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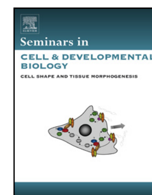

Review

Research into cancer metabolomics: Towards a clinical metamorphosis

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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 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 other 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 anticipations of research in the field.

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Abbreviations: ACSS, acetyl-CoA synthetase; ADI, arginine deiminase; 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; DMFO, difluoromethylornithine; FAS, fatty acid synthase; FDG, 18-fluoro-deoxyglucose; Fd-UMP, 5-fluoro-2′-deoxyuridine monophosphate; 18-F-FGln, 4-18-F-(2S,4R)-fluoro-glutamine; FH, fumarate hydratase; FLT, 18-fluoro-thymidine; 5FU, 5-fluorouracil; F-UTP, 5-fluorouridine triphosphate; GIST, gastrointestinal stromal tumours; GLDC, glycine decarboxylase; GLS, glutaminase; 2HG, 2-hydroxyglutarate; HIF, hypoxia-inducible factor; IDH, isocitrate dehydrogenase; KD, ketogenic diet; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NAPRT, nicotinic acid 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.

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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, it is known that the ability of cancer cells to adapt their metabolism in response to challenging conditions is 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]. The ongoing accumulation of knowledge raises hopes that understanding tumour metabolism will 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, while 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, where cancer cell specific products, termed "oncometabolites", may reach high levels. Examples are 2-hydroxyglutarate (2HG), produced due to oncogenic mutations in isocitrate dehydrogenase (IDH), or succinate and fumarate, which are elevated due to the loss of function of succinate dehydrogenase (SDH) or fumarate hydratase (FH), respectively [9,10]. These cancers are more likely to contain profound adaptations to their genetically imposed metabolic state, and so demonstrate 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 a number of drugs targeting metabolic pathways are employed to treat cancers. Still, many metabolism-specific cancer diagnostics and treatments are at a pre-clinical stage only, and routine targeting of metabolism as a general therapeutic strategy remains challenging. In this review, we present a comprehensive summary 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 overview of the bench-to-bedside studies of tumour metabolomes.

2. Currently approved clinical applications

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

2.1. Anti-metabolites

Compounds that inhibit 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, aka 5FU), nucleoside analogues (e.g. gemcitabine and cytarabine) and anti-folates (e.g. methotrexate) [14].

One of the most widely used "anti-metabolites" is 5FU, a synthetic analogue of the nucleic acid uracil, 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, the fluoride atom prevents the addition of a methyl group at carbon 5, thereby preventing further reactions. Thus, Fd-UMP and F-UTP inhibit thymidylate synthase activity *via* product inhibition, causing lack of the nucleotide deoxythymidine monophosphate. Consequently, DNA replication and cell proliferation are arrested. In addition, F-UTP is incorporated into RNA as a "false building block", impairing the synthesis and maturation of all the classes of RNA [15,16]. Due to its broad cytotoxic activity, 5FU and, increasingly, its orally available pro-drug capecitabine, are indicated for several malignancies including most gastrointestinal cancers [17,18]. Actual nucleoside analogues such as gemcitabine and cytarabine are routinely used alone or in combination with other drugs against a wide range of cancers. They are processed within the cell and incorporated into DNA thus impeding replication and proliferation [14].

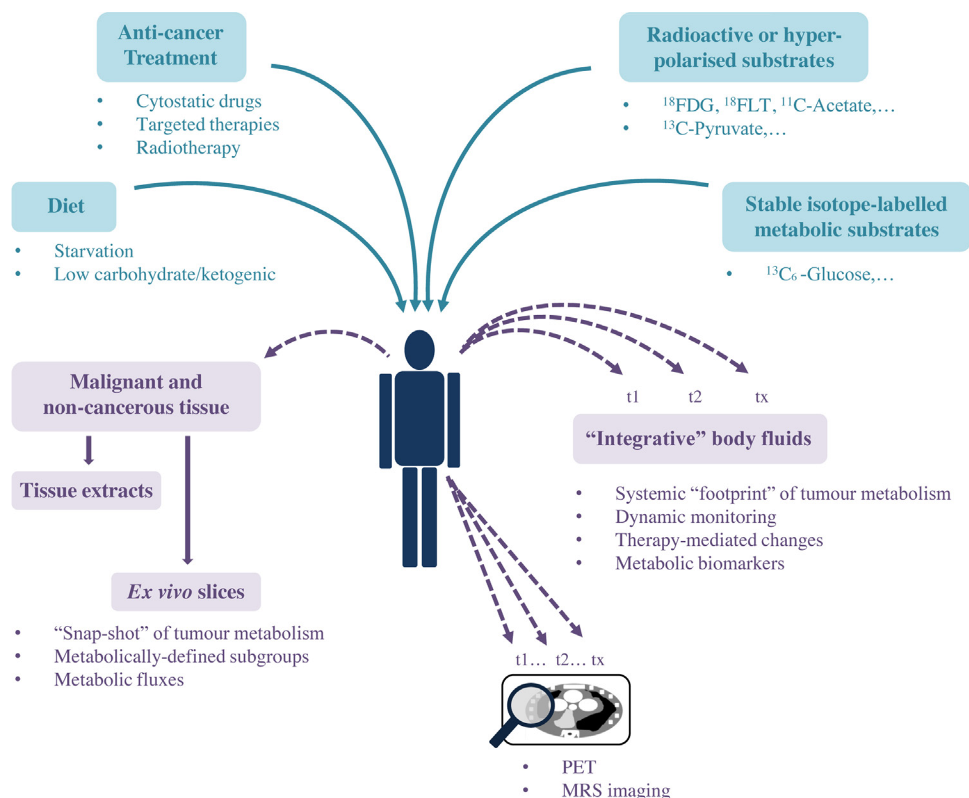

Fig. 1. Approaches to analytical metabolomics in cancer patients. Pharmacological and radiological anti-cancer therapies as well as intentional and accidental changes in dietary intake constitute rather general modifications of tumour cell metabolism and have to be taken into account for analytical considerations. 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; t_1–t_x, sequential time-points.

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 effective against acute lymphoblastic leukaemia (ALL) after the initial observation that low folate diets lowered the white blood cell counts of leukaemia patients [19]. With less toxic side effects and high efficiency, methotrexate has become a powerful standard treatment for ALL, lymphoma and more. Normally, folate is reduced by dihydrofolate reductase (DHFR) into dihydrofolate and then further into tetrahydrofolate (THF), a methyl group donor for many enzymes including thymidylate synthase. Anti-folates bind DHFR, diminishing the production of bases for DNA or RNA synthesis [14]. Importantly, methotrexate toxicity to normal tissues is prevented by administrating folinic acid starting 24 up to 42 h after methotrexate dosing [20].

2.2. Metabolic tracers for cancer detection

Some cancer-specific metabolic alterations are exploited as powerful diagnostic tools. By taking advantage of their enhanced glucose uptake, malignant glycolytic tissues are detected by administrating the radioactively labelled glucose analogue ^{18}fluoro-deoxyglucose (FDG) and measuring site-specific radioactive emission by positron emission tomography (PET). Developed back in 1978, FDG can be taken up by glycolytic cells and phosphorylated by hexokinase, but as it lacks a 2′ hydroxyl group needed for subsequent metabolism, it is trapped in the cells in its phosphorylated form [21]. FDG-PET detection is now clinically used for various lymphomas and is being evaluated in a variety of other cancer types [22]. However, the inherent limitations of this method (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 nutrients more specifically, and different cancer types may have distinct metabolite requirements. For instance, ALL cells were found to be dependent on exogenous supply ("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 date, 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 section describes advanced new investigational technologies, which are in the clinical application stage (illustrated in Fig. 1).

3.1. Broadening the array of cancer tracers

A new generation of metabolic PET-tracers, beyond FDG, is emerging and is currently being evaluated in clinical trials. Additionally, we are seeing rapid development of hyperpolarised ^{13}C-labelled metabolic tracers for magnetic resonance spectroscopy (MRS) imaging. 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 to produce acetyl-CoA, which follows either a catabolic route (feeding the TCA cycle), or an anabolic pathway (for fatty acid and, to a lesser extent, amino acid synthesis [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 tracing ^{11}C-labelled acetate have been published with promising results (e.g. [32]).

3.1.2. Tracing one carbon metabolism: choline and methionine

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

3.1.3. ^{18}Fluoro-thymidine (FLT)

A thymidine analogue, FLT is used to directly detect tumour cell proliferation, taking advantage of the high DNA replication and cell division rates of many malignant tumours [33]. Following its phosphorylation by thymidine kinase 1 (TK1), which shows increased activity during the 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 also allow the use of PET diagnostics for less avidly glycolytic cancers.

3.1.4. ^{18}Fluoro-glutamine

Glutamine – through its conversion to glutamate by glutaminases (GLS1 and GLS2) – fuels the tri-carboxylic acid (TCA) cycle, generates "reducing power" and contributes to the synthesis of non-essential amino acids *via* cytosolic or mitochondrial transaminases [34]. 4-^{18}F-(2S,4R)-fluoro-glutamine (^{18}F-FGln), designed to detect glutamine consumption and utilisation, 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. Hyperpolarised ^{13}C metabolic tracers

By dramatically increasing signal-to-noise-ratios, "hyperpolarisation" of the nuclear spins of ^{13}C-labelled metabolites has enabled MRS imaging of these isotopes *in vivo* [37]. In addition to evaluating their immediate transport, the metabolic fate of these labelled substrates can be traced in various tissues [38]. This principle has been applied in different cancer models to assess extracellular pH (using the ^{13}C-bicarbonate to $^{13}CO_2$ ratio), alterations in glycolytic flux (from ^{13}C-glucose to ^{13}C-lactate), production of the oncometabolite 2HG (from ^{13}C-glutamate), redox stress (*via* ^{13}C-dehydroascorbate), and for detecting cancer cells on a background of healthy mouse liver tissue (reading [1,3-$^{13}C_2$]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* (*via* its conversion to ^{13}C-lactate by lactate dehydrogenase (LDH)) and a good indicator of 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 degree of conversion of pyruvate to lactate was confirmed in the carcinomas compared to tumour-free tissues [45]. Moreover, this method appeared superior to standard ($^{+}$H-based) magnetic resonance imaging (MRI) for identifying the more aggressive carcinomas [45]. New hyperpolarised metabolic MRS imaging tracers are under development and the prospect of simultaneously hyperpolarising and tracking several molecules *in vivo* highlights 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

In this section we highlight the power of analytical metabolomic investigations in humans. The use of nuclear magnetic resonance (NMR) or mass spectrometry to provide medium- to high-throughput metabolic profiles, techniques collectively known as "metabolomics", is a powerful approach recently incorporated into the research arsenal in the clinic. Besides the increasing application of metabolism-based imaging techniques, four main approaches for investigating cancer-driven metabolic re-programming in humans exist: First, the comparing of tumour metabolomes to those of adjacent normal tissues after resection *in sano*. Second, the metabolic tracing in patients of systemically administered stable isotope-labelled substrates (e.g. ^{13}C-labelled glucose) can provide a more dynamic metabolic picture. Third, more detailed metabolic tracing can be performed in thin tissue slices derived from freshly resected tumours *ex vivo*. Last, 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. Together, these methods may provide valuable "integrative" insights into tumour metabolism and dynamics, and enable the discovery of biomarkers that quantitatively or qualitatively correlate with malignant features. Fig. 1 summarises different approaches to investigating cancer metabolism in patients; a selection of recent studies is discussed hereinafter.

3.2.1. Mechanistically informative metabolic biomarkers

In a metabolomic profiling study of untreated gliomas, 2HG was identified to be the major grade-discriminating metabolite in the low grade tumours [13]. Furthermore, in higher grade tumours, three metabolically defined subtypes ("anabolic", "energetic" and "phospholipid catabolic") were identified and correlated with the prognosis of the respective patients. These metabolic subgroups were largely independent from the gene expression profile-based tumour stratification [13]. Another integrative study, which metabolically characterised diffuse large B cell lymphoma, identified a subset of tumours with increased oxidative energy metabolism [11]. In both studies, the metabolic profiling provided informative signatures that helped characterise 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. Mayers et al. took advantage of a large number of blood samples acquired over multiple studies, and searched for potential diagnostic biomarkers by identifying early changes in systemic metabolite concentrations, which associated with the later development of

Table 1
Current clinical investigations of metabolism-related imaging compounds in cancer medicine.

Tracing	Entity	Trial number	Lead site
FDG-PET	Various	>250 Open trials	Various sites
FLT-PET	Various	>35 Open trials	Various sites
^{11}C-methionine- PET	Glioblastoma	NCT01867593	Massachusetts General Hospital, Boston, USA
		NCT01873469	Technische Universität Dresden, Dresden, Germany
	Brain tumours	JPRN-UMIN000003386	Tokushima University Hospital, Tokushima, Japan
	Brain metastases	NCT02433171	Yale University, New Haven, USA
	Lung cancer	JPRN-UMIN000005096	Osaka University Graduate School of Medicine, Osaka, Japan
	Thyroid carcinoma	NTR1937	University Medical Centre Groningen, Groningen, The Netherlands
	Various	NCT00840047	St. Jude Children's Research Hospital, Memphis, USA
		JPRN-UMIN000016128	Hokkaido University Hospital, Sapporo, Japan
		JPRN-UMIN000002048	Saitama Medical University, Saitama, Japan
		JPRN-UMIN000004043	Kyoto University Hospital, Kyoto, Japan
^{18}F-FGln-PET	Solid malignancies, lymphoma	NCT01697930	Memorial Sloan Kettering Cancer Center, New York, USA
^{11}C-acetate-PET	Prostate carcinoma	NCT01304485	Arizona Molecular Imaging Center, Phoenix, USA
		NCT01530269	Arizona Molecular Imaging Center, Phoenix, USA
		NCT01777061	University of Kansas Medical Center Research Institute, Fairway, USA
	Bladder carcinoma	NCT01918592	Turku University Hospital, Åbo, Finland
	Glioma	NCT01961934	Arizona Molecular Imaging Center, Phoenix, USA
	Multiple myeloma	NCT01691300	Chang Gung Memorial Hospital, Gueishan, Taiwan
^{11}C-choline-PET	Prostate carcinoma	NCT02260817	Decatur Memorial Hospital, Decatur, USA
		NCT02397408	University of California, San Francisco, USA
		NCT02232724	Odense University Hospital, Odense, Denmark
		NCT02232672	Odense University Hospital, Odense, Denmark
		JPRN-UMIN000013377	National Centre for Global Health and Medicine, Tokyo, Japan
		EUCTR2013-001330-17-IT	IRCCS Azienda Ospedaliera Universitaria San Martino – IST Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy
^{18}F-choline-PET	Prostate carcinoma	NCT01981707	University Hospital, Grenoble, France
		NCT02232685	Odense University Hospital, Odense, Denmark
		NCT02397408	University of California, San Francisco, USA
		NCT01751737	University of Michigan, Ann Arbour, USA
		NCT01804231	Lawson Health Research Institute, London, Canada
		NCT02131649	Lawson Health Research Institute, London, Canada
		EUCTR2009-014839-21-FR	CIS bio international, member of IBA group, France
		EUCTR2011-004951-38-IT	Programma Ricerca e Innovazione, Forli, Italy
		EUCTR2009-011189-27-IT	Istituti Fisioterapici Ospitalieri, Roma, Italy
		EUCTR2008-004236-20-IT	Azienda Ospedaliera Arcispedale Santa Maria Nuova, Reggio Emilia, Italy
	HCC	NCT01116804	University Hospital, Ghent, Belgium
	Breast cancer	NCT01956409	National Taiwan University Hospital, Taipei, Taiwan
	Glioma	EUCTR2009-011380-37-IT	Azienda Ospedaliera Arcispedale Santa Maria Nuova, Reggio Emilia, Italy
^{18}F-methylcholine- PET	Prostate carcinoma	NCT00928252	Queen's Medical Center, Honolulu, USA
		EUCTR2013-004397-99-ES	Instituto Tecnológico PET, S.A.U. (ITP), Madrid, Spain
		EUCTR2007-004419-69-FR	Assistance publique – Hôpitaux de Paris, Paris, France
		EUCTR2008-003337-24-IT	IRCCS CROB, Rionero In Vulture PZ, Italy
	HCC	NCT01395030	Queen's Medical Center, Honolulu, USA
	High grade glioma	NCT00628940	University Hospital, Ghent, Belgium
	Breast cancer	EUCTR2011-003036-31-IT	Medicina Nucleare Biomolecolare, Florence, Italy
^{18}F-ethylcholine- PET	HCC	NCT02238769	Peking Union Medical College Hospital, Beijing, China
	Prostate carcinoma	EUCTR2012-004297-26-IT	Ist. Medicina Nucleare, Roma, Italy
		EUCTR2006-003933-33-DE	Bundeswehr, Germany
	Endometrial carcinoma, cervical carcinoma	NCT01836484	Barts & The London NHS Trust, London, UK
^{18}F-FSPG-PET (BAY94-9392)	HCC	NCT02379377	Vanderbilt-Ingram Cancer Center, Nashville, USA
	Brain cancer	NCT02370563	Stanford University Medical Center, Stanford, USA
	Lung cancer	NCT02448225	Vanderbilt-Ingram Cancer Center, Nashville, USA
FET-PET	Glioblastoma	NCT02329795	Rigshospitalet, Copenhagen, Denmark
		NCT01756352	Brigham and Women's Hospital, Boston, USA
O-2-FET-PET	Low grade glioma	NCT02286531	University Hospital, Toulouse, France
2HG detection (MRS)	Glioma	NCT02388659	University of Texas Southwestern Medical Center, Dallas, USA
Hyperpolarised ^{13}C-Pyruvate	Malignant solid tumours	NCT02421380	Memorial Sloan Kettering Cancer Center, New York, USA
	Prostate carcinoma	NCT02450201	University of California, San Francisco, USA

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.

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 2–10 years prior to diagnosis with PDAC [47]. Their findings were affirmed in a mouse model of PDAC, but not in other KRAS-associated cancers, implying tumour-specific changes in systemic protein (amino acid) metabolism at very early stages of this malignancy. Thus a simple blood screening may detect PDAC at its initial stage, when complete surgical resection, the only curative option, is still possible. However, since elevated BCAAs levels were found to be associated with a twofold increase in PDAC risk and for a limited window prior to diagnosis only, other prognosticators are required in order to define the population that would benefit from intensified surveillance [47]. Further studies in PDAC metabolomics and the integration of results with genetic information, such as performed by Zhang et al., whose findings pointed towards

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 of more than 200 acute myeloid leukaemia (AML) patients, many plasma metabolites were found to be elevated in comparison to healthy controls [49]. Here, a 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 currently highly heterogeneous subgroup with regards to therapeutic outcome. Model-based investigation further implied that high glycolytic activity could be associated with resistance to cytarabine, a drug commonly used to treat AML, and that pharmacologically interfering with glycolysis may increase the 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 non-small cell lung cancer (NSCLC) specimens from over 80 patients, analysing metabolic adaptations [12,50]. They injected patients with uniformly ^{13}C-labelled glucose and, after resection, analysed its intra-tumoural metabolic fate. They found that tumours had a specific increased activity of pyruvate carboxylase (PC). PC is an anaplerotic enzyme that carboxylates pyruvate directly to oxaloacetate; hence its activity sustains TCA cycle metabolite levels. *Ex vivo* experiments comparing the metabolite labelling patterns of 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 of a tumour from its metabolic profile remains an improbable goal. However, genetic screening for changes in gene expression, promoter methylations or the copy number of "metabolic genes" may constitute a better strategy for arriving at a prognosis or for 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

The 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. To date IDH3 mutations in cancer have not been detected, whereas 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, pathological, catalytic activity namely the reduction of α-ketoglutarate to 2HG. By the competitive inhibition of demethylases (of the α-ketoglutarate-dependent dioxygenase family), 2HG alters the epigenome [57,58]. IDH-mutated tumours provide a rare opportunity to specifically target a "metabolic oncogene" without interfering with normal metabolism, and clinical trials in AML and glioma are currently testing small-compounds inhibitors of IDH (AG-120 against IDH1 or AG-221 against IDH2), or vaccine immunotherapy against IDH1 neoantigen [59] (Table 2). In addition, other IDH inhibitors as well as other IDH-mutated malignancies are being investigated (Table 2). It is noteworthy that in lower grade glioma, IDH1/2 gain of function mutations confer a relatively favourable prognosis [60,61], whereas data are much less clear for AML [62,63]. The mutational status of IDH1 and IDH2 and the degree of 2HG production are likely to be incorporated into routine diagnostic algorithms for glioma and AML [64,65].

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 the 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]. Families 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 carcinomas (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 a current RCC classification [69,70]. SDH inactivation also occurs in a fraction of gastrointestinal stromal tumours (GIST) without c-kit mutations [71,72]. Hence, the 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 acquire diagnostic relevance once 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 phaeochromocytoma 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. The increased PC activity compensates for and bypasses the TCA cycle block and partially sustains aspartate synthesis. Elevated PC protein expression was also detected in SDH-mutated RCC specimens when compared to same-patient adjacent non-cancerous kidney tissues. 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 in various solid tumours and AML renders them auxotrophic of this otherwise non-essential amino acid [7,78–80]. Mechanistically, lack of ASS1 activity supports proliferation because aspartate, one of its substrates, is then free for use in nucleotide biosynthesis [81]. Therapeutically, since ASS1 loss induces arginine auxotrophy, depleting plasma arginine using systemic administration of bacterial or bio-engineered arginase or arginine deiminases (ADI), is being tested in clinical trials, showing efficacy in these tumours ([79,82,83], Table 2).

4.4. Acetyl-CoA synthetase 2 (ACSS2) induction

The metabolic role of acetate in cancer was outlined in Section 3.1.1. Recently, acetate metabolism in cancer was revisited with a fresh look. By applying *in situ* tumour metabolomics directly in patients, Maher et al., provided evidence for high TCA cycle activity in glioblastomas [84]. Following intravenous infusion of uniformly

Table 2
Current clinical investigation of metabolism-related compounds as cancer treatment.

Target	Compound	Entity	Trial number	Lead site
MCT1	AZD3965	Advanced solid tumour, prostate carcinoma, gastric cancer, DLBCL	NCT01791595	Royal Marsden Hospital, London, UK
Polyamines	DMFO	Neuroblastoma	NCT02030964	Children's Hospital Los Angeles, Los Angeles, USA
			NCT02395666	Helen DeVos Children's Hospital, Grand Rapids, USA
			NCT02139397	Helen DeVos Children's Hospital, Grand Rapids, USA
		(Prevention of) CRC	NCT00983580	Mayo Clinic, Rochester, United States
			NCT01483144	Multinational
			NCT01349881	689 sites, USA
Arginine (plasma)	Pegylated arginase (BCT-100)	HCC	NCT02089763	Prince of Wales Hospital, Hong Kong, China
			NCT02089633	Queen Mary Hospital, Hong Kong, China
			NCT02285101	Loma Linda University Medical Center, Loma Linda, USA
		RCC, melanoma, prostate carcinoma	NCT02285101	Loma Linda University Medical Center, Loma Linda, USA
	Pegylated arginine deiminase (ADI-PEG 20)	Advanced solid tumours	NCT01497925	University of California at Davis Sacramento, USA
			NCT01665183	MD Anderson Cancer Center, Houston, USA
		(gastrointestinal)	NCT02102022	Memorial Sloan-Kettering Cancer Center, New York, USA
		Melanoma	NCT01665183	MD Anderson Cancer Center, Houston, USA
			NCT02029690	Centre for Experimental Cancer Medicine (CECM), London, UK
		Mesothelioma	NCT02029690	Centre for Experimental Cancer Medicine (CECM), London, UK
			EUCTR2006-004592-35-GB	Centre for Experimental Cancer Medicine (CECM), London, UK
		NSCLC	NCT02029690	Centre for Experimental Cancer Medicine (CECM), London, UK
		Glioma	NCT02029690	Centre for Experimental Cancer Medicine (CECM), London, UK
		HCC	NCT02029690	Centre for Experimental Cancer Medicine (CECM), London, UK
			NCT02101593	MD Anderson Cancer Center, Houston, USA
			NCT02006030	National Taiwan University Hospital, Taipei, Taiwan
			EUCTR2011-002041-36-GB	Multinational
		Breast cancer	NCT01948843	MD Anderson Cancer Center, Houston, USA
		Pancreatic carcinoma	NCT02101580	Memorial Sloan-Kettering Cancer Center, New York, USA
		NHL	NCT01910025	Chang Gung Medical Foundation-Kaohsiung, Kaohsiung, Taiwan
		AML	NCT01910012	MD Anderson Cancer Center, Houston, USA
Glutamine (plasma)	(PEG-) asparaginase	AML, ALL, NHL, pancreatic carcinoma	>50 Open trials	Various sites
Glutaminase	CB-839	AML, ALL	NCT02071927	Various sites, USA
		Multiple myeloma, NHL	NCT02071888	Various sites, USA
		Solid malignancies (including FH-, SDH-deficient and IDHmt tumours)	NCT02071862	Various sites, USA
mt. IDH1	AG-120	IDH1mt AML/haematological malignancies	NCT02074839	Various sites, USA and France
		IDH1 mt solid malignancies	NCT02073994	Various sites, USA and France
	IDH305	IDHR132 mt advanced malignancies	NCT02381886	Multinational
	Peptide vaccine	IDH1mt glioma grade III–IV	NCT02454634	National Centre for Tumour Diseases, Heidelberg, Germany
		IDH1mt Glioma grade II	NCT02193347	Duke Comprehensive Cancer Center, Durham, USA
mt. IDH2	AG-221	IDH2mt haematological malignancies	NCT01915498	Various sites, USA and France
		IDH2mt solid malignancies	NCT02273739	Various sites, USA and France
		IDH2mt AILD	NCT02273739	Various sites, USA and France
mt. IDH1/2	AG-881	IDH1/2mt haematological malignancies	NCT02492737	MD Anderson Cancer Center, Houston, USA
		IDH1/2mt solid tumours	NCT02481154	Various sites, USA
Glutathione	Dimethylfumarate	Glioblastoma	NCT02337426	Virginia Commonwealth University, Richmond, USA
		Cutaneous T-NHL	EUCTR2014-000924-11-DE	University Medical Centre Mannheim, Mannheim, Germany
mTOR, complex 1	Metformin	Various	>100 Open trials	Various sites
Cholesterol synthesis	Statins	Various	>20 Open trials	Various sites
General tumour metabolism	Modified diets	Various	>400 Studies	Various sites
	Ketogenic diets	Glioblastoma	NCT02286167	Mid-Atlantic Epilepsy and Sleep Center, Bethesda, USA
			NCT02302235	Mid-Atlantic Epilepsy and Sleep Center, Bethesda, USA
			NCT02046187	St. Joseph's Hospital and Medical Center, Phoenix, USA
			NCT01535911	Michigan State University/Sparrow Hospital, E. Lansing, USA
			NCT01754350	Johann Wolfgang Goethe University Hospital, Frankfurt, Germany
			NTR5167	Erasmus Medical Centre, Rotterdam, The Netherlands
		Rectum carcinoma	NCT02516501	Leopoldina Krankenhaus, Schweinfurt, Germany
		Breast carcinoma	NCT02516501	Leopoldina Krankenhaus, Schweinfurt, Germany
			NCT02092753	Rehaklinik Am Kurpark, Bad Kissingen, Germany
		Head and neck cancer	NCT02516501	Leopoldina Krankenhaus, Schweinfurt, Germany
			NCT01975766	Holden Comprehensive Cancer Center, Iowa City, USA
	"Atkins diet"	Glioblastoma	NCT02286167	The Johns Hopkins Hospital, Baltimore, USA
		Prostate carcinoma	NCT00932672	Duke University Medical Center, Durham, USA
	Fasting	Various	>150 Trials	Various sites

Even though Table 2 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.

labelled ^{13}C-glucose, MRS imaging of intra-tumoural metabolites indicated a source, in addition to glucose, of acetyl-CoA [84]. In a follow-up study, Mashimo and colleagues identified acetate as a “bioenergetic substrate” for glioblastoma and brain metastases by tracing the intracellular fate of ^{13}C-labelled acetate, following its administration to patients during surgery [30]. Together with recent work by Schug et al. [31] and Comerford et al. [85], these findings point at the acetate metabolising 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 was recently reported, with significantly higher ACSS2 mRNA and protein level associated with poorer prognosis [31]. These results accord 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.

4.5. Addiction to the serine and glycine biosynthetic pathway

D-3-phosphoglycerate dehydrogenase (PHGDH) catalyses the rate limiting step of serine biosynthesis, converting the glycolytic intermediate 3-phosphoglycerate to 3-phospho-hydroxypyruvate, and has been found to be genetically amplified, overexpressed or otherwise essential for the growth of melanoma, as well as colorectal and breast cancer [5,8,86]. The mitochondrial enzyme serine hydroxyl-methyl-transferase 2 (SHMT2) converts serine to glycine, thereby freeing one carbon unit that supports THF and nucleotide biosynthesis. Publically available gene expression data sets revealed *shmt2* copy-number gain and overexpression in breast and ovarian cancer and in 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 them with a metabolic advantage under hypoxia [90]. PHGDH, SHMT2 and GLDC may not only serve as prognostic markers, but, in the future, serine/glycine metabolism may be therapeutically targeted (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 (Section 2), cumulative understanding of metabolic transformation has 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 specifically designed to interfere with tumour metabolism that are either being currently assessed (or re-evaluated) in clinical trials (Table 2) or, to our knowledge, are in advanced stages of pharmacological and pre-clinical development.

5.1.1. 2-Deoxyglucose (2DG)

In the 1950s, 2DG, a glucose analogue that enters, but is not 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], causes growth arrest and apoptosis and thereby potentiates the activity of classical antineoplastic agents [93,94]. Unfortunately, clinical studies 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), but 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 many reactions of cellular metabolism and redox balance. In cancer cells NAD is mostly regenerated from nicotinamide-mononucleotide. *Nampt* encodes the rate-limiting enzyme of the NAD salvage pathway, and its increased gene expression has been observed in a variety of cancer entities ([97,98], reviewed in [99]). NAMPT activity increases the abundance of NAD^+, also a coenzyme of the deacetylase silent information regulator 1 (SIRT1), thereby activating the oncogene *c-myc* and inhibiting the senescence and apoptosis responses to oncogenic activation [100]. Moreover, NAMPT is actually transcriptionally activated by c-MYC [100]. Corroborating the vulnerability of tumour cells 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 partially explain the selective toxicity that NAMPT inhibitors inflict on cancer cells and suggests 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 to poorer outcome in relapsed multiple myeloma [104]. Accordingly, pre-clinical treatment with FK866, as well as its combination with bortezomib, has indicated beneficial effects 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 the drugs were used as single agents [107–109]. The development of more potent NAMPT inhibitors, a more thorough patient stratification strategy, drug administration earlier in the course of disease and identification of promising therapeutic combinations may provide better prospects for the use of NAMPT-targeting drugs.

5.1.3. Polyamines and arginine biosynthetic pathways

Despite being involved in virtually all cellular functions, polyamines (a class comprising three molecules produced successively from arginine and ornithine: putrescine, spermidine and spermine, and their acetylated derivates) are interesting targets for cancer-directed therapies due to their important role in cell proliferation specifically [110]. Several inhibitors of enzymes involved in polyamine synthesis, such as difluoromethylornithine (DFMO), were developed and tested in clinical trials either as chemopreventives or chemotherapeutics [111] (Table 2). Arginine itself can be converted to ornithine but, in addition to being a polyamine precursor, arginine seems to have other important roles in some cancers [80]. Arginine metabolism is highly connected the urea

cycle too and provides cancer cells with a way of disposing of nitrogen residues. Ornithine is a central intermediate at the "crossroad" of proline, urea and polyamine pathways. As discussed in Section 4.3, arginine-depleting approaches in *ASS1*-silenced tumours are demonstrating efficacy.

5.1.4. Glutaminase (GLS)

The idea of interrupting the supply or utilisation of the conditionally-essential amino acid glutamine in order to fight cancer dates back several decades and is based on its high concentration in plasma as well as the selective vulnerability of a variety of malignant cells to glutamine depletion [3,112,113]. Interestingly, the antineoplastic effects of asparaginase (discussed in Section 2.3) have been partly linked to its additional, inherent glutamine-depleting function [114]. Intracellular glutaminases catalyse the deamination of glutamine to glutamate, which can serve as a precursor for α-ketoglutarate (for anaplerosis) or for glutathione (antioxidant) [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 therapeutic activity in tumour models with elevated GLS activity without prohibitive systemic toxicity [4,119,120]. Additionally, metabolic profiling of IDH1/2-mutated glioma cells has shown 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 being 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 in conjunction with oxidising cytosolic NADH to NAD^+ hence supporting high glycolytic flux. LDHA is a direct target of the hypoxia inducible transcription factors (HIFs) and of several activated oncogenes, and consequently is overexpressed in a variety of malignancies [124]. Silencing 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 decrease in the conversion of ^{13}C-labelled glucose to lactate as well as in anaplerotic glutamine consumption [127]. Furthermore, following anti-angiogenic treatment, *in vivo* metabolic tracing of ^{13}C-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 neutralising a tumour escape mechanism from treatment with bevacizumab, an anti-vascular endothelial growth factor (VEGF) antibody. Several small molecule inhibitors of LDHA have been reported over 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, now being tested against advanced cancers in the UK (Table 2) [133]. Unfortunately, MCT1 inhibitors fail to block MCT4 [134,135] and 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 was 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 being assessed in clinical trials (Table 2).

5.2.1. Metformin

Metformin, an anti-diabetic drug, modulates both peripheral insulin-resistance and hepatic gluconeogenesis. It does so 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 energy-demanding anabolic processes and stimulate energy-generating catabolic processes. It does so partly *via* the inhibition of 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]). Disappointingly however, a recent large randomised trial in advanced pancreatic cancer showed no benefit [145].

5.2.2. Statins

Statins, which are 3-hydroxy-3-methylgutaryl-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 require cholesterol for stability [147], it was supposed that impeding cholesterol synthesis would affect tumour cell growth. Studies aiming at correlating tumour incidence or treatment outcomes to statin use were conducted for various cancers [148–150], but outcomes have remained largely inconclusive to date.

5.3. Addressing cancers with modified diets

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 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 KDs on two girls suffering from astrocytoma was published in 1995 [155]. Since then, KDs have demonstrated anti-tumour activity in cancer cells *in vitro* [152,156] and in 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 these introductory reports and to appreciate who may benefit from such an approach.

6. Future perspectives for metabolomics in cancer diagnosis and therapy

We herein provide an overview of current and possible future clinical applications of recent advances in metabolomics. Analytical metabolomics have by now added 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 in 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 structure and strength. 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 metabolic biomarkers that will enable better diagnosis and monitoring, we expect new metabolomics studies to provide new molecular targets and enable tailoring of treatments to patients. Relatively inexpensive and reproducible metabolomics analyses, combined with other "omics" approaches, may soon shape modern medicine. So, 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.

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