 2013; Yang et al., 2014). Recently, it has been shown that autophagy dependence in PDAC is part of a larger transcriptional program driven by microphthalmia/transcription factor E (MIT/TFE) proteins, which also activate lysosomal biogenesis and nutrient-scavenging pathways. MIT/TFE proteins are required for the maintenance of amino acid pools, and knockdown of these genes is strongly growth inhibitory (Perera et al., 2015).

Inhibition of autophagy in PDAC leads to decreased mitochondrial oxygen consumption and increased dependence on glycolysis to make ATP (Yang et al., 2011). It also leads to a disrupted metabolic crosstalk with non-malignant cells in the tumor microenvironment (Figure 3).

**Figure 3. Methods of Nutrient Acquisition Utilized by PDAC**

Pancreatic cancer cells engage in metabolic crosstalk with stromal cells by multiple avenues. Growth factors (GF) released from the PDAC cells can metabolically reprogram fibroblasts, which respond by the release of different GFs capable of reciprocal reprogramming of the epithelial cells. PDAC cells also induce autophagy in pancreatic stellate cells, stimulating the release of growth-promoting alanine (Ala). Metabolite exchange also occurs among cancer cells, as PDAC cells in hypoxic environments release lactate (Lac) which fuels proliferation in normoxic cancer cells. Pancreatic cancer cells are capable of utilizing recycling pathways and engage in multiple mechanisms of scavenging extracellular nutrient sources, including non-specific macrophagocytosis and lipid uptake, to obtain nutrients in the austere pancreatic tumor microenvironment.

Pancreatic cancer cells activate pathways that enable (1) recycling of intracellular nutrients, (2) access to non-traditional extracellular nutrients through scavenging of extracellular space, and (3) engage in
mechanisms of lipid import may prove to be attractive metabolic poor relative to surrounding normal tissue (Ma et al., 2011; Yabushita et al., 2013; Zhang et al., 2013), suggest that targeting mechanisms of lipid import may prove to be attractive metabolic vulnerabilities.

Metabolic Crosstalk

Intra-tumoral heterogeneity at the cellular and genetic level is well documented (Bailey et al., 2014, 2016; Collisson et al., 2011; Delgrosso et al., 2014; Li et al., 2007; Waddell et al., 2015; Westphalen et al., 2016). While less appreciated, metabolic heterogeneity also exists among cancer cells within the same tumor (Birsoy et al., 2014; Daemen et al., 2015); such as differences among quiescent, rapidly dividing, and invasive populations (LeBleu et al., 2014; Sancho et al., 2015; Viale et al., 2014). Additional metabolic heterogeneity also exists due to environmental factors within a tumor, such as local nutrient and oxygen concentrations.

Accordingly, metabolically distinct cell populations have evolved crossfeeding mechanisms in which metabolites from one population can be used to fuel growth of another. For example, hypoxic regions of pancreatic tumors express high levels of the lactate exporter monocarboxylate transporter 4 (MCT4), whereas normoxic regions surrounding the hypoxic regions overexpress the lactate importer MCT1 (Guillaumond et al., 2013). Lactate secreted by cancer cells grown in hypoxia is actively taken up by cancer cells grown in normoxia, and this increases their proliferation (Guillaumond et al., 2013) (Figure 3). Lactate secretion also has profound effects via epithelial-stromal crosstalk, as lactate secreted from PDAC cells contributes to polarization of immunosuppressive macrophages (Hutcheson et al., 2016).

In addition to the ability of cancer cells to share metabolites, growing evidence demonstrates that stromal cells impact metabolism of cancer cells. In primary tumors, cancer cells are outnumbered by stromal cells, which include immune cells and fibroblasts (Figure 1). Numerous studies have illustrated the role of growth factor and cytokine exchange between cancer cells and surrounding stromal cells (Baumgart et al., 2014; Gunderson et al., 2016; Iuchi et al., 2011; Incio et al., 2016; Lee et al., 2016; Lesina et al., 2011; Mathew et al., 2014, 2016; Pylayeva-Gupta et al., 2016; Sherman et al., 2014; Waghray et al., 2016). More recently, evidence has also emerged that mutant Kras-induced growth factors mediate reciprocal stromal-epithelial crosstalk, which drives PDAC metabolic reprogramming, including an increase in mitochondrial membrane potential and respiratory capacity (Tape et al., 2016).

Stromal-derived metabolites also provide nutrients to fuel biosynthesis in cancer cells. We recently found that an abundant stromal cell type found in pancreatic tumors, pancreatic stellate cells (PSCs), release NEAAs in response to culture with PDAC cells (Sousa et al., 2016). Among these NEAAs, PDAC cells avidly consume PSC-derived alanine (Ala) and use it to fuel diverse biosynthetic processes, including mitochondrial metabolism, as well as fatty acid and amino acid biosynthesis. More striking was the observation that Ala-derived carbon out-competed glucose and Gln carbon for incorporation into mitochondrial metabolism and thereby enabled these molecules to be used for other biosynthetic functions. Autophagy in the PSC compartment was required for Ala release, as inhibition of autophagy machinery ablated the ability of PSCs to excrete Ala. Together with the observation that cancer cells stimulated autophagy and Ala release, these results provided evidence for a new intra-tumoral metabolic crosstalk pathway (Figure 3).
The precise mechanism by which Ala is released from the stromal compartment is unclear. One source by which PDAC cells have been demonstrated to obtain PSC-derived Ala is through uptake of exosomes released by PSCs (Zhao et al., 2016). Unlike the Ala transfer mechanism described above (Sousa et al., 2016), exosome-mediated transfer is not specific to Ala, where numerous amino acids are exosomal cargo. In contrast to macropinocytosis, which has been exclusively described in PDAC cells bearing KRAS mutations, metabolic reprogramming via uptake of exosomal metabolites was found to be independent of KRAS mutational status (Zhao et al., 2016). Collectively, the description of these new intra-tumoral metabolic crosstalk pathways further illustrates the proficiency of pancreatic cancer cells to adopt unorthodox methods to thrive in a challenging environment.

Therapeutic Opportunities
Development of successful new treatment modalities for pancreatic cancer has been limited since the establishment of gemcitabine as standard of care two decades ago. Nanoparticle albumin-bound paclitaxel (nab-paclitaxel) represents one such success (Von Hoff et al., 2011, 2013), and it is tempting to speculate that this may owe its success to rewired metabolism of PDAC cells. Nab-paclitaxel is a formulation of the chemotherapy paclitaxel, which is stabilized through binding to albumin. Given that PDAC cells readily scavenge serum albumin by macropinocytosis, it is possible that the albumin-coated formulation of paclitaxel is delivered disproportional to PDAC cells (Commissio et al., 2013; Kamphorst et al., 2015), enhancing the therapeutic index. While a metabolic role for the efficacy of nab-paclitaxel may have been fortuitous, there are currently several other strategies underway to directly target PDAC by exploiting their altered metabolism.

Inhibitors of Intermediary Metabolism
Many PDAC cells are dependent on glucose flux through glycolysis to maintain bioenergetic balance, whereas normal cells are more reliant on mitochondrial ATP generation. The final step of glycolysis, conversion of pyruvate to lactate by lactate dehydrogenase (LDH), is required to regenerate NAD+ and thus to facilitate continued glycolysis (Figure 2). This makes LDH an attractive target for pancreatic cancer, as blocking lactate production inhibits glycolysis and results in redox imbalance. In line with this, FX11, a small-molecule inhibitor of LDH (Le et al., 2010), reduces growth and induces apoptosis in patient-derived pancreatic cancer xenografts (Rajeshkumar et al., 2015).

Interestingly, the efficacy of FX11 was linked to p53 function, in which wild-type p53 function coincided with a lack of response to FX11 treatment. The regulation of metabolism by p53 is an important and complex topic (Berkers et al., 2013) that has not been examined specifically in pancreatic cancer. However, as p53 activation has been shown to be important for cell survival in the face of glucose deprivation (Jones et al., 2005), it is possible that the p53 status may be predictive of PDAC response to treatments which modulate glucose metabolism. Variable responses of PDAC cells to LDH inhibition were also noted with the LDH inhibitor, GNE-140 (Boudreau et al., 2016). In this study, only a small subset of PDAC lines responded robustly to GNE-140 treatment, while the rest were able to compensate through increased OXPHOS.

In contrast to bulk tumor, pancreatic-tumor-initiating cells (Sancho et al., 2015) and cells that can survive inhibition of oncogenic signaling (Viale et al., 2014) depend on mitochondrial OXPHOS (Viale et al., 2015). As a result, these cells are very sensitive to treatment with the OXPHOS inhibitors metformin or oligomycin (Sancho et al., 2015; Viale et al., 2014). Tumor-initiating cells have also been reported to be responsible for disease relapse after debulking chemotherapy and radiation. Therefore, methods that target metabolism of both oncogene/glycolysis-dependent tumor bulk (e.g., Kras inhibition) and OXPHOS-dependent resistant cells (e.g., oligomycin) lead to more effective and durable therapeutic responses in murine tumor models (Viale et al., 2014). As a result, inhibitors of OXPHOS are set to be explored in clinical trials for patients with pancreatic and other cancers (Protopopova et al., 2016). These data have also provided a rationale to reconsider existing literature and utility of the OXPHOS inhibitor metformin.

Metformin, a biguanide prescribed for type 2 diabetes, disrupts mitochondrial bioenergetics and inhibits hepatic gluconeogenesis via LKB/AMP-dependent and -independent mechanisms (Andrzejewski et al., 2014; Cusi et al., 1996; Foretz et al., 2010; Madiraju et al., 2014; Shaw et al., 2005). The direct effects of metformin on disruption of mitochondrial bioenergetics are experienced only by cells that express drug transporters (e.g., liver cells), whereas the indirect effects of reduced circulating glucose and insulin may have important systemic antineoplastic functions (Pollak, 2012). Importantly, alterations in systemic glucose metabolism, either through obesity or diabetes, are known risk factors for pancreatic cancer (Bracci, 2012; Garcia-Jimenez et al., 2016; Garg et al., 2014).

Examination of diabetic patient populations on metformin treatment has led to conflicting reports regarding the impact of the treatment on the risk of developing PDAC or the prognostic value of treatment after diagnosis (Bodmer et al., 2012; Hwang et al., 2013; Nakai et al., 2013; Sadeghi et al., 2012; Singh et al., 2013; Suissa and Azoulay, 2012; van Staa et al., 2012; Walker et al., 2015). However, two recent phase 2 clinical trials have found no benefit of metformin treatment when administered at levels used for glycemic control in patients with advanced or metastatic cancers (Kordes et al., 2015; Reni et al., 2016). While these results are disappointing, there may still be potential benefit for metformin treatment in the neoadjuvant setting, as a maintenance therapy in patients with stabilized metastatic cancer (NCT0248834) (Yang and Rustgi, 2016), or if used at higher concentrations. In addition, more potent biguanides such as phenformin (NCT02475499) or metformin analogs may be more efficacious, as they do not require active transport and exhibit cell-autonomous and systemic antineoplastic activities (Cheng et al., 2016).

Cell-autonomous metabolic alterations observed in PDAC are present to varying degrees. Recent work has classified PDAC cell lines into three distinct metabolic subtypes: slow proliferating, glycolytic, and lipogenic (Daemen et al., 2015). Among these, the glycolytic subtype responded robustly to inhibition of glutaminase (GLS), the primary enzyme responsible for anaerobic entry of Gln into the TCA cycle in cell culture models (Figure 2). Despite success with GLS inhibitors in vitro studies (Daemen et al., 2015; Son et al., 2013), this has not yet been effective for treating PDAC in vivo (Chakrabarti et al., 2015). As
one of the primary roles for Gln in PDAC cells is to maintain redox homeostasis (Son et al., 2013), we tested combinatorial activity of GLS inhibition with a clinical agent that induces redox imbalance. β-Lapachones (β-laps) are a class of targetted cancer therapeutics that induce ROS formation and NADPH depletion in an NADPH:quinone oxidoreductase 1 (NQO1)-dependent fashion. NQO1 is highly upregulated in PDAC relative to normal pancreatic tissue and is the most widely recognized Nrf2 target, suggesting a further degree of selectivity for this treatment regimen. Indeed, subclinical doses of β-lap and a GLS1 inhibitor, which were non-toxic as single agents, exhibited tumoricidal activity and slowed tumor growth through on-target activity of both agents in vivo (Chakrabarti et al., 2015).

**Inhibitors Targeting Scavenging Pathways**

Hydroxychloroquine (HCQ), a lysosomotropic drug which prevents acidification of lysosomes, is a potent inhibitor of autophagy that prevents degradation of autophagosomes. The blockade of lysosomal acidification can also prevent uptake of nutrients via macropinocytosis, as lysosomal degradation is the terminal step in that pathway. Indeed, HCQ has moved forward in several clinical trials for pancreatic cancer patients, including single-agent HCQ in patients previously treated with standard of care for metastatic cancer (NCT01273805), as well as combination treatments with gemcitabine (NCT01128296) (Boone et al., 2015), gemcitabine/nab-paclitaxel (NCT01506973), or in combination with capecitabine and radiation or proton therapy (NCT01494155).

Despite its potential, clinical outcomes with HCQ have been limited by pharmacokinetic and pharmacodynamic shortcomings. Specifically, HCQ requires a long treatment regimen to reach a therapeutic steady state, which for rheumatoid arthritis patients has been found to take up to 6 months (Tett et al., 1988). As such, in its current dose formulation, HCQ may not be a sufficiently potent inhibitor of autophagy in patients. In a recent report on clinical trials (NCT01273805), only 10% of patients treated with HCQ demonstrated progression-free survival (Wolpin et al., 2014). However, patients treated with both HCQ and gemcitabine who met criteria for HCQ response via peripheral blood mononuclear cell LC3 staining demonstrated marked improvement in disease-free and overall survival (Boone et al., 2015). Accordingly, there are several strategies underway to develop new and more potent autophagy inhibitors which may yield better clinical results (Kulkarni et al., 2016; Liu et al., 2011; McAfee et al., 2012; Wang et al., 2015; Zhao et al., 2015).

While early attempts to treat PDAC by targeting tumor metabolism have only been met with moderate success, there is reason for continued optimism. Many recent preclinical studies, such as those detailed above, have revealed additional features of altered metabolism in PDAC with more tractable therapeutic targets. Nevertheless, a concern with designing therapies to target metabolic vulnerabilities is the development of resistance, which has plagued kinase-targeted therapies in pancreatic cancer (Alagesan et al., 2015). Indeed, like the redundancies in cell signaling, metabolic networks also have flexibility and recent reports have revealed that these can be rewired to evade targeted therapy (Boudreau et al., 2016; Davidson et al., 2016). Finally, as mentioned previously, the pancreatic stroma has traditionally presented a barrier to drug delivery (Olive et al., 2009) and, as such, effective metabolic inhibitors will need to be designed with this in mind.

**PET Imaging**

PET is a non-invasive imaging approach in which gamma rays are detected from positron-emitting isotopes. As detection of an emission source limits spatial resolution, PET is often combined with computed tomography (CT). The workhorse imaging agent in clinical oncology for PET is the glucose analog [18F]fluorodeoxyglucose (FDG), which capitalizes on the observation that glucose uptake is increased in cancer, including PDAC. Upon uptake into cells, both glucose and FDG are phosphorylated by HK to prevent their release from the cell. However, in contrast to glucose, FDG lacks a 2′-hydroxyl group which prevents it from being further metabolized. The end result is FDG accumulation in cells proportional to the amount of uptake and HK activity (Figure 4A).

The ability of FDG-PET imaging to detect PDAC was reported early in the development of the technology (Friess et al., 1995; Zimny et al., 1997). However, there are mixed reports on the utility of FDG-PET compared with CT, MRI, and endoscopic ultrasound techniques. The most useful aspect of FDG-PET for PDAC appears to be the strong correlation between levels of FDG uptake and tumor aggressiveness in terms of pathological grade (Ann et al., 2014; Chen et al., 2016), prediction of distant metastasis (Shinoto et al., 2013), and survival (Chen et al., 2016; Kitasato et al., 2014; Lee et al., 2014; Yamamoto et al., 2015). The strongest critique against use of FDG-PET in PDAC is the observation that it is no more effective than conventional imaging techniques to diagnose early stage pancreatic cancer or to detect small metastases (Matsumoto et al., 2013; Rijkers et al., 2014). In addition, there is concern over the ability of FDG-PET to distinguish between PDAC and pancreatitis (Kato et al., 2013), given that mass-forming pancreatitis also shows an increase in FDG uptake (Kamisawa et al., 2010).

As PET imaging with FDG in PDAC is limited to prognostic value, other PET strategies are currently being developed. In addition to glucose-based PET-imaging agents, 18F-labeled Gln analogs have been created (Figure 4A) (Lieberman et al., 2011; Ploessl et al., 2012; Qu et al., 2011b; Wu et al., 2014). Gln-based PET agents have demonstrated the ability to selectively detect tumors in preclinical animal models (Lieberman et al., 2011; Ploessl et al., 2012; Venneti et al., 2015; Wu et al., 2014) and have been successfully tested in human glioblastoma patients (Venneti et al., 2015). Because of the
Alterations in Gln metabolism described in detail above, 18F-labeled Gln analogs could potentially be useful for detection and monitoring of PDAC.

Hyperpolarized MRS

MRS has been used extensively for in vitro metabolism studies with non-radioactive 13C-labeled metabolites; however, in vivo imaging of metabolic pathways using 13C-labeled metabolites has remained a challenge due to both low sensitivity of 13C-MRS and the inability to deliver a sufficient amount of labeled isotope. Recent advances in biomedical imaging have addressed limitations in sensitivity using 13C isotopes and now allow for 10,000-fold enhancements in signal (Ardenkjaer-Larsen et al., 2003; Keshari and Wilson, 2014). This process is referred to as hyperpolarization (HP) and possesses several significant advantages over traditional imaging technologies. First, the 13C-labeled imaging agent is the parent molecule; that is, it is structurally identical to its non-labeled counterpart. Accordingly, labeled metabolite can be detected directly and, like traditional imaging agents, provides readout of tissue avidity. However, unlike traditional probes, 13C-labeled metabolites, once taken into a cell, can also participate and inform of the altered metabolism within a cancer cell. An example of this as applied to a preclinical PDAC model revealed that conversion of 1-13C-labeled pyruvate to lactate could distinguish cancer from normal tissue and even stage disease (Figure 4B) (Serrao et al., 2015).

A limitation of this technology concerns the short half-life of the probe, which is typically on the order of 1 min. Despite this temporal constraint, application of HP-MRS probes as imaging agents in vivo is feasible (Golman et al., 2003). Metabolic imaging via HP-MRS began with the development of HP-pyruvate (Golman et al., 2006), which has now been successfully used in preclinical models to assess tumor metabolism (Dutta et al., 2013; Harris et al., 2009; Hu et al., 2011; Keshari et al., 2013b), correlate pyruvate metabolism to tumor grade (Albers et al., 2008), and monitor tumor response to therapeutics (Day et al., 2007, 2011; Park et al., 2011; Sandulache et al., 2014; Sourbier et al., 2014). HP-pyruvate imaging has also been used in preclinical models of PDAC, including autochthonous mouse models (Serrao et al., 2015), and patient-derived xenografts (Rajeshkumar et al., 2015). Importantly, feasibility and safety of HP-pyruvate imaging in human patients has been demonstrated in a clinical trial for prostate cancer (Nelson et al., 2013).

Other hyperpolarized metabolic probes have exhibited promising results (Salamanca-Cardona and Keshari, 2015) that are of particular interest for the study of pancreatic cancer metabolism.
(Figure 4B). HP-Gln probes have been developed (Cabella et al., 2013; Gallagher et al., 2008, 2011; Qu et al., 2011a) that would be useful in identification of Kras-rewired Gln metabolism in vivo. HP-glucose probes have also been described (Allouche-Amon et al., 2013; Christensen et al., 2014; Harris et al., 2013; Rodrigues et al., 2014; Timm et al., 2015) that would allow identification of changes in glycolytic metabolites and the PPP. Lastly, HP-dehydroascorbate has been used to measure the redox state and inform on the level of ROS in tumors (Bohndiek et al., 2011; Keshari et al., 2011, 2013a), which could be used to select treatment modalities and monitor therapeutic response. Despite recent progress in defining the genetic landscape of pancreatic cancer (Bailey et al., 2016; Moffitt et al., 2015; Waddell et al., 2015), genetic approaches to predict treatment response have thus far not provided new treatment options for the majority of patients. In light of this, profiling the metabolism of tumors in vivo represents a promising new avenue to help inform treatment options for these patients.

Conclusion and Future Directions
While altered metabolism has long been recognized as a central hallmark of cancer, we have only recently begun to evolve a sufficient mechanistic understanding of these processes to begin to exploit differences between cancer cells and normal cells. Pancreatic cancer metabolism is dramatically rewired by oncogenic Kras, presenting several opportunities for selective targeting. Furthermore, the austere microenvironment forces pancreatic cancer cells to rely on alternative sources of nutrients and to utilize unique methods to obtain them. The need for new therapies in pancreatic cancer treatment is clear. While a heroic effort is being made to target Kras and Kras-surrogate signaling (Cox et al., 2014), there are no clinical Kras inhibitors and few clinically viable Ras-effector treatments have emerged, due largely to compensatory signaling from single agents or toxicity of combination strategies. As outlined in this review, it is our belief that targeting tumor metabolism may overcome these limitations.

Metabolic changes in pancreatic tumors can also provide diagnostic information that is not available with traditional imaging methods. This is particularly true of HP-MRS imaging, which with more routine use may be able to stratify tumors for treatment based on metabolic signatures. Furthermore, the ability to trace tumor metabolism in vivo will be critical to verify that information gathered from preclinical models and studies are applicable in PDAC patients.

Finally, immunotherapy has emerged as a promising treatment option for several cancers (Brahmer et al., 2012; Javel et al., 2016). Accordingly, there has been substantial interest in dissecting immune response to PDAC and translating these findings into successful treatments (Bayne et al., 2012; Beatty et al., 2011; Pilyateva-Gupta et al., 2012; Soares et al., 2015; Winograd et al., 2015; Zhu et al., 2014). However, it is important to note that immune cell metabolism is also important to the activation and differentiation of these cells (Maclver et al., 2013; Wahl et al., 2012). As such, metabolic inhibitors targeting tumor cells may either interfere or enhance the immune response to therapy. Future studies will be needed to gather a more detailed understanding of the tumor immune response to metabolic perturbation.

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