

Research into Cancer Metabolomics: Towards a Clinical Metamorphosis

Orianne Olivares^{1,*}, J. Henry M. Däbritz^{2,*}, Ayala King¹, Eyal Gottlieb^{2,#} and Christina Halsey^{1,#}

1: Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, College of Medical Veterinary and Life Sciences, University of Glasgow, Garscube Estate, Switchback Road, Glasgow, UK

2: Cancer Research UK Beatson Institute, Garscube Estate, Switchback Road, Glasgow, UK

*: These authors contributed equally to the writing process

#: Corresponding authors: Prof. Eyal Gottlieb, e-mail: e.gottlieb@beatson.gla.ac.uk , tel: +44 (0) 141 330 3662, Dr. Christina Halsey, e-mail chris.halsey@glasgow.ac.uk, Tel: +44 (0)141 330 8135, Fax: +44 (0)141 942 6521

Abstract

The acknowledgement that metabolic reprogramming is a central feature of cancer has generated high expectations for major advances in both diagnosis and treatment of malignancies through addressing metabolism. These hopes have so far only been partially fulfilled, with only a few clinical applications. However, numerous diagnostic and therapeutic compounds are currently being evaluated in either clinical trials or pre-clinical models and new discoveries of alterations in metabolic genes indicate future prognostic or other applicable relevance. Altogether, these metabolic approaches now stand alongside current available measures providing hopes for the prospects of metabolomics in the clinic. Here we present a comprehensive overview of both ongoing and emerging clinical, pre-clinical and technical strategies for exploiting unique tumour metabolic traits, highlighting the current promises and hopes of research in the field.

Keywords: Biomarkers, Cancer Metabolism, Clinical Trial, Imaging, Targeted Therapy

Highlights:

- Metabolism-related tools are employed to diagnose and treat cancer.
- Promising metabolic tracers and anti-cancer drugs are in clinical trials.
- Metabolomics provides new information on the vulnerabilities of malignant diseases.

Abbreviations:

ACSS, Acetyl-CoA synthetase; ADI, arginine deiminases; AML, acute myeloid leukaemia; AMPK, AMP-activated protein kinase; ALL, acute lymphoblastic leukaemia; ASS, argininosuccinate synthase; BCAAs, branched-chain amino acids; BPTES, bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulphide; 2DG, 2-deoxyglucose; DHFR, dihydrofolate reductase; fas, difluoromethylornithine; FAS, fatty acid synthase; FDG, 18-fluoro-deoxyglucose; Fd-UMP, 5-fluoro-2'-deoxyuridine monophosphate; 18-F-FGln, 4-18-F-(2S,4R)-fluoroglutamine; FH, fumarate hydratase; FLT, 18-fluoro-thymidine; 5FU, 5-fluorouracil; F-UTP, 5-fluorouridine triphosphate; GIST, gastrointestinal stroma tumours; GLDC, glycine decarboxylase; GLS, glutaminases; 2HG, 2-hydroxyglutarate; HIF, hypoxia-inducible factor; IDH, isocitrate dehydrogenase; KD, ketogenic diets; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter, MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; NAPRT, nicotinic acid phosphoribosyltransferase; NAMPT, nicotinamide phosphoribosyltransferase; NMR, nuclear magnetic resonance; NSCLC, non-small cell lung cancer; PARP1, Poly [ADP-ribose] polymerase 1; PC, pyruvate carboxylase; PDAC, pancreatic ductal adenocarcinoma; PET, positron- emission tomography; PHGDH; D-3-phosphoglycerate dehydrogenase; RCC, renal cell carcinoma; SDH, succinate dehydrogenase; SHMT, serine hydroxyl-methyl-transferase; SIRT1; silent information regulator 1; TCA, tri-carboxylic acid; THF, tetrahydrofolate; TK1, thymidine kinase 1

1) Introduction

A metabolic rewiring whereby cells flexibly use alternative metabolic pathways depending on their needs - is now believed to occur in virtually all types of cancer [1]. The addiction of malignant cells to glucose was first discovered in the early 20th century [2], and by now, the ability of cancer cells to adapt their metabolism in response to challenging conditions is known to be a much more general phenomenon than previously thought. Besides glucose, a major role for glutamine as a metabolic substrate has been highlighted in several malignancies [3, 4]. The importance of non-essential amino acids like serine, proline and arginine has also been appreciated recently [5-8]. This accumulating knowledge raises the hopes that understanding tumour metabolism would provide new ways for predicting, diagnosing, and even treating cancers. However, to what extent has our current understanding in the field actually been converted into clinical applications?

Targeting tumour-specific metabolism in patients avoiding dose-limiting systemic toxicity has so far proved a difficult task since many metabolic alterations in cancers are quantitative differences in flux through physiological pathways. More promising targets may be found in tumours carrying mutations in genes encoding metabolic enzymes, resulting in the production of cancer cell-specific products, termed “oncometabolites”, such as 2-hydroxyglutarate (2HG), produced due to oncogenic mutations in isocitrate dehydrogenase (IDH), or succinate and fumarate, produced due to loss of function of succinate dehydrogenase (SDH) or fumarate hydratase (FH), respectively [9, 10]. These cancers are more likely to demonstrate profound adaptations to their genetically-imposed metabolic state which would create cancer-specific addictions and vulnerabilities.

Distinct metabolic profiles have already been described for certain tumours [11-13], several metabolic biomarkers are used in the clinic, and drugs targeting metabolic pathways are employed to treat cancers. However, many metabolism-specific cancer diagnostics and treatments are still at a pre-clinical stage, and routine targeting of metabolism as a general therapeutic strategy remains challenging. In this review, we present a comprehensive overview of the current clinical applications of metabolomics, of areas in advanced stages of development and of those that remain putative, thus

providing the reader with a timely clear view of the past, present and future of the bench-to-bedside studies of tumour metabolomes.

2) Currently approved clinical applications

In this chapter we review confirmed metabolic approaches firmly established as strategies for clinical management of cancer patients.

2.1) Anti-metabolites

Compounds that are potent inhibitors of cell proliferation through interference with RNA and DNA synthesis have been in use as anti-neoplastic agents for many decades. Known under the broad (and confusing) name “anti-metabolites” – the term refers *stricto sensu* to drugs that block nucleotide biosynthesis, affecting RNA/DNA metabolism – these drugs can be divided into several subgroups: anti-purines (*e.g.* 6-thioguanine), anti-pyrimidines (*e.g.* 5-fluorouracil - 5FU), nucleoside analogues (*e.g.* gemcitabine and cytarabine) and anti-folates (*e.g.* methotrexate) [14].

One of the most widely-used “anti-metabolite” is 5FU, a synthetic uracil analogue containing a fluoride atom at the fifth carbon position. 5FU is converted by thymidylate synthase into two active metabolites: 5-fluoro-2'-deoxyuridine monophosphate (Fd-UMP) and 5-fluorouridine triphosphate (F-UTP). However, as the fluoride atom prevents the addition of a methyl group at carbon 5 (necessary for further reactions), Fd-UMP and F-UTP inhibit thymidylate synthase activity via product inhibition, causing a lack of the nucleotide deoxythymidine monophosphate. Consequently, DNA replication is arrested, as is cell proliferation. In addition, F-UTP is incorporated into RNA as a “false building block”, leading to impairment of synthesis and maturation of all different classes of RNA [15, 16]. Due to its broad cytotoxic activity, 5FU and, increasingly, its orally available pro-drug capecitabine, is indicated for several malignancies, including most gastrointestinal cancers [17, 18]. Nucleoside analogues such as gemcitabine and cytarabine are routinely used as single agents or in combination with other drugs against a wide range of cancers,. As with 5FU, they are processed within the cell and incorporated into DNA thus impeding replication and proliferation [14].

Another class of widely used anti-metabolites comprises the anti-folates (*e.g.* methotrexate), antagonists of folic acid (vitamin B9) metabolism. In the 1940s, a folate antagonist (aminopterin) was found to be effective against acute lymphoblastic leukaemia (ALL) after the initial observation that low folate diets were able to lower the white blood cells counts of leukaemic patients [19]. With few toxic side effects and high efficiency, methotrexate was developed into a powerful standard treatment for ALL, lymphoma and more. Folate is normally transformed in cells by dihydrofolate reductase (DHFR) into dihydrofolate which is then reduced into tetrahydrofolate (THF), a methyl group donor to many enzymes, including thymidylate synthase. Anti-folates bind DHFR, diminishing the production of bases for DNA or RNA synthesis [14]. Of note, methotrexate toxicity to normal tissues is prevented by administering folinic acid starting from 24 up to 42 hours after methotrexate dosing [20].

2.2) Metabolic tracers for cancer detection

Some cancer-specific metabolic alterations are used as powerful diagnostic tools. Exploiting the enhanced glucose uptake by cancer cells, malignant glycolytic tissues are detected by administering the radioactively-labelled glucose analogue ¹⁸fluoro-deoxyglucose (FDG) and measuring site-specific radioactive emission by positron emission tomography (PET). Developed in 1978, FDG can be taken up by glycolytic cells and phosphorylated intracellularly by hexokinase, but as it lacks a 2' hydroxyl group needed for subsequent metabolism, it is retained in the cells in its phosphorylated form [21]. This detection method is now clinically used for various lymphomas and is being evaluated in a variety of other cancer types [22]. However, the inherent limitations of FDG-PET (costs, short half-life and “false positive” signals due to inflammatory processes), prevent across the board application [23].

2.3) Interfering with metabolite availability

Apart from glucose, tumours may require other specific nutrients, and different cancer types may have distinct metabolite requirements. For instance, ALL cells were found to be dependent on exogenous (“auxotrophic”) for both asparagine and glutamine [24], leading to the use of asparaginases as part of multimodal treatment approaches [25]. By hydrolysing asparagine to aspartate, asparaginases deplete

serum asparagine (as well as glutamine due to cross reactivity [26]), thus sensitising the weakened ALL cells to conventional chemotherapy. To our knowledge, the use of asparaginase for ALL remains the only case of direct translation of “pure scientific metabolic knowledge” to potent approved cancer treatment.

3) Emerging clinical metabolic technologies

This chapter describes advanced new investigational technologies which are in clinical application stages (illustrated in Figure 1).

3.1) Broadening the array of cancer tracers

A new generation of metabolic PET-tracers, beyond FDG, is emerging and are currently being evaluated in clinical trials. Additionally, hyperpolarised ^{13}C -labeled metabolic tracers for magnetic resonance spectroscopy (MRS) imaging are in rapid development. Table 1 summarises current clinical trials investigating metabolism-based approaches to cancer imaging.

3.1.1) ^{11}C -acetate

Systemically-available acetate is taken up by cells and transformed into acetyl-CoA, following either a catabolic route - feeding the TCA cycle - or an anabolic pathway for synthesis of fatty acid and, to a lesser extent, amino acids [27]. Indeed, many cancer types show high expression of fatty acid synthase (FAS) [28]. Yoshimoto *et al.* published the first study highlighting the metabolic role of acetate in malignant tumours. They showed acetate-derived ^{14}C accumulation in lipid membranes as well as a correlation between acetate consumption and the growth rate of cancer cell lines [27]. The metabolic importance of acetate was further emphasised in multiple malignancies [29-31]. Several studies using labelled (^{11}C) acetate as an imaging tracer have been published with promising results (e.g. [32]). It is important to note that since this, and other potentially potent PET tracers described below, are evaluated in clinical trials only (Table 1), they should be considered investigational.

3.1.2) Tracing one carbon metabolism? choline and methionine

Notably, ^{11}C -methionine (for brain tumours) and ^{11}C - and ^{18}F -choline derivatives (prostate and urological tumours, glioma and hepatocellular carcinoma) are in advanced investigational clinical use [23]. Methionine and choline are both involved in important inter-connected pathways of one carbon metabolism, including DNA methylation and THF production. Therefore, these tracers allow powerful detection for a range of malignancies.

3.1.3) ^{18}F -thymidine (FLT)

Taking advantage of the high DNA replication and cell division rates of many malignant tumours, a thymidine analogue, FLT, is used to directly detect tumour cell proliferation [33]. Following its phosphorylation by thymidine kinase 1 (TK1), which shows increased activity during S-phase of the cell cycle, FLT is trapped within dividing cells enabling their imaging [23]. Therefore, FLT-PET holds promise for distinguishing malignancy from inflammation and may allow the use of PET diagnostics for less avidly glycolytic cancers.

3.1.4) ^{18}F -glutamine

Glutamine, through its transformation to glutamate by glutaminases (GLS1 and GLS2), fuels the tri-carboxylic acid (TCA) cycle, contributes to the synthesis of non-essential amino acids via cytosolic or mitochondrial transaminases, and generates of “reducing power” [34]. Thus, 4- ^{18}F -(2S,4R)-fluoro-glutamine (^{18}F -FGln), designed to detect glutamine consumption and utilization, has been suggested as a tumour-specific tracer in both mouse brain tumour models and glioma patients, and is currently being clinically evaluated in other malignancies too [35, 36].

3.1.5) Hyperpolarise ^{13}C metabolic tracers

By dramatically increasing signal-to-noise-ratios, “hyperpolarisation” of nuclear spins of ^{13}C -labels metabolites has enabled MRS imaging of these isotopes *in vivo* [37]. Beyond evaluating their immediate transport, the metabolic fate of labelled substrates can be traced in various tissues [38]. This principle has been applied in different cancer models to assess extracellular pH (^{13}C -bicarbonate to $^{13}\text{CO}_2$ ratio), alterations in glycolytic flux (^{13}C -glucose to ^{13}C -lactate), production of the oncometabolite 2HG (from ^{13}C -glutamate), redox stress (^{13}C -dehydroascorbate), and for the detection

of cancer cells in a background of healthy mouse liver tissue ([1,3- $^{13}\text{C}_2$]ethylacetoacetate) [39-42]. Pre-clinical results with MRS imaging indicated that, due to its favourable hyperpolarisation characteristics and broad metabolic utilisation in malignant cells, ^{13}C -pyruvate is a lead candidate for tracing cancer metabolism *in situ* (conversion to ^{13}C -lactate by lactate dehydrogenase - LDH) and a good indicator for treatment-induced metabolic alterations [43, 44]. In line with this, Nelsen *et al.* recently reported the first clinical study employing hyperpolarised ^{13}C -pyruvate for tumour imaging. In 31 patients with localised prostate cancer, a higher pyruvate to lactate conversion in carcinomas compared to tumour-free tissues was confirmed [45]. Moreover, this method appeared superior to standard (^1H -based) magnetic resonance imaging (MRI) in diagnosing more aggressive carcinoma [45]. New metabolic MRS imaging hyperpolarised tracers are under development and the prospect of simultaneously hyperpolarising and tracking several molecules *in vivo*, highlight molecular metabolic imaging as one of the most rapidly advancing fields in cancer diagnostics [46].

3.2) Applied analytical metabolomics in the clinic: from biomarkers to novel molecular targets

The use of nuclear magnetic resonance (NMR) or mass-spectrometer techniques to provide medium- to high-throughput metabolic profiles, collectively known as ‘metabolomics’, is a powerful technology that has been recently incorporated into the arsenal of research tools in the clinic. In this chapter we demonstrate the power of analytical metabolomic investigations in humans. Besides the increasing application of metabolism-based imaging techniques described above, there are four main approaches for investigating cancer-driven metabolic re-programming in humans: First, tumour metabolomes can be compared to adjacent normal tissues after resection *in sano*. Second, metabolic tracing in patients after systemic administration of stable isotope-labelled substrates (*e.g.* ^{13}C -labelled glucose) can provide a more dynamic metabolic picture. Thirdly, more detailed metabolic tracing can be performed in thin tissue slices derived from freshly-resected tumours *ex vivo*. Lastly, body fluids such as blood, urine and saliva can be easily and repeatedly analysed for their composition at different stages of malignancy, in response to treatment or after administration of a labelled metabolite. This may provide valuable “integrative” insights into tumour metabolism and its dynamics, and enable the discovery of biomarkers that quantitatively or qualitatively correlate with certain malignancies.

Figure 1 summarises different approaches to investigating cancer metabolism in patients; a selection of recent studies is discussed below.

3.2.1) *Mechanistically-informative metabolic biomarkers*

In a metabolomics profiling study of untreated gliomas, 2HG was identified to be the major grade-discriminating metabolite being highly present in low grade tumours [13]. Furthermore, in higher grade tumours, three metabolically-defined subtypes (“anabolic”, “energetic” and “phospholipid catabolism”) were identified and correlated with prognostic outcome of the respective patients. These metabolic subgroups were largely independent from gene expression profile-based tumour stratification [13]. Another integrative study which metabolically characterized diffuse large B cell lymphoma confirmed a subset of tumours which exhibit increased oxidative energy metabolism [11]. In both studies, tumours’ metabolic profiling provided informative signatures that helped characterising these tumours, and suggested potential liabilities for future investigation.

Pancreatic cancer remains a rapidly fatal disease for most patients, partly since it is most often detected at an advanced stage. Taking advantage of a large number of blood samples acquired over multiple studies, Mayers *et al* searched for potential diagnostic biomarkers by identifying early changes in systemic metabolite concentrations, associated with the later development of pancreatic ductal adenocarcinoma (PDAC) [47]. Employing targeted metabolic profiling, they found that branched-chain amino acids (BCAAs) were elevated in the plasma of affected individuals two to ten years prior to cancer diagnosis with PDAC [47]. These findings were confirmed in a mouse model of PDAC, but not in other KRAS-associated cancers, implying profound tumour-specific changes in systemic protein (amino acid) metabolism at very preliminary stages of this malignancy. Thus a simple blood screening could detect PDAC at its initial stage, when complete surgical resection, the only curative option, is still possible. However, since elevated BCAAs levels were associated with “only” a two fold increase in PDAC risk and for a limited window prior to diagnosis, other prognosticators are required in order to define the population that would benefit from intensified surveillance [47]. Further studies of PDAC metabolomics and integration of results with genetic information, such as these performed by Zhang *et al.*, whose findings pointed toward de-regulated

fatty acid metabolism in PDAC [48], are likely to provide new insights and pave the way to new therapeutic strategies, urgently needed for this disease.

In another biomarker-driven study with more than 200 acute myeloid leukaemia (AML) patients many plasma metabolites identified to be elevated in comparison to healthy controls [49] and the profile of six central metabolites (lactate, α -ketoglutarate, pyruvate, 2HG, glycerol-3-phosphate and citrate) was sufficient for prognostic patient stratification in cytogenetically normal AML, a highly heterogenic subgroup with regards to therapeutic outcome. Model-based investigation further implied that high glycolytic activity could be associated with resistance to cytarabine, commonly used to treat AML, and that pharmacologically interfering with glycolysis may increase efficacy of cytarabine treatment in AML patients [49].

3.2.2) *Metabolomics-driven target identification: pyruvate carboxylase activity in lung cancer*

Combining *in vivo* and *ex vivo* approaches, the group of Theresa Fan studied metabolic adaptations in non-small cell lung cancer (NSCLC) specimens from over 80 patients [12, 50]. They injected uniformly ^{13}C -labelled glucose and analysed its intra-tumoural metabolic fate after resection. An increased activity of pyruvate carboxylase (PC) was observed in tumours when compared to non-cancerous lung tissues. PC is an anaplerotic enzyme that carboxylates pyruvate directly to oxaloacetate; hence its activity supports TCA cycle metabolite levels. *Ex vivo* experiments comparing metabolite labelling patterns between malignant and non-cancerous tissue slices from the same patients confirmed the *in vivo* results. In line with this, genetic PC silencing in different NSCLC models exerted growth-inhibitory effects [12].

4) Genetic biomarkers for future diagnosis, novel therapeutic strategies and patient stratification

Direct deduction of a genetic status from a metabolic profile remains an improbable goal. However, mutations, and/or changes in gene expression, promoter methylation or copy number of “metabolic genes” are increasingly observed in certain types of cancer. In these cases, genetic screening of metabolic genes may constitute a better strategy for prognosis or specific treatment allocation than

metabolomics data. Examples of the most promising clinically relevant alterations in metabolic genes are given below.

4.1) IDH oncogenic mutations

IDH enzyme exists as three distinct isoforms: IDH1 (cytosol and peroxisomes), IDH2 (mitochondria), IDH3 (mitochondria - TCA cycle), and normally catalyses the oxidative decarboxylation of isocitrate to α -ketoglutarate. Whereas IDH3 has, to date, never been found to be mutated in cancer, point mutations in the catalytic sites of both IDH1 and IDH2 are found in glioma, AML and several other cancers [51-56]. These mutations result in a new catalytic activity namely the reduction of α -ketoglutarate to 2HG. By competitive inhibition of demethylases (of the α -ketoglutarate-dependent dioxygenase family), 2HG alters the epigenome [57, 58] and is likely to become soon an accepted biomarker for glioma and AML [59, 60]. IDH-mutated tumours provide a rare opportunity to specifically target these “metabolic oncogenes” without interfering with normal metabolism, and clinical trials in AML and glioma are currently testing small compounds inhibitors of (AG-120 against IDH1 or AG-221 against IDH2) (Table 2), or vaccine immunotherapy against IDH1 neoantigen [61]. (Table 2). In addition, Other IDH inhibitors as well as other IDH-mutated malignancies are also being investigated (Table 2). It is noteworthy that in lower grade glioma, IDH1/2 gain of function mutations confer a relatively favourable prognosis [62, 63], whereas data are much less clear for AML ([64, 65]). The mutational status of IDH1 and IDH2 and 2HG production are likely to be incorporated into routine diagnostic algorithms for glioma and AML.

4.2) SDH and FH loss-of-function mutations

Two other metabolic enzymes, FH and SDH, are mutated in some rare cancers and, loss of their enzymatic activity provokes accumulation of fumarate and succinate, respectively. These metabolites inhibit a plethora of dioxygenases and so act as oncometabolites (recently reviewed in [66]). Typically, inactivation of either of the metabolic tumour suppressors SDH and FH occurs by loss of heterozygosity in patients with a familial predisposition to cancer due to a mono-allelic germline mutation in one of these genes [67]. People at risk are relatively easily identified and screened regularly. The detection of germline mutations in FH and SDH guides patient management, and

therefore they can be considered as clinical biomarkers. Renal cell carcinoma (RCC) due to either mutation are aggressive and metastatic [68] and they are accepted (FH) or provisionally considered (SDH) to comprise distinct tumour subtypes in current RCC classification [69, 70]. SDH inactivation also occurs in a fraction of gastrointestinal stroma tumours (GIST) [71, 72]. Thus, use of c-kit directed tyrosine kinase inhibitors, a therapeutic standard in advanced GIST, may be ineffective in SDH-deficient cases. SDH inactivation is increasingly observed in other malignancies [73-75] and may reach diagnostic relevance as soon as compounds that interfere with the resulting epigenetic [76] or metabolic [77] rewiring become available.

A recent work has uncovered an essential role of PC in SDH-deficient tumours [77]. Using metabolic profiling of primary human pheochromocytoma and paraganglioma, Cardaci *et al.* found significantly decreased aspartate levels in SDH-mutated cases when compared to SDH-proficient neoplasms. Loss of SDH activity and therefore TCA cycle truncation, results in a decrease of oxaloacetate synthesis, and consequently, lower aspartate levels. Increased PC activity bypasses the TCA cycle block and partially sustains aspartate synthesis. Elevated protein expression of PC was also detected in SDH-mutated RCC specimens when compared to adjacent non-cancerous kidney tissues from the same patients. Moreover, genetic silencing of PC limited the proliferation and tumorigenic capacity of SDH-mutated cells. Therefore, it is now tempting to speculate that pharmacological targeting of PC may offer an effective therapeutic strategy for SDH-deficient tumours (and NSCLC – see above).

4.3) Argininosuccinate synthetase 1 (ASS1) deficiency

ASS1 is a urea cycle enzyme which catalyses one of the key steps of arginine biosynthesis in mammals. Genetic or epigenetic silencing of ASS1 has been recognised in various solid tumours and AML, rendering them dependent on exogenous supply of this non-essential amino acid (“auxotrophic”) [7, 78-80]. Mechanistically, it was recently demonstrated that ASS1 loss supports proliferation by sparing aspartate (one of its substrates) and re-routing it for nucleotide biosynthesis [81]. Therapeutically, since ASS1 loss induces arginine auxotrophy, depleting plasma arginine through the systemic application of bacterial or bio-engineered arginase or arginine deiminases (ADI), is now in clinical trials, and showing efficacy in these tumours ([79, 82, 83], Table 2).

4.4) Acetyl-CoA synthetase 2 (ACSS2) induction

The metabolic role of acetate in different cancer entities has been outlined in chapter 3.1.1. Nevertheless, more recently acetate metabolism in cancer was revisited with a fresh look. By applying *in situ* tumour metabolomics in patients, Maher *et al.*, provided evidence for high TCA cycle activity in glioblastomas [84]. MRS imaging of intra-tumoural metabolites following intravenous infusion of uniformly-labelled ^{13}C -glucose pointed towards an additional source, beyond glucose, of acetyl-CoA, central for oxidative energy metabolism and anabolic processes [84]. In a follow-up study, Mashimo and colleagues have identified acetate as a “bioenergetic substrate” for glioblastoma and brain metastases by tracing the intracellular fate of ^{13}C -labelled acetate, following its systemic administration to patients during surgery [30]. Together with recent work by Schug *et al.* [31] and Comerford *et al.* [85], these findings indicate the acetate metabolizing enzyme, ACSS2, as a potential therapeutic target.

ACSS2 converts acetate into its metabolically active form acetyl-CoA. Gain in copy number of the ACSS2 locus in breast cancer has been recently revealed and significantly higher ACSS2 protein level was associated with poorer prognosis [31]. In line with this, ACSS2 mRNA expression is increased at progressive stages of invasive ductal breast cancer, and is particularly high at a metastatic stage [31]. These results are consistent with other studies in triple-negative breast cancer, primary human ovarian and lung tumours, metastatic prostate carcinomas and glioma patients [30, 31, 85]. Furthermore, patient-derived models of glioma and brain metastases of other cancers revealed that ACSS2 upregulation causes preferential metabolic utilisation of acetate by malignant tumours in the central nervous system [30]. These findings suggest that ACSS2 expression levels may help identify high-risk patients and treat them accordingly. This however requires validation in prospective trials.

4.5) Addiction to the serine and glycine biosynthetic pathway

D-3-phosphoglycerate dehydrogenase (PHGDH) converts the glycolytic intermediate 3-phosphoglycerate to 3-phospho-hydroxy-pyruvate as the rate-limiting step of serine biosynthesis and has been found to be genetically amplified, overexpressed or otherwise essential for malignant growth in melanoma, colorectal and breast cancer [5, 8, 86]. Together with PHGDH, the mitochondrial

enzyme serine hydroxyl-methyl-transferase 2 (SHMT2) directs serine towards nucleotide metabolism. Publically available gene expression data sets revealed *shmt2* overexpression in a variety of human cancer entities and *shmt2* copy-number gain in breast and ovarian cancer as well as NSCLC and melanoma [87, 88]. Elevated SHMT2 expression has also been linked to an adverse patient outcome in neuroblastoma, NSCLC and breast cancer [87-89]. Co-expression of SHMT2 and glycine decarboxylase (GLDC), which prevents the toxic accumulation of glycine in SHMT2 expressing cells, was specifically detected in hypoxic regions of gliomas, thereby providing a metabolic advantage under hypoxia [90]. PHGDH and SHMT2 may not only serve as prognostic markers, but, in the future, serine metabolism may be therapeutically exploited (e.g. patent applications US 2014/0087970 A1 and US20150011611 A1).

5) Developing therapeutic metabolic modalities in the clinic

Beyond the few approved tools in the clinic (chapter 2), cumulative mechanistic understanding of metabolic transformation led to the development, repositioning or reassessment of metabolic-specific drugs and therapeutic approaches (Table 2).

5.1) Searching for direct interference with metabolic pathways

Here we describe drugs that specifically designed to interfere with tumour metabolism and are either currently assessed (or re-evaluated) in clinical trials (Table 2) or, to our knowledge, are in advanced stage of pharmacological and pre-clinical development.

5.1.1) 2-deoxyglucose (2DG)

In the 1950's, 2DG, a glucose analogue able to enter, but not to be metabolised by cells was designed to target glycolytic tumour cells by competing with glucose [91]. Once phosphorylated by the first glycolytic enzyme, hexokinase, the accumulated 2DG-6-phosphate inhibits glycolysis [92]. It causes growth arrest and apoptosis and thereby potentiates the activity of classical antineoplastic agents [93, 94]. Unfortunately, clinical studies have revealed limiting systemic toxicity of 2DG leading to use of lower (and therefore less efficient) doses [95]. More recently, combinations of 2DG with chemotherapeutic drugs were tested in solid tumours (e.g. trial NCT00096707), although we are not

aware of currently recruiting trials. Lonidamine, another glycolytic inhibitor, has also been evaluated in clinical trials, but like 2DG, has failed to provide a therapeutic benefit to date [96].

5.1.2) Nicotinamide phosphoribosyltransferase (NAMPT)

Nicotinamide adenine dinucleotide (NAD) is a central coenzyme required for the regulation of cellular metabolism and redox balance. In cancer cells NAD is mostly regenerated from nicotinamide-mononucleotide. Increased gene expression of *nampt*, encoding the rate-limiting enzyme of the NAD salvage pathway, has been observed in a variety of cancer entities ([97, 98], reviewed in [99]). NAMPT activity increases the abundance of NAD⁺, a coenzyme of the deacetylase silent information regulator 1 (SIRT1), thereby activating the oncogene *c-myc* and diminishing both apoptotic and senescence responses to oncogenic activation [100]. Moreover, NAMPT is transcriptionally activated by c-MYC [100]. Consistent with their vulnerability to NAD⁺ depletion, a specific, non-competitive inhibitor of NAMPT, FK866, was shown to induce apoptosis in a variety of tumour cell lines [101]. The high NAD⁺ demand of the DNA repair and apoptosis regulator Poly [ADP-ribose] polymerase 1 (PARP1) may partly explain the selective toxicity of NAMPT inhibition in cancer models and suggests that combining NAMPT inhibition with DNA-damaging agents as therapeutic strategies [101-103]. Moreover, high *nampt* gene expression has been linked to resistance to the proteasome-inhibitor bortezomib, and poorer outcome in relapsed multiple myeloma [104]. Pre-clinical treatment with FK866 as well as its combination with bortezomib has suggested beneficial effect in this disease [105]. An alternative way to re-generate NAD⁺ in malignant cells (potentially generating resistance to NAMPT inhibition) relies on the enzyme nicotinic acid phosphoribosyltransferase (NAPRT) [99]. Therefore, NAPRT-deficient tumours such as a substantial fraction of glioblastomas, neuroblastomas and sarcomas may be particularly sensitive to NAMPT inhibition [106].

Despite the convincing biological rationale for the use of NAMPT inhibitors depicted above, early clinical trials for advanced solid malignancies revealed substantial side effects (mainly haematological and gastrointestinal) and suggested only modest anti-tumour efficacy when used as single agents [107-109]. Development of more potent NAMPT inhibitors, a more thorough patient stratification

strategy, administration earlier in the course of disease and identification of promising therapeutic combinations may provide better results using NAMPT-targeting drugs for cancer treatment.

5.1.3) Polyamines and arginine biosynthetic pathways

Despite being involved in virtually all cellular functions, the polyamines (a class comprising three molecules: putrescine, spermine and spermidine, and their acetylated derivatives) represent interesting targets for cancer-directed therapies due to their important role in cell proliferation [110]. Several inhibitors of enzymes involved in polyamine metabolism, such as difluoromethylornithine (DMFO), were developed and have been tested in clinical trials either as chemopreventives or chemotherapeutics [111] (Table 2). Along with polyamines, arginine appears important in some cancers [80]. Arginine metabolism is highly connected to both the urea cycle and polyamines and provides cancer cells with a way to dispose of nitrogen residues as well as the generation of ornithine, a central intermediate at the “crossroad” of proline, urea and polyamine pathways. As discussed in chapter 4.3, arginine-depleting approaches are demonstrating efficacy in targeting *ASS1*-silenced tumours.

5.1.4) Glutaminase (GLS)

The idea of interrupting the supply or utilisation of the conditionally-essential amino acid glutamine to fight cancer dates back several decades and is based on its high concentration in plasma as well as the selective vulnerability of a wide variety of malignant cells to glutamine depletion [3, 112, 113]. Interestingly, the antineoplastic effects of therapeutically-employed asparaginase (discussed in chapter 2.3) have been partly linked to their inherent additional glutamine-depleting function [114]. Intracellular glutaminases catalyse the deamination of glutamine to glutamate, which can serve as a precursor for glucose-independent anaplerotic α -ketoglutarate or for glutathione biosynthesis [113, 115]. Of the two human glutaminase genes, *gls2* expression is mainly restricted to the liver, whereas the product of *gls1* (kidney-type GLS) can be found in most tissues [116]. In line with a pivotal role in metabolic rewiring during tumorigenesis, GLSs are differentially regulated by tumour suppressors and oncogenes [117, 118]. GLS inhibitors such as bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES) have shown remarkable therapeutic activity in tumour models with elevated GLS

activity without over systemic toxicity [4, 119, 120]. Additionally, metabolic profiling of IDH1/2-mutated glioma cells has revealed a decrease in α -ketoglutarate abundance, and consequently, GLS inhibition exhibited selective toxicity in these gliomas [121, 122]. Currently, clinical phase I trials with the orally available inhibitor of GLS1, CB-839 [123], are conducted in both solid and haematological malignancies, but, to the best of our knowledge, results have not yet been reported in peer-reviewed publications.

5.1.5) Lactate metabolism

Due to increased aerobic glycolysis in many malignancies, pyruvate is largely transformed into lactate by LDH and exported to the micro-environment by Monocarboxylate transporters (MCTs). The A subunit of LDH favours the reduction of pyruvate to lactate while oxidizing cytosolic NADH to NAD⁺ hence supporting high glycolytic flux. LDHA is a direct target of the hypoxia inducible transcription factors (HIFs) and several activated oncogenes, and is consequently overexpressed in a variety of malignancies [124]. Silencing of LDHA in hypoxic breast cancer models decreased tumorigenicity [125]. Additionally, genetic or pharmacologic LDHA inhibition elevated mitochondrial reactive oxygen species, induced cell death [126-128] and selectively impaired the growth of patient-derived xenografts [129]. In a model of NSCLC, LDHA inhibition was toxic to tumour-initiating cells, and primary human NSCLC tissue slices *ex vivo* showed a decreased conversion of ¹³C-labelled glucose to lactate as well as less anaplerotic glutamine consumption [127]. Furthermore, following anti-angiogenic treatment, *in vivo* metabolic tracing of ¹³C-glucose in patient-derived glioblastoma xenografts revealed increased lactate production (a switch to anaerobic metabolism), and immunohistochemical analyses of human glioblastoma biopsies confirmed higher expression of LDHA [130]. Therefore, LDHA inhibition may help neutralise a tumour escape mechanism to treatment with bevacizumab, an antibody to the vascular endothelial growth factor (VEGF).

Several small molecule inhibitors of LDHA have been reported over the past recent years, and some are in advanced stage of pharmacological development (e.g. [131]). Nevertheless, to our knowledge, none of these drugs is currently under clinical evaluation.

Once produced by LDH in glycolytic tumours, lactate must be secreted from cells via MCTs. High levels of MCT1 are expressed in various cancer types and MCT4 expression is induced under hypoxic conditions, typically observed in solid tumours [132]. Therefore, MCTs represent interesting therapeutic targets, with the MCT1 inhibitor, AZD3965, is now being tested against advanced cancers in the UK (Table 2) and MCT4 is in the pre-clinical pipeline [133]. Unfortunately, MCT1 inhibitors fail to block MCT4 [134, 135]. Thus, MCT4 expression, particularly under hypoxia, overcomes MCT1 inhibition [134, 136]. Furthermore, high MCT4 expression is associated with a poor prognosis in many solid tumours [137-141]. Genetic interference with MCT4 or its chaperone CD147 were effective in different cancer models [137, 138, 142], and recently, a specific MCT4 inhibitor (AZ93) was reported [133]. Data from pre-clinical studies are eagerly awaited.

5.2) Repositioning non-cancer drugs to anti-cancer strategies

Some drugs which are already in use for other medical conditions could potentially serve as effective anti-cancer agents and are currently assessed in clinical trials (Table 2).

5.2.1) Metformin

Metformin, an anti-diabetic drug, modulates both peripheral insulin-resistance and hepatic neogluconeogenesis by indirectly activating AMP-activated protein kinase (AMPK) through inhibition of the mitochondrial respiratory chain, thus increasing the AMP/ATP ratio in cells. Activated AMPK inhibits mammalian Target of Rapamycin (mTOR) which is involved in regulation of cell proliferation [143]. Metformin is the subject of many ongoing clinical trials with non-diabetic cancer patients (reviewed in [144] and Table 2). Disappointingly however, a recent large randomized trial in advanced pancreatic cancer showed no benefit [145].

5.2.2) Statins

Statins, which are 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, target a key enzyme in the mevalonate synthetic pathway (necessary for cholesterol synthesis). They are conventionally used to decrease blood cholesterol (reviewed in [146]). As cellular membranes need cholesterol for stability [147], it was supposed that impeding cholesterol synthesis would negatively impact tumour cell

growth. Studies aiming at correlating tumour incidence or treatment outcomes to statin use have been conducted for various cancers [148-150], but outcomes have remained largely inconclusive to date.

5.3) Tackling cancer with modified diets: plenty of food for thought

Tumour cells compete with normal cells for energy and biomass supplies. Therefore, limiting nutrient delivery to malignant tissues may be a potential therapeutic strategy and controlling a patient's nutrition may help to achieve this goal. Reports suggest that some tumours lack the ability to use ketone bodies (mainly aceto-acetate and β -hydroxy-butyrate) or fatty acids as energy sources, with the latter even being toxic to some malignant cells [151-153]. Hence, ketogenic diets (KD) were investigated with regards to their effects on cancer progression. Successfully employed to decrease the likelihood of seizures in epilepsy patients since the early 1920s (e.g. [154]), the first case-report showing a beneficial effect of KD on two girls suffering from astrocytoma was published in 1995 [155]. Since then, KD have demonstrated anti-tumour activity in cancer cells *in vitro* [152, 156], on xenografts [151, 157], and have been tested in pilot studies on patients with advanced cancers [158, 159]. Nevertheless, large clinical studies are required to substantiate those anecdotal reports and to appreciate who may benefit from such an approach.

6) Future perspectives for metabolomics in cancer diagnosis and therapy

We herein provided an overview of current and possible future clinical applications of recent advances in metabolomics. Analytical metabolomics have contributed to our understanding of cancer pathogenesis. Some metabolic biomarkers are already routinely employed to diagnose malignant diseases and metabolic alterations of tumours are increasingly being validated as therapeutic targets. The importance of altered glucose and glutamine metabolism in different cancers is well documented. The study of the metabolic role of non-essential amino acids in malignant tumours is gaining momentum [5-8, 160]. New metabolic tracers which may improve diagnostic accuracy and monitoring of tumours by functional imaging are at different stages of development [161], and strategies combining established therapies with new metabolism-related approaches are being tested. Further studies in computerised modelling of metabolic networks, pre-clinical models, and

investigation of primary human tumours, are likely to identify new metabolic vulnerabilities [162]. However, due to impediments such as poor specificity of tool compounds, the presence of compensatory metabolic pathways, unforeseen side effects and imprecise patient stratification, several seemingly-promising metabolism-guided strategies have only yielded modest results so far.

The concept of “precision medicine” in cancer care is gradually gaining force and form. In the future, in-depth analyses of genomic, proteomic and metabolic alterations of an individual malignant disease, as well as prospective assessment of therapeutic options in mice bearing patient-derived tumour xenografts, are likely to guide clinical strategies. In addition personalised medicine strongly emphasises the use of predictive biomarkers and will thereby facilitate the integration of metabolomics into clinical patient management. Besides discovering and validating new metabolic biomarkers that will enable better diagnosis and monitoring, we expect new metabolomics studies to provide new molecular therapeutic targets and enable tailoring of treatments to patients. Relatively inexpensive and reproducible metabolomics analyses, combined with other “omics” approaches, may soon shape modern medicine. Despite obvious obstacles, the increasing use of metabolomics in clinical research may soon turn it into one of the most powerful tools used to identify, monitor and fight cancer.

References

- [1] Cairns RA, Harris IS, Mak TW. Regulation of cancer cell metabolism. *Nat Rev Cancer* 2011; 11:85-95.
- [2] Warburg O, Wind F, Negelein E. The Metabolism of Tumors in the Body. *J Gen Physiol* 1927; 8:519-30.
- [3] Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. *Nature* 2013; 496:101-5.
- [4] Xiang Y, Stine ZE, Xia J, Lu Y, O'Connor RS, Altman BJ, et al. Targeted inhibition of tumor-specific glutaminase diminishes cell-autonomous tumorigenesis. *J Clin Invest* 2015; 125:2293-306.
- [5] Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, et al. Functional genomics reveal that the serine synthesis pathway is essential in breast cancer. *Nature* 2011; 476:346-50.
- [6] Liu W, Le A, Hancock C, Lane AN, Dang CV, Fan TW, et al. Reprogramming of proline and glutamine metabolism contributes to the proliferative and metabolic responses regulated by oncogenic transcription factor c-MYC. *Proc Natl Acad Sci U S A* 2012; 109:8983-8.
- [7] Mussai F, Egan S, Higginbotham-Jones J, Perry T, Beggs A, Odintsova E, et al. Arginine dependence of acute myeloid leukemia blast proliferation: a novel therapeutic target. *Blood* 2015; 125:2386-96.
- [8] Locasale JW, Grassian AR, Melman T, Lyssiotis CA, Mattaini KR, Bass AJ, et al. Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. *Nat Genet* 2011; 43:869-74.
- [9] Frezza C, Pollard PJ, Gottlieb E. Inborn and acquired metabolic defects in cancer. *J Mol Med (Berl)* 2011; 89:213-20.
- [10] Yang M, Soga T, Pollard PJ. Oncometabolites: linking altered metabolism with cancer. *J Clin Invest* 2013; 123:3652-8.
- [11] Caro P, Kishan AU, Norberg E, Stanley IA, Chapuy B, Ficarro SB, et al. Metabolic signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma. *Cancer Cell* 2012; 22:547-60.
- [12] Sellers K, Fox MP, Bousamra M, 2nd, Slone SP, Higashi RM, Miller DM, et al. Pyruvate carboxylase is critical for non-small-cell lung cancer proliferation. *J Clin Invest* 2015; 125:687-98.
- [13] Chinnaiyan P, Kensicki E, Bloom G, Prabhu A, Sarcar B, Kahali S, et al. The metabolomic signature of malignant glioma reflects accelerated anabolic metabolism. *Cancer Res* 2012; 72:5878-88.
- [14] Kaye SB. New antimetabolites in cancer chemotherapy and their clinical impact. *Br J Cancer* 1998; 78 Suppl 3:1-7.
- [15] Heidelberger C, Chaudhuri NK, Danneberg P, Mooren D, Griesbach L, Duschinsky R, et al. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature* 1957; 179:663-6.
- [16] Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003; 3:330-8.
- [17] Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* 2006; 355:11-20.
- [18] Midgley R, Kerr DJ. Capecitabine: have we got the dose right? *Nat Clin Pract Oncol* 2009; 6:17-24.
- [19] Farber S, Diamond LK. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. *N Engl J Med* 1948; 238:787-93.
- [20] Joerger M, Huitema AD, Illerhaus G, Ferreri AJ. Rational administration schedule for high-dose methotrexate in patients with primary central nervous system lymphoma. *Leuk Lymphoma* 2012; 53:1867-75.
- [21] Zhu A, Lee D, Shim H. Metabolic positron emission tomography imaging in cancer detection and therapy response. *Semin Oncol* 2011; 38:55-69.

- [22] Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol* 2014; 32:3059-68.
- [23] Groves AM, Win T, Haim SB, Ell PJ. Non-[¹⁸F]FDG PET in clinical oncology. *Lancet Oncol* 2007; 8:822-30.
- [24] Schrek R, Holcenberg JS, Batra KV, Roberts J, Dolowy WC. Effect of asparagine and glutamine deficiency on normal and leukemic cells. *J Natl Cancer Inst* 1973; 51:1103-7.
- [25] Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med* 2006; 354:166-78.
- [26] Derst C, Henseling J, Rohm KH. Engineering the substrate specificity of *Escherichia coli* asparaginase. II. Selective reduction of glutaminase activity by amino acid replacements at position 248. *Protein Sci* 2000; 9:2009-17.
- [27] Yoshimoto M, Waki A, Yonekura Y, Sadato N, Murata T, Omata N, et al. Characterization of acetate metabolism in tumor cells in relation to cell proliferation: acetate metabolism in tumor cells. *Nucl Med Biol* 2001; 28:117-22.
- [28] Schug ZT, Frezza C, Galbraith LC, Gottlieb E. The music of lipids: how lipid composition orchestrates cellular behaviour. *Acta Oncol* 2012; 51:301-10.
- [29] Lyssiotis CA, Cantley LC. Acetate fuels the cancer engine. *Cell* 2014; 159:1492-4.
- [30] Mashimo T, Pichumani K, Vemireddy V, Hatanpaa KJ, Singh DK, Sirasanagandla S, et al. Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. *Cell* 2014; 159:1603-14.
- [31] Schug ZT, Peck B, Jones DT, Zhang Q, Grosskurth S, Alam IS, et al. Acetyl-CoA Synthetase 2 Promotes Acetate Utilization and Maintains Cancer Cell Growth under Metabolic Stress. *Cancer Cell* 2015; 27:57-71.
- [32] Mena E, Turkbey B, Mani H, Adler S, Valera VA, Bernardo M, et al. ¹¹C-Acetate PET/CT in localized prostate cancer: a study with MRI and histopathologic correlation. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine* 2012; 53:538-45.
- [33] Shields AF, Grierson JR, Dohmen BM, Machulla HJ, Stayanoff JC, Lawhorn-Crews JM, et al. Imaging proliferation in vivo with [¹⁸F]FLT and positron emission tomography. *Nat Med* 1998; 4:1334-6.
- [34] Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. *Trends Biochem Sci* 2010; 35:427-33.
- [35] Lieberman BP, Ploessl K, Wang L, Qu W, Zha Z, Wise DR, et al. PET imaging of glutaminolysis in tumors by ¹⁸F-(2S,4R)4-fluoroglutamine. *J Nucl Med* 2011; 52:1947-55.
- [36] Venneti S, Dunphy MP, Zhang H, Pitter KL, Zanzonico P, Campos C, et al. Glutamine-based PET imaging facilitates enhanced metabolic evaluation of gliomas in vivo. *Sci Transl Med* 2015; 7:274ra17.
- [37] Gallagher FA, Bohndiek SE, Kettunen MI, Lewis DY, Soloviev D, Brindle KM. Hyperpolarized ¹³C MRI and PET: in vivo tumor biochemistry. *J Nucl Med* 2011; 52:1333-6.
- [38] Golman K, Ardenkjaer-Larsen JH, Petersson JS, Mansson S, Leunbach I. Molecular imaging with endogenous substances. *Proc Natl Acad Sci U S A* 2003; 100:10435-9.
- [39] Gallagher FA, Kettunen MI, Day SE, Hu DE, Ardenkjaer-Larsen JH, Zandt R, et al. Magnetic resonance imaging of pH in vivo using hyperpolarized ¹³C-labelled bicarbonate. *Nature* 2008; 453:940-3.
- [40] Rodrigues TB, Serrao EM, Kennedy BW, Hu DE, Kettunen MI, Brindle KM. Magnetic resonance imaging of tumor glycolysis using hyperpolarized ¹³C-labeled glucose. *Nat Med* 2014; 20:93-7.
- [41] Chaumeil MM, Larson PE, Woods SM, Cai L, Eriksson P, Robinson AE, et al. Hyperpolarized [¹³C] glutamate: a metabolic imaging biomarker of IDH1 mutational status in glioma. *Cancer Res* 2014; 74:4247-57.
- [42] Keshari KR, Kurhanewicz J, Bok R, Larson PE, Vigneron DB, Wilson DM. Hyperpolarized ¹³C dehydroascorbate as an endogenous redox sensor for in vivo metabolic imaging. *Proc Natl Acad Sci U S A* 2011; 108:18606-11.

- [43] Day SE, Kettunen MI, Gallagher FA, Hu DE, Lerche M, Wolber J, et al. Detecting tumor response to treatment using hyperpolarized ^{13}C magnetic resonance imaging and spectroscopy. *Nat Med* 2007; 13:1382-7.
- [44] Hu S, Balakrishnan A, Bok RA, Anderton B, Larson PE, Nelson SJ, et al. ^{13}C -pyruvate imaging reveals alterations in glycolysis that precede c-Myc-induced tumor formation and regression. *Cell Metab* 2011; 14:131-42.
- [45] Nelson SJ, Kurhanewicz J, Vigneron DB, Larson PE, Harzstark AL, Ferrone M, et al. Metabolic imaging of patients with prostate cancer using hyperpolarized $[1-(1)^{13}\text{C}]$ pyruvate. *Sci Transl Med* 2013; 5:198ra08.
- [46] Wilson DM, Keshari KR, Larson PE, Chen AP, Hu S, Van Criekinge M, et al. Multi-compound polarization by DNP allows simultaneous assessment of multiple enzymatic activities in vivo. *J Magn Reson* 2010; 205:141-7.
- [47] Mayers JR, Wu C, Clish CB, Kraft P, Torrence ME, Fiske BP, et al. Elevation of circulating branched-chain amino acids is an early event in human pancreatic adenocarcinoma development. *Nat Med* 2014; 20:1193-8.
- [48] Zhang G, He P, Tan H, Budhu A, Gaedcke J, Ghadimi BM, et al. Integration of metabolomics and transcriptomics revealed a fatty acid network exerting growth inhibitory effects in human pancreatic cancer. *Clin Cancer Res* 2013; 19:4983-93.
- [49] Chen WL, Wang JH, Zhao AH, Xu X, Wang YH, Chen TL, et al. A distinct glucose metabolism signature of acute myeloid leukemia with prognostic value. *Blood* 2014; 124:1645-54.
- [50] Fan TW, Lane AN, Higashi RM, Farag MA, Gao H, Bousamra M, et al. Altered regulation of metabolic pathways in human lung cancer discerned by (^{13}C) stable isotope-resolved metabolomics (SIRM). *Mol Cancer* 2009; 8:41.
- [51] Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science* 2008; 321:1807-12.
- [52] Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med* 2009; 360:765-73.
- [53] Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Collier HA, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* 2010; 17:225-34.
- [54] Ghiam AF, Cairns RA, Thoms J, Dal Pra A, Ahmed O, Meng A, et al. IDH mutation status in prostate cancer. *Oncogene* 2012; 31:3826.
- [55] Terunuma A, Putluri N, Mishra P, Mathe EA, Dorsey TH, Yi M, et al. MYC-driven accumulation of 2-hydroxyglutarate is associated with breast cancer prognosis. *J Clin Invest* 2014; 124:398-412.
- [56] Fujimoto A, Furuta M, Shiraishi Y, Gotoh K, Kawakami Y, Arihiro K, et al. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. *Nat Commun* 2015; 6:6120.
- [57] Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer Cell* 2011; 19:17-30.
- [58] Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. *Nature* 2012; 483:474-8.
- [59] Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG, et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *J Exp Med* 2010; 207:339-44.
- [60] Esmaeili M, Vettukattil R, Bathen TF. 2-hydroxyglutarate as a magnetic resonance biomarker for glioma subtyping. *Transl Oncol* 2013; 6:92-8.
- [61] Dimitrov L, Hong CS, Yang C, Zhuang Z, Heiss JD. New developments in the pathogenesis and therapeutic targeting of the IDH1 mutation in glioma. *Int J Med Sci* 2015; 12:201-13.
- [62] Eckel-Passow JE, Lachance DH, Molinaro AM, Walsh KM, Decker PA, Sicotte H, et al. Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. *N Engl J Med* 2015; 372:2499-508.

- [63] Cancer Genome Atlas Research N, Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, et al. Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med* 2015; 372:2481-98.
- [64] Marcucci G, Maharry K, Wu YZ, Radmacher MD, Mrozek K, Margeson D, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol* 2010; 28:2348-55.
- [65] Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med* 2012; 366:1079-89.
- [66] Nowicki S, Gottlieb E. Oncometabolites: Tailoring our Genes. *FEBS J* 2015; 282:2796-805.
- [67] Gottlieb E, Tomlinson IP. Mitochondrial tumour suppressors: a genetic and biochemical update. *Nat Rev Cancer* 2005; 5:857-66.
- [68] Linehan WM, Srinivasan R, Garcia JA. Non-clear cell renal cancer: disease-based management and opportunities for targeted therapeutic approaches. *Semin Oncol* 2013; 40:511-20.
- [69] Escudier B, Porta C, Schmidinger M, Algaba F, Patard JJ, Khoo V, et al. Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2014; 25 Suppl 3:iii49-56.
- [70] Srigley JR, Delahunt B, Eble JN, Egevad L, Epstein JI, Grignon D, et al. The International Society of Urological Pathology (ISUP) Vancouver Classification of Renal Neoplasia. *Am J Surg Pathol* 2013; 37:1469-89.
- [71] Killian JK, Miettinen M, Walker RL, Wang Y, Zhu YJ, Waterfall JJ, et al. Recurrent epimutation of SDHC in gastrointestinal stromal tumors. *Sci Transl Med* 2014; 6:268ra177.
- [72] Janeway KA, Kim SY, Lodish M, Nose V, Rustin P, Gaal J, et al. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. *Proc Natl Acad Sci U S A* 2011; 108:314-8.
- [73] Weinhold N, Jacobsen A, Schultz N, Sander C, Lee W. Genome-wide analysis of noncoding regulatory mutations in cancer. *Nat Genet* 2014; 46:1160-5.
- [74] Castro-Vega LJ, Letouze E, Burnichon N, Buffet A, Disderot PH, Khalifa E, et al. Multi-omics analysis defines core genomic alterations in pheochromocytomas and paragangliomas. *Nat Commun* 2015; 6:6044.
- [75] Aspuria PJ, Lunt SY, Varemo L, Vergnes L, Gozo M, Beach JA, et al. Succinate dehydrogenase inhibition leads to epithelial-mesenchymal transition and reprogrammed carbon metabolism. *Cancer Metab* 2014; 2:21.
- [76] Letouze E, Martinelli C, Lorient C, Burnichon N, Abermil N, Ottolenghi C, et al. SDH mutations establish a hypermethylator phenotype in paraganglioma. *Cancer Cell* 2013; 23:739-52.
- [77] Cardaci S, Zheng L, MacKay G, van den Broek NJF, MacKenzie ED, Nixon C, et al. Pyruvate carboxylation enables growth of SDH-deficient cells by supporting aspartate biosynthesis. *Nat Cell Biol*; In Press, doi: 10.1038/ncb3233.
- [78] Qiu F, Chen YR, Liu X, Chu CY, Shen LJ, Xu J, et al. Arginine starvation impairs mitochondrial respiratory function in ASS1-deficient breast cancer cells. *Sci Signal* 2014; 7:ra31.
- [79] Szlosarek PW, Luong P, Phillips MM, Baccharini M, Stephen E, Szyszko T, et al. Metabolic response to pegylated arginine deiminase in mesothelioma with promoter methylation of argininosuccinate synthetase. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2013; 31:e111-3.
- [80] Qiu F, Huang J, Sui M. Targeting arginine metabolism pathway to treat arginine-dependent cancers. *Cancer Lett* 2015; 364:1-7.
- [81] Rabinovich S, Adler L, Yizhak K, Sarver A, Silberman A, Agron S, et al. Diversion of aspartate in ASS1-deficient tumors fosters de novo pyrimidine synthesis. *Nature*; In Press, DOI: .
- [82] Ascierto PA, Scala S, Castello G, Daponte A, Simeone E, Ottaiano A, et al. Pegylated arginine deiminase treatment of patients with metastatic melanoma: results from phase I and II studies. *J Clin Oncol* 2005; 23:7660-8.

- [83] Glazer ES, Piccirillo M, Albino V, Di Giacomo R, Palaia R, Mastro AA, et al. Phase II study of pegylated arginine deiminase for nonresectable and metastatic hepatocellular carcinoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010; 28:2220-6.
- [84] Maher EA, Marin-Valencia I, Bachoo RM, Mashimo T, Raisanen J, Hatanpaa KJ, et al. Metabolism of [U-13 C]glucose in human brain tumors in vivo. *NMR Biomed* 2012; 25:1234-44.
- [85] Comerford SA, Huang Z, Du X, Wang Y, Cai L, Witkiewicz AK, et al. Acetate dependence of tumors. *Cell* 2014; 159:1591-602.
- [86] Snell K, Natsumeda Y, Eble JN, Glover JL, Weber G. Enzymic imbalance in serine metabolism in human colon carcinoma and rat sarcoma. *Br J Cancer* 1988; 57:87-90.
- [87] Ye J, Fan J, Venneti S, Wan YW, Pawel BR, Zhang J, et al. Serine catabolism regulates mitochondrial redox control during hypoxia. *Cancer Discov* 2014; 4:1406-17.
- [88] Lee GY, Haverty PM, Li L, Kljavin NM, Bourgon R, Lee J, et al. Comparative oncogenomics identifies PSMB4 and SHMT2 as potential cancer driver genes. *Cancer Res* 2014; 74:3114-26.
- [89] Antonov A, Agostini M, Morello M, Minieri M, Melino G, Amelio I. Bioinformatics analysis of the serine and glycine pathway in cancer cells. *Oncotarget* 2014; 5:11004-13.
- [90] Kim D, Fiske BP, Birsoy K, Freinkman E, Kami K, Possemato RL, et al. SHMT2 drives glioma cell survival in ischaemia but imposes a dependence on glycine clearance. *Nature* 2015; 520:363-7.
- [91] Landau BR, Laszlo J, Stengle J, Burk D. Certain metabolic and pharmacologic effects in cancer patients given infusions of 2-deoxy-D-glucose. *J Natl Cancer Inst* 1958; 21:485-94.
- [92] Wick AN, Drury DR, Nakada HI, Wolfe JB. Localization of the primary metabolic block produced by 2-deoxyglucose. *The Journal of biological chemistry* 1957; 224:963-9.
- [93] Kaplan O, Navon G, Lyon RC, Faustino PJ, Straka EJ, Cohen JS. Effects of 2-deoxyglucose on drug-sensitive and drug-resistant human breast cancer cells: toxicity and magnetic resonance spectroscopy studies of metabolism. *Cancer Res* 1990; 50:544-51.
- [94] Lin X, Zhang F, Bradbury CM, Kaushal A, Li L, Spitz DR, et al. 2-Deoxy-D-glucose-induced cytotoxicity and radiosensitization in tumor cells is mediated via disruptions in thiol metabolism. *Cancer Res* 2003; 63:3413-7.
- [95] Dwarakanath B, Jain V. Targeting glucose metabolism with 2-deoxy-D-glucose for improving cancer therapy. *Future Oncol* 2009; 5:581-5.
- [96] Papaldo P, Lopez M, Cortesi E, Cammilluzzi E, Antimi M, Terzoli E, et al. Addition of either lonidamine or granulocyte colony-stimulating factor does not improve survival in early breast cancer patients treated with high-dose epirubicin and cyclophosphamide. *J Clin Oncol* 2003; 21:3462-8.
- [97] Wang B, Hasan MK, Alvarado E, Yuan H, Wu H, Chen WY. NAMPT overexpression in prostate cancer and its contribution to tumor cell survival and stress response. *Oncogene* 2011; 30:907-21.
- [98] Bi TQ, Che XM, Liao XH, Zhang DJ, Long HL, Li HJ, et al. Overexpression of Nampt in gastric cancer and chemopotentiating effects of the Nampt inhibitor FK866 in combination with fluorouracil. *Oncol Rep* 2011; 26:1251-7.
- [99] Sampath D, Zabka TS, Misner DL, O'Brien T, Dragovich PS. Inhibition of nicotinamide phosphoribosyltransferase (NAMPT) as a therapeutic strategy in cancer. *Pharmacol Ther* 2015; 151:16-31.
- [100] Menssen A, Hydbring P, Kapelle K, Vervoorts J, Diebold J, Luscher B, et al. The c-MYC oncoprotein, the NAMPT enzyme, the SIRT1-inhibitor DBC1, and the SIRT1 deacetylase form a positive feedback loop. *Proc Natl Acad Sci U S A* 2012; 109:E187-96.
- [101] Hasmann M, Schemainda I. FK866, a highly specific noncompetitive inhibitor of nicotinamide phosphoribosyltransferase, represents a novel mechanism for induction of tumor cell apoptosis. *Cancer Res* 2003; 63:7436-42.
- [102] Gehrke I, Bouchard ED, Beiggi S, Poepl AG, Johnston JB, Gibson SB, et al. On-target effect of FK866, a nicotinamide phosphoribosyl transferase inhibitor, by apoptosis-mediated death in chronic lymphocytic leukemia cells. *Clin Cancer Res* 2014; 20:4861-72.

- [103] Moore Z, Chakrabarti G, Luo X, Ali A, Hu Z, Fattah FJ, et al. NAMPT inhibition sensitizes pancreatic adenocarcinoma cells to tumor-selective, PAR-independent metabolic catastrophe and cell death induced by beta-lapachone. *Cell Death Dis* 2015; 6:e1599.
- [104] Cagnetta A, Cea M, Calimeri T, Acharya C, Fulciniti M, Tai YT, et al. Intracellular NAD(+) depletion enhances bortezomib-induced anti-myeloma activity. *Blood* 2013; 122:1243-55.
- [105] Cea M, Cagnetta A, Fulciniti M, Tai YT, Hideshima T, Chauhan D, et al. Targeting NAD+ salvage pathway induces autophagy in multiple myeloma cells via mTORC1 and extracellular signal-regulated kinase (ERK1/2) inhibition. *Blood* 2012; 120:3519-29.
- [106] Watson M, Roulston A, Belec L, Billot X, Marcellus R, Bedard D, et al. The small molecule GMX1778 is a potent inhibitor of NAD+ biosynthesis: strategy for enhanced therapy in nicotinic acid phosphoribosyltransferase 1-deficient tumors. *Mol Cell Biol* 2009; 29:5872-88.
- [107] von Heideman A, Berglund A, Larsson R, Nygren P. Safety and efficacy of NAD depleting cancer drugs: results of a phase I clinical trial of CHS 828 and overview of published data. *Cancer Chemother Pharmacol* 2010; 65:1165-72.
- [108] Hovstadius P, Larsson R, Jonsson E, Skov T, Kissmeyer AM, Krasilnikoff K, et al. A Phase I study of CHS 828 in patients with solid tumor malignancy. *Clin Cancer Res* 2002; 8:2843-50.
- [109] Holen K, Saltz LB, Hollywood E, Burk K, Hanauske AR. The pharmacokinetics, toxicities, and biologic effects of FK866, a nicotinamide adenine dinucleotide biosynthesis inhibitor. *Invest New Drugs* 2008; 26:45-51.
- [110] Casero RA, Jr., Marton LJ. Targeting polyamine metabolism and function in cancer and other hyperproliferative diseases. *Nat Rev Drug Discov* 2007; 6:373-90.
- [111] Gerner EW, Meyskens FL, Jr. Polyamines and cancer: old molecules, new understanding. *Nat Rev Cancer* 2004; 4:781-92.
- [112] Roberts J, Holcenberg JS, Dolowy WC. Antineoplastic activity of highly purified bacterial glutaminases. *Nature* 1970; 227:1136-7.
- [113] Hensley CT, Wasti AT, DeBerardinis RJ. Glutamine and cancer: cell biology, physiology, and clinical opportunities. *J Clin Invest* 2013; 123:3678-84.
- [114] Willems L, Jacque N, Jacquelin A, Neveux N, Maciel TT, Lambert M, et al. Inhibiting glutamine uptake represents an attractive new strategy for treating acute myeloid leukemia. *Blood* 2013; 122:3521-32.
- [115] Zhao Y, Butler EB, Tan M. Targeting cellular metabolism to improve cancer therapeutics. *Cell Death Dis* 2013; 4:e532.
- [116] Katt WP, Cerione RA. Glutaminase regulation in cancer cells: a druggable chain of events. *Drug Discov Today* 2014; 19:450-7.
- [117] Suzuki S, Tanaka T, Poyurovsky MV, Nagano H, Mayama T, Ohkubo S, et al. Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. *Proc Natl Acad Sci U S A* 2010; 107:7461-6.
- [118] Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, et al. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* 2009; 458:762-5.
- [119] Baenke F, Chaneton B, Smith M, Van Den Broek N, Hogan K, Tang H, et al. Resistance to BRAF inhibitors induces glutamine dependency in melanoma cells. *Molecular Oncology*; In Press, doi.org/10.1016/j.molonc.2015.08.003.
- [120] Jacque N, Ronchetti AM, Larrue C, Meunier G, Birsén R, Willems L, et al. Targeting glutaminolysis has anti-leukemic activity in acute myeloid leukemia and synergizes with BCL-2 inhibition. *Blood*; In Press, DOI 10.1182/blood-2015-01-621870.
- [121] Reitman ZJ, Jin G, Karoly ED, Spasojevic I, Yang J, Kinzler KW, et al. Profiling the effects of isocitrate dehydrogenase 1 and 2 mutations on the cellular metabolome. *Proc Natl Acad Sci U S A* 2011; 108:3270-5.

- [122] Seltzer MJ, Bennett BD, Joshi AD, Gao P, Thomas AG, Ferraris DV, et al. Inhibition of glutaminase preferentially slows growth of glioma cells with mutant IDH1. *Cancer Res* 2010; 70:8981-7.
- [123] Gross MI, Demo SD, Dennison JB, Chen L, Chernov-Rogan T, Goyal B, et al. Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. *Mol Cancer Ther* 2014; 13:890-901.
- [124] Doherty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. *J Clin Invest* 2013; 123:3685-92.
- [125] Fantin VR, St-Pierre J, Leder P. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* 2006; 9:425-34.
- [126] Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, Deck LM, et al. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *Proc Natl Acad Sci U S A* 2010; 107:2037-42.
- [127] Xie H, Hanai J, Ren JG, Kats L, Burgess K, Bhargava P, et al. Targeting lactate dehydrogenase--a inhibits tumorigenesis and tumor progression in mouse models of lung cancer and impacts tumor-initiating cells. *Cell Metab* 2014; 19:795-809.
- [128] Billiard J, Dennison JB, Briand J, Annan RS, Chai D, Colon M, et al. Quinoline 3-sulfonamides inhibit lactate dehydrogenase A and reverse aerobic glycolysis in cancer cells. *Cancer Metab* 2013; 1:19.
- [129] Rajeshkumar NV, Dutta P, Yabuuchi S, de Wilde RF, Matrinez GV, Le A, et al. Therapeutic targeting of the Warburg effect in pancreatic cancer relies on an absence of p53 function. *Cancer Res* 2015.
- [130] Fack F, Espedal H, Keunen O, Golebiewska A, Obad N, Harter PN, et al. Bevacizumab treatment induces metabolic adaptation toward anaerobic metabolism in glioblastomas. *Acta Neuropathol* 2015; 129:115-31.
- [131] Labadie S, Dragovich PS, Chen J, Fauber BP, Boggs J, Corson LB, et al. Optimization of 5-(2,6-dichlorophenyl)-3-hydroxy-2-mercaptocyclohex-2-enones as potent inhibitors of human lactate dehydrogenase. *Bioorg Med Chem Lett* 2015; 25:75-82.
- [132] Parks SK, Chiche J, Pouyssegur J. Disrupting proton dynamics and energy metabolism for cancer therapy. *Nat Rev Cancer* 2013; 13:611-23.
- [133] Marchiq I, Pouyssegur J. Hypoxia, cancer metabolism and the therapeutic benefit of targeting lactate/H symporters. *J Mol Med (Berl)*; In Press, DOI 10.1007/s00109-015-1307-x.
- [134] Ovens MJ, Davies AJ, Wilson MC, Murray CM, Halestrap AP. AR-C155858 is a potent inhibitor of monocarboxylate transporters MCT1 and MCT2 that binds to an intracellular site involving transmembrane helices 7-10. *Biochem J* 2010; 425:523-30.
- [135] Murray CM, Hutchinson R, Bantick JR, Belfield GP, Benjamin AD, Brazma D, et al. Monocarboxylate transporter MCT1 is a target for immunosuppression. *Nat Chem Biol* 2005; 1:371-6.
- [136] Polanski R, Hodgkinson CL, Fusi A, Nonaka D, Priest L, Kelly P, et al. Activity of the monocarboxylate transporter 1 inhibitor AZD3965 in small cell lung cancer. *Clin Cancer Res* 2014; 20:926-37.
- [137] Baek G, Tse YF, Hu Z, Cox D, Buboltz N, McCue P, et al. MCT4 defines a glycolytic subtype of pancreatic cancer with poor prognosis and unique metabolic dependencies. *Cell Rep* 2014; 9:2233-49.
- [138] Lim KS, Lim KJ, Price AC, Orr BA, Eberhart CG, Bar EE. Inhibition of monocarboxylate transporter-4 depletes stem-like glioblastoma cells and inhibits HIF transcriptional response in a lactate-independent manner. *Oncogene* 2014; 33:4433-41.
- [139] Doyen J, Trastour C, Ettore F, Peyrottes I, Toussant N, Gal J, et al. Expression of the hypoxia-inducible monocarboxylate transporter MCT4 is increased in triple negative breast cancer and correlates independently with clinical outcome. *Biochem Biophys Res Commun* 2014; 451:54-61.

- [140] Fisel P, Kruck S, Winter S, Bedke J, Hennenlotter J, Nies AT, et al. DNA methylation of the SLC16A3 promoter regulates expression of the human lactate transporter MCT4 in renal cancer with consequences for clinical outcome. *Clin Cancer Res* 2013; 19:5170-81.
- [141] Pertega-Gomes N, Vizcaino JR, Miranda-Goncalves V, Pinheiro C, Silva J, Pereira H, et al. Monocarboxylate transporter 4 (MCT4) and CD147 overexpression is associated with poor prognosis in prostate cancer. *BMC Cancer* 2011; 11:312.
- [142] Marchiq I, Le Floch R, Roux D, Simon MP, Pouyssegur J. Genetic disruption of lactate/H⁺ symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin. *Cancer Res* 2015; 75:171-80.
- [143] Dowling RJ, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. *Cancer Res* 2007; 67:10804-12.
- [144] Salani B, Del Rio A, Marini C, Sambuceti G, Cordera R, Maggi D. Metformin, cancer and glucose metabolism. *Endocr Relat Cancer* 2014; 21:R461-71.
- [145] Kordes S, Pollak MN, Zwinderman AH, Mathot RA, Weterman MJ, Beeker A, et al. Metformin in patients with advanced pancreatic cancer: a double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Oncol* 2015; 16:839-47.
- [146] Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell* 2015; 161:161-72.
- [147] Edwards PA, Ericsson J. Sterols and isoprenoids: signaling molecules derived from the cholesterol biosynthetic pathway. *Annu Rev Biochem* 1999; 68:157-85.
- [148] Mondul AM, Han M, Humphreys EB, Meinhold CL, Walsh PC, Platz EA. Association of statin use with pathological tumor characteristics and prostate cancer recurrence after surgery. *J Urol* 2011; 185:1268-73.
- [149] Brewer TM, Masuda H, Liu DD, Shen Y, Liu P, Iwamoto T, et al. Statin use in primary inflammatory breast cancer: a cohort study. *Br J Cancer* 2013; 109:318-24.
- [150] Nowakowski GS, Maurer MJ, Habermann TM, Ansell SM, Macon WR, Ristow KM, et al. Statin use and prognosis in patients with diffuse large B-cell lymphoma and follicular lymphoma in the rituximab era. *J Clin Oncol* 2010; 28:412-7.
- [151] Zhou W, Mukherjee P, Kiebish MA, Markis WT, Mantis JG, Seyfried TN. The calorically restricted ketogenic diet, an effective alternative therapy for malignant brain cancer. *Nutr Metab (Lond)* 2007; 4:5.
- [152] Skinner R, Trujillo A, Ma X, Beierle EA. Ketone bodies inhibit the viability of human neuroblastoma cells. *J Pediatr Surg* 2009; 44:212-6; discussion 6.
- [153] Holm E, Hagmuller E, Staedt U, Schlickeiser G, Gunther HJ, Leweling H, et al. Substrate balances across colonic carcinomas in humans. *Cancer Res* 1995; 55:1373-8.
- [154] Neal EG, Chaffe H, Schwartz RH, Lawson MS, Edwards N, Fitzsimmons G, et al. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *Lancet Neurol* 2008; 7:500-6.
- [155] Nebeling LC, Miraldi F, Shurin SB, Lerner E. Effects of a ketogenic diet on tumor metabolism and nutritional status in pediatric oncology patients: two case reports. *J Am Coll Nutr* 1995; 14:202-8.
- [156] Fine EJ, Miller A, Quadros EV, Sequeira JM, Feinman RD. Acetoacetate reduces growth and ATP concentration in cancer cell lines which over-express uncoupling protein 2. *Cancer Cell Int* 2009; 9:14.
- [157] Hardman WE. Dietary canola oil suppressed growth of implanted MDA-MB 231 human breast tumors in nude mice. *Nutr Cancer* 2007; 57:177-83.
- [158] Schmidt M, Pfetzer N, Schwab M, Strauss I, Kammerer U. Effects of a ketogenic diet on the quality of life in 16 patients with advanced cancer: A pilot trial. *Nutr Metab (Lond)* 2011; 8:54.

- [159] Schwartz K, Chang HT, Nikolai M, Pernicone J, Rhee S, Olson K, et al. Treatment of glioma patients with ketogenic diets: report of two cases treated with an IRB-approved energy-restricted ketogenic diet protocol and review of the literature. *Cancer Metab* 2015; 3:3.
- [160] Phillips MM, Sheaff MT, Szlosarek PW. Targeting arginine-dependent cancers with arginine-degrading enzymes: opportunities and challenges. *Cancer Res Treat* 2013; 45:251-62.
- [161] Brindle K. Watching tumours gasp and die with MRI: the promise of hyperpolarised ¹³C MR spectroscopic imaging. *Br J Radiol* 2012; 85:697-708.
- [162] Yizhak K, Chaneton B, Gottlieb E, Ruppin E. Modeling cancer metabolism on a genome scale. *Mol Syst Biol* 2015; 11:817.

Figure and table legends

Figure 1: Approaches to analytical metabolomics in cancer patients. Pharmacological and radiological anti-cancer therapies as well as intentional and accidental changes in dietary intake constitute rather general modifications of tumour cell metabolism and have to be taken into account for analytical considerations. In order to track their metabolic fates (by liquid or gas chromatography-coupled mass spectrometry) in excised malignant as well as non-cancerous tissues or body fluids, specific stable isotope-labelled metabolic precursors can be administered to a patient. Body fluids that can be repeatedly sampled by non- or minimally-invasive procedures, and therefore enable dynamic monitoring over time, reflect an “integrative” picture of tumour-derived alterations of systemic metabolism. In contrast, comparative analyses of resected cancerous and non-malignant tissues, either immediately prepared for metabolomic processing or monitored as *ex vivo* cultures may provide direct insights into tumour-specific metabolic changes. Modern imaging techniques including positron emission tomography (PET) and nuclear magnetic resonance detection based on radioactively-labelled (PET) or ^{13}C -hyperpolarised (MRS imaging) metabolites are increasingly employed to functionally monitor malignant lesions and therapeutic responses. FDG: fluorodeoxyglucose; FLT: fluorothymidine; t1-tx, sequential time-points.

Table 1: Current clinical investigation of metabolism-related imaging compounds in cancer medicine. Even though table 1 states the lead site for most trials, many trials are conducted at several sites (sources: www.clinicaltrials.gov [only open clinical trials with reported status were included] and <http://apps.who.int/trialsearch> [only recruiting trials were included]). FDG, ^{18}F -fluorodeoxyglucose; FET, ^{18}F -fluoroethyltyrosine; ^{18}F -FGln, 4- ^{18}F -(2S, 4R)-fluoroglutamine; FLT, ^{18}F -fluorothymidine; ^{18}F -FSPG, ^{18}F -fluoro-L-glutamate derivative BAY94-9392; HCC, hepatocellular carcinoma; 2HG, 2-hydroxyglutarate; MRS, magnetic resonance spectroscopy; PET, positron emission tomography.

Table 2: Current clinical investigation of metabolism-related compounds as cancer treatment. Even though table 1 states the main site for most trials, many trials are conducted at several sites

(sources: www.clinicaltrials.gov [Only open clinical trials with reported status were included] and <http://apps.who.int/trialsearch> [only recruiting trials were included]). ADI, arginine deiminase; AILD, angioimmunoblastic T-cell lymphoma; AML, acute myeloid leukaemia, ALL, acute lymphoblastic leukaemia; CRC, colorectal cancer, DMFO, difluoromethylornithine, FH, fumarate hydratase; HCC, hepatocellular carcinoma; MDS, myelodysplastic syndrome; MCT, monocarboxylate transporter; mt, mutated; mTOR, mammalian Target of Rapamycin; NHL, Non-Hodgkin's lymphoma; PEG, polyethyleneglycol; RCC, renal cell carcinoma, SDH, succinate dehydrogenase.