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Stable Isotope Tracing in Cancer Metabolism

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Stable Isotope-Resolved Metabolomics (SIRM)

The use of tracers in metabolism has a long history, with radioisotopes being instrumental in deciphering metabolic pathways of both central (e.g., the Krebs cycle) and secondary metabolism. With the awakening of inadequate understanding of cell metabolism in various biological systems, greatly improved analytical techniques (especially NMR and mass spectrometry), and the dramatically increased availability of stable isotope-enriched precursors, there has been a resurgence of interest in utilizing stable isotope tracers in metabolic studies.

Since 2000, more than 1,100 articles on cancer metabolism with stable isotope tracing have been published. Stable isotopes have the advantage of being non-hazardous and are thus easier to handle than radiotracers and commensurate with human studies. Furthermore, the additional neutron(s) in the nucleus of a rare stable isotope enables very simple detection by low-resolution mass spectrometry. For example, of the three natural isotopes of carbon, nitrogen, and hydrogen, the rare (1.1 % abundance) ^{13}C atom is 1 amu heavier than the abundant ^{12}C atom (99.9%), and similarly for ^{15}N (0.35%) versus ^{14}N and ^{2}H (0.02%) versus ^{1}H. With the advances in ultrahigh resolution mass spectrometry (UHRMS), double- or triple-tracer experiments are now feasible, as the additional neutron mass in ^{13}C is different from that in ^{15}N or ^{2}H, so that isotopologues (identical compounds differing by the number of tracer atoms) containing these tracer atoms are distinguishable by UHRMS. Stable isotopes such as ^{13}C can also be detected directly by NMR, but ^{12}C or radioactive ^{14}C is invisible. In fact, radioactive isotopes are generally unsuited for NMR detection except for ^{3}H. More importantly, ^{15}N and ^{13}C can be observed indirectly by the attached proton, thereby affording much higher sensitivity than direct detection. As these nuclei resonate at widely different radiofrequencies (known as chemic shifts), multiplexed stable isotope resolved metabolomics (mSIRM) experiments can be readily performed.

The power of NMR and MS techniques in metabolic studies is that it is practical to determine not only the metabolite structures but also the number and position of heavy atoms present. The two techniques are complementary, as NMR can more readily provide information on heavy atom position than MS and vice versa for the number of heavy atoms in given metabolites. Both pieces of information exquisitely reveal the routes of metabolic transformations and with the appropriate experimental deign, detailed metabolic fluxes. This can all be achieved in unfractionated extracts of cultured cells, organs or tissues. Furthermore, it is possible to measure metabolic transformations and fluxes *in vivo* by NMR, especially using the new hyperpolarization methods (e.g. DNP).[1,2]

The most commonly used stable isotope in metabolic studies, most notably cancer metabolism research, is ^{13}C. There are numerous ^{13}C-enriched precursors commercially available, including several isotopomers of D-glucose (e.g. [U-$^{13}C_6$], [1,2-$^{13}C_2$], [1,6-$^{13}C_2$], [3,4-$^{13}C_2$]) for probing different routes of glucose metabolism, ^{13}C-octanoic,[3,4] ^{13}C-palmitic and ^{13}C-acetate for probing fatty acid metabolism and fate of intracellular ^{13}C-acetyl CoA,[5] ^{13}C-fructose, and amino acids, which can be obtained as ^{13}C only, ^{15}N only, or doubly labeled versions.[6] Such versatility of stable isotope-labeled resources opens a rich avenue of experimental designs for interrogating the vast and intricate metabolic networks in mammalian systems.

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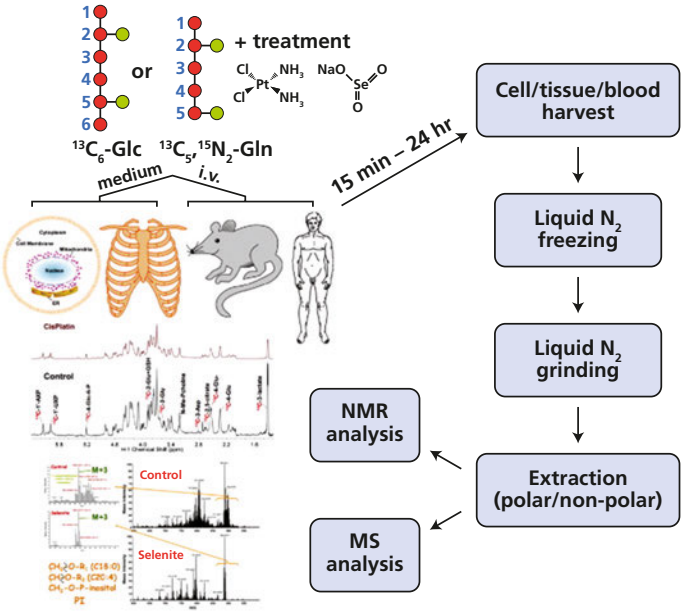
Cancer Metabolism

As indicated above, cancer metabolic studies have most frequently taken advantage of the stable isotope tracer approach, and as such metabolic reprogramming is now recognized as a hallmark of human cancer.[7] The transformation of a normal cell to a highly proliferative cancer state is associated with substantial perturbations in metabolism, which are often interactively linked to changes in the transcriptional program driven by alterations of critical oncoproteins and/or tumor suppressors. The increasingly hostile tumor microenvironment during growth can further confound metabolic reprogramming and tumor progression.[8-10] Most solid tumors up-regulate glycolysis and convert a high fraction of the glucose consumed to lactate (i.e., the Warburg effect), which is excreted along with a proton acidifying the extracellular space. In order to support cell growth, survival, and eventual metastasis, other parts of the metabolism must also be up-regulated, especially the synthesis of precursors for nucleic acids, lipids, complex carbohydrates and proteins (i.e. non-essential amino acids such as Ala, Glu, proline[11]), serine, and glycine (for purine biosynthesis, one-carbon metabolism), aspartate (for pyrimidine biosynthesis), and glutamate (for antioxidant glutathione synthesis). It is also becoming clear that the metabolism of essential amino acids, particularly the branched chain amino acids Ile, Leu, and Val are important in the development of some cancers.[12]

Enhanced lactic fermentation is readily determined using ^{13}C-glucose, by tracking the ^{13}C fate in cell culture and organ systems from the uptake of ^{13}C-glucose to the excretion of ^{13}C-lactate in terms of both levels and fractional enrichment in lactate, as well as the fraction of glucose consumed that is converted to lactate. The excretion ^{13}C-Ala and ^{13}C-Glu (due to exchange with extracellular cystine) can also be observed in the ^{13}C-glucose tracer studies of cancer cells and tissues, which informs the status of Ala metabolism via the alanine aminotransferase (ALT) activity and the demand for glutathione (cysteine being a precursor). The fate of individual ^{13}C atoms of glucose into intracellular metabolites can also be traced to map both central catabolic and anabolic pathways that are keys to supporting cancer cell proliferation and survival. These include, but are not limited to, the pentose phosphate pathway (PPP), the Krebs cycle, Ser-Gly one-carbon metabolism, and the synthesis of glutathione, non-essential amino acids, purine/pyrimidine nucleotides, nucleotide sugars (e.g., UDP *N*-acetylglucosamine or UDP-GlcNAc), and lipids. Moreover, the incorporation of newly synthesized non-essential amino acids into proteins and nucleotides into RNA can be determined following careful hydrolysis of the macromolecules.[13,14]

The PPP is important both for generating cytoplasmic NADPH required for anabolic and detoxification metabolism, as well as ribose for nucleotide synthesis. Thus, proliferating cells such as cancer cells have a strong requirement for the PPP. However, it is often unclear the extent of which the PPP contributes to NADPH production via the oxidative branch in cancer cells. It is feasible to discriminate the activity of the oxidative from non oxidative branches using specific ^{13}C isotopomers of glucose, such as [1,2-^{13}C]-glucose via mass spectrometry[15] or a mixture of [1-^{13}C]- and [2-^{13}C]-glucose via NMR analysis.[4]

In addition to the common adaptation of accelerated aerobic glycolysis in cancer cells, other adaptations that generate metabolic energy and anabolic precursors are certainly important. It has long been thought that mitochondria are non-functional in cancers. However, tracer studies with ^{13}C-glucose, ^{13}C-Gln, and ^{13}C-fatty acids clearly demonstrated that cancer cells in general oxidize a wide range of substrates in the mitochondria, even under hypoxia. Indeed, many established cancer cells have a high requirement for glutamine which also serves as the nitrogen donor in important amidotransferase reactions such as those in nucleotide[16] and UDP-GlcNAc biosynthesis, and as a source of Glu which is the most common substrate for aminotransferases. We have shown in SIRM studies using NMR and MS that many aspects of the mitochondrial metabolism is activated *in vivo*[17,18] and *ex vivo* in freshly resected human lung cancer tissues.[10,17)]



Stable isotope-resolved metabolomics (SIRM) pipeline. (Adapted from Fan, T.W.-M. et al. **2012**. *Pharmacol Ther, 113(3),* 366-391.

Mitochondrial metabolism and glycolysis together are essential to fueling nucleotide biosynthesis, which is fundamental to cell proliferation.[19] As indicated above, the ribose subunit of the free nucleotides is derived from glucose (or glycogen) as shown by tracer studies with ^{13}C-glucose and other ^{13}C-enriched sources.[14] The nucleobases, however, derive from multiple sources. Uracil (and subsequently cytosine) derives primarily for aspartate, which is mainly synthesized *de novo* in the mitochondria of cells and tissues as the extracellular concentration of Asp is very low, and it is not efficiently transported. Asp can also be synthesized via transamination of oxaloacetate with Glu in the cytoplasm. Using [U-^{13}C,^{15}N]-Gln as the tracer, the elevated presence of the m+5 (i.e., $^{13}C_4$,$^{15}N_1$) isotopologue of Asp[20] is evidence for the activation of the aminotransferases GOT1/2.[21] This transamination is also part of the aspartate/malate shuttle system for transferring reducing equivalents from the cytoplasm to the mitochondria for oxidation via the electron transport chain. Under respiration

continued ➤

Stable Isotope Tracing in Cancer Metabolism *(continued)*

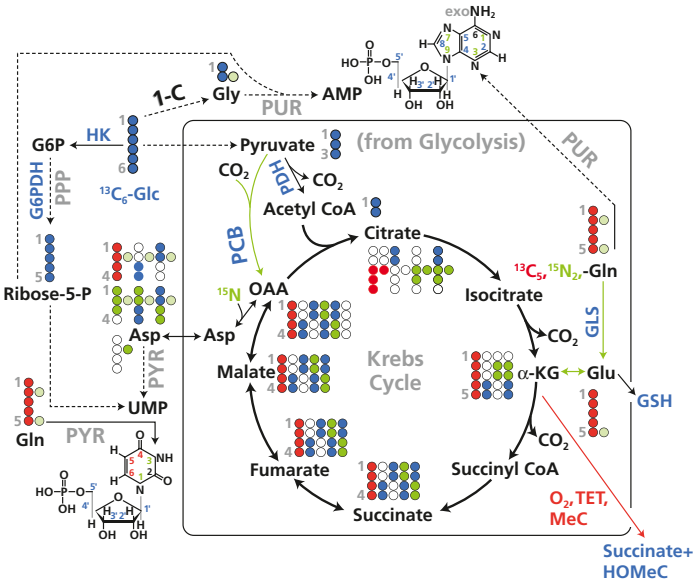
deficiency, pyruvate may act as an alternative electron acceptor and utilize pyruvate carboxylase to maintain Asp synthesis.[22-24] Indeed, we determined that pyruvate carboxylase is activated *in vivo* in human lung cancer by employing $^{13}C_6$-glucose infusion into patients coupled with SIRM analysis.[17] We also determined that Gln is a better substrate for Asp and Uracil ring synthesis than glucose by comparing ^{13}C incorporation from $^{13}C_6$-glucose and [U-^{13}C,^{15}N]-Gln into the two products.[14]

Glycine-derived one-carbon metabolism plays a central role in cell cycle control both in replication and cell growth.[25] Purines require glycine as a direct donor of two carbons and one nitrogen. Glycine is also the source of the one-carbon unit in methylene tetrahydrofolate (THF) that supplies two additional purine ring carbons. Although glycine is freely available in the serum, it is also synthesized in cells from serine, which in turn is made from the glycolytic intermediate 3-phosphoglycerate. This pathway is up-regulated in human cancer,[25,26] and as much as 8-9% of the glucose flux may be shunted to serine synthesis.[26] In addition, purine synthesis require Gln, and we have shown that the three-ring nitrogen atoms in purine nucleotides are derived from Gln by using [U-^{13}C,^{15}N]-Gln as a tracer. Two derive from the amidotransferase reactions, and the third is from Asp, which must have received its amino nitrogen by transamination from Gln-derived Glu.[20] Other than fueling nucleotide biosynthesis, one-carbon metabolites such as 5-methyl THF is the source of methyl groups for replenishing S-adenosylmethionine (SAM) from S-adenosylhomocysteine (SAH). SAM is the universal methyl donor for a wide range of methyl transferases involved in epigenetic methylation of histones as well as cytosine in DNA.

Although many cancers are driven by alterations in oncoproteins and tumor suppressors, which often lead to reprogramming of cell metabolism, there are also cases where loss or gain of function directly in enzymes is key to carcinogenesis. For example, loss-of-function alterations in fumarate hydratase (FH) and succinate dehydrogenase (SDH) are important to the development of several aggressive familial cancers of the kidney. Such loss of enzyme activity results in the accumulation of high levels of fumarate or succinate, respectively, which are derived predominantly from glutamine based on ^{13}C tracer studies in cell cultures.[27,28] Cells expressing these inactive enzymes display substantial reductive carboxylation via a reversal of the isocitrate dehydrogenase (IDH) reaction to support lipogenesis. Both metabolites are potent inhibitors of the α-ketoglutarate-dependent dioxygenases, including prolyl hydroxylases (e.g. those hydroxylate hypoxia-inducible factor Hif1α for proteosomal degradation) and the epigenetic modulating enzymes Ten-eleven translocation enzyme (TET) and jumonji-domain histone K/R demethylases.

In addition to loss-of-function enzyme variants, there are rare gain-of-function mutations in the genes encoding IDH1 and 2, which are NADPH-dependent cytoplasmic and mitochondrial isoforms, respectively. These mutations alter the enzyme activity from oxidative decarboxylation of isocitrate to α- ketoglutarate (α-KG) to reduction of α-KG to 2-hydroxyglutarate (2HG) with NADPH. 2HG is now known as an oncometabolite that accumulates to high levels in certain gliomas[29] and in acute myeloid leukemia (AML). This has the combined effect of depleting NADPH (which is needed both for anabolic purposes and for detoxifying excess H_2O_2 production) and α-KG (which inhibits the α-KG-dependent dioxygenases.

NADPH is a key coenzyme and the NADPH/NADP+ ratio is tightly regulated in cells. The possible sources of the hydride have been elegantly determined using different deuterated substrates, resulting in the production of NADPD.[30,31] When combined with genetic modification of specific enzymes, this can also provide information about compartmentation of different NADPH-producing pathways,[31] and is requiring reassessment of NADPH production in cancer cells.



Metabolism and epigenetics: ^{13}C tracing from glucose (blue) or glutamine (red) in the Krebs cycle and linkage to nucleotide synthesis and epigenetic modulations.

IDH1,2 variants -> 2HG

SDH, FH mutations -> succinate, fumarate inhibitors of α-KG-dependent dioxygenases such as TET

In most cases, metabolic reprogramming in human cancer is quantitative rather than qualitative. Regardless of the nature of the reprogrammed events, stable isotope tracing has opened many new avenues for quantifying metabolic fates in atomic details that are crucial to deciphering reprogrammed metabolic pathways required for supporting cancer cell proliferation, survival, and metastasis. Tracer data are also essential for rigorous metabolic flux analysis, which can inform the changes in the kinetics of individual enzymes and/or transporters in given metabolic networks *in vivo,* in response to disease development or therapeutics. This information can be readily linked to functional genomic data for elucidating altered metabolic regulatory network.[32,33] We fully expect that the application of stable isotope tracers to functional analysis of cancers or other metabolic diseases *in vitro* and *in vivo* to flourish in the future.

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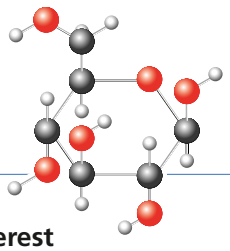


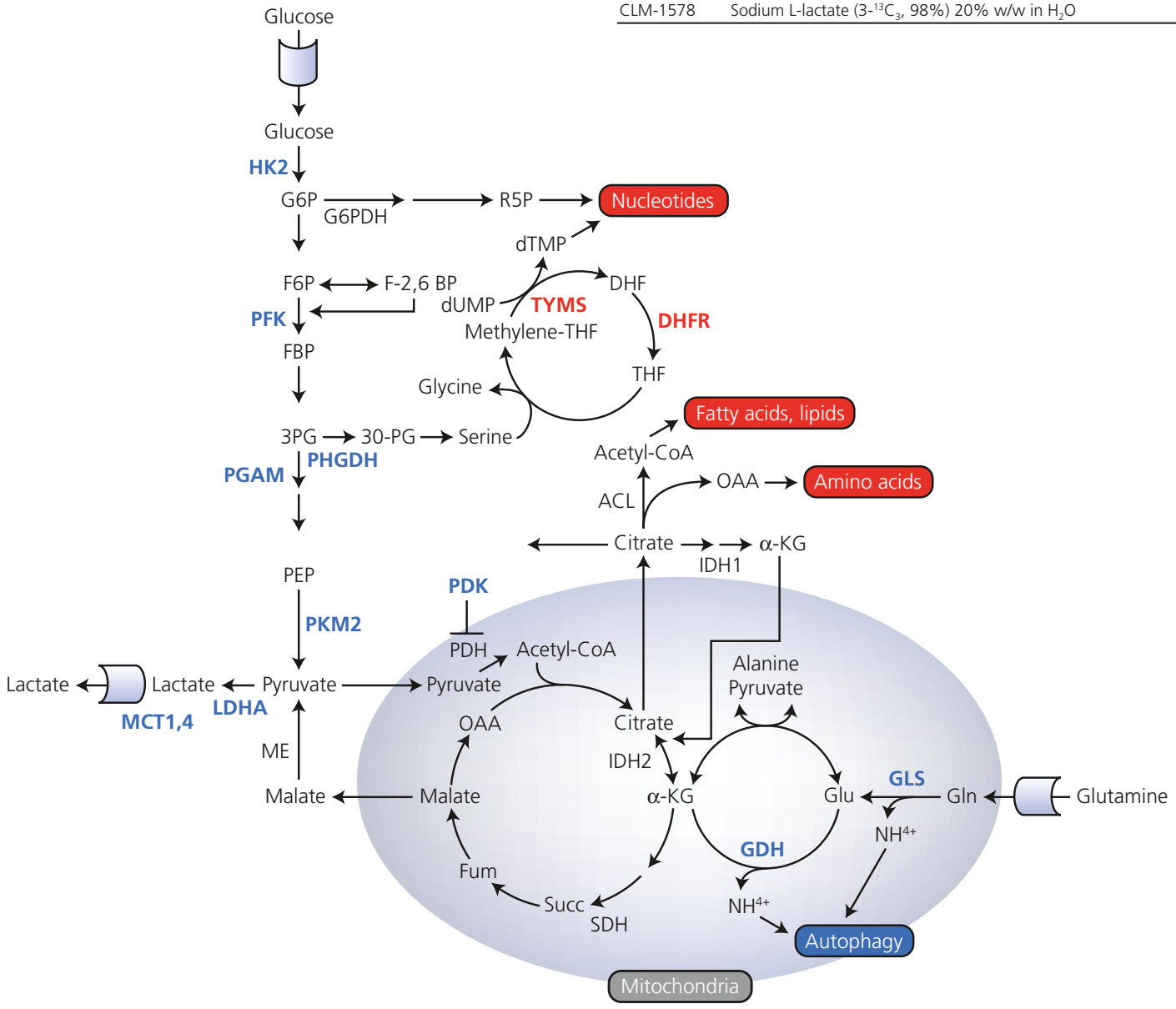
D-Glucose

Glucose

Cancer displays an up-regulated glycolysis pathway and TCA cycle. Glucose-$^{13}C_6$ is commonly used by researchers to measure flux of metabolites through these pathways. When analyzing the data using isotopomer analysis, it could lead to discoveries in novel pathways and/or important metabolic characteristics that could be exploited as a drugable target. CIL offers a wide variety of glucoses labeled with either deuterium and/or ^{13}C.

Products of Interest

Catalog No.	Description
CLM-420	D-Glucose (1-^{13}C, 98-99%)
CLM-746	D-Glucose (2-^{13}C, 99%)
CLM-504	D-Glucose (1,2-$^{13}C_2$, 99%)
CLM-2717	D-Glucose (1-^{13}C, 99%; 6-^{13}C, 97%+)
CLM-6750	D-Glucose (3,4-$^{13}C_2$, 99%)
CLM-4673	D-Glucose (1,2,3-$^{13}C_3$, 99%)
CLM-1396	D-Glucose ($^{13}C_6$, 99%)
DLM-349	D-Glucose (6,6-D_2, 99%)
DLM-2062	D-Glucose (1,2,3,4,5,6,6-D_7, 97-98%)
CDLM-3813	D-Glucose ($^{13}C_6$, 99%; 1,2,3,4,5,6,6-D_7, 97-98%)
CLM-1579	Sodium L-lactate ($^{13}C_3$, 98%) 20% w/w in H_2O
CLM-1578	Sodium L-lactate (3-$^{13}C_3$, 98%) 20% w/w in H_2O



Glucose

Pyruvate Carboxylase Is Critical for Non-Small-Cell Lung Cancer Proliferation

Sellers, K.; Fox, M.P.; Bousamra, M. 2nd.; Slone, S.P.; Higashi, R.M.; Miller, D.M.; Wang, Y.; Yan, J.; Yuneva, M.O.; Deshpande, R.; Lane, A.N.; Fan, T.W.
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2015. *J Clin Invest, 125(2),* 687-698. PMID: 25607840

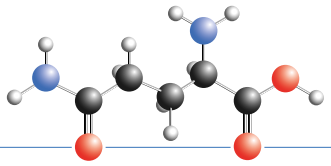
ABSTRACT Anabolic biosynthesis requires precursors supplied by the Krebs cycle, which in turn requires anaplerosis to replenish precursor intermediates. The major anaplerotic sources are pyruvate and glutamine, which require the activity of pyruvate carboxylase (PC) and glutaminase 1 (GLS1), respectively. Due to their rapid proliferation, cancer cells have increased anabolic and energy demands; however, different cancer cell types exhibit differential requirements for PC- and GLS-mediated pathways for anaplerosis and cell proliferation. Here, we infused patients with early-stage non-small-cell lung cancer (NSCLC) with uniformly ^{13}C-labeled glucose before tissue resection and determined that the cancerous tissues in these patients had enhanced PC activity. Freshly resected paired lung tissue slices cultured in $^{13}C_6$-glucose or $^{13}C_5$,$^{15}N_2$-glutamine tracers confirmed selective activation of PC over GLS in NSCLC. Compared with noncancerous tissues, PC expression was greatly enhanced in cancerous tissues, whereas GLS1 expression showed no trend. Moreover, immunohistochemical analysis of paired lung tissues showed PC overexpression in cancer cells rather than in stromal cells of tumor tissues. PC knockdown induced multinucleation, decreased cell proliferation and colony formation in human NSCLC cells, and reduced tumor growth in a mouse xenograft model. Growth inhibition was accompanied by perturbed Krebs cycle activity, inhibition of lipid and nucleotide biosynthesis, and altered glutathione homeostasis. These findings indicate that PC-mediated anaplerosis in early-stage NSCLC is required for tumor survival and proliferation.

Metabolism of [U-^{13}C]Glucose in Human Brain Tumors *in vivo*

Maher, E.A.; Marin-Valencia, I.; Bachoo, R.M.; Mashimo, T.; Raisanen, J.; Hatanpaa, K.J.; Jindal, A.; Jeffrey, F.M.; Choi, C.; Madden, C.; Mathews, D.; Pascual, J.M.; Mickey, B.E.; Malloy, C.R.; DeBerardinis, R.J.
Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas USA
2012. *NMR Biomed, 25(11),* 1234-1244. PMID: 22419606

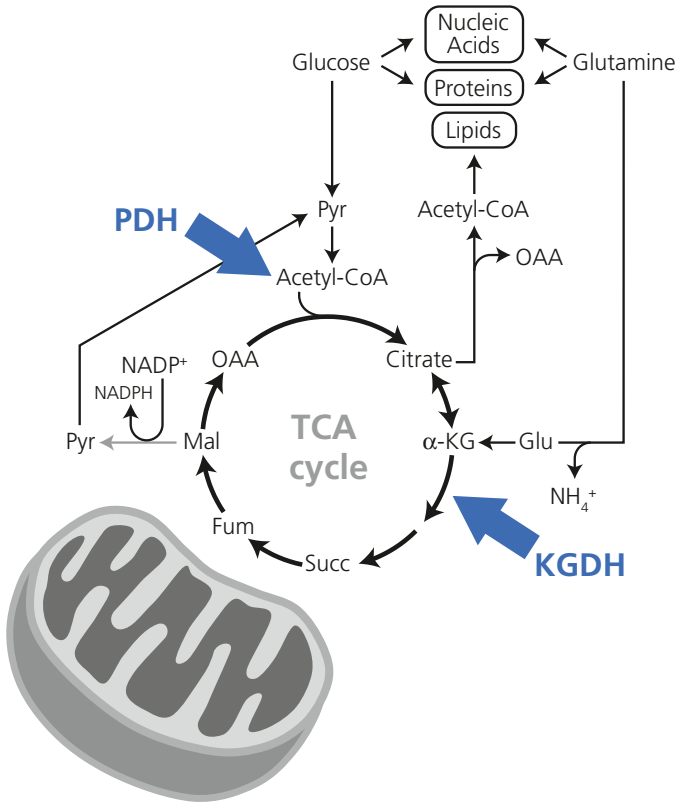
ABSTRACT Glioblastomas and brain metastases demonstrate avid uptake of 2-[^{18}-F]fluoro-2-deoxyglucose by positron emission tomography and display perturbations of intracellular metabolite pools by ^{1}H MRS. These observations suggest that metabolic reprogramming contributes to brain tumor growth *in vivo*. The Warburg effect, excess metabolism of glucose to lactate in the presence of oxygen, is a hallmark of cancer cells in culture. 2-[^{18}-F] fluoro-2-deoxyglucose-positive tumors are assumed to metabolize glucose in a similar manner, with high rates of lactate formation relative to mitochondrial glucose oxidation, but few studies have specifically examined the metabolic fates of glucose *in vivo*. In particular, the capacity of human brain cancers to oxidize glucose in the tricarboxylic acid cycle is unknown. Here, we studied the metabolism of human brain tumors in situ. [U-^{13}C]glucose (uniformly labeled glucose, i.e. D-glucose labeled with ^{13}C in all six carbons) was infused during surgical resection, and tumor samples were subsequently subjected to ^{13}C NMR spectroscopy. The analysis of tumor metabolites revealed lactate production, as expected. We also determined that pyruvate dehydrogenase, turnover of the tricarboxylic acid cycle, anaplerosis and *de novo* glutamine and glycine synthesis contributed significantly to the ultimate disposition of glucose carbon. Surprisingly, less than 50% of the acetyl-coenzyme A pool was derived from blood-borne glucose, suggesting that additional substrates contribute to tumor bioenergetics. This study illustrates a convenient approach that capitalizes on the high information content of ^{13}C NMR spectroscopy and enables the analysis of intermediary metabolism in diverse cancers growing in their native microenvironment.

Glutamine



Glutamine

It is a well recognized that glutamine metabolism is up-regulated in cancer cells. Glutamine is important to cancer cells because it creates glutamate, which can be used in synthesizing other compounds: TCA intermediates, amino acids, fatty acids, and lipids to supply necessary intermediates and energy for the rapidly dividing cells. Glutamine and glutamate can also serve as nitrogen sources for the synthesis of other amino acids such as serine, alanine, aspartate, asparagine, proline, and arginine. Tracing the glutamine metabolism with stable isotope tracers helps determine how cancer alters metabolism and possibly how it can be used to guide therapeutic discovery.



Products of Interest

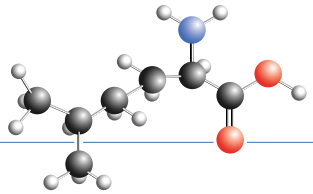
Catalog No.	Description
CLM-3612	L-Glutamine (1-^{13}C, 99%)
CLM-1822-H	L-Glutamine ($^{13}C_5$, 99%)
NLM-1016	L-Glutamine (α-^{15}N, 98%)
NLM-557	L-Glutamine (amide-^{15}N, 98%+)
NLM-1328	L-Glutamine ($^{15}N_2$, 98%)
CNLM-1275-H	L-Glutamine ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
DNLM-6997	L-Glutamine (2,3,3,4,4-D_5, 97-98%; $^{15}N_2$, 97-98%)
CDNLM-6805	L-Glutamine ($^{13}C_5$, 97-99%; D_5, 97-99%; $^{15}N_2$, 97-99%)

Glutamine Supports Pancreatic Cancer Growth through a KRAS-Regulated Metabolic Pathway

Son, J.; Lyssiotis, C.A.; Ying, H.; Wang, X.; Hua, S.; Ligorio, M.; Perera, R.M.; Ferrone, C.R.; Mullarky, E.; Shyh-Chang, N.; Kang, Y.; Fleming, J.B.; Bardeesy, N.; Asara, J.M.; Haigis, M.C.; DePinho, R.A.; Cantley, L.C.; Kimmelman, A.C.
Division of Genomic Stability and DNA Repair, Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts USA
2013. *Nature, 496(7443),* 101-105. PMID: 23535601

ABSTRACT Cancer cells have metabolic dependencies that distinguish them from their normal counterparts.[1] Among these dependencies is an increased use of the amino acid glutamine to fuel anabolic processes.[2] Indeed, the spectrum of glutamine-dependent tumours and the mechanisms whereby glutamine supports cancer metabolism remain areas of active investigation. Here we report the identification of a non-canonical pathway of glutamine use in human pancreatic ductal adenocarcinoma (PDAC) cells that is required for tumour growth. Whereas most cells use glutamate dehydrogenase (GLUD1) to convert glutamine-derived glutamate into α-ketoglutarate in the mitochondria to fuel the tricarboxylic acid cycle, PDAC relies on a distinct pathway in which glutamine-derived aspartate is transported into the cytoplasm where it can be converted into oxaloacetate by aspartate transaminase (GOT1). Subsequently, this oxaloacetate is converted into malate and then pyruvate, ostensibly increasing the NADPH/NADP$^+$ ratio which can potentially maintain the cellular redox state. Importantly, PDAC cells are strongly dependent on this series of reactions, as glutamine deprivation or genetic inhibition of any enzyme in this pathway leads to an increase in reactive oxygen species and a reduction in reduced glutathione. Moreover, knockdown of any component enzyme in this series of reactions also results in a pronounced suppression of PDAC growth *in vitro* and *in vivo*. Furthermore, we establish that the reprogramming of glutamine metabolism is mediated by oncogenic KRAS, the signature genetic alteration in PDAC, through the transcriptional upregulation and repression of key metabolic enzymes in this pathway. The essentiality of this pathway in PDAC and the fact that it is dispensable in normal cells may provide novel therapeutic approaches to treat these refractory tumours.

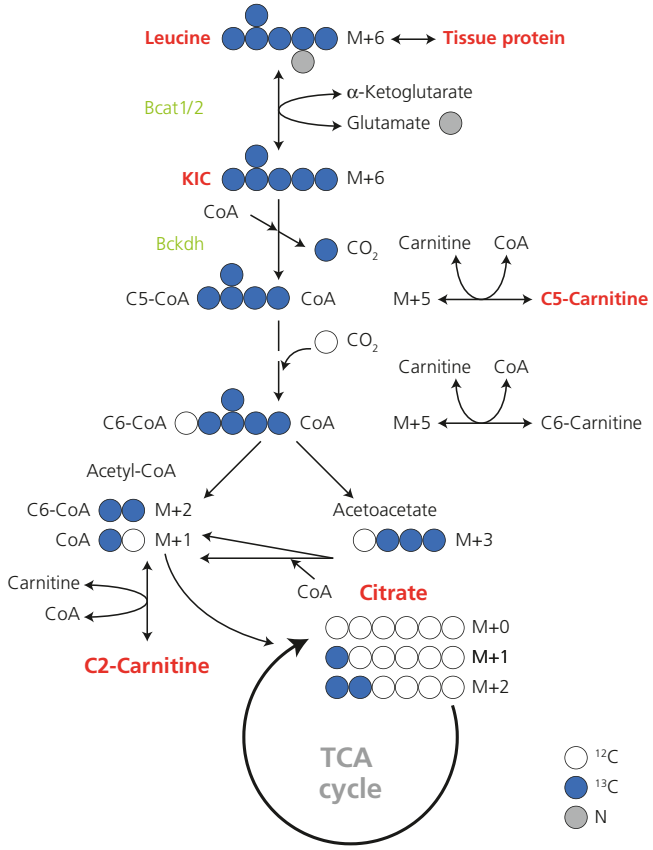
Branched-Chain Amino Acids



Branched-chain amino acids (BCAAs) are the essential amino acids of leucine, valine, and isoleucine. BCAAs are not only used in protein synthesis, but also as building blocks for sterols, ketone bodies, and glucose biosynthesis. Leucine works as a signaling molecule affecting the mTOR pathway as well. Because the BCAAs are used in so many cellular building blocks and signaling pathways, there has been quite a bit of interest in understanding their role in cancer metabolism.

Products of Interest

Catalog No.	Description
CLM-2248-H	L-Isoleucine ($^{13}C_6$, 99%)
NLM-292	L-Isoleucine (^{15}N, 98%)
CNLM-561-H	L-Isoleucine ($^{13}C_6$, 99%; ^{15}N, 99%)
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
DLM-1259	L-Leucine (5,5,5-D_3, 99%)
NLM-142	L-Leucine (^{15}N, 98%)
CNLM-281-H	L-Leucine ($^{13}C_6$, 99%; ^{15}N, 99%)
CLM-2249-H	L-Valine ($^{13}C_5$, 99%)
DLM-488	L-Valine (D_8, 98%)
NLM-316	L-Valine (^{15}N, 98%)
CNLM-442-H	L-Valine ($^{13}C_5$, 99%; ^{15}N, 99%)



Right: Diagram of the leucine catabolic pathway. Red labels indicate metabolites measured by mass spectrometry. Blue circles indicate ^{13}C-labeled carbons. KIC = α-ketoisocaproate.

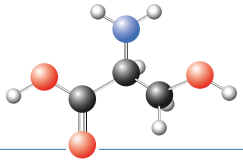
Tissue of Origin Dictates Branched-Chain Amino Acid Metabolism in Mutant *Kras*-Driven Cancers

Mayers, J.R.; Torrence, M.E.; Danai, L.V.; Papagiannakopoulos, T.; Davidson, S.M.; Bauer, M.R.; Lau, A.N.; Ji, B.W.; Dixit, P.D.; Hosios, A.M.; Muir, A.; Chin, C.R.; Freinkman, E.; Jacks, T.; Wolpin, B.M.; Vitkup, D.; Vander Heiden, M.G.
Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts USA
2016. *Science, 353(6304)*, 1161-1165. PMID: 27609895

ABSTRACT Tumor genetics guides patient selection for many new therapies, and cell culture studies have demonstrated that specific mutations can promote metabolic phenotypes. However, whether tissue context defines cancer dependence on specific metabolic pathways is unknown. *Kras* activation and *Trp53* deletion in the pancreas or the lung result in pancreatic ductal adenocarinoma (PDAC) or non-small cell lung carcinoma (NSCLC), respectively, but despite the same initiating events, these tumors utilize branched-chain amino acids (BCAAs) differently. NSCLC tumors incorporate free BCAAs into tissue protein and use BCAAs as a nitrogen source, while PDAC tumors have decreased BCAA uptake. These differences are reflected in expression levels of BCAA catabolic enzymes in both mice and humans. Loss of Bcat1 and Bcat2, the enzymes responsible for BCAA utilization, impairs NSCLC tumor formation, but these enzymes are not required for PDAC tumor formation, arguing that tissue-of-origin is an important determinant of how cancers satisfy their metabolic requirements.

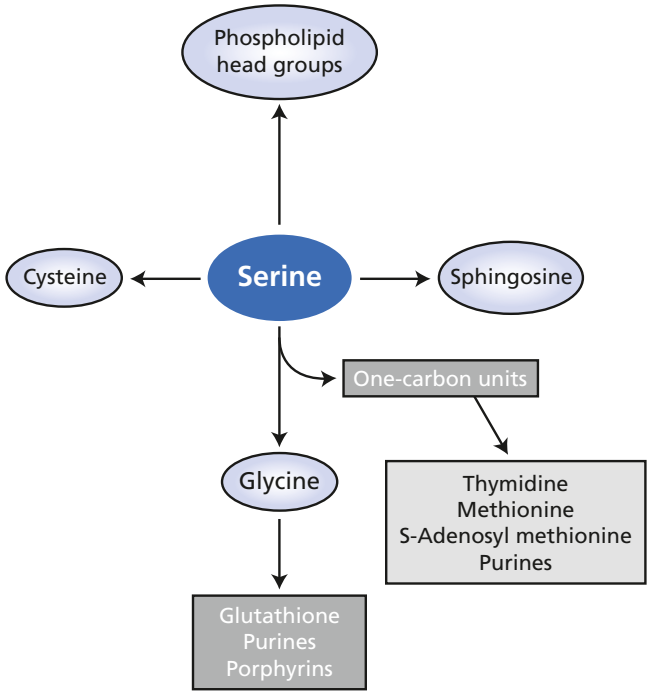
Graphic from above paper.

Serine



Serine

Since the early 1970s, it has been known that serine metabolism is different in cancer cells. Serine is important because it is a precursor for many different metabolites such as glycine, cysteine, porphyrins, nucleotide bases, shingolipids, and contributes to the one-carbon pool. With serine being linked to so many compounds, understanding its metabolism is very important. CIL offers a variety of labeled serines to probe cancer metabolism.



Products of Interest

Catalog No.	Description
CLM-1573	L-Serine (1-^{13}C, 99%)
CLM-1574-H	L-Serine ($^{13}C_3$, 99%)
DLM-161	L-Serine (3,3-D_2, 98%)
DLM-582	L-Serine (2,3,3-D_3, 98%)
NLM-2036	L-Serine (^{15}N, 98%)
CNLM-474-H	L-Serine ($^{13}C_3$, 99%; ^{15}N, 99%)
DNLM-6863	L-Serine (2,3,3-D_3, 98%; ^{15}N, 98%)
OLM-9660	L-Serine (carboxyl-$^{18}O_2$, 95%)

Serine One-Carbon Catabolism with Formate Overflow

Meiser, J.; Tumanov, S.; Maddocks, O.; Labuschagne, C.F.; Athineos, D.; Van Den Broek, N.; Mackay, G.M.; Gottlieb, E.; Blyth, K.; Vousden, K.; Kamphorst, J.J.; Vazquez, A.

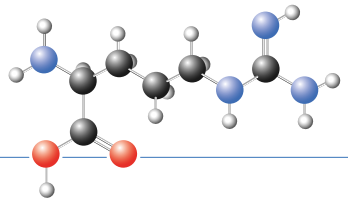
Cancer Research UK Beatson Institute, Glasgow, UK

2016. *Sci Adv, 2(10),* e1601273. PMID: 27819051

ABSTRACT Serine catabolism to glycine and a one-carbon unit has been linked to the anabolic requirements of proliferating mammalian cells. However, genome-scale modeling predicts a catabolic role with one-carbon release as formate. We experimentally prove that in cultured cancer cells and nontransformed fibroblasts, most of the serine-derived one-carbon units are released from cells as formate, and that formate release is dependent on mitochondrial reverse 10-CHO-THF synthetase activity. We also show that in cancer cells, formate release is coupled to mitochondrial complex I activity, whereas in nontransformed fibroblasts, it is partially insensitive to inhibition of complex I activity. We demonstrate that in mice, about 50% of plasma formate is derived from serine and that serine starvation or complex I inhibition reduces formate synthesis *in vivo*. These observations transform our understanding of one-carbon metabolism and have implications for the treatment of diabetes and cancer with complex I inhibitors.

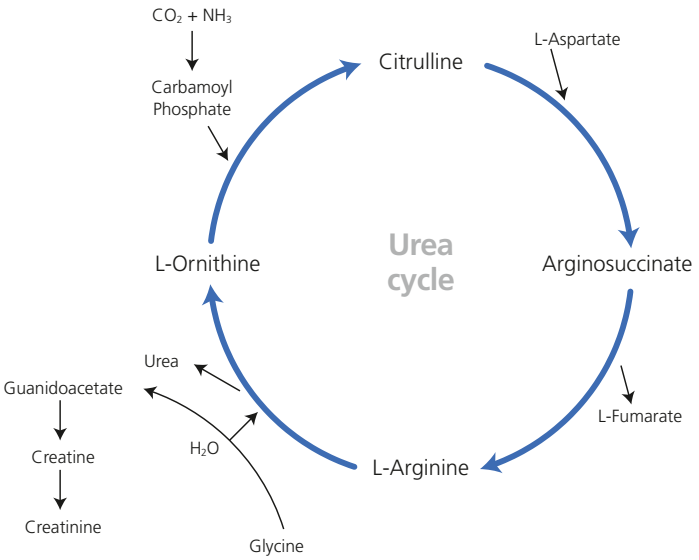
Graphic from above paper.

Arginine



Arginine

The significance of cancer metabolism on the urea cycle is not yet fully understood. The main purpose of the urea cycle is the removal of nitrogenous waste in the form of urea. The urea cycle intermediate, ornithine, is used to synthesize the polyamines used for cell proliferation. Disrupting the arginine availability for cancer metabolism could lead to a drugable target and possibly improve patient outcome. CIL has the widest range of label patterns of citrulline, ornithine, and arginine for tracing the urea cycle.



Products of Interest

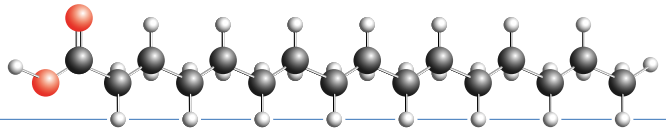
Catalog No.	Description
DLM-7476	ADMA·HCl·XH_2O (asymmetric dimethylarginine) (2,3,3,4,4,5,5-D_7, 98%) may be hydrate
CLM-2265-H	L-Arginine·HCl ($^{13}C_6$, 99%)
NLM-395	L-Arginine·HCl (guanido-$^{15}N_2$, 98%+)
NLM-396	L-Arginine·HCl ($^{15}N_4$, 98%)
CNLM-539-H	L-Arginine·HCl ($^{13}C_6$, 99%; $^{15}N_4$, 99%)
CNLM-9007-CA	L-Argininosuccinic acid barium salt·$2H_2O$ (arginine-$^{13}C_6$, 99%; $^{15}N_4$, 99%) CP 90%+
CLM-8699	L-Asparagine·H_2O ($^{13}C_4$, 99%)
CLM-1801	L-Aspartic acid ($^{13}C_4$, 99%)
CLM-4899	L-Citrulline (ureido-^{13}C, 99%)
DLM-6039	L-Citrulline (4,4,5,5-D_4, 95%)
CDLM-8808	L-Citrulline (ureido-^{13}C, 99%; 3,3,4-D_3, 98%)
CLM-7933	Creatine (guanidino-^{13}C, 99%)
DLM-1302	Creatine (methyl-D_3, 98%) CP 97%
DLM-3653	Creatinine (*N*-methyl-D_3, 98%)
CLM-1822-H	L-Glutamine ($^{13}C_5$, 99%)
NLM-1328	L-Glutamine ($^{15}N_2$, 98%)
NLM-823	Nitric oxide (^{15}N, 98%+)
CLM-4724-H	L-Ornithine·HCl ($^{13}C_5$, 99%)
DLM-6046	L-Ornithine·HCl (4,4,5,5-D_4, 95%)
DLM-2969	L-Ornithine·HCl (3,3,4,4,5,5-D_6, 98%)
NLM-3610	L-Ornithine·HCl ($^{15}N_2$, 98%)
CDLM-3873	L-Ornithine·HCl (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)
CNLM-7578-H	L-Ornithine·HCl ($^{13}C_5$, 99%; $^{15}N_2$, 99%)
CLM-311	Urea (^{13}C, 99%)
NLM-233	Urea ($^{15}N_2$, 98%+)
CNLM-234	Urea (^{13}C, 99%; $^{15}N_2$, 98%+)

Reduced Arginine Availability and Nitric Oxide Synthesis in Cancer is Related to Impaired Endogenous Arginine Synthesis

Engelen, M.P.; Safar, A.M.; Bartter, T.; Koeman, F.; Deutz, N.E.
Center for Translational Research in Aging & Longevity, Health and Kinesiology, Texas A&M University, College Station, Texas USA
Department of Geriatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas USA
2016. *Clin Sci, 130(14)*, 1185-1195. PMID: 27129191

ABSTRACT Reduced plasma arginine (ARG) concentrations are found in various types of cancer. ARG and its product nitric oxide (NO) are important mediators in the immune function and the defense against tumour cells. It remains unclear whether the diminished systemic ARG availability in cancer is related to insufficient endogenous ARG synthesis, negatively affecting NO synthesis, and whether a dietary amino acid mixture is able to restore this. In 13 patients with advanced non-small cell lung cancer (NSCLC) and 11 healthy controls, whole body ARG and CIT (citrulline) rates of appearance were measured by stable isotope methodology before and after intake of a mixture of amino acids as present in whey protein. The conversions of CIT to ARG (indicator of *de novo* ARG synthesis) and ARG to CIT (marker of NO synthesis), and ARG clearance (reflecting ARG disposal capacity) were calculated. Plasma isotopic enrichments and amino acid concentrations were measured by LC-MS/MS. Conversions of CIT to ARG and ARG to CIT ($P<0.05$), and CIT rate of appearance ($P=0.07$) were lower in NSCLC. ARG rate of appearance and clearance were comparable suggesting no enhanced systemic ARG production and disposal capacity in NSCLC. After intake of the mixture, ARG rate of appearance and concentration increased ($P<0.001$), and ARG to CIT conversion was restored in NSCLC. In conclusion, an impaired endogenous ARG synthesis plays a role in the reduced systemic ARG availability and NO synthesis in advanced NSCLC. Nutritional approaches may restore systemic ARG availability and NO synthesis in cancer, but the clinical implication remains unclear.

Fatty Acid Synthesis

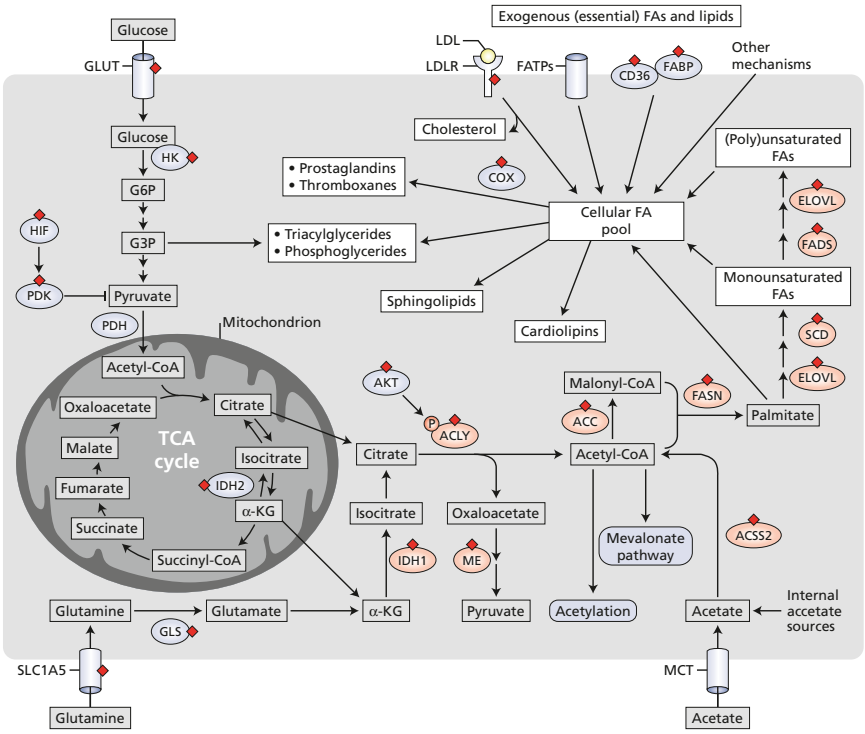


Palmitic acid

It is well-known that synthesis is impacted by cancer. Lipids are important to cancer cells to provide cellular components for the rapidly dividing cells. Fatty acids also serve as an alternative source of ATP (other than glucose), to meet the increased requirement for energy. *De novo* synthesis of fatty acids can be measured using sodium acetate (1-^{13}C) or deuterium oxide. The impact on the pathways is not fully understood, or if it could be a possible drugable target in cancer metabolism.

Products of Interest

Catalog No.	Description
DLM-4-70	Deuterium oxide (D, 70%)
DLM-4-99	Deuterium oxide (D, 99%)
CLM-1396	D-Glucose ($^{13}C_6$, 99%)
CLM-2248-H	L-Isoleucine ($^{13}C_6$, 99%)
CLM-2262-H	L-Leucine ($^{13}C_6$, 99%)
CLM-460	Oleic acid ($^{13}C_{18}$, 98%)
CLM-7896	Palmitic acid (1,2,3,4-$^{13}C_4$, 99%)
CLM-409	Palmitic acid ($^{13}C_{16}$, 98%)
DLM-215	Palmitic acid (D_{31}, 98%)
CLM-6865	Potassium palmitate (1,2,3,4-$^{13}C_4$, 99%)
CLM-3943	Potassium palmitate ($^{13}C_{16}$, 98%+)
CLM-156	Sodium acetate (1-^{13}C, 99%)
CLM-6990	Stearic acid ($^{13}C_{18}$, 98%) CP 97%)
DLM-379	Stearic acid (D_{35}, 98%)
CLM-2249-H	L-Valine ($^{13}C_5$, 99%)



Inhibition of Acetyl-CoA Carboxylase Suppresses Fatty Acid Synthesis and Tumor Growth of non-Small-Cell Lung Cancer in Preclinical Models

Svensson, R.U.; Parker, S.J.; Eichner, L.J.; Kolar, M.J.; Wallace, M.; Brun, S.N.; Lombardo, P.S.; Van Nostrand, J.L.; Hutchins, A.; Vera, L.; Gerken, L.; Greenwood, J.; Bhat, S.; Harriman, G.; Westlin, W.F.; Harwood, H.J., Jr.; Saghatelian, A.; Kapeller, R.; Metallo, C.M.; Shaw, R.J.

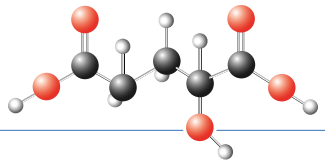
Department of Molecular and Cell Biology, Salk Institute for Biological Studies, San Diego, La Jolla, California USA

2016. *Nat Med, 22(10),* 1108-1119. PMID: 27643638

ABSTRACT Continuous *de novo* fatty acid synthesis is a common feature of cancer that is required to meet the biosynthetic demands of a growing tumor. This process is controlled by the rate-limiting enzyme acetyl-CoA carboxylase (ACC), an attractive but traditionally intractable drug target. Here we provide genetic and pharmacological evidence that in preclinical models ACC is required to maintain the *de novo* fatty acid synthesis needed for growth and viability of non-small-cell lung cancer (NSCLC) cells. We describe the ability of ND-646-an allosteric inhibitor of the ACC enzymes ACC1 and ACC2 that prevents ACC subunit dimerization-to suppress fatty acid synthesis *in vitro* and *in vivo*. Chronic ND-646 treatment of xenograft and genetically engineered mouse models of NSCLC inhibited tumor growth. When administered as a single agent or in combination with the standard-of-care drug carboplatin, ND-646 markedly suppressed lung tumor growth in the Kras;Trp53$^{-/-}$ (also known as KRAS p53) and Kras;Stk11$^{-/-}$ (also known as KRAS Lkb1) mouse models of NSCLC. These findings demonstrate that ACC mediates a metabolic liability of NSCLC and that ACC inhibition by ND-646 is detrimental to NSCLC growth, supporting further examination of the use of ACC inhibitors in oncology.

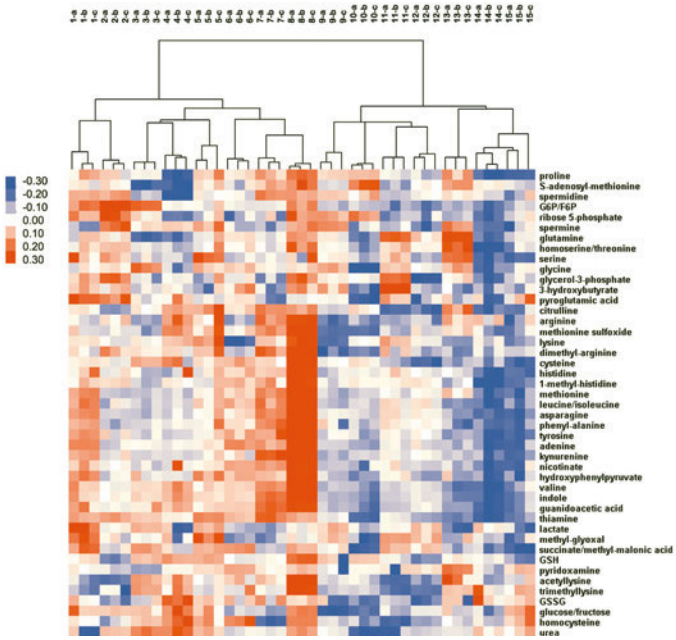
Graphic: The multifaceted roles of fatty acid synthesis in cancer. 2016. *Nature Reviews Cancer.*

Metabolomics/Biomarkers



DL-2-Hydroxyglutaric acid

Quite a bit of research has been done on untargeted and targeted metabolomics. The goal of the studies is to determine possible biomarker(s) for cancer, which could lead to earlier detection and treatment, thus leading to better patient outcome. Because cancer metabolism influences so many different pathways, it is proving difficult to determine specific markers that are elevated in a particular disease state. CIL offers a wide range of products to aid in quantitation of metabolites, including individual chemicals, internal standard mixtures, and biological extracts.



Products of Interest

Catalog No.	Description
CLM-10351	DL-2-Hydroxyglutaric acid, disodium salt ($^{13}C_5$, 99%)
DLM-9104	(RS)-2-Hydroxyglutaric acid, disodium salt (2,3,3-D_3; OD, 98%) CP 95%
MSK-CRED-KIT	Credentialed *E. coli* Cell Extract Kit (in solution) (^{13}C-labeled and unlabeled)
MSK-CRED-DD-KIT	Credentialed *E. coli* Cell Extract Kit (dried down) (^{13}C-labeled and unlabeled)
MSK-A2-1.2	Metabolomics Amino Acid Mix Standard
MSK-A2-US-1.2	Metabolomics Amino Acid Mix Unlabeled Standard
MSK-QC-KIT	Metabolomics QC Kit
NSK-A	Labeled Amino Acid Standards Set A
NSK-B	Labeled Carnitine Standards Set B
NSK-B-G1	Labeled Carnitine Standards Supplement to NSK-B
ISO1	Metabolite Yeast Extract (U-^{13}C, 98%)

Please visit **isotope.com** for a complete listing of isotope-labeled products.

Procedures for Large-Scale Metabolic Profiling of Serum and Plasma Using Gas Chromatography and Liquid Chromatography Coupled to Mass Spectrometry

Dunn, W.B.; Broadhurst, D.; Begley, P.; Zelena, E.; Francis-McIntyre, S.; Anderson, N.; Brown, M.; Knowles, J.D.; Halsall, A.; Haselden, J.N.; Nicholls, A.W.; Wilson, I.D.; Kell, D.B.; Goodacre, R.; and the Human Serum Metabolome (HUSERMET) Consortium.

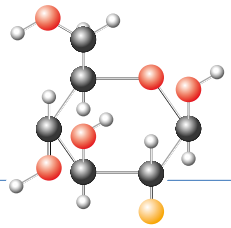
Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, University of Manchester, Manchester, UK

2011. *Nat Protoc, 6(7),* 1060-1083. PMID: 21720319

ABSTRACT Metabolism has an essential role in biological systems. Identification and quantitation of the compounds in the metabolome is defined as metabolic profiling, and it is applied to define metabolic changes related to genetic differences, environmental influences, and disease or drug perturbations. Chromatography-mass spectrometry (MS) platforms are frequently used to provide the sensitive and reproducible detection of hundreds to thousands of metabolites in a single biofluid or tissue sample. Here we describe the experimental workflow for long-term and large-scale metabolomic studies involving thousands of human samples with data acquired for multiple analytical batches over many months and years. Protocols for serum- and plasma-based metabolic profiling applying gas chromatography-MS (GC-MS) and ultraperformance liquid chromatography-MS (UPLC-MS) are described. These include biofluid collection, sample preparation, data acquisition, data pre-processing, and quality assurance. Methods for quality control-based robust LOESS signal correction to provide signal correction and integration of data from multiple analytical batches are also described.

Image courtesy of Ralph DeBerardinis

Positron Emission Tomography (PET)



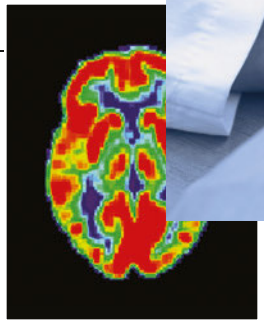
2-Fluoro-2-deoxy-D-glucose (FDG)

CIL has been dedicated to the PET market since the completion of its first ^{18}O-separation facility in 2001. CIL then expanded its ^{18}O-separation capabilities in 2003 and 2012. CIL also purchased Germany-based Advanced Biochemical Compounds (ABX) in 2006. The addition of this company allowed for a complete solution offering for the PET community. ABX specializes in the reagents, precursors, kits, and targets used during the manufacture of PET probes.

Products of Interest

Catalog No.	Description
OLM-240	Water-^{18}O (97%)
OLM-212	Oxygen ($^{18}O_2$, 97%) CP >99.8%
CGM-P39	Cyclotron Target Gas Mixture 39 ($^{15}N_2$, 99%:1 O_2) (CODE I-460) CP 99.99%

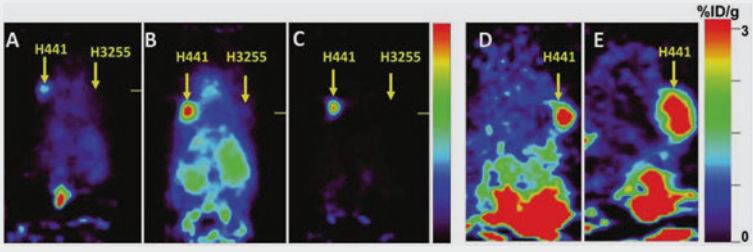
ABX
advanced biochemical compounds



Positron Emission Tomography (PET) Imaging with ^{18}F-Based Radiotracers

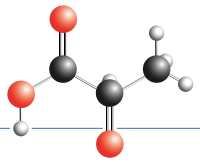
Alauddin, M.
University of California, Department of Radiology, Davis, California USA
2017. *J Med Imaging (Bellingham), 4(1),* 011013. PMID: 28401173

ABSTRACT Positron emission tomography (PET) is a nuclear medicine imaging technique that is widely used in early detection and treatment followup of many diseases, including cancer. This modality requires positron-emitting isotope-labeled biomolecules, which are synthesized prior to performing imaging studies. Fluorine-18 is one of the several isotopes of fluorine that is routinely used in radiolabeling of biomolecules for PET, because of its positron-emitting property and favorable half-life of 109.8 min. The biologically active molecule most commonly used for PET is 2-deoxy-2-(18)F-fluoro-β-D-glucose ((18)F-FDG), an analogue of glucose, for early detection of tumors. The concentrations of tracer accumulation (PET image) demonstrate the metabolic activity of tissues in terms of regional glucose metabolism and accumulation. Other tracers are also used in PET to image the tissue concentration. In this review, information on fluorination and radiofluorination reactions, radiofluorinating agents, and radiolabeling of various compounds and their application in PET imaging is presented.



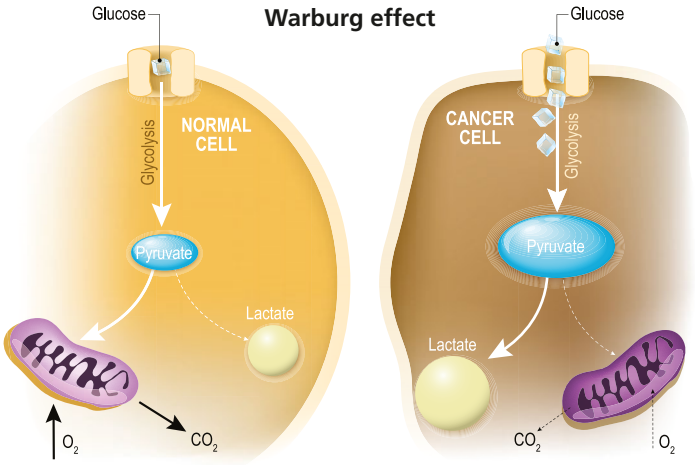
PET images of tumor-bearing mice using A) ^{18}F-L-FMAU, B) ^{18}F-D-FMAU, C) ^{18}F-FLT, D) N^3-^{18}F-FET, and E) N^3-^{18}F-FPrT.

Hyperpolarization



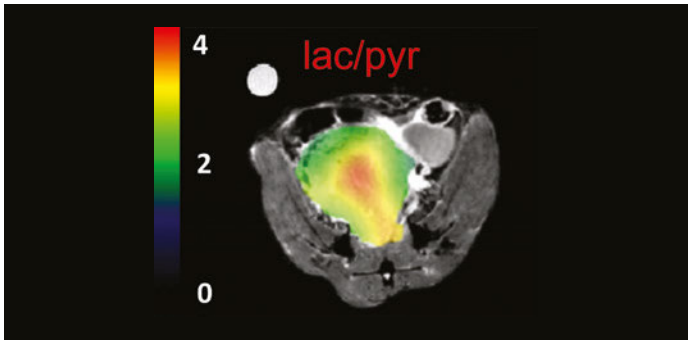
Pyruvic acid

A huge limitation to ^{13}C magnetic resonance spectroscopy (MRS) has been the sensitivity of the detection. Hyperpolarization overcomes this shortcoming by increasing signal-to-noise ratios by up to 100,000 times for a short period of time, allowing metabolism to be measured. Currently, researchers are taking advantage of the Warburg effect and measuring the lactate-to-pyruvate ratio to determine disease state or treatment response. Other applications for this technique are probing different metabolic and physiological conditions such as pH, perfusion, redox state, cardiac infarctions, gluconeogenesis, and renal function.



Products of Interest

Catalog No.	Description
CLM-3085	L-Ascorbic acid (1-^{13}C, 99%)
CDLM-6062	Fumaric acid (1-^{13}C, 99%; 2,3-D_2, 98%)
CDLM-8473	Fumaric acid (1,4-$^{13}C_2$, 99%; 2,3-D_2, 98%)
CDLM-3813	D-Glucose ($^{13}C_6$, 99%; 1,2,3,4,5,6,6-D_7, 97-98%)
CLM-1166	L-Glutamine (5-^{13}C, 99%)
CLM-8077	Pyruvic acid (1-^{13}C, 99%)
CLM-8849	Pyruvic acid (2-^{13}C, 99%) CP 95%
CLM-9505	Pyruvic acid (1,2-$^{13}C_2$, 99%)
CLM-1577	Sodium L-lactate (1-^{13}C, 99%) 20% w/w in H_2O
CLM-311	Urea (^{13}C, 99%)
CNLM-234	Urea (^{13}C, 99%; $^{15}N_2$, 98%+)



Metabolic Imaging of Patients with Prostate Cancer Using Hyperpolarized [1-^{13}C]Pyruvate

Nelson, S.J.; Kurhanewicz, J.; Vigneron, D.B.; Larson, P.E.Z.; Harzstark, A.L.; Ferrone, M.; van Criekinge, M.; Chang, J.W.; Bok, R.; Park, I.; Reed, G.; Carvajal, L.; Small, E.J.; Munster, P.; Weinberg, V.K.; Ardenkjaer-Larsen, J.H.; Chen, A.P.; Hurd, R.E.; Odegardstuen, L-I; Robb, F.J.; Tropp, J.; Murray, J.A.

Surbeck Laboratory of Advanced Imaging, Department of Radiology and Biomedical Imaging, University of California, San Francisco, California USA

2013. *Sci Transl Med, 5(198)*. PMID: 23946197

ABSTRACT This first-in-man imaging study evaluated the safety and feasibility of hyperpolarized [1-^{13}C]pyruvate as an agent for noninvasively characterizing alterations in tumor metabolism for patients with prostate cancer. Imaging living systems with hyperpolarized agents can result in more than 10,000-fold enhancement in signal relative to conventional magnetic resonance (MR) imaging. When combined with the rapid acquisition of *in vivo* ^{13}C MR data, it is possible to evaluate the distribution of agents such as [1-^{13}C]pyruvate and its metabolic products lactate, alanine, and bicarbonate in a matter of seconds. Preclinical studies in cancer models have detected elevated levels of hyperpolarized [1-^{13}C]lactate in tumor, with the ratio of [1-^{13}C]lactate/[1-^{13}C]pyruvate being increased in high-grade tumors and decreased after successful treatment. Translation of this technology into humans was achieved by modifying the instrument that generates the hyperpolarized agent, constructing specialized radio frequency coils to detect ^{13}C nuclei, and developing new pulse sequences to efficiently capture the signal. The study population comprised patients with biopsy-proven prostate cancer, with 31 subjects being injected with hyperpolarized [1-^{13}C]pyruvate. The median time to deliver the agent was 66 s, and uptake was observed about 20 s after injection. No dose-limiting toxicities were observed, and the highest dose (0.43 mL/kg of 230 mM agent) gave the best signal-to-noise ratio for hyperpolarized [1-^{13}C]pyruvate. The results were extremely promising in not only confirming the safety of the agent but also showing elevated [1-^{13}C]lactate/[1-^{13}C]pyruvate in regions of biopsy-proven cancer. These findings will be valuable for noninvasive cancer diagnosis and treatment monitoring in future clinical trials.

Above-right image courtesy of John Kurhanewicz

Research Use of CIL Products

CIL manufactures highly pure research biochemicals that are produced for research applications. As a service to our customers, some of these materials have been tested for the presence of *S.aureus, P. aeruginosa, E. coli, Salmonella sp,* aerobic bacteria, yeast and mold as well as, the presence of endotoxin in the bulk material by taking a random sample of the bulk product. Subsequent aliquots are not retested. Presence of endotoxin is assessed by determining endotoxin content following established protocols and standardized limulus amebocyte lysate (LAL) reagents. These tests are provided at no charge for any materials listed in our catalog or website that is designated as "MPT" (microbiologically and pyrogen tested) in the item product number (i.e, DLM-349-MPT).

CIL is able to provide microbiological testing for other products. Depending on the compound and the quantity ordered, an additional charge may apply. Please note that microbiological-tested products are not guaranteed to be sterile and pyrogen free when received by the customer, and microbiological testing does not imply suitability for any desired use. If the product must be sterile and pyrogen-free for a desired application, CIL recommends that the product be packaged or formulated into its ultimate dose form by the customer or appropriate local facility. The product should always be tested by a qualified pharmacy/facility prior to actual use.

CIL research products are labeled "For research use only. Not for use in diagnostic procedures." Persons intending to use CIL products in applications involving humans are responsible for complying with all applicable laws and regulations including but not limited to the US FDA, other local regulatory authorities and institutional review boards concerning their specific application or desired use.

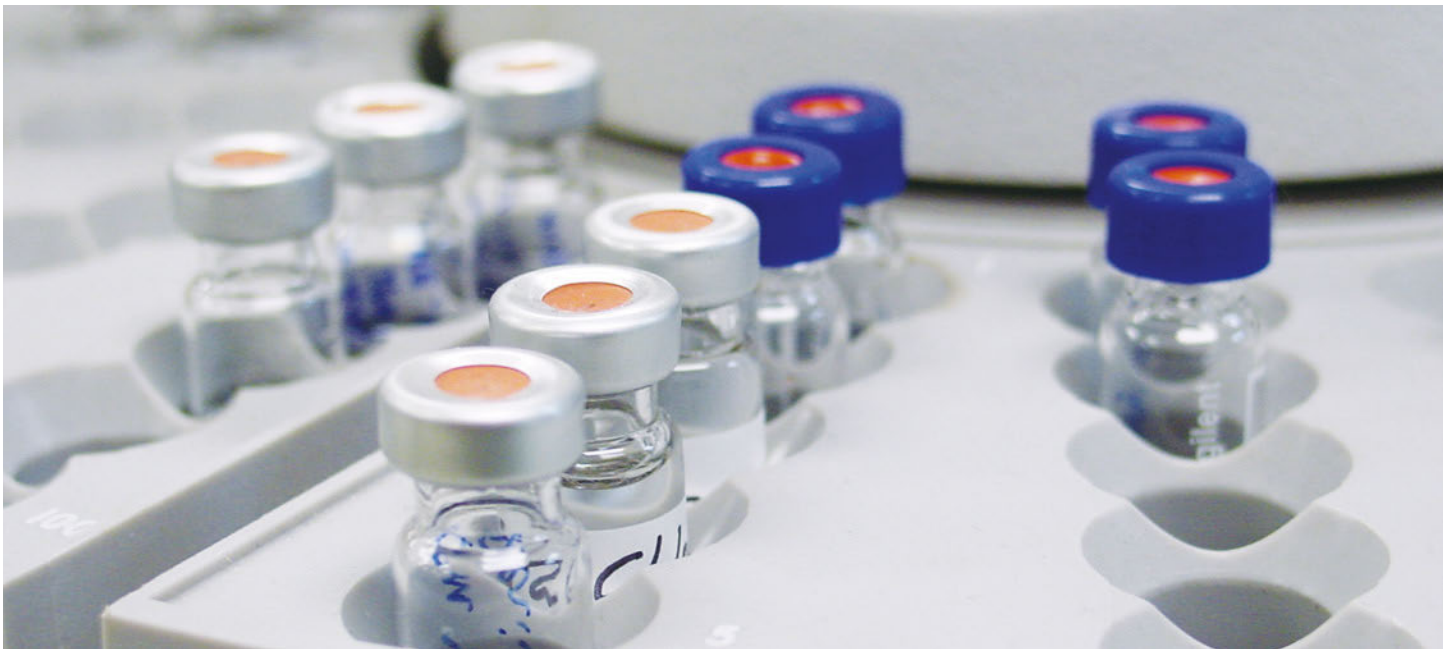
It may be necessary to obtain approval for using these research products in humans from the US FDA or the comparable governmental agency in the country of use. CIL will provide supporting information, such as lot-specific analytical data and test method protocols, to assist medical research groups in obtaining approval for the desired use. An Enhanced Technical Data Package (EDP) is also available.

CIL will allocate a specific lot of a product to customers who are starting long-term projects requiring large amounts of material. Benefits from this type of arrangement include experimental consistency arising from use of only one lot, no delay in shipments, and guaranteed stock. Please note that some CIL products have a specific shelf life and cannot be held indefinitely. If interested, please contact your sales manager for further details.

Because of increasing regulatory requirements, CIL manufactures different grades of materials to help researchers with those requirements. Listed below are the grades of materials that CIL currently manufactures:

Catalog No.	Description
CLM-XXX-**PK**	Research grade
CLM-XXX-**MPT**	Microbiologically and Pyrogen Tested
CLM-XXX-**CTM**	Manufactured following ICH Q7, Section XIX
CLM-XXX-**GMP**	Good Manufacturing Practices grade

For more information on controls in manufacturing and testing of the different grades, go to: Search → Literature → Product Quality Designations from the isotope.com home page.



CIL's cGMP Production Capabilities

With increasing requirements from institutional review boards (IRBs) and governmental agencies, partnering with CIL for your next stable isotope cGMP (current good manufacturing practices) project can help ensure your regulatory compliance. With the world's largest ^{13}C and ^{18}O isotope-separation plants, CIL is able to provide the raw materials necessary for your project. Your compound of interest most likely already appears in CIL's extensive list of research compounds – if not, CIL's team of PhD chemists can determine the best method of synthesis for incorporating ^{13}C, ^{15}N, D, ^{17}O, and/or ^{18}O into your compound.

CIL has manufactured bulk active pharmaceutical ingredients (APIs) since 1994. It recently added a 15,000-square-foot, state-of-the-art cGMP facility to complement its existing cGMP facilities. An additional team of experts – specializing in synthetic chemistry, customer support, quality control, and quality assurance – serves to provide technical guidance from beginning to end of your project.

Partner with CIL to help you meet your increasing regulatory compliance requirements.

Products of Interest

Catalog No.	Description
CLM-804-CTM	Cholesterol (3,4-$^{13}C_2$)
DLM-4-70-CTM	Deuterium oxide (D, 70%)
CLM-1396-CST	D-Glucose ($^{13}C_6$)
CLM-420-CST	D-Glucose (1-^{13}C)
DLM-349-CTM	D-Glucose (6,6-D_2)
DLM-1229-CST	Glycerol (1,1,2,3,3-D_5)
CLM-2262-CTM	L-Leucine ($^{13}C_6$)
DLM-1259-CTM	L-Leucine (5,5,5-D_3)
CLM-762-CTM	L-Phenylalanine (1-^{13}C)
CLM-8077-CTM	Pyruvic acid (1-^{13}C)
CLM-156-CTM	Sodium acetate (1-^{13}C)
CLM-440-CTM	Sodium acetate (1,2-$^{13}C_2$)
CLM-3276-GMP	Uracil (2-^{13}C)
CLM-311-GMP	Urea (^{13}C)

Other products may be available as CTM/cGMP. Please inquire for details.

Manufacturing Capabilities

- Dedicated development facility
- Five production and two isolation suites
- Dedicated packaging room
- Production scale from milligrams to multikilograms
- Clinical trials to bulk API
- Customizable projects to meet your needs

Analytical Services

- Fully equipped, cGMP-dedicated analytical facility
- Method development and validation
- Raw material and final product testing
- Wet chemistry and compendial methods
- Stability studies and chambers
- Analytical instrumentation:
  - High-field NMR (^1H, D, ^{13}C, ^{15}N, multinuclear)
  - HPLC with UV, RI, ELSD, DA, Pickering, and MS detection
  - GC with FID, ECD, and MS detection
  - KF
  - FT-IR
  - Polarimetery
  - TOC

Quality and Compliance

- Drug master files
- FDA-audited facility
- QA release of API product
- Follows FDA and ICH guidances
- CMC sections for NDA or IND

Enhanced Technical Data Package (EDP)

CIL offers the option of an Enhanced Technical Data Package (EDP). This data package is available for most MPT products. It includes all of the data currently included with the MPT products, as well as the additional information listed below. You have the option of purchasing this package at the time of order or at a later date.

Please note that if you choose to purchase at a later date, some of the information listed below may not be available. Also, the EDP may not be available for all lots. In some cases, only a partial EDP may be available. Please confirm availability and content prior to order.

Enhanced Technical Data Package Contents

- Additional testing data; products with an EDP have been tested to the specifications/monograph similar to those detailed in the USP or EP, but not using compendia methods.
- Product description: structural formula, stereochemical description, molecular formula.
- Product physical properties: melting point, pH, optical rotation (mix of literature or measured values).
- Outline of the synthesis route, including details of solvents used.
- Data used to confirm structure and chemical purity.
- Impurities: available data on impurities detected and identified together with the method of detection and the cutoff applied.
- Residual solvents: measured residual solvents from the final synthetic step and purification.
- Certificates of Analysis if raw materials where appropriate.
- Informal stability data: estimated and measured.
  - This will be either actual shelf life data, if it can be obtained from CIL history or by analysis of in-stock batches, or
  - If no data is available, CIL will commit to assaying the batch provided after six months and one year. Data will be provided after one year, unless the batch fails assay after six months. This option will not be available if the Enhanced Data Package is ordered at a later date.

CIL products are labeled "For Research Use Only. Not for use in diagnostic procedures."

CIL Application Notes of Interest

Application Note 44

Pathway-Targeted Metabolomic Analysis in Oral/Head and Neck Cancer Cells Using Ion Chromatography-Mass Spectrometry

Metabolomics aims to measure a wide breadth of small molecules (metabolome) in the context of physiological stimuli or disease states. The general problems encountered when characterizing the metabolome are the highly complex nature and the wide concentration range of the compounds. Separation science plays an important role in metabolomics by reducing the sample complexity to achieve a comprehensive profiling analysis. The strength of mass spectrometry (MS)-based metabolomics is best realized when coupled to a separation technique such as capillary electrophoresis, gas chromatography (GC), or liquid chromatography (LC). Ion chromatography (IC) or ion-exchange chromatography offers an excellent complementary platform for separation of charged and polar compounds. Because of its unique selectivity, IC has been coupled with MS for targeted screening and quantification of metabolites such as carbohydrates, organic acids, sugar phosphates, and nucleotides in biological samples. Metabolomics is now widely used in the characterization and diagnostic research of an ever-increasing number of diseases. *Read more at isotope.com*.

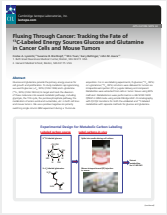
Application Note 43

Analysis of Whole-Body Branched-Chain Amino Acid Metabolism in Mice Utilizing 20% Leucine $^{13}C_6$ and 20% Valine $^{13}C_5$ Mouse Feed

Cancer cells have altered metabolism relative to normal cells. To date, most cancer metabolism research has focused on understanding the mechanisms of cell autonomous metabolic alterations such as the influence of different oncogenic signals on nutrient utilization and the effects of altered regulation of specific enzymes on metabolic fluxes through different pathways (Cairns, et al., 2011). While these studies have provided insight into metabolic needs of proliferating cancer cells (Vander Heiden, et al., 2009), they do not address potential interactions between tumor and normal tissues. Research on whole-body metabolic alterations associated with type 2 diabetes (T2DM) provides insight into how altered metabolite sensing can affect the metabolism of specific tissues. Intriguingly, there are clear epidemiological connections between diabetes and several types of cancer, especially pancreatic adenocarcinoma (PDAC) (Everhart and Wright, 1995; Wang, et al., 2003). Indeed, epidemiologic evidence indicates that pancreatic cancer can be both a consequence of longstanding diabetes (Ben, et al., 2011) and cause of new-onset cases (Huxley, et al., 2005). Methods to study metabolism across tissues are needed to understand how whole-body metabolic alterations influence tumor metabolism, and to understand the systemic changes associated with metabolic disease. *Read more at isotope.com*.

Application Note 34

Fluxing Through Cancer: Tracking the Fate of ^{13}C-Labeled Energy Sources Glucose and Glutamine in Cancer Cells and Mouse Tumors



Glucose and glutamine provide the primary energy sources for cell growth and proliferation. To study metabolic reprogramming, we used D-glucose ($^{13}C_6$, 99%) (CLM-1396) and L-glutamine ($^{13}C_5$, 99%) (CLM-1822-H) to target and track the diversion of these molecules into several metabolic pathways, including glycolysis, the TCA cycle, the pentose phosphate pathway, the metabolism of amino acids and nucleotides, etc. in both cell lines and mouse tumors. We use a positive/negative ion polarity switching single column SRM experiment during a 15-minute acquisition. For *in vivo* labeling experiments, D-glucose ($^{13}C_6$, 99%) or L-glutamine ($^{13}C_5$, 99%) solutions were delivered to tumors via intraperitoneal injection (IP) or jugular delivery and compared. Metabolites were extracted from cells or tumor tissues using 80% methanol. Metabolomics were performed on a SCIEX QTRAP® 5500 in SRM mode using amide XBridge HILIC chromatography with Q1/Q3 transitions for both the unlabeled and ^{13}C-labeled metabolites with separate methods for glucose and glutamine. *Read more at isotope.com*.

Please visit isotope.com for a complete list of isotope-labeled compounds.

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email: cilsales@isotope.com | telephone: 1.978.749.8000 | 1.800.322.1174 (North America)

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These articles do not imply that materials produced by CIL are suitable for any intended purpose.