UNIVERSITÀ DI PARMA
ARCHIVIO DELLA RICERCA

University of Parma Research Repository

Impairing energy metabolism in solid tumors through agents targeting oncogenic signaling pathways

This is the peer reviewd version of the followng article:

Original
Impairing energy metabolism in solid tumors through agents targeting oncogenic signaling pathways / Fumarola, Claudia; Petronini, Pier Giorgio; Alfieri, Roberta. - In: BIOCHEMICAL PHARMACOLOGY. - ISSN 0006-2952. - 151(2018), pp. 114-125-125. [10.1016/j.bcp.2018.03.006]

Availability:
This version is available at: 11381/2841816 since: 2022-01-07T20:29:19Z

Publisher:

Published
DOI:10.1016/j.bcp.2018.03.006

Terms of use:
openAccess

Anyone can freely access the full text of works made available as "Open Access". Works made available

Publisher copyright

(Article begins on next page)

13 October 2022

Commentary

Impairing energy metabolism in solid tumors through agents targeting oncogenic signaling pathways

Claudia Fumarola

claudia.fumarola@unipr.it

Pier Giorgio Petronini

piergiorgio.petronini@unipr.it

Roberta Alfieri*

roberta.alfieri@unipr.it

Department of Medicine and Surgery, University of Parma, Parma, Italy

*Corresponding author at: Department of Medicine and Surgery, University of Parma, Via Volturno, 39, 43126 Parma, Italy.

Abstract

Cell metabolic reprogramming is one of the main hallmarks of cancer and many oncogenic pathways that drive the cancer-promoting signals also drive the altered metabolism.

This review focuses on recent data on the use of oncogene-targeting agents as potential modulators of deregulated metabolism in different solid cancers. Many drugs, originally designed to inhibit a specific target, then have turned out to have different effects involving also cell metabolism, which may contribute to the mechanisms underlying the growth inhibitory activity of these drugs. Metabolic reprogramming may also represent a way by which cancer cells escape from the selective pressure of targeted drugs and become resistant. Here we discuss how targeting metabolism could emerge as a new effective strategy to overcome such resistance.

Finally, accumulating evidence indicates that cancer metabolic rewiring may have profound effects on tumor-infiltrating immune cells. Modulating cancer metabolic pathways through oncogene-targeting agents may not only restore more favorable conditions for proper lymphocytes activation, but also increase the persistence of memory T cells, thereby improving the efficacy of immune-surveillance.

Abbreviations: ACC, acetyl-CoA carboxylase; ALDOA, aldolase A; AIs, aromatase inhibitors; AMPK, AMP-activated protein kinase; BC, breast cancer; CML, chronic myelogenous leukemia; CT, computed tomography; CPT1, carnitine palmitoyltransferase 1; CRC, colorectal cancer; 2-DG, 2-deoxyglucose; EGFR, epidermal growth factor receptor; EML4-ALK, echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase; FA, fatty acids; FAO, fatty acid β-oxidation; FASN, FA synthase; FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography; FGFR1, fibroblast growth factor receptor type 1; GIST, gastrointestinal stromal tumors; GLS, glutaminase; GLUD1, glutamate dehydrogenase; GLUT, glucose transporter; HCC, hepatocellular carcinoma; HIF-1, hypoxia-inducible factor 1; HNSCC, head and neck squamous cell carcinoma; HK, exokinase; HP-MRS, hyperpolarized magnetic resonance spectroscopy; IDO, indoleamine 23-dioxygenase; LDH, lactate hydrogenase; LDs, lipid droplets; MDM2, murine double minute 2; MDSCs, myeloid-derived suppressor cells; NSCLC, Non-Small Cell Lung Cancer; OXPHOS, oxidative phosphorylation; PC, prostate cancer; PDC, pyruvate dehydrogenase enzyme complex; PDH, pyruvate dehydrogenase; PDHK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PKM, pyruvate kinase M; PPAR-δ, peroxisome proliferator–activated receptor-δ; PPP, pentose phosphate pathway; RCC, renal cancer carcinoma; ROS, reactive oxygen species; SCD, stearoyl CoA desaturase; SREBP1, Sterol regulatory element binding protein 1; SQCLC, squamous cell lung carcinoma; TCA, tricarboxylic acid; TIGAR, TP53-induced glycolysis and apoptosis regulator; TKI, tyrosine kinase inhibitor; TME, tumor microenvironment; TNBC, triple negative breast cancer cells

Keywords: Metabolism; Target therapy; Cancer; Resistance; Immunity

1 Introduction

Energy metabolic reprogramming is one of the hallmarks of cancer cells. Together with aerobic glycolysis, the best known metabolic feature of tumor cells, glutamine addiction and increased lipid metabolism have been also added as important components of metabolic transformation.

1.1 Aerobic glycolysis

Aerobic glycolysis was firstly reported in the 1920s by Otto Warburg, who observed that cancer cells limited their energy metabolism largely to glycolysis producing large amounts of lactate even under fully oxygenated

conditions.

This metabolic feature, known as the “Warburg effect”, has represented the basis for the development of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET), a non-invasive imaging technique that, providing an accurate assessment of tumor glucose utilization, is widely exploited in the clinic for initial diagnosis, staging disease, and monitoring tumor responses to therapies. The glycolytic switch in cancer cells allows to maximize the diversion of glycolytic intermediates into multiple branching pathways, that provide substrates for the biosynthesis of macromolecules (lipids, nucleic acids, and proteins) required for rapid tumor growth and proliferation [1]. In addition, an increased flux through the cytosolic oxidative pentose phosphate pathway (PPP) provides cytosolic NADPH to balance the intracellular redox potential and neutralize the excessive levels of reactive oxygen species (ROS) resulting from the enhanced metabolic activity of cancer cells [2]. It is worth noting that, despite the increased glycolytic rates, some glucose transitions to the tricarboxylic acid (TCA) cycle in mitochondria are still maintained in cancer cells for both ATP production and generation of citrate for lipid synthesis [1].

Alterations in oncogenic signaling transduction pathways and loss of tumor suppressor genes, by affecting the regulation of transporters and enzymes, drive cancer cells to aerobic glycolysis. Over-expression of the facilitative glucose transporter 1 (GLUT-1) promotes an increased glucose uptake in a variety of cancer cell types, and has been associated with the constitutive activation of oncogenes such as *KRAS*, *AKT*, *src*, and *Myc* [3] or with the loss of the tumor suppressor *PTEN* [4]. Activated AKT may also favor cancer glucose uptake by promoting the cell surface localization of GLUT-1 and attenuating its internalization [5]. In addition, aberrant activity of the PI3K/AKT/mTORC1 pathway has been shown to enhance the expression of Hexokinase II (HKII), the first rate-limiting enzyme of glycolysis, which mediates the rapid phosphorylation of glucose upon its entrance into the cell [6]. Conversely, miR-124 overexpression has been demonstrated to hinder glycolysis by decreasing AKT-induced expression of both GLUT-1 and HKII in non-small cell lung cancer (NSCLC) cells [7]. Interestingly, PI3K has been shown to activate an AKT-independent Rac-dependent mechanism that promotes the release of filamentous actin-bound aldolase A (ALDOA), thus increasing ALDOA activity and enhancing the flux through glycolysis [8]. AKT may also induce the rate-controlling enzyme phosphofructokinase-1 (PFK1) by directly phosphorylating and activating phosphofructokinase-2 (PFK2) [9]; AKT-dependent modulation of cancer glucose metabolism may be indirectly mediated by the downstream target mTORC1, which in turn can activate the hypoxia-inducible factor 1 (HIF-1) even under normoxic conditions. HIF-1 is consider as a master regulator of cancer metabolic reprogramming towards glycolysis, being responsible for the induction of a variety of genes encoding for glucose transporters and glycolytic enzymes [10]. Not only AKT/mTORC1 signaling, but also deregulated Myc can stabilize HIF-1 protein under normoxic conditions [11]. HIF-1 and Myc have been shown to cooperatively induce HKII and pyruvate dehydrogenase kinase 1 (PDHK1). PDHK1 in turn inactivates the pyruvate dehydrogenase (PDH) and hence the pyruvate dehydrogenase enzyme complex (PDC) responsible for the conversion of pyruvate to acetyl-coenzyme A, thereby inhibiting pyruvate entry in the tricarboxylic acid (TCA) cycle and diminishing mitochondrial respiration [12]. In addition, both HIF-1 and Myc enhance the transcription of lactate dehydrogenase A (LDHA), which catalyzes the conversion of pyruvate into lactate, further contributing to the glycolytic cancer phenotype in Myc over-expressing cancers [12]. HIF-1 and Myc positively regulate the expression of pyruvate kinase M2 (PKM2), the final rate-limiting enzyme of glycolysis, which catalyzes the conversion of phosphoenolpyruvate to pyruvate. Over-expression of PKM2 has been found in multiple human cancers, including lung, breast, and colon, compared to matched normal tissues, and is considered as a major contributor to altered cancer metabolism [13]. PKM2 exists as a tetramer, showing high catalytic activity, or as a dimeric less active form, and the conversion between these two forms is dynamically regulated. In cancer cells, a number of post-translational modifications, such as fibroblast growth factor receptor type 1 (FGFR1)-mediated phosphorylation at Tyr105, promote the tetramer-to-dimer switching, thus reducing pyruvate kinase activity and favoring the diversion of glycolytic intermediates towards collateral pathways, such as PPP and serine biosynthesis [14]. PKM2 can facilitate the Warburg effect also through non canonical functions, that are independent of its pyruvate kinase activity, and involve its ability to directly transactivate HIF-1α or indirectly activate Myc via ßcatenin [15].

Besides activation of oncogenes, an important mechanism contributing to cancer metabolic reprogramming involves the loss of p53 function, which occurs in 50% of all human cancers due to gene mutation or deletion.

As a tumor suppressor, p53 counteracts the Warburg effect by limiting glycolysis through multiple mechanisms, such as transcriptional repression of GLUT-1 and GLUT-4, and induction of TP53-induced glycolysis and apoptosis regulator (TIGAR), that functions as a fructose-2,6-bisphosphatase, reducing the activity of PFK1 and favoring the diversion of glycolytic intermediates towards PPP to provide nucleotides for DNA repair and NADPH for ROS scavenging [16,17]. In addition, p53 maintains the mitochondrial integrity and positively regulates OXPHOS, up-regulating the expression of Synthesis of Cytochrome *c* Oxidase 2 (SCO2), and apoptosis-inducing factor (AIF), two factors essential for the assembly of complexes of the electron transport chain [18]. Under stress conditions, such as nutrient and energy limitations, p53 induces $p21^{cip1}$ and represses mTORC1 signaling to coordinately inhibit cell proliferation and cell growth in biomass, respectively [19]. p53-dependent inhibition of mTORC1 may be mediated by different mechanisms, including transcription of PTEN, and activation of the energy sensor AMP-activated protein kinase (AMPK), which plays a key role in restoring energy homeostasis by switching on the catabolic pathways and switching off the anabolic pathways [20]. It is worth noting that stress conditions that alter the energy status of the cell, such as glucose starvation, can directly activate AMPK, which in turn can stabilize and activate p53 protein, establishing a mutual regulation that reinforce their tumor suppressive functions [21]. AMPK can contribute to p53-dependent induction of autophagy, through mTORC1 inhibition or direct activation of the autophagy machinery [22]. p53-mediated autophagy can have a pro-survival adaptive function, but can also facilitate the induction of apoptosis when the cells are exposed to high stress or irreparable damage. In these conditions p53 may promote cell death also by activating pro-oxidant functions, which result in increased ROS production [23].

Taking into account the lines of evidence here reported, loss of p53 may effectively contribute to cancer development by facilitating the metabolic transformation. In addition, stimulation of the Warburg effect has recently

emerged as a novel gain of function of mutant p53 [18]. However, considering that some metabolic functions of p53 allow pro-survival adaptations to stress conditions, in some circumstances loss of p53 may increase the vulnerability of cancers to metabolic stress [24].

1.2 Glutamine metabolism

Glutamine is the most abundant free amino acid and is involved in multiple fundamental processes, such as energy generation, synthesis of macromolecules, redox homeostasis, and signal transduction [25].

Glutamine is taken up from the extracellular milieu via transporters such as ASCT2 (Na+-dependent neutral amino acid transporter), and is mainly catabolized into glutamate by glutaminase (GLS). Glutamate is a precursor of glutathione, the major cellular antioxidant, and provides amino groups for nonessential amino acids, such as serine and glycine, necessary for macromolecular syntheses. In addition, glutamate, converted to α-ketoglutarate by glutamate dehydrogenase (GLUD1), acts as an anaplerotic nutrient that refills the TCA cycle to supply intermediates for further biosynthetic pathways. This process is particularly relevant for cancer cells, in which the flux of pyruvate entering the TCA cycle is reduced as a consequence of aerobic glycolysis. Moreover, glutamate can support the production of NADPH. Glutamine can be also used directly as a carbon source to feed anaplerotic reactions or as a nitrogen source for nucleotide and hexosamine synthesis.

Many signaling pathways involved in tumorigenesis can reprogram glutamine metabolism, however deregulated Myc has been proved to play a major role in this regard, rendering cancer cells addicted to glutamine and therefore especially sensitive to glutamine deprivation [26]. Myc enhances glutamine utilization by activating the expression of genes involved in glutamine uptake, including *SLC1A5* (ASCT2 encoding gene) or catabolism such as *GLS* [27].

In addition, oncogenic KRAS mediates reprogramming of glutamine metabolism in pancreatic cancer, by up-regulating aspartate transaminase (GOT1) and inhibiting *GLUD1* transcription; this results in an increased dependence on glutamine for NADPH production to ensure the maintenance of redox balance essential for cancer survival [28].

1.3 Lipid metabolism

Increased lipid metabolism is common in cancer cells and is generally accomplished via up-regulation of lipid endogenous synthesis, although exogenous lipid uptake and consumption (through fatty acid β-oxidation, FAO) appear essential for survival and proliferation of some non-glycolytic tumor types, such as prostate cancer (PC), and may become a relevant mechanism of adaptation under conditions of metabolic stress [29]. Fatty acids (FA) are building blocks for the synthesis of phospholipids and glycolipids that serve as components of biological membranes, are precursors of lipids with signaling function, and, in form of triacylglycerides, are stored in lipid droplets (LDs) together with cholesterylesters. LDs accumulation in cancer cells has been associated with increased aggressiveness [30].

Cancer cells activate *de novo* biosynthesis of FA by increasing the expression of lipogenic enzymes, including FA synthase (FASN), acetyl-CoA carboxylase (ACC), and ATP citrate lyase (ACLY) that promotes also cholesterol synthesis [31]. Transcription of the corresponding genes is mediated by Sterol regulatory element binding protein 1 (SREBP-1), whose activation in cancer cells can be promoted through different mechanisms [32]. mTORC1 is essential for nuclear accumulation of SREBP-1 in AKT-activated cancer cells [33], whereas mTORC1-independent activation of SREBP-1 has been reported in human glioblastoma multiforme with altered epidermal growth factor receptor (EGFR) signaling [34]; SREBP-1 can also be activated by SIRT1 deacetylase and SIRT1-dependent induction of lipogenesis has been shown to promote the growth of endometrial tumor cells [35].

2 Oncogene-targeting agents and energy metabolism

It is now clear that oncogenes that drive the cancer-promoting signals also drive the altered metabolism. This chapter of the review is focused on recent data regarding the use of drugs directed against oncogenic signaling pathways (Fig. 1) as potential modulators of deregulated metabolism in different solid cancers and in chronic myelogenous leukemia (CML) (Table1).

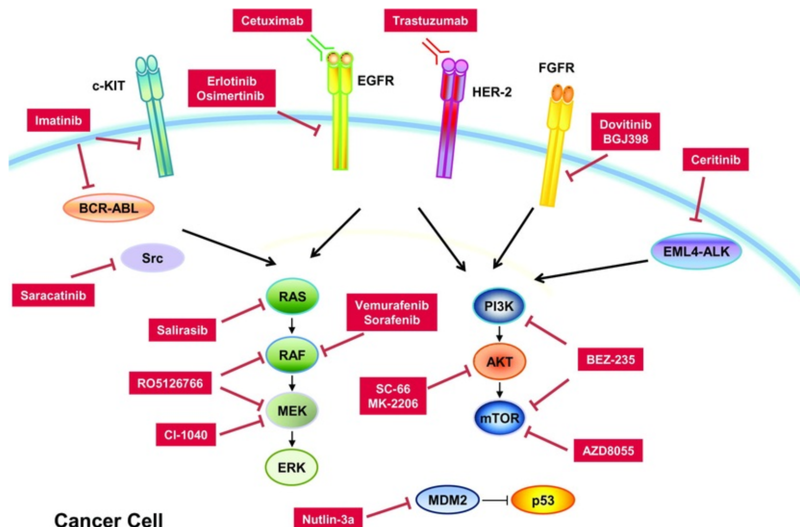


Fig. 1 Drugs directed against oncogenic signaling pathways involved in the modulation of cancer cell metabolism.

Table 1 Effect of targeted agents on energy metabolism.

Drugs	Specific target	Metabolic targets	Metabolic effects	Cancer types	Refs
Erlotinib	EGFR	GLUT-1 HKII, PKM2 Mitoch. complex subunits GLS	↓ Glycolysis ↑ OXPHOS ↑ OXPHOS ↓ Glutaminolysis	NSCLC	[36,37] [38] [38] [40]
Osimertinib	EGFR	HKI/II ALDOA, LDHA-B, PDHK1, PFKB4, PKM2 ; TXNIP	↓ Glycolysis	NSCLC	[39]
Cetuximab	EGFR	LDHA ASCT2	↓ Glycolysis ↓ Gln and Cys uptake	HNSCC	[41] [42,43]
Trastuzumab	HER-2	GLS1	↓ Glutaminolysis	BC	[44]
Dovitinib	FGFR	PKM2 PDHK1 GLUT-1	↑ OXPHOS ↑ OXPHOS ↓ Glycolysis	NSCLC SQCLC	[45] [46] [47]
BGJ398	FGFR	GLUT-1	↓ Glycolysis	SQCLC	[47]
Ceritinib	EML4-ALK	HKII	↓ Glycolysis	NSCLC	[49]
SC-66	AKT	GLUT-1, GLUT-4	↓ Glycolysis	Cervical cancer	[50]
MK-2206	AKT 1-2-3		↓ Glycolysis	BC, PC	[51,52]
BEZ-235	PI3K/mTORC1-C2		↓ Glycolysis	BC	[51]
AZD8055	mTOR	CPT1A	↓ Lipogenesis ↓ FAO	BC	[54]
Salirasib	KRAS	FASN, SCD	↓ Lipogenesis	NSCLC	[57]

RO5126766	RAF/MEK	GLUT-1	↓Glycolysis	Colon cancer	[57]
CI-1040	MEK1/2	HKII	↓Glycolysis	Melanoma	[59]
Vemurafenib	BRAF V600E	GLUT-1, GLUT-3, HKII	↓Glycolysis	Melanoma	[60]
Saracatinib	Src	GLUT-1, HKII, LDHA	↓Glycolysis	BC	[61]
Imatinib	BCR-ABL/KIT	GLUT-1 PKM2	↓Glycolysis	CML CML, GIST	[62–64] [45,65]
Sorafenib	Multi TKI	Mitochondrial complex I GLUT3, PFKP, PDH GLUT-1	↓OXPHOS ↑Glycolysis ↑/↓Glycolysis	HCC, neuroblast, BC HCC BC	[67–70] [70] [69]
Nutlin 3a	MDM2	GLUT-1	↓Glycolysis	BC, glioma	[75]

2.1 Inhibitors of EGFR family

A variety of evidence indicates that oncogenic mutated EGFR promotes a metabolic reprogramming to glycolysis, which may be reverted by EGFR tyrosine-kinase inhibitors (TKIs), contributing to cancer cell growth inhibition.

In a previous study, we reported that the first-generation EGFR TKI erlotinib down-regulated the glucose uptake in sensitive NSCLC cell lines, an effect occurring before the appearance of inhibitory effects on cell proliferation and associated with inhibition of GLUT-1 protein expression [36]. We also demonstrated that downstream of EGFR, inhibition of the PI3K/AKT/mTOR and not the MAPK pathway plays a role in erlotinib-mediated down-regulation of glucose transport activity.

Treatment with erlotinib or osimertinib, a third-generation irreversible EGFR TKI, decreased lactate production, glucose consumption, and the extracellular acidification rate in lung adenocarcinoma EGFR mutant sensitive cells and in T790M resistant mutant cells, respectively [37]. In addition, a metabolomics analysis revealed that treatment with the EGFR TKIs reduced the amount of early intermediate metabolites of both glycolysis and PPP, thereby hindering also *de novo* pyrimidine biosynthesis. The underlying mechanism involved the inhibition of PI3K/AKT/mTOR signaling, which, in contrast with our findings [36], caused a reduction of membrane-bound GLUT-1 without affecting its total expression.

Interestingly, distinct mechanisms were activated in NSCLC cells *in vitro* and *in vivo* after a long-term incubation (48–72 h) with erlotinib or WZ4002, a specific inhibitor of EGFR-T790M. Indeed, in these conditions EGFR inhibition promoted a switch from aerobic glycolysis to mitochondrial oxidative phosphorylation (OXPHOS) by reducing HKII and p-PKM2 Tyr105 levels, and up-regulating the expression of specific mitochondrial complexes subunits. These effects were attributed to the inhibition of ERK1/2 and AKT, respectively [38].

Also osimertinib reduced HKI/II activity in osimertinib-sensitive cells, and down-regulated the expression of other key regulators of glycolysis (ALDOA, LDHA-B, PDHK1, PFKB4, PKM2), while increasing the expression of thioredoxin interacting protein (TXNIP), a negative regulator of glycolysis [39].

Besides glycolysis and PPP, EGFR inhibition has been shown to affect additional metabolic pathways, resulting in profound alterations of the metabolic profile in EGFR-mutated lung adenocarcinoma cells. Indeed, erlotinib treatment in these cells hindered glutaminolysis and lowered the expression of GLS, induced an increase of proline and aspartate, and decreased the levels of NADH and glutathione [40].

The EGFR-blocking monoclonal antibody cetuximab has been reported to affect both glucose and glutamine metabolism in head and neck squamous cell carcinoma (HNSCC). In particular, treatment with cetuximab in sensitive HNSCC cells suppressed the expression and activity of LDHA through downregulation of HIF-1α, with consequent decrease of glucose consumption, lactate production, and ATP intracellular levels [41]. By reducing the supply needed to generate biomass, cetuximab arrested cell proliferation and cell cycle at G1 phase, without evident signs of apoptosis. However, a further down-regulation of LDHA by RNA silencing or oxamate treatment induced pro-apoptotic effects, thus enhancing the therapeutic efficacy of cetuximab.

In more recent studies performed in EGFR-overexpressing HNSCC cells, EGFR was found to be physically associated with ASCT2 transporter, which plays a key role in glutamine uptake in rapidly proliferating cells, including cancer cells [42]. By inducing EGFR endocytosis, cetuximab mediated the internalization of EGFR-ASCT2 complex, which includes the adaptor-related protein complex 1 gamma 1 subunit (AP1G1), a component of clathrin-coated vesicles involved in membrane protein sorting [43]. ASCT2 internalization reduced the uptake of glutamine and cysteine, which are essential precursors for the biosynthesis of the anti-oxidant glutathione. The resulting decrease of glutathione supply rendered the cells susceptible to ROS-induced apoptosis, a finding with important clinical implications, considering that cetuximab is currently approved for treatment of HNSCC in combination with radiotherapy or

cisplatin, which are known to increase ROS production. The therapeutic efficacy of such combinations may indeed rely on cetuximab-mediated sensitization to radiation- or cisplatin-induced oxidative stress.

A functional relationship between ErbB2 and glutamine metabolism has been demonstrated in breast cancer (BC) tumorigenesis. ErbB2 activation has been shown to promote glutamine utilization during oncogenic and metabolic transformation of breast epithelial cells, by up-regulating GLS1 expression through NF-kB signaling. Conversely, the ErbB2-blocking monoclonal antibody trastuzumab inhibited the increased GLS1 expression and activity in ErbB2-expressing BC cell lines [44]. In these models, GLS1 knockdown, as well as treatment with BPTES, a selective inhibitor of GLS1 activity, suppressed cell proliferation, suggesting that the enhanced GLS1 activity in ErbB2-positive BC is not a mere epiphenomenon, but is instead a key event in the process of malignant transformation.

2.2 FGFR inhibitors

Dovitinib (TKI258), a multiple receptor TKI with activity against FGFR1/3 among its targets, has been demonstrated to inhibit FGFR1-mediated phosphorylation of PKM2 in FGFR1-overexpressing NSCLC cells, with the result of enhancing PKM2 enzymatic activity [45]. This mechanism may contribute to dovitinib anti-tumor activity, as suggested by the observation that the induced expression of a catalytically more active form of PKM2 increased OXPHOS and decreased proliferation of NSCLC cells under hypoxic conditions, and also reduced tumor growth *in vivo*. In addition, FGFR1 has been shown to co-localize with PDHK1 in the mitochondria and to induce PDHK1 through tyrosine phosphorylation at multiple sites, with consequent inhibition of PDC [46]. Dovitinib inhibited also FGFR1-dependent phosphorylation/activation of PDHK1 in FGFR1-overexpressing NSCLC cells, thus relieving PDHK1-mediated inhibition of PDC and favoring the reversion of the Warburg effect.

Besides the effects on PKM2 and PDHK1, we have recently demonstrated that FGFR1 can regulate glucose metabolism in FGFR1 amplified/over-expressing squamous cell lung carcinoma (SQCLC) cell lines through a mechanism that involves AKT/mTOR-dependent HIF-1α accumulation and up-regulation of GLUT-1 [47]. Treatment with dovitinib or with the selective FGFR inhibitor BGJ398 under serum deprivation prevented FGF2-mediated stimulation of FGFR1/AKT/mTOR signaling, thereby reducing GLUT-1 expression, glucose uptake, glycolysis, and lactate production. As a consequence cell proliferation and viability were significantly impaired, under both normoxic and hypoxic conditions. In presence of serum, FGFR1 targeting decreased glucose metabolism only when AKT/mTOR signaling was inhibited. In cell models in which FGFR1 selective inhibition failed to down-regulate the AKT/mTOR pathway due to the activation of alternative signaling pathways, the anti-proliferative effects of BGJ398 were significantly enhanced by the combination with AKT/mTOR inhibitors. Interestingly, co-targeted inhibition of FGFR1 and AKT/mTOR by BGJ398 and BEZ235 (dual PI3K/mTOR inhibitor) reduced GLUT-1 mRNA and protein expression in xenografts and led to superior anti-tumor effects as compared with individual treatments [47].

2.3 Ceritinib (ALK inhibitor)

The echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion oncogene is a driving factor of tumorigenesis, whose expression defines a specific clinicopathologic subset of NSCLC characterized by an increased glycolytic phenotype compared with EML4-ALK-negative NSCLC [48]. EML4-ALK rearrangement in NSCLC cell lines was shown to induce a high glucose metabolism through HIF1α-dependent up-regulation of HKII expression not associated with increased expression of GLUT-1 or LDHA proteins [49]. EML4-ALK modulated HIF1α at the level of transcription and translation, with downstream PI3K/AKT/mTOR pathway playing a major role in sustaining HIF1α protein synthesis. Ceritinib, a second-generation ALK inhibitor approved for the treatment of ALK-positive NSCLC, hindered glycolysis and lactate production in EML4-ALK-rearranged NSCLC cells by reducing both mRNA and protein expression of HIF1α with consequent down-regulation of HKII. Downstream of EML4-ALK, ceritinib inhibited the PI3K/AKT/mTOR pathway, thus blocking its contribution to enhance HIF1α protein synthesis. Interestingly, a brief treatment with ceritinib (2–3 days) strongly decreased the glycolytic rate in EML4-ALK-positive xenografts, as indicated by FDG-PET scans in live animals [49]. This finding suggests that FDG-PET might represent an useful tool to quickly determine the ALK status and to identify the patients likely to respond to ALK inhibitors, especially in those part of the world where ALK testing has not been introduced into routine diagnostics.

2.4 Inhibitors of PI3K/AKT/mTOR pathway

The PI3K/AKT/mTOR inhibitors have been widely used in the literature to investigate the involvement of PI3K, AKT, and mTOR, alone or in cascade, in the control of cancer metabolic reprogramming downstream of altered receptors or along other signaling axes (e.g. (AMPK)/mTORC1 signaling). In addition, these inhibitors have served as valuable tools to provide mechanistic explanations for the metabolic effects of other targeted drugs. Here, we present some of the studies that have been designed to assess the impact of PI3K/AKT/mTOR inhibition on cancer cell metabolism in tumors carrying specific oncogenic alterations of this pathway.

The allosteric AKT inhibitor SC-66 induced non-apoptotic cell death in a cell model of cervical cancer carrying both activating *PIK3CA* and inactivating *PTEN* mutation [50]. Mechanistically, inhibition of AKT/mTOR signaling following treatment with this drug reduced the translocation of GLUT-1 and GLUT-4 to the cell surface, thus decreasing glucose uptake and glycolysis. SC-66-mediated cytotoxicity was further enhanced by the combination with the competitive HK inhibitor 2-deoxyglucose (2-DG), that exacerbated the inhibitory effects on glycolysis.

MK-2206, a highly selective inhibitor of AKT 1-2-3 isoforms, and BEZ-235, a dual PI3K and mTORC1-C2 inhibitor, were shown to down-regulate AKT phosphorylation and to inhibit cell growth in basal-like BC xenografts bearing an increased PI3K signaling [51]. These effects were associated with decreased concentration levels of lactate and increased levels of phosphocholine, while glucose and glycerophosphocholine concentrations were increased. In

contrast, no metabolic change was detected in basal-like BC xenografts that expressed lower baseline levels of AKT activation in comparison with basal-like xenografts, and resulted insensitive to treatment. The interest aspect emerging from this study is that lactate, phosphocholine and glycerophosphocholine may represent novel potential metabolic biomarkers for response to therapies with PI3K/mTOR inhibitors in basal-like BCs.

The potential usefulness of metabolic changes as biomarkers of responsiveness to PI3K/mTOR inhibitors has been also proposed for other types of cancer. By using hyperpolarized magnetic resonance spectroscopy (HP-MRS) to monitor the conversion of hyperpolarized [1-^{13}C] pyruvate into lactate, MK-2206 was demonstrated to reduce aerobic glycolysis in spheroids derived from PTEN-mutated or PTEN-deleted PC cell lines [52]. Such metabolic alteration occurred early upon AKT inhibition, and was followed by significant down-regulation of cell growth. This suggested that HP-MRS may be used to measure the metabolic flux after treatment with AKT inhibitors in patient-derived tumor organoids, providing a non-invasive method for early evaluation of the therapeutic efficacy of these drugs in PC patients.

Similarly, studies performed in a PTEN-null glioblastoma xenograft model suggested that FDG-PET may be a valuable marker for response to the mTORC1/mTORC2 inhibitor AZD8055 in glioblastoma patients [53]. In BC cells, the anti-tumor efficacy of AZD8055 was affected by its ability to suppress lipid metabolism [54]. Indeed, AZD8055 induced cell death only in HER2+/PIK3CA mutant cell lines, in which the treatment produced a significant decrease of *de novo* lipid synthesis and also down-regulated the lipid catabolism by reducing the levels of carnitine palmitoyltransferase 1A (CPT1A), a rate-limiting enzyme for FAO. In contrast, in HER2-/PIK3CA wt cells AZD8055 failed to alter the lipid metabolism and its effects were cytostatic.

2.5 Inhibitors of RAS/RAF/MEK pathway

2.5.1 KRAS inhibitor (Salirasib)

Reprogramming of tumor metabolism by oncogenic KRAS is essential to support cell growth in a variety of cancers, including glioblastoma and pancreatic cancer; in particular, previous studies have shown that activated KRAS increased glucose consumption and lactate production, decreased oxidative TCA cycle flux, and stimulated the utilization of glutamine for anabolic pathways [28,55,56]. Results from a very recent work adds further complexity to KRAS-driven cancer metabolism, demonstrating the existence of a causal link between KRAS activation and a specific lipogenesis gene signature in NSCLC [57]. In this study, KRAS has been shown to promote lipogenesis via ERK2-mediated induction of FASN and stearoyl CoA desaturase (SCD). Selective inhibition of KRAS with salirasib resulted in the decrease of both *FASN* and *SCD* gene expression in mutated KRAS NSCLC cell lines; on the other hand proliferation of these cells was efficiently slowed down by the FASN inhibitor cerulenin, suggesting that lipogenesis may be a relevant therapeutic target for treating KRAS-induced lung cancer.

2.5.2 RAF/MEK inhibitors

The dual RAF/MEK kinase Inhibitor RO5126766 was shown to decrease FDG uptake in KRAS and BRAF mutant colon cancer xenografts [58]. This metabolic change was associated with a reduced surface expression of GLUT-1 levels and correlated with a decreased expression of the marker of proliferation ki67, confirming the relevance of FDG-PET as a surrogate marker for the efficacy of RAF/MEK inhibitors.

Impairment of glucose metabolism following treatment with BRAF inhibitors was also demonstrated in BRAF-driven melanoma cancer. Using HP-MRS, the MEK1/2-selective inhibitor CI-1040 was shown to reduce lactate production in BRAF mutant melanoma cells *in vitro* and in xenografts *ex vivo* but not in BRAFwt melanoma cells [59]. The underlying mechanism involved the down-regulation of HKII expression, which was a likely consequence of Myc down-regulation. Comparable findings were obtained in BRAF mutant melanoma cells with vemurafenib, a selective inhibitor of mutated BRAFV600E, that down-regulated *HKII*, *GLUT-1*, and *GLUT-3* genes, and hence hindered glucose metabolism via concerted inhibition of Myc and HIF-1α, and activation of MONDOA, a transcriptional factor involved in the negative regulation of glycolysis [60].

2.6 Intracellular TK inhibitors

2.6.1 Src inhibitor (saracatinib)

The role of src in regulating cancer glucose metabolism and the effects of its selective inhibition by saracatinib have been investigated in premalignant estrogen receptor (ER)-negative mammary epithelial cells modified to overexpress ErbB2 and exhibiting ErbB2-dependent src activation [61]. These cells displayed an increased glucose utilization, which was inhibited by treatment with saracatinib. In particular, saracatinib reduced GLUT-1 mRNA and protein, and inhibited glucose uptake, thus affecting the synthesis of early intermediate metabolites of both glycolysis and PPP, such as glucose 6-phoshate and fructose 6-phosphate. HKII and LDHA expressions were moderately inhibited, while no change was observed for PFK1. These effects were associated with saracatinib-mediated inhibition of the ERK1/2-MNK1-e-IF4E pathway, that resulted in a reduced cap-dependent translation of Myc. By hampering glucose metabolism, saracatinib inhibited cell proliferation, induced apoptosis, and prevented a disorganized acinar growth in 3D culture; in addition, saracatinib markedly prevented the development of mammary premalignant lesions and delayed tumor onset *in vivo*.

2.6.2 BCR-ABL and KIT inhibitor (imatinib)

In CML, the BCR-ABL oncogene redirects the energy metabolism towards glycolysis as a mechanism contributing to its transforming activity. The BCR-ABL inhibitor imatinib, currently used for treatment of CML as well as acute lymphocytic

leukemia and gastrointestinal stromal tumors (GIST), can efficiently hamper glucose metabolism through different mechanisms, including reduction of surface localization of GLUT-1 and inhibition of BCR-ABL-mediated phosphorylation of PKM2 [45,62,63]. In GIST imatinib dramatically reduces FDG uptake, providing an early (24 h) indicator of treatment response [64]. More recently, imatinib has been shown to promote the switching from PKM2 to PKM1 isoform, by inducing miR-124 expression, which in turn down-regulates polypyrimidine tract-binding protein 1 (PTBP1), an alternative splicing repressor of PKM1 [65].

2.7 Sorafenib (multi-kinase inhibitor)

Sorafenib is a multi-kinase inhibitor, targeting V600E mutant BRAF as well as a number of TK receptors, including vascular endothelial growth factor receptors (VEGFR)-2/3, and Platelet-derived growth factor receptor (PDGFR)-ß [66]. Apart from the metabolic effects deriving from its antiangiogenic properties, sorafenib has been shown to affect cancer energy metabolism by directly targeting the mitochondrial electron transport chain complex I, thus inhibiting OXPHOS and impairing mitochondrial energy production in hepatocellular carcinoma (HCC) and in neuroblastoma cells [67,68].

In BC cells we demonstrated that sorafenib caused an early mitochondrial dysfunction associated with drop of intracellular ATP levels, ROS production, and activation of the energy sensor AMPK [69]. As a result of AMPK activation, sorafenib induced an increase of GLUT-1 expression, glucose uptake, aerobic glycolysis and lactate production, at least at early time points, in ER + and ER-/ErbB2 + BC cells. Comparable effects were described in HCC cell lines [70], where sorafenib up-regulated genes involved in the glycolytic pathway, such as GLUT-3 and PFKP (platelet isoform), while reducing the expression of PDH. However, AMPK activation produced different outcomes in the two types of cancer. Indeed, BC cells were not able to cope with sorafenib-induced stress, despite the initial attempt to restore the energy balance, and AMPK activation persisted during sorafenib treatment, finally leading to a decreased glucose utilization also in a triple negative BC (TNBC) high glycolytic cell model. This long-term inhibitory effect on glucose metabolism was mediated by AMPK-dependent inhibition of the mTORC1 pathway and contributed to the anti-proliferative and pro-apoptotic action of sorafenib [69]. In contrast, activated AMPK exerted a cytoprotective role in HCC cells [70], presumably via autophagy induction, and combination with glucose deprivation or the glycolytic inhibitor 2-DG was required for sorafenib to produce cytotoxic effects.

In addition with glycolysis inhibitors, other combinatorial strategies targeting cancer energy metabolism were demonstrated to improve the efficacy of sorafenib. For example, the anti-tumor activity of sorafenib associated with ROS production was significantly potentiated in HCC cells by disturbing the redox balance through combination with oxythiamine, inhibitor of the PPP enzyme transketolase [71]. Moreover, combination of sorafenib with the AMPK activator metformin in NSCLC promoted a synergistic activation of AMPK and inhibition of downstream mTOR signaling, leading to a synergistic growth inhibition both *in vitro* and *in vivo* [72]. The efficacy of such combination was also demonstrated in cholangiocarcinoma cells [73].

2.8 Nutlin-3a (p53 activator)

Due to the frequent loss of p53 in human cancers, considerable effort has been made to develop novel therapeutic strategies aimed at restoring p53 function. One of these strategies is based on the use of drugs directed against murine double minute 2 (MDM2), a negative regulator of p53, to activate wild-type p53 in tumors that have lost its expression as a consequence of MDM2 overexpression/amplification [74]. Combining the MDM2 antagonist Nutlin-3a with the V-ATPase inhibitor archazolid was shown to exert synergistic pro-apoptotic effects in p53 wild type BC cells and to reduce tumor growth in a mouse xenograft glioma model [75]. The rationale for such combination came from the suggestion that p53 restoration could improve the efficacy of archazolid by counteracting its stimulating effects on glycolysis. Indeed, combination of the two drugs resulted in a significant up-regulation of TIGAR associated with down-regulation of GLUT-1 expression and glucose uptake.

3 Alteration of metabolism as a mechanism of resistance to targeted agents

Intrinsic or acquired resistance to targeted agents are still the main limits for their efficacy.

Preventing or overcoming such resistance is currently one of the most important research aim in the field of anti-cancer therapy.

Metabolic reprogramming may represent a way by which cancer cells escape from the selective pressure of drugs and become resistant (Table 2).

Table 2 Metabolic reprogramming as a resistance mechanism to targeted agents.

Metabolic reprogramming	Drug resistance	Mechanism of resistance	Overcoming resistance	Cancer types	Refs
Glucose metabolism					
	Sorafenib or sunitinib	GLUT 1 overexpression	mTOR inhibition	RCC	[76]

	Axitinib	GLUT1 overexpression Increased glycolysis	AKT inhibition	Pancreatic cancer	[77]
	Erlotinib	GLUT1 overexpression	HKII inhibition Glucose starvation/autophagy inhibition	NSCLC	[36] [78]
	Lapatinib	Glycolytic Enzymes phosphorylation	HKII inhibition	BC	[79]
	Tamoxifen	Increased glycolysis	HKII inhibition	BC	[80]
	BEZ235	Increased glycolysis Reduced OXPHOS	Glycolysis inhibition	NSCLC	[81]
OXPHOS					
	Aromatase inhibitors	miR-155	miR-155 targeting	BC	[82]
	Imatinib	Increased expression of mitochondrial enzymes	OXPHOS inhibition Mitoch. translation inhibition	GIST CML	[83] [84]
Lipid metabolism					
	Imatinib	FAO	FA derivative	CLM	[65]
	Gefitinib	FASN	FASN inhibitor	NSCLC	[87]
Glutamine metabolism ***(Please delete bold characters for "Glutamine metabolism")***					
	Aromatase inhibitors	SLC1A5 overexpression	GPNA inhibitor GLS inhibition	BC	[88]
	mTOR inhibitors	GLS overexpression	GLS inhibition	Glioblastoma	[89]
	Erlotinib	GLS overexpression	GLS inhibition	NSCLC	[90]
	Vemurafenib		GLS inhibition	Melanoma	[91]
	Sorafenib	PPARδ up-regulation	GLS or PPAR inhibition	HCC	[92]
	Cetuximab	SLC1A5 overexpression	GPNA inhibitor	CRC	[93]

3.1 Glucose metabolism

As assessed by FDG-PET integrated with computed tomography (CT), early acceleration of glucose uptake and accumulation, associated with activation of the mTOR pathway, indicated resistance to the antiangiogenic inhibitors sorafenib or sunitinib in renal cell carcinoma (RCC) patients. Treatment with the mTOR inhibitor everolimus decreased the max SUVmax in all the treated resistant patients [76]. FDG-PET assessment after 2 days of erlotinib treatment has been proposed as an useful method to identify early resistant NSCLC patients and may also predict survival in unselected NSCLC pre-treated population [36]. These reports indicate the usefulness of FDG PET/CT to monitor tumor response sequentially during TKI therapy, for an early evaluation of resistance acquisition to TKI treatment.

Along this line, increased glucose metabolism has been associated with resistance to different TKIs in several tumor types. Resistance to the anti-angiogenic multi-kinase inhibitor axitinib was associated with increased GLUT-1 cell surface expression, glucose uptake, and glycolysis rate in pancreatic adenocarcinoma cells. In these resistant cells, the AKT inhibitor MK-2206 restored the sensitivity to axitinib by reverting the translocation of GLUT-1 into the plasma membrane and normalizing the glycolytic flux [77]. Similarly, HCC827-ER6 NSCLC cells resistant to erlotinib [78] exhibited a high glycolytic rate, dependent on GLUT-1 over-expression, together with a mitochondrial dysfunction. However, in this resistant cell model, AKT inhibition failed to decrease cell viability, suggesting that up-regulation of GLUT-1 might be independent from AKT signaling. In addition, HCC827-ER6 cells were more sensitive to 2-DG treatment or glucose starvation than parental cells; however, these stressful conditions activated autophagy, and combination with an autophagy inhibitor was required for overcoming resistance to erlotinib.

Interestingly, in BC cells resistant to the EGFR/ErbB2 inhibitor lapatinib, an extensive reprogramming of glycolytic activity mediated by post-translational regulation through phosphorylation has been recently described [79]. Tyrosine-phosphorylation of LDHA, enolase (ENO1), PKM and phosphoglycerate mutase 1 (PGAM1), and serine-phosphorylation of ALDOA, PFKP, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were significantly increased, leading to enzymatic activation and hence to significant changes in the intracellular level of glycolytic metabolites, such as glucose-6-phosphate, glyceraldehyde-3-phosphate, and phosphoenolpyruvate. Therefore, not a single kinase but multiple activation of different kinases was involved in lapatinib resistance. Nevertheless, this increased glycolytic activity in resistant cells was suppressed by the competitive HK inhibitor 2-DG. Similarly, the inhibition of HKII was sufficient to overcome resistance to tamoxifen in tamoxifen-resistant human BC cell lines derived from MCF7 cells [80].

In H1975 NSCLC cells, resistance to the PI3K/mTOR inhibitor BEZ235 was associated with high glycolysis and low mitochondrial respiration rates mediated by the presence of a mitochondrial DNA MT-C01 variant. Depletion of mitochondrial DNA in sensitive cells induced increased glycolysis and resistance to BEZ235 and other PI3K/mTOR inhibitors. The combination of BEZ235 with the glycolysis inhibitor 3-bromopyruvate exerted a synergistic anti-proliferative effect in resistant cells [81].

3.2 OXPHOS

A very interesting example of metabolic plasticity, able to sustain resistance despite glycolysis inhibition, has been reported in BC cells resistant to aromatase inhibitors (AIs). In this cell model, glycolysis targeting by 2-DG was ineffective in reverting resistance to AIs, due to a shift from glycolysis to OXPHOS. miR-155 was found to be associated with this change in the metabolic phenotype, revealing a dynamism that allows resistant tumor cells to further modify energy metabolism in the presence of specific metabolic blockade [82]. miR-155 targeting may therefore have a potential therapeutic implication in AIs-resistant tumors.

Also during prolonged imatinib treatment, the metabolic phenotype of GIST shifted from glycolysis to OXPHOS by increasing the expression of key mitochondrial enzymes, including cytochrome *c* oxidase 1, 2, and 4, and mitochondrial transcription factor A. A mitochondrial OXPHOS inhibitor (VLX-600) increased tumor cell dependence on glycolysis, enhancing the anti-glycolytic and apoptotic effects of imatinib either *in vitro* and *in vivo* [83]. Therefore, inhibiting mitochondrial OXPHOS increased the efficacy of imatinib, highlighting an important limitation of imatinib when administered as monotherapy.

A recent study on CML disclosed that primitive CML stem and progenitor cells, known to be resistant to imatinib, were highly susceptible to inhibition of OXPHOS by the FDA-approved mitochondrial translation inhibitor tigecycline [84]. These findings suggest that restraining mitochondrial functions may represent a new therapeutic option when combined with imatinib to treat imatinib resistance.

This conclusion finds further confirmation in the results from another study, showing that low-dose oligomycin-A (inhibitor of mitochondrial ATP-synthase) synergizes with imatinib in killing CML cells both *in vitro* and *in vivo*, by inducing a rapid mitochondrial membrane depolarization associated with reduced ATP levels and enhanced superoxide production [85]. The greater efficacy of such combination relies on the increased dependence on mitochondrial pyruvate oxidation, required to compensate for the inhibition of BCR-ABL-driven glycolysis and satisfy the energetic and anabolic demands for cell maintenance. An interesting implication emerging from this study is that targeting mitochondrial functions together with imatinib might help in preventing the acquisition of imatinib resistance in CML.

3.3 Lipid metabolism

Several studies have demonstrated that FAO inhibition is a potential strategy to overcome chemoresistance in tumors. Regarding the resistance to targeted agents, a correlation has been reported between high CPT1 expression (the rate-limiting enzyme of FAO) and rapamycin resistance in mouse xenograft models [86]. Also the efficacy of imatinib was shown to be reduced by the activation of CPT1 in CML cells [65]. In fact, suppression of glycolysis by imatinib stimulated compensatory FAO via up-regulation of the AMPK/ACC signaling, that activates CPT1. Activation of this mechanism, observed also in BCR-ABL-positive acute lymphoblastic leukemia stem cells, contributed to the acquisition of insensitivity to imatinib. Combination of imatinib with AIC-47, a novel anti-cancer fatty acid derivative able to inhibit the expression of CPT1, demonstrated a significant synergistic cytotoxicity, suggesting that tackling on energy metabolism at multiple levels may be a superior strategy for therapeutic intervention in BCR-ABL-driven cancers

Very recently [87], a novel EGFR/FASN signaling axis has been documented in NSCLC with acquired gefitinib-resistance independent of T790M mutation. The palmitoylation of EGFR induced its nuclear translocation and supported growth of TKI-resistant EGFR mutated NSCLC cells. Pharmacological inhibition of FASN with orlistat, a FDA-approved anti-obesity drug, induced EGFR ubiquitination and cell death *in vitro* and reduced tumor growth in both xenografts and transgenic mouse models. This result provides the rationale for blocking the FA metabolic pathway in TKI-resistant T790M negative NSCLC patients.

3.4 Glutamine metabolism

In many tumors, the intrinsic metabolism of resistant cells is reprogrammed to preferential activation of glutamine-dependent pathways, indicating that glutamine addiction may be considered a common feature of targeted agent-resistant cells.

ASCT2 was significantly up-regulated by Myc activation in BC cells resistant to AIs [88], and inhibition of Myc, SLC1A5 and GLS significantly decreased cell proliferation in resistant cells, suggesting that targeting glutamine metabolism is a potential therapeutic strategy in the treatment of AIs-resistant BC.

Increased GLS expression promoted acquired resistance to mTOR-targeted therapy in glioblastoma [89]. Suppression of GLS expression with either RNA interference or the GLS inhibitor compound 968 sensitized resistant glioblastoma cells and xenograft models to mTOR-targeted therapies.

The inhibition of GAC (a splice variant of GLS1) activity has also been proposed as a new strategy to overcome erlotinib resistance in NSCLC, being NSCLC resistant cells dependent for their growth to glutamine synthesis and disposition. Interestingly, the combination of compound 968 with erlotinib suppressed not only glutamine metabolism, but also glycolysis [90]. Also melanoma cells with acquired resistance to vemurafenib showed an increased sensitivity to glutamine deficiency or GLS inhibitors in respect to parental cells both *in vitro* and *in vivo*. Acquired mutations in *NRAS* contributed for reprogramming metabolism to glutamine dependence and were responsible for vemurafenib resistance [91].

It has been recently demonstrated that up-regulation of peroxisome proliferator–activated receptor-δ (PPAR-δ) contributed to sorafenib resistance in HCC. PPAR-δ promoted reductive glutamine metabolism and reductive glutamine carboxylation, which induced proliferation of resistant cells. Inhibition of GLS1 or PPAR-δ activity reversed the glutamine metabolic reprogramming in these cells and restored their sensitivity to sorafenib [92].

Very recently [93], the expression of SLC1A5 in samples of patients with colorectal cancer (CRC) resistant to cetuximab was demonstrated to be significantly higher than in responders. Pharmacological inhibition of SLC1A5 with GPNA inhibited the growth and enhanced the efficacy of cetuximab in CRC cell lines both *in vitro* and *in vivo*. This work also reveals a new function of SLC1A5. Indeed SLC1A5 was found to induce the EGFR degradation and to reduce its nuclear expression.

4 Effects of oncogene-targeting agents on the metabolism-immune system link

Metabolic reprogramming has emerged as a critical requisite for immune cells to sustain differentiation, proliferation, and proper effector function. However, within the tumor microenvironment (TME), the ability of these cells to satisfy the increased demands for energy and nutrients is challenged by a severe competition with cancer cells, that show the same metabolic requirements. The enhanced acquisition of both extracellular energy and nutrient resources by tumor cells creates metabolic-restricted conditions that may severely affect the immune response, contributing to immune escape. Due to the complex metabolic interplay between cancer and immune cells, it is reasonable consider that oncogenic signaling, by controlling cancer metabolic rewiring, may also regulate the immune escape function [94]. Therefore, targeted inhibition of specific oncogenic pathways may in principle not only dampen cancer cell metabolism, but also indirectly reduce the tumor-imposed metabolic restrictions, thus restoring the effectiveness of immune cells.

The triggering of T cell effector functions is associated with a transition from OXPHOS, typical of naïve and memory T cells, to a biosynthetic and anabolic metabolism, that requires an increased uptake of nutrients from the environment. This switch results in aerobic glycolysis, with a concomitant increased glutamine metabolism and consumption of oxygen [95]. As previously discussed, many oncogene-targeting agents can reduce cancer glucose uptake and utilization, thus potentially restoring its availability for T cells. However, some of these drugs, such as PI3K/AKT/mTOR inhibitors, may act not only on cancer cells but also on T cells that use the same pathways to sustain their metabolic reprogramming. The resulting decrease of T cell glucose metabolism may therefore limits anti-tumor immunity, unless a therapeutic window is find in which this metabolic effect may instead reinforce the immunologic anti-tumor activity through the generation of memory T cells. Indeed, loss or reduction of AKT signaling does not compromise T cell proliferation or survival, but causes differentiated cytotoxic T cells to transcriptionally reprogram from an effector to a memory phenotype. To support metabolic demands, memory CD8+ T cells engage a catabolic metabolism, including increased OXPHOS and FAO, in contrast with effector CD8+ T cells that, as previously discussed, preferentially utilize anabolic metabolism [96]. Pharmacological inhibition of AKT has been associated with enhanced FAO in human cytotoxic tumor-specific lymphocytes and with induction of memory cell characteristics in melanoma [97]. Also mTOR inhibiting compounds, such as rapamycin, have metabolism-targeting effects on T cells, promoting memory CD8+ T cell formation [98]. Therefore, despite rapamycin is known to reduce activation, proliferation, and effector function of CD8+ T cells, its ability to stimulate the generation of memory cells may ultimately enhance tumor clearance and prevent tumor recurrence [99].

Besides reducing glucose availability, a consequence of cancer aerobic glycolysis, possibly affecting T cell function, is the release of lactate in the extracellular milieu.

Lactic acid produced by tumor cells acidifies the TME, and both the lower pH or the direct effect of lactate/lactic acid can modulate the immune cells, weakening T cell effector functions [100]. Indeed, a reduction in the proliferation of cytotoxic T lymphocytes, as well as a dramatically reduced availability of IL-2 and IFN-γ, and intracellular perforin and granzyme-B, were observed upon exposure to external lactic acid [101].

In TNBC cells, EGF signaling mediated aerobic glycolysis through up-regulation of HKII expression and down-regulation of PKM2 activity, leading to an accumulation of metabolic intermediates and extracellular lactate responsible for cancer cell immune escape. Also in this case, the reduced cytotoxic T cell activation was accompanied by a decrease in INFγ and IL-2 production. Therefore, co-targeting EGFR with gefitinib and glycolysis with 2-DG has been suggested as a new therapeutic strategy to treat TNBC and other EGFR-expressing cancers [102].

The altered nutrient state of the TME, related not only to glucose metabolism but also to amino acid deprivation, impairs anti-tumor immune responses in different ways (Fig. 2).

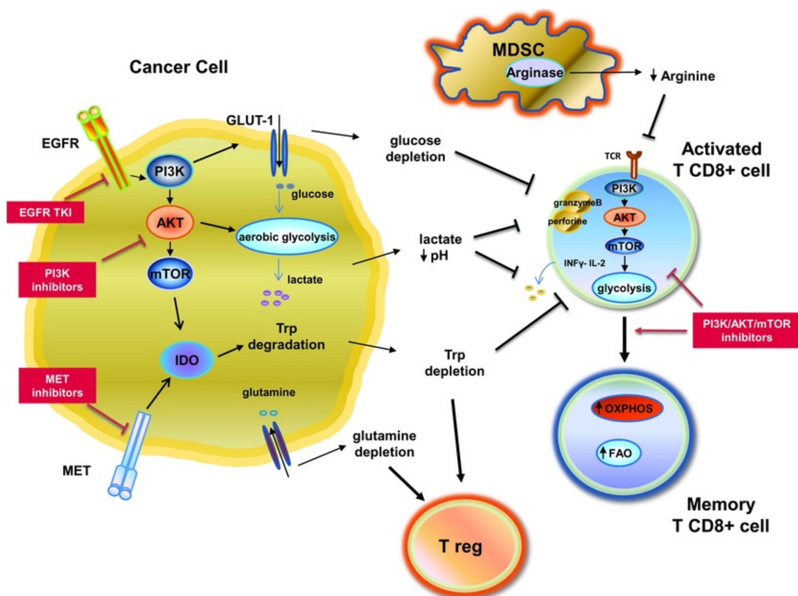


Fig. 2 Targeting the link between cancer metabolism and immune system. By increasing lactate production and depleting the microenvironment of nutrients, such as glucose and amino acids, cancer cells can alter the activation status of lymphocytes and ultimately impair immune function. By modulating cancer metabolism, oncogene-targeting agents not only may affect cancer growth directly, but may also restore more favorable conditions for effective immune-surveillance.

L-arginine is essential for T cell functions and its shortage determines a down-regulation of surface T cell receptors, thus inhibiting the antigen-specific T cell responses. Arginase I and NOS are the two enzymes involved in arginine catabolism, and when expressed at high levels within the TME lead to a deficiency in the availability of arginine and a decrease in immune cell function. The L-arginine analogue N-hydroxy-nor-L-arginine, a well-known inhibitor of arginase activity, was shown to successfully inhibit primary tumor growth in Lewis lung carcinoma through a myeloid cell-mediated mechanism [103]. Further investigations are critical to determine whether known oncogene-targeting drugs could restore the availability of arginine in the TME.

It has been demonstrated that T lymphocytes exposed to tryptophan starvation undergo proliferation arrest. Indoleamine 2,3-dioxygenase (IDO), an enzyme that catalyzes tryptophan degradation and is expressed in cancer cells or in dendritic cells, has been shown to induce immunosuppression. This inhibitory effect was also mediated by an increased differentiation of regulatory T cells (Tregs) at the tumor site. Tregs further recruited and activated myeloid-derived suppressor cells (MDSCs), thus enhancing the immunosuppressive status. *In vivo* treatment with the selective IDO inhibitor indoximod reversed this tumor-associated immunosuppression by decreasing tumor-infiltrating MDSCs and Tregs and abolishing their suppressive function [104]. Interestingly, it has been reported that PHA-665752 (c-Met TK inhibitor) and LY294002 (PI3K inhibitor) suppressed IDO expression [105], although their role in the immune context has not been investigated yet.

Glutamine deprivation, which occurs in TME due to the high glutamine uptake by cancer cells, shifts the balance of the immune response to become more suppressive. Glutamine-deficient TME is a critical factor in enforcing differentiation of Treg cells [106], and, in this context, agents such as erlotinib and trastuzumab (see Table 1), through their inhibitory activity on glutaminolysis, may provide a novel approach to decrease the persistence of Tregs in the TME.

5 Conclusions and future directions

The renewed interest emerged in recent years towards cancer metabolic reprogramming has allowed to expand our knowledge on the mechanisms underlying tumor initiation and progression, thus opening new opportunities for the development of effective therapeutic strategies against cancer.

To date, a multitude of drugs directly targeting peculiar cancer metabolic pathways have been proposed for cancer therapy. However, despite the encouraging results obtained in preclinical models, very few of them are

currently in clinical trials. Indeed, one major challenge with such agents is to achieve a therapeutic window that allows to efficiently target cancer cells, with minimal toxicity for normal proliferating cells, such as immune cells, that share common metabolic requirements with cancer cells.

An alternative approach to tackling on cancer metabolism, which may potentially limit the inhibitory effects to cancer cells, is to target the oncogenic signaling pathways responsible for cancer metabolic reprogramming. The variety of studies discussed in this review sustain the validity of such an approach. However, another important issue concerns the metabolic plasticity that allows cancer cells to adapt to drug-mediated inhibition of their preferential metabolic pathway by shifting their metabolic demands to alternative fuel sources. Therefore, combining oncogenic signaling-targeted drugs with agents directed against distinct metabolic pathways may offer a superior therapeutic option, with the advantage of reducing the doses of both drugs and limiting the side effects.

Activation of compensatory metabolic pathways is considered as one of the mechanisms involved in the acquisition of drug resistance. However, a causal link between altered cancer metabolism and drug resistance may be difficult to determine. Indeed, activation of metabolic pathways in cancer resistant cells may emerge simply as a consequence of their restored capability to proliferate in the presence of the drug, which in turn may depend on the adaptive activation of oncogenic signaling, such as the PI3K/AKT/mTOR pathway, impacting also on cancer metabolism. However, the identification of molecular and genetic rearrangements in metabolic pathways in cancer resistant cells have clarified their direct and essential role in the acquisition of drug resistance.

Although acquired resistance can arise from multiple mechanisms, targeting a common deregulated metabolic pathway across different types of cancer can result in overcoming resistance to different drugs. Just as an example, the pharmacological inhibition of GLS (Table 2) has demonstrated a great efficacy in overcoming resistance to different targeted agents (AIs, mTOR inhibitors, erlotinib, vemurafenib, sorafenib) in different tumor cells (BC, glioblastoma, NSCLC, melanoma, HCC) in preclinical studies and CB-839, a potent and selective inhibitor of GLS is now tested in Phase 1 study in patients with solid tumors (NCT02071862).

Emerging evidence indicates that cancer metabolic reprogramming may have a profound impact on immune anti-tumor response, affecting both T cell activation/differentiation and the induction of a tumor-specific immunological memory. Therefore, a more deepened knowledge of the effects of oncogene-targeting agents on cancer metabolism and their consequence on TME components may contribute to find better therapeutic anti-cancer treatments. In this context, the ultimate goal may be to design combination based-strategies using oncogenic-targeted agents with known metabolic effects and drugs capable of improving T cell reactivation, to promote effective and sustainable anti-tumor responses.

Disclosure

None of the authors have any financial conflict of interest.

Acknowledgements

We thank “Associazione Augusto per la Vita”, Novellara (RE).

References

[1] R.J. DeBerardinis and N.S. Chandel, Fundamentals of cancer metabolism, *Sci. Adv.* **2** (5), 2016, e1600200.

[2] P. Jiang, W. Du and M. Wu, Regulation of the pentose phosphate pathway in cancer, *Protein Cell* **5** (8), 2014, 592–602.

[3] C.V. Dang, Links between metabolism and cancer, *Genes Dev.* **26** (9), 2012, 877–890.

[4] F. Morani, S. Phadngam, C. Follo, R. Titone, G. Aimaretti, A. Galetto, O. Alabiso and C. Isidoro, PTEN regulates plasma membrane expression of glucose transporter 1 and glucose uptake in thyroid cancer cells, *J. Mol. Endocrinol.* **53** (2), 2014, 247–258.

[5] H.L. Wieman, J.A. Wofford and J.C. Rathmell, Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking, *Mol. Biol. Cell* **18** (4), 2007, 1437–1446.

[6] L. Wang, H. Xiong, F. Wu, Y. Zhang, J. Wang, L. Zhao, X. Guo, L.J. Chang, M.J. You, S. Koochekpour, M. Saleem, H. Huang, J. Lu and Y. Deng, Hexokinase 2-mediated Warburg effect is required for PTEN- and p53-deficiency-driven prostate cancer growth, *Cell Rep.* **8** (5), 2014, 1461–1474.

[7] X. Zhao, C. Lu, W. Chu, B. Zhang, Q. Zhen, R. Wang, Y. Zhang, Z. Li, B. Lv, H. Li and J. Liu, MicroRNA-124 suppresses proliferation and glycolysis in non-small cell lung cancer cells by targeting AKT-GLUT1/HKII, *Tumour Biol.* **39** (5), 2017, 1010428317706215.

[8] H. Hu, A. Juvekar, C.A. Lyssiotis, E.C. Lien, J.G. Albeck, D. Oh, G. Varma, Y.P. Hung, S. Ullas, J. Lauring, P. Seth, M.R. Lundquist, D.R. Tolan, A.K. Grant, D.J. Needleman, J.M. Asara, L.C. Cantley and G.M. Wulf, Phosphoinositide 3-kinase regulates glycolysis through mobilization of aldolase from the actin cytoskeleton, *Cell* **164** (3), 2016, 433–446.

[9] R.A. Cairns, I. Harris, S. McCracken and T.W. Mak, Cancer cell metabolism, *Cold Spring Harbor Symp. Quant. Biol.* **76**, 2011, 299–311.

[10] G.L. Semenza, HIF-1: upstream and downstream of cancer metabolism, *Curr. Opin. Genet. Dev.* **20** (1), 2010, 51–56.

[11] M.R. Doe, J.M. Ascano, M. Kaur and M.D. Cole, Myc posttranscriptionally induces HIF1 protein and target gene expression in normal and cancer cells, *Cancer Res.* **72** (4), 2012, 949–957.

[12] C.V. Dang, J.W. Kim, P. Gao and J. Yustein, The interplay between MYC and HIF in cancer, *Nat. Rev. Cancer* **8** (1), 2008, 51–56.

[13] S. Desai, M. Ding, B. Wang, Z. Lu, Q. Zhao, K. Shaw, W.K. Yung, J.N. Weinstein, M. Tan and J. Yao, Tissue-specific isoform switch and DNA hypomethylation of the pyruvate kinase PKM gene in human cancers, *Oncotarget* **5** (18), 2014, 8202–8210.

[14] T.L. Dayton, T. Jacks and M.G. Vander, Heiden, PKM2, cancer metabolism, and the road ahead, *EMBO Rep.* **17** (12), 2016, 1721–1730.

[15] W. Yang and Z. Lu, Pyruvate kinase M2 at a glance, *J. Cell Sci.* **128** (9), 2015, 1655–1660.

[16] F. Schwartzenberg-Bar-Yoseph, M. Armoni and E. Karnieli, The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression, *Cancer Res.* **64** (7), 2004, 2627–2633.

[17] K. Bensaad, A. Tsuruta, M.A. Selak, M.N. Vidal, K. Nakano, R. Bartrons, E. Gottlieb and K.H. Vousden, TIGAR, a p53-inducible regulator of glycolysis and apoptosis, *Cell* **126** (1), 2006, 107–120.

[18] J. Liu, C. Zhang, W. Hu and Z. Feng, Tumor suppressor p53 and its mutants in cancer metabolism, *Cancer Lett.* **356** (2 Pt A), 2015, 197–203.

[19] F. Kruiswijk, C.F. Labuschagne and K.H. Vousden, p53 in survival, death and metabolic health: a lifeguard with a licence to kill, *Nat. Rev. Mol. Cell Biol.* **16** (7), 2015, 393–405.

[20] Z. Feng, W. Hu, E. de Stanchina, A.K. Teresky, S. Jin, S. Lowe and A.J. Levine, The regulation of AMPK beta1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways, *Cancer Res.* **67** (7), 2007, 3043–3053.

[21] I.A. Olovnikov, J.E. Kravchenko and P.M. Chumakov, Homeostatic functions of the p53 tumor suppressor: regulation of energy metabolism and antioxidant defense, *Semin. Cancer Biol.* **19** (1), 2009, 32–41.

[22] S. Herzig and R.J. Shaw, AMPK: guardian of metabolism and mitochondrial homeostasis, *Nat. Rev. Mol. Cell Biol.* **19** (2), 2018, 121–135.

[23] K. Bensaad and K.H. Vousden, Savior and slayer: the two faces of p53, *Nat. Med.* **11** (12), 2005, 1278–1279.

[24] E. Gurpinar and K.H. Vousden, Hitting cancers' weak spots: vulnerabilities imposed by p53 mutation, *Trends Cell Biol.* **25** (8), 2015, 486–495.

[25] C.T. Hensley, A.T. Wasti and R.J. DeBerardinis, Glutamine and cancer: cell biology, physiology, and clinical opportunities, *J. Clin. Invest.* **123** (9), 2013, 3678–3684.

[26] B.J. Altman, Z.E. Stine and C.V. Dang, From Krebs to clinic: glutamine metabolism to cancer therapy, *Nat. Rev. Cancer* **16** (10), 2016, 619–634.

[27] D.R. Wise, R.J. DeBerardinis, A. Mancuso, N. Sayed, X.Y. Zhang, H.K. Pfeiffer, I. Nissim, E. Daikhin, M. Yudkoff, S.B. McMahon and C.B. Thompson, Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction, *Proc. Natl. Acad. Sci. U.S.A.* **105** (48), 2008, 18782–18787.

[28] J. Son, C.A. Lyssiotis, H. Ying, X. Wang, S. Hua, M. Ligorio, R.M. Perera, C.R. Ferrone, E. Mullarky, N. Shyh-Chang, Y. Kang, J.B. Fleming, N. Bardeesy, J.M. Asara, M.C. Haigis, R.A. DePinho, L.C. Cantley and A.C. Kimmelman, Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway, *Nature* **496** (7443), 2013, 101–105.

[29] L.K. Boroughs and R.J. DeBerardinis, Metabolic pathways promoting cancer cell survival and growth, *Nat. Cell Biol.* **17** (4), 2015, 351–359.

[30] D. de Gonzalo-Calvo, L. Lopez-Vilaro, L. Nasarre, M. Perez-Olabarria, T. Vazquez, D. Escuin, L. Badimon, A. Barnadas, E. Lerma and V. Llorente-Cortes, Intratumor cholesteryl ester accumulation is associated with human breast cancer proliferation and aggressive potential: a molecular and clinicopathological study, *BMC Cancer* **15**, 2015, 460.

[31] S. Beloribi-Djefaflia, S. Vasseur and F. Guillaumond, Lipid metabolic reprogramming in cancer cells, *Oncogenesis* **5**, 2016, e189.

[32] Z. Li and H. Zhang, Reprogramming of glucose, fatty acid and amino acid metabolism for cancer progression, *Cell Mol. Life Sci.* **73** (2), 2016, 377–392.

[33] T. Porstmann, C.R. Santos, B. Griffiths, M. Cully, M. Wu, S. Leevers, J.R. Griffiths, Y.L. Chung and A. Schulze, SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth, *Cell Metab.* **8** (3), 2008, 224–236.

[34] D. Guo, R.M. Prins, J. Dang, D. Kuga, A. Iwanami, H. Soto, K.Y. Lin, T.T. Huang, D. Akhavan, M.B. Hock, S. Zhu, A.A. Kofman, S.J. Bensinger, W.H. Yong, H.V. Vinters, S. Horvath, A.D. Watson, J.G. Kuhn, H.I. Robins, M.P. Mehta, P.Y. Wen, L.M. DeAngelis, M.D. Prados, I.K. Mellinghoff, T.F. Cloughesy and P.S. Mischel, EGFR signaling through an Akt-SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to antilipogenic therapy, *Sci. Signaling* **2** (101), 2009, ra82.

[35] L. Lin, X. Zheng, C. Qiu, S. Dongol, Q. Lv, J. Jiang, B. Kong and C. Wang, SIRT1 promotes endometrial tumor growth by targeting SREBP1 and lipogenesis, *Oncol Rep.* **32** (6), 2014, 2831–2835.

[36] M. Tiseo, M. Ippolito, M. Scarlattei, P. Spadaro, S. Cosentino, F. Latteri, L. Ruffini, M. Bartolotti, B. Bortesi, C. Fumarola, C. Caffarra, A. Cavazzoni, R.R. Alfieri, P.G. Petronini, R. Bordonaro, P. Bruzzi, A. Ardizzoni and H.J. Soto, Parra, Predictive and prognostic value of early response assessment using 18FDG-PET in advanced non-small cell lung cancer patients treated with erlotinib, *Cancer Chemother. Pharmacol.* **73** (2), 2014, 299–307.

[37] H. Makinoshima, M. Takita, K. Saruwatari, S. Umemura, Y. Obata, G. Ishii, S. Matsumoto, E. Sugiyama, A. Ochiai, R. Abe, K. Goto, H. Esumi and K. Tsuchihara, Signaling through the phosphatidylinositol 3-Kinase (PI3K)/mammalian target of rapamycin (mTOR) axis is responsible for aerobic glycolysis mediated by glucose transporter in epidermal growth factor receptor (EGFR)-mutated lung adenocarcinoma, *J. Biol. Chem.* **290** (28), 2015, 17495–17504.

[38] V. De Rosa, F. Iommelli, M. Monti, R. Fonti, G. Votta, M.P. Stoppelli and S. Del Vecchio, Reversal of warburg effect and reactivation of oxidative phosphorylation by differential inhibition of EGFR signaling pathways in non-small cell lung cancer, *Clin. Cancer Res.* **21** (22), 2015, 5110–5120.

[39] M.J. Martin, C. Eberlein, M. Taylor, S. Ashton, D. Robinson and D. Cross, Inhibition of oxidative phosphorylation suppresses the development of osimertinib resistance in a preclinical model of EGFR-driven lung adenocarcinoma, *Oncotarget* **7** (52), 2016, 86313–86325.

[40] H. Makinoshima, M. Takita, S. Matsumoto, A. Yagishita, S. Owada, H. Esumi and K. Tsuchihara, Epidermal growth factor receptor (EGFR) signaling regulates global metabolic pathways in EGFR-mutated lung adenocarcinoma, *J. Biol. Chem.* **289** (30), 2014, 20813–20823.

[41] H. Lu, X. Li, Z. Luo, J. Liu and Z. Fan, Cetuximab reverses the Warburg effect by inhibiting HIF-1-regulated LDH-A, *Mol. Cancer Ther.* **12** (10), 2013, 2187–2199.

[42] H. Lu, X. Li, Y. Lu, S. Qiu and Z. Fan, ASCT2 (SLC1A5) is an EGFR-associated protein that can be co-targeted by cetuximab to sensitize cancer cells to ROS-induced apoptosis, *Cancer Lett.* **381** (1), 2016, 23–30.

[43] X. Tao, Y. Lu, S. Qiu, Y. Wang, J. Qin and Z. Fan, AP1G1 is involved in cetuximab-mediated downregulation of ASCT2-EGFR complex and sensitization of human head and neck squamous cell carcinoma cells to ROS-induced apoptosis, *Cancer Lett.* **408**, 2017, 33–42.

[44] S. Qie, C. Chu, W. Li, C. Wang and N. Sang, ErbB2 activation upregulates glutaminase 1 expression which promotes breast cancer cell proliferation, *J. Cell. Biochem.* **115** (3), 2014, 498–509.

[45] T. Hitosugi, S. Kang, M.G. Vander Heiden, T.W. Chung, S. Elf, K. Lythgoe, S. Dong, S. Lonial, X. Wang, G.Z. Chen, J. Xie, T.L. Gu, R.D. Polakiewicz, J.L. Roesel, T.J. Boggon, F.R. Khuri, D.G. Gilliland, L.C. Cantley, J. Kaufman and J. Chen, Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth, *Sci. Signaling* **2** (97), 2009, ra73.

[46] T. Hitosugi, J. Fan, T.W. Chung, K. Lythgoe, X. Wang, J. Xie, Q. Ge, T.L. Gu, R.D. Polakiewicz, J.L. Roesel, G.Z. Chen, T.J. Boggon, S. Lonial, H. Fu, F.R. Khuri, S. Kang and J. Chen, Tyrosine phosphorylation of mitochondrial pyruvate dehydrogenase kinase 1 is important for cancer metabolism, *Mol. Cell* **44** (6), 2011, 864–877.

[47] C. Fumarola, D. Cretella, S. La Monica, M.A. Bonelli, R. Alfieri, C. Caffarra, F. Quaini, D. Madeddu, A. Falco, A. Cavazzoni, G. Digiacomo, G. Mazzaschi, V. Vivo, E. Barocelli, M. Tiseo, P.G. Petronini and A. Ardizzoni, Enhancement of the anti-tumor activity of FGFR1 inhibition in squamous cell lung cancer by targeting downstream signaling involved in glucose metabolism, *Oncotarget* **8** (54), 2017, 91841–91859.

[48] H. Choi, J.C. Paeng, D.W. Kim, J.K. Lee, C.M. Park, K.W. Kang, J.K. Chung and D.S. Lee, Metabolic and metastatic characteristics of ALK-rearranged lung adenocarcinoma on FDG PET/CT, *Lung Cancer* **79** (3), 2013, 242–247.

[49] Y. Ma, C. Yu, E.M. Mohamed, H. Shao, L. Wang, G. Sundaresan, J. Zweit, M. Idowu and X. Fang, A causal link from ALK to hexokinase II overexpression and hyperactive glycolysis in EML4-ALK-positive lung cancer,

Oncogene **35** (47), 2016, 6132–6142.

[50] R. Rashmi, C. DeSelm, C. Helms, A. Bowcock, B.E. Rogers, J.L. Rader, P.W. Grigsby and J.K. Schwarz, AKT inhibitors promote cell death in cervical cancer through disruption of mTOR signaling and glucose uptake, *PLoS ONE* **9** (4), 2014, e92948.

[51] S.A. Moestue, C.G. Dam, S.S. Gorad, A. Kristian, A. Bofin, G.M. Maelandsmo, O. Engebraten, I.S. Gribbestad and G. Bjorkoy, Metabolic biomarkers for response to PI3K inhibition in basal-like breast cancer, *Breast Cancer Res.* **15** (1), 2013, R16.

[52] S.S. Tee, I. Suster, S. Truong, S. Jeong, R. Eskandari, V. Di Gialleonardo, J.A. Alvarez, H.N. Aldeborgh and K. Keshari, Targeted AKT inhibition in prostate cancer cells and spheroids reduces aerobic glycolysis and generation of hyperpolarized [1-(13)C] lactate, *Mol. Cancer Res.* 2018.

[53] H.G. Keen, S.A. Ricketts, J. Maynard, A. Logie, R. Odedra, A.M. Shannon, S.R. Wedge and S.M. Guichard, Examining changes in [18 F]FDG and [18 F]FLT uptake in U87-MG glioma xenografts as early response biomarkers to treatment with the dual mTOR1/2 inhibitor AZD8055, *Mol. Imaging Biol.* **16** (3), 2014, 421–430.

[54] J. Qian, Y. Chen, T. Meng, L. Ma, L. Meng, X. Wang, T. Yu, A. Zask, J. Shen and K. Yu, Molecular regulation of apoptotic machinery and lipid metabolism by mTORC1/mTORC2 dual inhibitors in preclinical models of HER2+/PIK3CAmut breast cancer, *Oncotarget* **7** (41), 2016, 67071–67086.

[55] H. Ying, A.C. Kimmelman, C.A. Lyssiotis, S. Hua, G.C. Chu, E. Fletcher-Sananikone, J.W. Locasale, J. Son, H. Zhang, J.L. Coloff, H. Yan, W. Wang, S. Chen, A. Viale, H. Zheng, J.H. Paik, C. Lim, A.R. Guimaraes, E.S. Martin, J. Chang A.F. Hezel, S.R. Perry, J. Hu, B. Gan, Y. Xiao, J.M. Asara, R. Weissleder, Y.A. Wang, L. Chin, L.C. Cantley and R.A. DePinho, Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism, *Cell* **149** (3), 2012, 656–670.

[56] D. Gaglio, C.M. Metallo, P.A. Gameiro, K. Hiller, L.S. Danna, C. Balestrieri, L. Alberghina, G. Stephanopoulos and F. Chiaradonna, Oncogenic K-Ras decouples glucose and glutamine metabolism to support cancer cell growth, *Mol. Syst. Biol.* **7**, 2011, 523.

[57] A.M. Gouw, L.S. Eberlin, K. Margulis, D.K. Sullivan, G.G. Toal, L. Tong, R.N. Zare and D.W. Felsher, Oncogene KRAS activates fatty acid synthase, resulting in specific ERK and lipid signatures associated with lung adenocarcinoma, *Proc. Natl. Acad. Sci. U.S.A.* **114** (17), 2017, 4300–4305.

[58] T. Tegnebratt, L. Lu, L. Lee, V. Meresse, J. Tessier, N. Ishii, N. Harada, P. Pisa and S. Stone-Elander, [18 F]FDG-PET imaging is an early non-invasive pharmacodynamic biomarker for a first-in-class dual MEK/Raf inhibitor, RO5126766 (CH5126766), in preclinical xenograft models, *EJNMMI Res.* **3** (1), 2013, 67.

[59] M. Falck Miniotis, V. Arunan, T.R. Eykyn, R. Marais, P. Workman, M.O. Leach and M. Beloueche-Babari, MEK1/2 inhibition decreases lactate in BRAF-driven human cancer cells, *Cancer Res.* **73** (13), 2013, 4039–4049.

[60] T.J. Parmenter, M. Kleinschmidt, K.M. Kinross, S.T. Bond, J. Li, M.R. Kaadige, A. Rao, K.E. Sheppard, W. Hugo, G.M. Pupo, R.B. Pearson, S.L. McGee, G.V. Long, R.A. Scolyer, H. Rizos, R.S. Lo, C. Cullinane, D.E. Ayer, A. Ribas, R.W. Johnstone, R.J. Hicks and G.A. McArthur, Response of BRAF-mutant melanoma to BRAF inhibition is mediated by a network of transcriptional regulators of glycolysis, *Cancer Discov.* **4** (4), 2014, 423–433.

[61] S. Jain, X. Wang, C.C. Chang, C. Ibarra-Drendall, H. Wang, Q. Zhang, S.W. Brady, P. Li, H. Zhao, J. Dobbs, M. Kyrish, T.S. Tkaczyk, A. Ambrose, C. Sistrunk, B.K. Arun, R. Richards-Kortum, W. Jia, V.L. Seewaldt and D. Yu, Src inhibition blocks c-myc translation and glucose metabolism to prevent the development of breast cancer, *Cancer Res.* **75** (22), 2015, 4863–4875.

[62] J.F. Barger, C.A. Gallo, P. Tandon, H. Liu, A. Sullivan, H.L. Grimes and D.R. Plas, S6K1 determines the metabolic requirements for BCR-ABL survival, *Oncogene* **32** (4), 2013, 453–461.

[63] S. Gottschalk, N. Anderson, C. Hainz, S.G. Eckhardt and N.J. Serkova, Imatinib (STI571)-mediated changes in glucose metabolism in human leukemia BCR-ABL-positive cells, *Clin. Cancer Res.* **10** (19), 2004, 6661–6668.

[64] A.D. Van den Abbeele, The lessons of GIST–PET and PET/CT: a new paradigm for imaging, *Oncologist* **13** (Suppl 2), 2008, 8–13.

[65] H. Shinohara, M. Kumazaki, Y. Minami, Y. Ito, N. Sugito, Y. Kuranaga, K. Taniguchi, N. Yamada, Y. Otsuki, T. Naoe and Y. Akao, Perturbation of energy metabolism by fatty-acid derivative AIC-47 and imatinib in BCR-ABL-harboring leukemic cells, *Cancer Lett.* **371** (1), 2016, 1–11.

[66] G. Gadaleta-Caldarola, S. Infusino, R. Divella, E. Ferraro, A. Mazzocca, F. De Rose, G. Filippelli, I. Abbate and M. Brandi, Sorafenib: 10 years after the first pivotal trial, *Future Oncol.* **11** (13), 2015, 1863–1880.

[67] L. Fiume, M. Manerba, M. Vettraino and G. Di Stefano, Effect of sorafenib on the energy metabolism of hepatocellular carcinoma cells, *Eur. J. Pharmacol.* **670** (1), 2011, 39–43.

[68] V.H. Bull, K. Rajalingam and B. Thiede, Sorafenib-induced mitochondrial complex I inactivation and cell death in human neuroblastoma cells, *J. Proteome Res.* **11** (3), 2012, 1609–1620.

[69] C. Fumarola, C. Caffarra, S. La Monica, M. Galetti, R.R. Alfieri, A. Cavazzoni, E. Galvani, D. Generali, P.G. Petronini and M.A. Bonelli, Effects of sorafenib on energy metabolism in breast cancer cells: role of AMPK-mTORC1 signaling, *Breast Cancer Res. Treat* **141** (1), 2013, 67–78.

[70] V. Tesori, A.C. Piscaglia, D. Samengo, M. Barba, C. Bernardini, R. Scatena, A. Pontoglio, L. Castellini, J.N. Spelbrink, G. Maulucci, M.A. Puglisi, G. Pani and A. Gasbarrini, The multikinase inhibitor Sorafenib enhances glycolysis and synergizes with glycolysis blockade for cancer cell killing, *Sci. Rep.* **5**, 2015, 9149.

[71] I.M. Xu, R.K. Lai, S.H. Lin, A.P. Tse, D.K. Chiu, H.Y. Koh, C.T. Law, C.M. Wong, Z. Cai, C.C. Wong and I.O. Ng, Transketolase counteracts oxidative stress to drive cancer development, *Proc. Natl. Acad. Sci. U.S.A.* **113** (6), 2016, E725–E734.

[72] F.H. Groenendijk, W.W. Mellema, E. van der Burg, E. Schut, M. Hauptmann, H.M. Horlings, S.M. Willems, M.M. van den Heuvel, J. Jonkers, E.F. Smit and R. Bernards, Sorafenib synergizes with metformin in NSCLC through AMPK pathway activation, *Int. J. Cancer* **136** (6), 2015, 1434–1444.

[73] S. Ling, T. Feng, Q. Ke, N. Fan, L. Li, Z. Li, C. Dong, C. Wang, F. Xu, Y. Li and L. Wang, Metformin inhibits proliferation and enhances chemosensitivity of intrahepatic cholangiocarcinoma cell lines, *Oncol. Rep.* **31** (6), 2014, 2611–2618.

[74] J. Floter, I. Kaymak and A. Schulze, Regulation of metabolic activity by p53, *Metabolites* **7** (2), 2017.

[75] L.S. Schneider, M. Ulrich, T. Lehr, D. Menche, R. Muller and K. von Schwarzenberg, MDM2 antagonist nutlin-3a sensitizes tumors to V-ATPase inhibition, *Mol. Oncol.* **10** (7), 2016, 1054–1062.

[76] N. Nakaigawa, K. Kondo, D. Ueno, K. Namura, K. Makiyama, K. Kobayashi, K. Shioi, I. Ikeda, T. Kishida, T. Kaneta, R. Minamimoto, U. Tateishi, T. Inoue and M. Yao, The acceleration of glucose accumulation in renal cell carcinoma assessed by FDG PET/CT demonstrated acquisition of resistance to tyrosine kinase inhibitor therapy, *BMC Cancer* **17** (1), 2017, 39.

[77] C.D. Hudson, T. Hagemann, S.J. Mather and N. Avril, Resistance to the tyrosine kinase inhibitor axitinib is associated with increased glucose metabolism in pancreatic adenocarcinoma, *Cell Death Dis.* **5**, 2014, e1160.

[78] M. Ye, S. Wang, T. Wan, R. Jiang, Y. Qiu, L. Pei, N. Pang, Y. Huang, Z. Zhang and L. Yang, Combined inhibitions of glycolysis and AKT/autophagy can overcome resistance to EGFR-targeted therapy of lung cancer, *J. Cancer* **8** (18), 2017, 3774–3784.

[79] B. Ruprecht, E.A. Zaal, J. Zecha, W. Wu, C.R. Berkers, B. Kuster and S. Lemeer, Lapatinib resistance in breast cancer cells is accompanied by phosphorylation-mediated reprogramming of glycolysis, *Cancer Res.* **77** (8), 2017, 1842–1853.

[80] Y.M. Woo, Y. Shin, E.J. Lee, S. Lee, S.H. Jeong, H.K. Kong, E.Y. Park, H.K. Kim, J. Han, M. Chang and J.H. Park, Inhibition of aerobic glycolysis represses Akt/mTOR/HIF-1alpha axis and restores tamoxifen sensitivity in antiestrogen-resistant breast cancer cells, *PLoS ONE* **10** (7), 2015, e0132285.

[81] K.X. Koh, G.H. Tan, S.H. Hui Low, M.F. Mohd Omar, M.J. Han, B. Iacopetta, R. Soo, M. Beloueche-Babari, B. Bhattacharya and R. Soong, Acquired resistance to PI3K/mTOR inhibition is associated with mitochondrial DNA mutation and glycolysis, *Oncotarget* **8** (66), 2017, 110133–110144.

[82] M. Bacci, E. Giannoni, A. Fearns, R. Ribas, Q. Gao, M.L. Taddei, G. Pintus, M. Dowsett, C.M. Isacke, L.A. Martin, P. Chiarugi and A. Morandi, miR-155 drives metabolic reprogramming of ER+ breast cancer cells following long-term estrogen deprivation and predicts clinical response to aromatase inhibitors, *Cancer Res.* **76** (6), 2016, 1615–1626.

[83] G.A. Vitiello, B.D. Medina, S. Zeng, T.G. Bowler, J.Q. Zhang, J.K. Loo, N.J. Param, M. Liu, J.A. Moral, J.N. Zhao, F. Rossi, C.R. Antonescu, V.P. Balachandran, J.R. Cross and R.P. DeMatteo, Mitochondrial inhibition augments the efficacy of imatinib by resetting the metabolic phenotype of gastrointestinal stromal tumor, *Clin. Cancer Res.* 2017.

[84] E.M. Kuntz, P. Baquero, A.M. Michie, K. Dunn, S. Tardito, T.L. Holyoake, G.V. Helgason and E. Gottlieb, Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells, *Nat. Med.* **23** (10), 2017, 1234–1240.

[85] F. Alvarez-Calderon, M.A. Gregory, C. Pham-Danis, D. DeRyckere, B.M. Stevens, V. Zaberezhnyy, A.A. Hill, L. Gemta, A. Kumar, V. Kumar, M.F. Wempe, D.A. Pollyea, C.T. Jordan, N.J. Serkova, D.K. Graham and J. DeGregori, Tyrosine kinase inhibition in leukemia induces an altered metabolic state sensitive to mitochondrial perturbations, *Clin. Cancer Res.* **21** (6), 2015, 1360–1372.

[86] K. Zaugg, Y. Yao, P.T. Reilly, K. Kannan, R. Kiarash, J. Mason, P. Huang, S.K. Sawyer, B. Fuerth, B. Faubert, T. Kalliomaki, A. Elia, X. Luo, V. Nadeem, D. Bungard, S. Yalavarthi, J.D. Growney, A. Wakeham, Y. Moolani, J. Silvester, A.Y. Ten, W. Bakker, K. Tsuchihara, S.L. Berger, R.P. Hill, R.G. Jones, M. Tsao, M.O. Robinson, C.B. Thompson, G. Pan and T.W. Mak, Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress, *Genes Dev.* **25** (10), 2011, 1041–1051.

[87] A. Ali, E. Levantini, J.T. Teo, J. Goggi, J.G. Clohessy, C.S. Wu, L. Chen, H. Yang, I. Krishnan, O. Kocher, J. Zhang, R.A. Soo, K. Bhakoo, T.M. Chin and D.G. Tenen, Fatty acid synthase mediates EGFR palmitoylation in EGFR mutated non-small cell lung cancer, *EMBO Mol. Med.* 2018.

[88] Z. Chen, Y. Wang, C. Warden and S. Chen, Cross-talk between ER and HER2 regulates c-MYC-mediated glutamine metabolism in aromatase inhibitor resistant breast cancer cells, *J. Steroid Biochem. Mol. Biol.* **149**, 2015, 118–127.

[89] K. Tanaka, T. Sasayama, Y. Irino, K. Takata, H. Nagashima, N. Satoh, K. Kyotani, T. Mizowaki, T. Imahori, Y. Ejima, K. Masui, B. Gini, H. Yang, K. Hosoda, R. Sasaki, P.S. Mischel and E. Kohmura, Compensatory glutamine metabolism promotes glioblastoma resistance to mTOR inhibitor treatment, *J. Clin. Invest.* **125** (4), 2015, 1591–1602.

[90] C. Xie, J. Jin, X. Bao, W.H. Zhan, T.Y. Han, M. Gan, C. Zhang and J. Wang, Inhibition of mitochondrial glutaminase activity reverses acquired erlotinib resistance in non-small cell lung cancer, *Oncotarget* **7** (1), 2016, 610–621.

[91] J.E. Hernandez-Davies, T.Q. Tran, M.A. Reid, K.R. Rosales, X.H. Lowman, M. Pan, G. Moriceau, Y. Yang, J. Wu, R.S. Lo and M. Kong, Vemurafenib resistance reprograms melanoma cells towards glutamine dependence, *J. Transl. Med.* **13**, 2015, 210.

[92] M.J. Kim, Y.K. Choi, S.Y. Park, S.Y. Jang, J.Y. Lee, H.J. Ham, B.G. Kim, H.J. Jeon, J.H. Kim, J.G. Kim, I.K. Lee and K.G. Park, PPARdelta reprograms glutamine metabolism in sorafenib-resistant HCC, *Mol. Cancer Res.* **15** (9), 2017, 1230–1242.

[93] H. Ma, Z. Wu, J. Peng, Y. Li, H. Huang, Y. Liao, M. Zhou, L. Sun, N. Huang, M. Shi, J. Bin, J. Rao, L. Wang and W. Liao, Inhibition of SLC1A5 sensitizes colorectal cancer to cetuximab, *Int. J. Cancer* 2018.

[94] S. Kouidhi, A.B. Elgaaied and S. Chouaib, Impact of metabolism on T-cell differentiation and function and cross talk with tumor microenvironment, *Front. Immunol.* **8**, 2017, 270.

[95] N.J. MacIver, R.D. Michalek and J.C. Rathmell, Metabolic regulation of T lymphocytes, *Annu. Rev. Immunol.* **31**, 2013, 259–283.

[96] G.J. van der Windt, B. Everts, C.H. Chang, J.D. Curtis, T.C. Freitas, E. Amiel, E.J. Pearce and E.L. Pearce, Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development, *Immunity* **36** (1), 2012, 68–78.

[97] J.G. Crompton, M. Sukumar, R. Roychoudhuri, D. Clever, A. Gros, R.L. Eil, E. Tran, K. Hanada, Z. Yu, D.C. Palmer, S.P. Kerkar, R.D. Michalek, T. Upham, A. Leonardi, N. Acquavella, E. Wang, F.M. Marincola, L. Gattinoni, P. Muranski, M.S. Sundrud, C.A. Klebanoff, S.A. Rosenberg, D.T. Fearon and N.P. Restifo, Akt inhibition enhances expansion of potent tumor-specific lymphocytes with memory cell characteristics, *Cancer Res.* **75** (2), 2015, 296–305.

[98] S. He, K. Kato, J. Jiang, D.R. Wahl, S. Mineishi, E.M. Fisher, D.M. Murasko, G.D. Glick and Y. Zhang, Characterization of the metabolic phenotype of rapamycin-treated CD8+ T cells with augmented ability to generate long-lasting memory cells, *PLoS ONE* **6** (5), 2011, e20107.

[99] Q. Li, R. Rao, J. Vazzana, P. Goedegebuure, K. Odunsi, W. Gillanders and P.A. Shrikant, Regulating mammalian target of rapamycin to tune vaccination-induced CD8(+) T cell responses for tumor immunity, *J. Immunol.* **188** (7), 2012, 3080–3087.

[100] W. Droge, S. Roth, A. Altmann and S. Mihm, Regulation of T-cell functions by L-lactate, *Cell Immunol.* **108** (2), 1987, 405–416.

[101] K. Fischer, P. Hoffmann, S. Voelkl, N. Meidenbauer, J. Ammer, M. Edinger, E. Gottfried, S. Schwarz, G. Rothe, S. Hoves, K. Renner, B. Timischl, A. Mackensen, L. Kunz-Schughart, R. Andreesen, S.W. Krause and M. Kreutz, Inhibitory effect of tumor cell-derived lactic acid on human T cells, *Blood* **109** (9), 2007, 3812–3819.

[102] S.O. Lim, C.W. Li, W. Xia, H.H. Lee, S.S. Chang, J. Shen, J.L. Hsu, D. Raftery, D. Djukovic, H. Gu, W.C. Chang, H.L. Wang, M.L. Chen, L. Huo, C.H. Chen, Y. Wu, A. Sahin, S.M. Hanash, G.N. Hortobagyi and M.C. Hung, EGFR signaling enhances aerobic glycolysis in triple-negative breast cancer cells to promote tumor growth and immune escape, *Cancer Res.* **76** (5), 2016, 1284–1296.

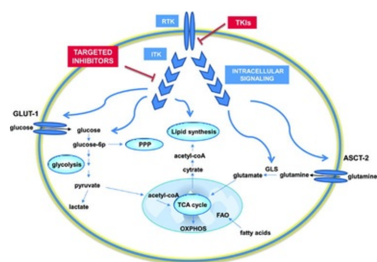
[103] V. Bronte and P. Zanovello, Regulation of immune responses by L-arginine metabolism, *Nat. Rev. Immunol.* **5** (8), 2005, 641–654.

[104] R.B. Holmgaard, D. Zamarin, Y. Li, B. Gasmi, D.H. Munn, J.P. Allison, T. Merghoub and J.D. Wolchok, Tumor-expressed IDO recruits and activates MDSCs in a treg-dependent manner, *Cell Rep.* **13** (2), 2015, 412–424.

[105] D. Wang, Y. Saga, N. Sato, T. Nakamura, O. Takikawa, H. Mizukami, S. Matsubara and H. Fujiwara, The hepatocyte growth factor antagonist NK4 inhibits indoleamine-2,3-dioxygenase expression via the c-Met-phosphatidylinositol 3-kinase-AKT signaling pathway, *Int. J. Oncol.* **48** (6), 2016, 2303–2309.

[106] D. Klysz, X. Tai, P.A. Robert, M. Craveiro, G. Cretenet, L. Oburoglu, C. Mongellaz, S. Floess, V. Fritz, M.I. Matias, C. Yong, N. Surh, J.C. Marie, J. Huehn, V. Zimmermann, S. Kinet, V. Dardalhon and N. Taylor, Glutamine-dependent alpha-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation, *Sci. Signaling* **8** (396), 2015, ra97.

Graphical abstract



Queries and Answers

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact PA.Crabtr@elsevier.com immediately prior to returning your corrections.
Answer: Yes

Query: The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.
Answer: Yes