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Targeting OXPHOS and the electron transport chain in cancer; Molecular and therapeutic implications

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ABSTRACT

Oxidative phosphorylation (OXPHOS) takes place in mitochondria and is the process whereby cells use carbon fuels and oxygen to generate ATP. Formerly OXPHOS was thought to be reduced in tumours and that glycolysis was the critical pathway for generation of ATP but it is now clear that OXPHOS, at least in many tumour types, plays a critical role in delivering the bioenergetic and macromolecular anabolic requirements of cancer cells. There is now great interest in targeting the OXPHOS and the electron transport chain for cancer therapy and in this review article we describe current therapeutic approaches and challenges.

1. Introduction

Active metabolism is essential for tumour growth. Mitochondria are key organelles found in most cells of eukaryotic organisms and functioning mitochondria are required for cancer cell survival. They are often referred to as the 'energy producing powerhouses' of cells although there has been a growing understanding in recent decades of their important role in marshalling macromolecular synthesis and cell signalling. It is now understood that all three of these mitochondrial functions play a role in the survival and propagation of cancer cells.

Three metabolic pathways generate energy in human cells, oxidative phosphorylation (OXPHOS), glycolysis and fatty oxidation. All three of these pathways are commonly dysregulated in cancer cells and are potential targets for therapy, however in this review we shall focus on the OXPHOS pathway. The OXPHOS metabolic pathway has two key functions in driving tumour cell proliferation. It provides the bioenergetic requirements in the form of ATP and funnels carbon from glucose for macromolecular synthesis, acting as a hub for both catabolic and anabolic metabolism. The enzymes of the tricarboxylic acid cycle (TCA) within the mitochondrial matrix and transmembrane protein complexes of the electron transport chain (ETC) are central to this process. Feeding carbon fuels into the TCA cycle generates the electron donors, NADH and $FADH_2$ which supply electrons to the ETC complexes I to IV. As electrons pass along these complexes, protons are pumped out into the intermembrane space by complexes I, III and IV. The generation of this proton motive force and subsequent flow of protons back into the mitochondrial matrix through Complex V (ATP synthase) generates ATP with oxygen acting as the final electron acceptor (see Fig. 1). The metabolic intermediates that are funnelled into the TCA cycle, thereby act as biosynthetic intermediates and carbon sources for a wide range of macromolecules required for cell proliferation including lipids, amino acids and nucleotides.

1.1. OXPHOS and energy metabolism in cancer

It was in the 1920′s that Otto Warburg first observed that tumour cells had high rates of glycolysis, 'the Warburg effect', even under oxygen rich conditions, and for several decades subsequently the canonical view was that mitochondria were wholly dysfunctional in cancer with low levels of OXPHOS [1]. Indeed there are a number of cancers including melanoma and renal cancer in which OXPHOS is downregulated and this may be related to mutations of mitochondrial DNA (mtDNA) or reduced mtDNA content [2]. Alternatively, other genetic markers such as LKB1 loss in non-small cell lung cancer (NSCLC) may define reduced OXPHOS [3]. However, this concept has been challenged since the 1950′s, with certain cancer cells demonstrating upregulated OXPHOS, and similar levels of TCA cycle intermediates and ATP as non-transformed cells [4,5]. Mounting evidence now suggests that mitochondria can be reprogrammed in proliferating cancers to ensure that the high energy requirements for cell division, migration and invasion are met [6]. Targeting of OXPHOS specifically to induce an energy stress in cancer cells may not be a successful strategy as studies

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have shown that cultured cancer cells which have defects in ETC function are unable to generate mitochondrial ATP, however they are still able to proliferate using ATP generated from glycolysis [7,8]. It has now been shown that there are some tumours that are highly dependent on OXPHOS for ATP and these tumours may be identifiable by certain genetic alterations, such as SMARCA4 mutant tumours [9]. Some tumours may alternatively have critical bioenergetic reliance on mitochondrial fatty acid oxidation to sustain tumour growth, for example Myc driven triple negative breast cancer (TNBC) [10]. Pancreatic cancer is an example of a high OXPHOS tumour that has high expression of mitochondrial respiratory complex I components at both the protein and transcriptomic level and for which, complex I inhibitors have demonstrated activity when combined with standard chemotherapy agents [11]. Similarly, melanomas with high PGC1alpha expression have been shown to have increased mitochondrial capacity [12]. For further detailed description of tumour types with reduced or increased OXPHOS activity, we direct the reader to the review by Ashton et al. [13].

1.2. OXPHOS and carbon metabolism in cancer

The high rates of glycolysis observed in tumour cells are now considered necessary, not to directly produce ATP and compensate for defective mitochondrial function, but rather to provide material for anabolic metabolism. Glycolytic intermediates can be funnelled through the TCA cycle in mitochondria toward the synthesis of fatty acids, nucleotides, and amino acids to ensure availability of macromolecules for cell proliferation (see Fig. 2). Besides glucose, glutamine is an important carbon source for cancer cells and indeed it has been shown that common genetic drivers in cancer, including Myc and KRAS can lead to glutamine addiction [14,15]. In the context of hypoxic conditions or genetic and pharmacological mitochondrial disruption, tumour cells may switch to glutamine over glucose as the main substrate for anabolic synthesis, using the 'reductive carboxylation' pathway to, in effect, bypass the TCA cycle [7,8]. Recent work has shown that aspartate levels are a key marker of ETC integrity and that the ETC plays a key role in maintaining aspartate synthesis and by virtue, cancer cell proliferation [16].

These fundamental principles are now being exploited to repurpose a number of drugs that interfere with OXPHOS, in particular anti-diabetic biguanides and anti-parasitic agents, alongside novel drug development programmes of small molecules designed to target OXPHOS in a highly specific manner. Here, we detail the current status of drug development programmes to target OXPHOS, discuss therapeutic opportunities, and describe current approaches to patient selection and drug combination.

2. Repurposing drugs that target OXPHOS for cancer

There is growing interest in repurposing therapies that target mitochondrial metabolism for cancer treatment (see Fig. 1 and Table 1). Many of the same principles of preclinical and clinical drug development are as relevant for drug repurposing as when exploring a novel molecule. For example, dose optimisation, assessment of pharmacodynamic effect in tumour tissue and patient selection, should all be carefully assessed in preclinical and/or clinical studies.

2.1. Biguanides

Metformin was first developed in the 1950′s to treat hyperglycaemia

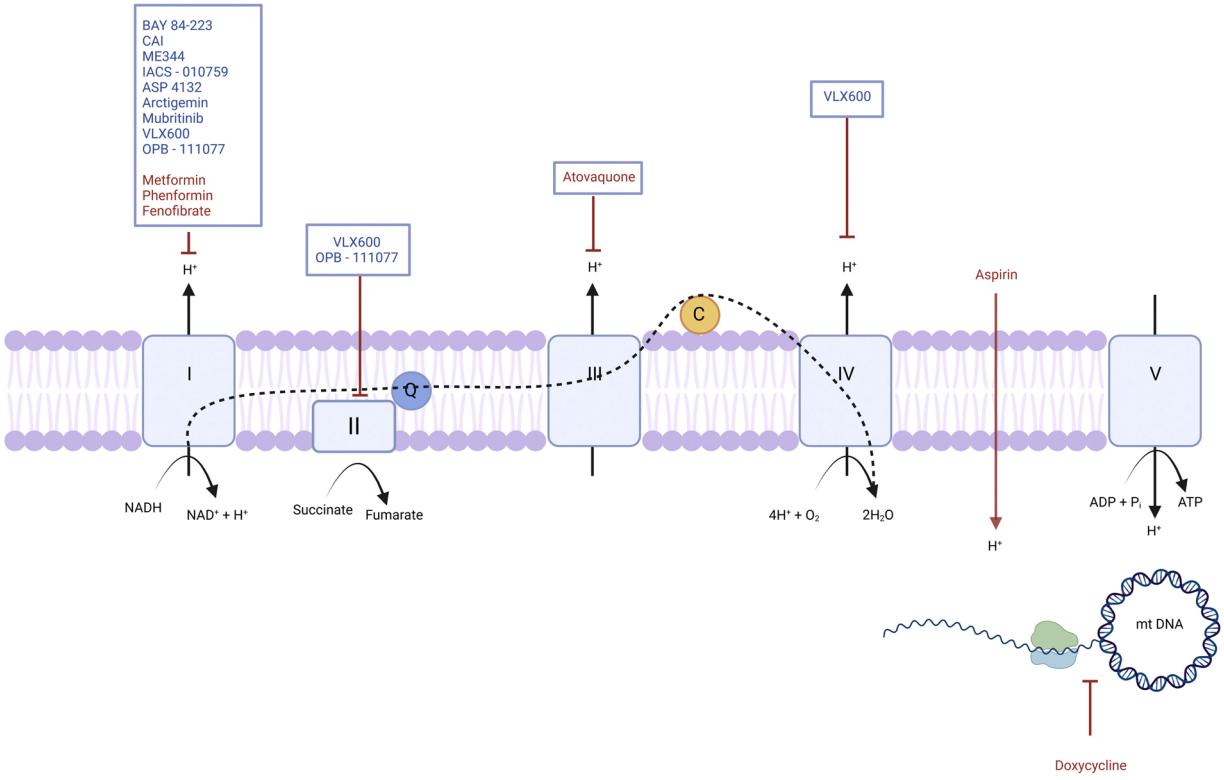

Fig. 1. Mammalian Complex 1 is an L-shaped protein with a hydrophobic domain anchored within the mitochondrial inner membrane and hydrophilic domain protruding into the mitochondrial matrix. Flavin mononucleotide (FMN) accepts electrons from NADH before transfer through 7 iron-sulphur clusters (N3, N1b, N4, N5, N6a, N6b and N2) to the electron carrier Coenzyme Q (also known as ubiquinone). Coenzyme Q then shuttles electrons to the other members of the electron transport chain. The energy released by electron transfer facilitates the pumping of protons across the mitochondrial inner membrane creating an electrochemical gradient that facilitates the conversion of ADP to ATP synthase. Several drugs that target the electron transport chain have been identified with potential to repurpose as cancer therapeutics (shown in red) and other novel compounds are in development (shown in blue). Abbreviations: CAI, Carboxyamidotriazole. Figure adapted from Ashton et al. [13].

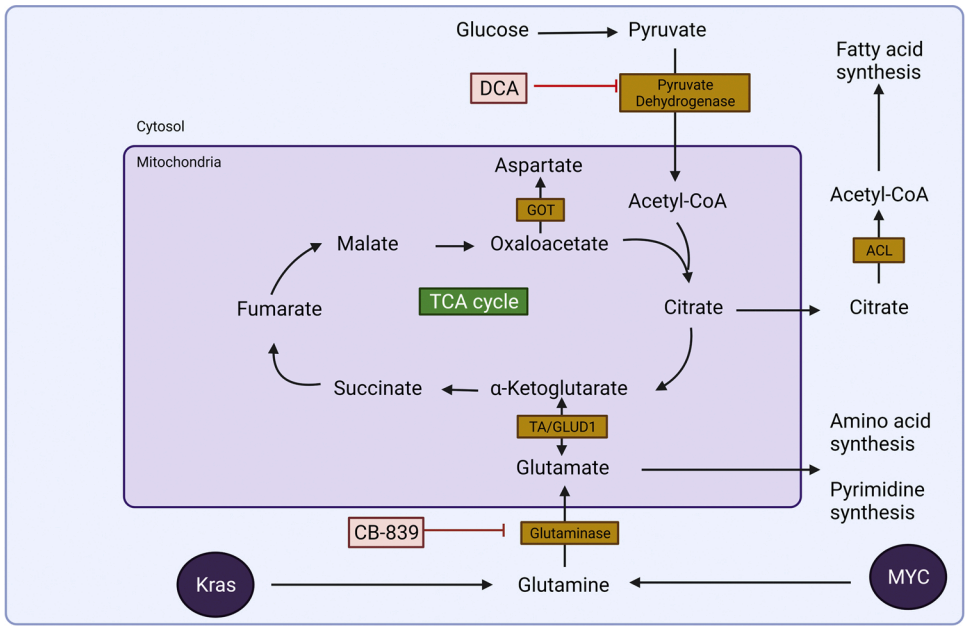

Fig. 2. Glucose and glutamine are the main sources of carbon for both macromolecular synthesis and ATP production. The TCA cycle coordinates these processes and hence plays a critical role in maintaining cancer cell proliferation. Oncogenic genetic alterations can drive metabolic rewiring and hence can also act as markers for metabolic vulnerabilities for therapeutic targeting. Potential drug combinations include the targeting of carbon input pathways into the TCA cycle. For example, dichloroacetate (DCA) which targets pyruvate dehydrogenase, and CB – 839, a small molecule glutaminase 1 inhibitor. Abbreviations: GOT, Glutamic Oxaloacetic Transaminase; TA/GLUD1, Transaminase/Glutamate Dehydrogenase 1; ACL, ATP Citrate Lyase.

in type 2 diabetes mellitus and is now believed to be the most widely prescribed diabetes drug worldwide. Growing evidence indicates the potential of metformin as a cancer preventative agent, initially sparked by a series of epidemiological studies of diabetic patients that suggested it had a protective effect against cancer incidence [17–19]. A subsequent meta-analysis of these studies put the reduction in relative risk in the order of 31 % [20].

The mechanisms that may lie behind metformin's anti-cancer properties are still not fully understood, but two hypotheses predominate. Firstly, metformin has been shown to inhibit mitochondrial complex 1 of the ETC [21–23] and several studies using cell lines both *in vitro* and *in vivo* have shown that metformin can inhibit tumour cell proliferation by disrupting their high energy and anabolic requirements [24] (see Fig. 1). AMP-activated protein kinase (AMPK) is a key regulator of energy homeostasis and is activated under energy stress conditions, coordinating metabolic function and nutrient supply. AMPK has been shown to be activated in a manner dependent on metformin's effects on mitochondrial respiration and the downstream effects include inhibition of mTOR and fatty acid synthesis signalling, putting a break on cell proliferation [25]. However, the doses used to elicit these effects in the laboratory have typically been 10- to 1000- fold greater than peak plasma levels [26] and so it remains a matter of debate as to the clinical relevance in patients. To assess the pharmacodynamic effects of metformin in patients, we have recently published data from a clinical study in breast cancer in which we showed, using RNA sequencing of primary breast tumour samples pre- and post-drug, that treatment with metformin at standard clinical doses led to the activation at the transcriptomic level of multiple mitochondrial metabolic pathways [27]. The degree of change in expression of a mitochondrial OXPHOS gene signature linked to change in a well-validated transcriptomic proliferation signature. This study provided clinical evidence that metformin targets mitochondrial metabolism at clinical doses and we hypothesised that the ability of breast tumours to increase OXPHOS gene transcription was a marker of resistance to metformin.

Other investigators consider that metformin's anti-cancer effects are indirect and driven by modulation of host metabolism, more specifically by reducing circulating insulin and IGF1 levels by targeting liver mitochondria and hence inhibiting of hepatic gluconeogenesis. The PI3K-AKT-mTOR pathway which is implicated in the pathogenesis of many human cancers is directly downstream of insulin and IGF1 receptor signalling and hence a reduction in stimulation by these growth factors might be expected to lead to reduced cell proliferation, cell motility and anabolic metabolism. There are now multiple clinical trials looking to assess the potential to repurpose metformin as a cancer therapy, although results from small randomised studies in a number of tumour types have to date been disappointing [28–31].

Phenformin is a biguanide that targets complex 1 and is under consideration for repurposing as a cancer therapy. Phenformin has greater potency and lipid solubility than metformin, and therefore may have advantages in the context of cancer therapy [32]. However, this drug is no longer licensed for use as an anti-diabetic treatment in most countries, due to the much higher risk of potentially fatal systemic lactic acidosis compared to metformin [33]. Phenformin has been shown to have anti-cancer properties in a number of preclinical models [3,34] and cancer cell lines exposed to low levels of glucose more akin to the tumour microenvironment have been shown to be especially susceptible to phenformin activity [35].

2.2. *Other agents that target OXPHOS with potential to repurpose*

Numerous studies have supported the use of regular aspirin as a means to reduce cancer risk [36]. Its principal mode of action is to inhibit the enzyme cyclooxygenase, suppressing prostanoid synthesis. This is understood to lead to a reduction in oxidative stress and ROS metabolism, and inhibit ROS-mediated DNA damage, and the canonical view is that this pathway drives a reduction in cancer risk. However, aspirin also appears be an uncoupler of OXPHOS with subsequent AMPK activation and mTOR inhibition [37,38]. In preclinical models of PI3K-mutant breast cancer, the combination of aspirin and PI3K inhibition led to enhanced activation of AMPK, inhibition of mTORC1 and induction of cell death [39]. Several drugs that are used to treat lipid metabolism have been shown to inhibit OXPHOS including statins, fibrates and thiazolidinediones [37]. Fenofibrate, a peroxisome proliferator-activated receptor (PPARa) agonist has also been shown to target complex 1 of the ETC and has demonstrated activity in glioblastoma and gastric cancer models [40–42].

A number of anti-parasitic drugs derive their anti-microbial properties by targeting mitochondrial function and have displayed promising activity in a number of preclinical cancer models. Ivermectin is FDA-approved for the treatment of onchocerciasis and intestinal strongyloidiasis. It has shown activity in chronic myeloid leukaemia (CML) by inhibiting mitochondrial complex I activity, leading to energy crisis, and

Table 1
Drugs in clinical development as cancer therapeutics that target the electron transport chain and OXPHOS.

Drug name	Mechanism of action	Status of development	ClinicalTrials.gov identifier
Metformin	Inhibits mitochondrial Complex 1	Clinical trials to repurpose	Multiple – over 390 trials
Phenformin	Inhibits mitochondrial Complex 1	Clinical trials to repurpose	NCT03026517 (currently recruiting)
Atovaquone	Mitochondrial Complex III inhibitor	Clinical trials to repurpose	NCT02628080 [87]; NCT04648033 (currently recruiting)
BAY 87−2243	Small molecule inhibitor of mitochondrial complex I	Phase 1 (clinical programme abandoned)	NCT01297530
ASP4132	Selective inhibitor of complex I	Phase I	NCT02383368 [75]
ME-344	Synthetic small molecule and demethylated metabolite of NV-128	Phase I	NCT01544322 [61]; NCT02100007 [62]; NCT02806817 [64]
IACS-010,759	Small inhibitor of mitochondrial complex I	Phase I	NCT03291938 [67]; NCT02882321 (currently active)
VLX600	Iron-chelating inhibitor of ETC subunits	Phase I	NCT02222363 [72]
OPB-111,077	Inhibitor of STAT3 mediated mitochondrial metabolism	Phase I	NCT01711034 [73]; NCT03197714 [74]; NCT04049825 (currently recruiting)
GBS-01 /Arctigenin	Inhibits mitochondrial complex 1	Phase I	University Hospital Medical Information Network Clinical Trials Registry Japan (UMIN CTR) UMIN000005787 [135]
Mubritinib	HER-2 directed tyrosine kinase inhibitor and inhibitor of complex I	Phase 1 (clinical programme abandoned)	NCT00034281
Carboxyamidotriazole (CAI)	Inhibitor of non-voltage operated calcium channels and Complex 1	Phase 2	NCT00019461 [58]

oxidative stress in CML preclinical models [43]. It has also been shown to cause apoptosis and suppress cellular proliferation in a variety of renal cell cancer (RCC) cell lines and impair tumour growth in a RCC xenograft mouse model [44]. Ivermectin combined with anti-PD1 antibodies has demonstrated significant activity in breast cancer leading to cell death and tumour regression in animal models possibly via differential ATP/P2 × 7-dependent cytotoxicity [45]. Proguanil, a biguanide derivative, was first developed as an anti-malarial drug for the management of acute P. vivax malaria. It is a complex I inhibitor and combined with atovaquone, is an effective anti-malarial. Its penetration into mitochondria is limited as a single agent, but proguanil enhances the activity of atovaquone in collapsing mitochondrial membrane potential and causing mitochondrial dysfunction [46]. Proguanil has demonstrated activity as a single agent in growth inhibition of colon and bladder cancer cell lines [47]. Doxycycline, a synthetically derived tetracycline, and commonly used antibiotic is known to be an effective agent for the prevention of malaria [48]. It targets the small mitochondrial ribosome (28S) and, as a consequence, is an inhibitor of mitochondrial protein translation. Doxycycline has been shown to re-sensitise certain cancer cell lines (colon and pancreatic cells) to gemcitabine [49]. The mechanism of action appears to be through doxycycline-induced inhibition of mitochondrial protein synthesis which decreases mitochondrial ATP generation, leading to a slower proliferation rate and therefore improving the efficacy of gemcitabine.

Targeting ATP synthase or Complex V of the electron transport chain may also represent another therapeutic opportunity. Oligomycin is a Complex V inhibitor and has shown modest activity as an anti-cancer agent in glioblastoma multiforme cell lines but synergy when combined with the inhibitor of glycolysis, 2-deoxyglucose (2-DG) [50]. Bedaquiline is also a Complex V inhibitor and has FDA approval for the treatment of multi-drug resistant tuberculosis. Bedaquiline has been shown to inhibit the expansion of cancer stem cells in preclinical models and development of an inhalable version of bedaquiline is currently being explored in NSCLC [51,52].

3. Novel drugs in development that target OXPHOS for cancer

In part, as a result of the interest in the anti-cancer properties of biguanide drugs, several novel compounds that target OXPHOS and the ETC are being developed that target complex I in cancer, a number of which are now being evaluated in the clinic (see Table 1).

BAY 87−2243 is a potent and selective small molecule inhibitor of mitochondrial complex I [53]. BAY 87−2243 displayed anti-tumour activity in an *in vivo* NSCLC xenograft model [54] and in melanoma cell lines. Here, it induced stimulation of mitochondrial ROS production, leading to oxidative damage and subsequent cell death [53]. In animal studies using head and neck cancer tumour models, the addition of BAY 87−2243 to radiotherapy improved local tumour control after fractionated radiation [55]. BAY 87−2243 has been shown to reduce hypoxia-inducible factor (HIF) gene activation and a reduction in tumour hypoxia is understood to be the radiosensitising mechanism of BAY-87−2243. However, clinical development of this compound is not progressing.

Carboxyamidotriazole (CAI) is a cytostatic inhibitor of non-voltage operated calcium channels and calcium channel mediated signalling pathways [56]. CAI has been shown to reduce mitochondrial oxygen consumption in cancer cells secondary to complex I inhibition [57]. As monotherapy, CAI has shown some clinical activity in relapsed heavily pre-treated ovarian cancer with mild toxicities reported in a phase II trial [58]. In combination with other agents, it has demonstrated activity in chemotherapy resistant pancreatic cancer combined with the glycolysis inhibitor 2-DG [59].

ME-344 is a synthetic small molecule and demethylated metabolite of the isoflavone derivative, NV-128, a complex 1 inhibitor that is now being studied in early phase clinical trials [60]. In phase I studies in ovarian, lung and cervical cancer, ME-344 showed good tolerability, but only limited clinical activity has been observed [61,62]. Anti-angiogenics induce vascular normalisation leading to reoxygenation and suppression of glycolysis and upregulation of mitochondrial respiration. Preclinical data, in which ME-344 was combined with either of the tyrosine kinase inhibitors regorafenib or nintedanib in a breast cancer mouse model demonstrated that ME-344 could sensitise tumours to anti-angiogenic therapy [63]. Subsequently, in an early phase pharmacodynamic window study in patients with HER-2 negative breast cancer, the combination of bevacizumab with ME-344 was shown to significantly reduce Ki67 expression compared to bevacizumab alone [64].

IACS-010,759 is a potent oral selective inhibitor of mitochondrial complex I. Treatment resistant leukaemic cells have been shown to have

an upregulated OXPHOS phenotype and IACS-010,759 has shown encouraging pre-clinical results in acute myeloid leukaemia (AML) [65]. In TNBC, the combination of IACS-010,759 and palbociclib, a CDK4/6 inhibitor demonstrated antitumour activity *in vitro* and *in vivo* [66]. Ongoing phase I trials are exploring the use of this drug with evidence of clinical activity in solid tumours [67].

Iron is a necessary component of haem and iron-sulfur clusters, present in numerous enzymes involved in OXPHOS and the Krebs cycle, and several iron chelators have been shown to possess anti-cancer activity [68,69]. VLX600 is an iron-chelating ETC inhibitor that has been shown to have anti-cancer activity in preclinical models [70]. The addition of VLX600 enhances the anti-tumour effect of irinotecan in colon cancer cells and also potentiates the effect of radiation in tumour cells [71]. In a phase I trial exploring the safety and tolerability of VLX600 in patients with treatment refractory advanced solid tumours, treatment was well tolerated as a single agent although no objective response were observed [72].

OPB-111,077 is an inhibitor of STAT3 and hence mitochondrial STAT3-mediated inhibition of complex 1 and complex 2. It has shown activity in preclinical models and a phase I trial has shown modest tumour responses in solid tumours [73]. A further study in a cohort of patients with AML has shown encouraging clinical activity with a number of clinical responses and was well tolerated [74]. A phase I trial combining OPB-111,077 with chemotherapy in relapsed lymphoma is ongoing, as is a trial with decitabine and venetoclax in AML (ClinicalTrials.gov: NCT04049825).

ASP4132, is a selective inhibitor of complex I that has now entered clinical trials. However, in a first in human study, no objective responses were observed and at higher doses, multiple dose limiting toxicities occurred included lactic acidosis, enteritis and posterior reversible encephalopathy syndrome [75].

Mubritinib, a selective TKI that targets HER-2 was shown in a screen of primary AML samples to have significant anti-leukaemic activity and, in the context of AML, function through inhibition of Complex I [76].

Arctigenin or GBS-01, is an extract from the fruit of Arctium lappa L, which has been shown to inhibit complex I. A phase 1 study of GBS-01 showed some evidence clinical activity in a cohort of patients with gemcitabine refractory pancreatic cancer [77,78]. Lastly, attempts are being made to modify antibiotics that target mitochondrial protein translation with a view to more specific accumulation within cancer mitochondria [79].

4. Therapeutic opportunities for drugs that target OXPHOS

4.1. Targeting tumour hypoxia

Tumour hypoxia occurs due to an imbalance between oxygen demand and supply, facilitated by inadequate and chaotic tumour vasculature [80]. Hypoxic tumours undergo metabolic and transcriptomic reprogramming promoting a more invasive, metastatic and angiogenic phenotype. Hypoxia is associated with poorer clinical outcome and resistance to treatment [81]. In particular, hypoxia is associated with loss of radiosensitivity and consequently has become a therapeutic target of interest in its own right [82]. The Danish Head and Neck Cancer Study (DAHANCA) Protocol 5–85 demonstrated that intervention with the oxygen-mimicking radiosensitizer nimorazole, which on its own has no activity against tumour cells, improved locoregional control compared to radiotherapy alone in patients with supraglottic larynx or pharynx tumours [83]. This study gave encouragement to the concept of using hypoxia modifiers in combination with radiotherapy and a number of clinical studies are now assessing the potential of drugs targeting OXPHOS and oxygen consumption. Atovaquone is a hydroxy-1, 4-naphthoquinone analogue of ubiquinone, also known as Co-enzyme Q10 (CoQ10). Atovaquone is indicated for acute treatment of mild to moderate Pneumocystis jirovecii and also used in combination with proguanil for malaria prophylaxis. Mechanistically, it is thought to act as a potent and selective OXPHOS inhibitor, by targeting the CoQ10-dependence of mitochondrial complex III [84]. *in vitro* studies have demonstrated that atovaquone at clinically relevant concentrations significantly reduces oxygen consumption in a number of tumour cell lines by more than 80 % at pharmacological concentrations. By reducing tumour hypoxia atovaquone acts as a radiosensitiser, whilst the induction of oxidative stress has been shown to potentiate the effect of platinum in lung cancer cell lines [85,86]. The ATOM (Atovaquone as Tumour HypOxia Modifier) trial was a pharmacodynamic window study which randomised participants with NSCLC to treatment with atovaquone or no intervention just prior to surgery [87]. Functional imaging using FMISO PET-CT provided proof of principle that atovaquone could reduce tumour hypoxia at clinical doses. The ongoing ARCADIAN trial is combining atovaquone with concurrent chemoradiotherapy in patients with locally advanced NSCLC (ClinicalTrials.gov: NCT04648033). Similarly, metformin has been shown to reduce oxygen consumption and tumour hypoxia [88] and metformin combined with radiation has been shown to enhance the radio-sensitising effect of cisplatin in NSCLC models [89]. Preclinical studies have also suggested that metformin may sensitise tumour cells to ionising radiation through AMPK activation [90–92].

Hypoxia driven immune escape of tumours is now well described [93]. Low oxygen levels may impair the function of immune cells of the innate and adaptive immune systems and also increase tumour cell resistance to immune effectors. Hence, the combination of anti-mitochondrial therapies with checkpoint immunotherapy is attractive and a preclinical combination with metformin has demonstrated improved intratumoural T-cell function and tumour cell clearance [94]. A clinical trial combining metformin and pembrolizumab in head and neck cancer is currently recruiting patients (ClinicalTrials.gov: NCT04414540).

4.2. Targeting OXPHOS for cancer prevention

As described above several epidemiological studies have shown the potential of metformin as a cancer preventative agent in patients with type 2 diabetes and the effect of metformin in several models of carcinogenesis has been investigated. A mouse model of lung carcinogenesis using the agent 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) showed that oral metformin reduced tumour burden but that intraperitoneal administration (with associated higher plasma levels of metformin) was even more effective [95]. Anisimov et al. demonstrated that metformin treatment of transgenic HER2/neu mice led to a significant reduction in the incidence, time to development and size of mammary adenocarcinomas [96]. For mice heterozygous for the tumour suppressor, PTEN, metformin delayed tumour onset [97]. A study of a cohort of mice treated with metformin from young or ‘middle age’ demonstrated both increased lifespan and reduced tumour incidence (by 22 and 25 %, respectively) [98]. A small clinical study from Japan reported that metformin had a chemo-preventative effect on the formation of aberrant crypt foci (considered a marker of colorectal cancer risk) and reduced colonic epithelial proliferation [99].

LFS is a hereditary cancer predisposition syndrome, secondary to germline TP53 mutation, that is associated with a very high lifetime risk of cancer [100]. It is now well established that TP53 plays a role in regulating mitochondrial metabolism and glycolysis [101,102] and a later study showed that the retention of the metabolic activities of TP53, but not its effects on the cell cycle and apoptosis, was sufficient to suppress tumorigenesis in mice [103]. Later, it was established, that lymphocytes and myoblasts taken from LFS patients have higher levels of oxygen consumption consistent with upregulated oxidative metabolism [104]. Further work identified that metformin could reduce levels of oxidative mitochondrial activity in LFS patients and that genetic or pharmacological (metformin) inhibition of mitochondrial respiration improved cancer free survival of a mouse model of LFS [105]. Randomised trials are now in development to address whether

metformin can reduce cancer incidence in patients with LFS, for example the UK Metformin in Li Fraumeni or MILI trial.

4.3. Drug combinations

Targeting the glycolytic pathway in conjunction with ETC inhibition has been postulated as a therapeutic strategy. Dichloroacetate (DCA) is a small molecule that targets pyruvate dehydrogenase kinase (see Fig. 2), the gatekeeper for pyruvate's entry into the TCA cycle and DCA has shown anti-cancer activity in preclinical models [106] although clinical studies have been limited. A number of studies in tumour models have now shown the potential to combine metformin with DCA in preclinical models [107,108]. However, the combination of drugs targeting both mitochondrial function and glycolysis may prove too toxic in the clinic.

Studies of human melanoma cell lines and patient samples have demonstrated an increased OXPHOS phenotype in $BRAF^{V600E}$ mutant melanoma following treatment with BRAF inhibitors [109]. Phenformin has been found to have synergy with BRAF inhibitors in $BRAF^{V600E}$ mutant melanoma models [110]. Similarly, treatment with the complex 1 inhibitor IACS-010,759 inhibited OXPHOS and *in vivo* growth of multiple MAPK inhibitor-resistant BRAF-mutant melanoma models with high OXPHOS levels [111]. *in vivo* tumour regression with single-agent treatment correlated with inhibition of both MAPK and mTOR complex I activity [65]. Genetic profiling of lymphoid cell lines and patient samples that had developed resistance to BCL-2 inhibition with ibrutinib demonstrated metabolic reprogramming with upregulated OXPHOS and susceptibility to treatment with the complex 1 inhibitor, IACS-010,759 [112,113].

Cells in culture with a disrupted ETC rely on exogenous glutamine to maintain cell proliferation and this represents a metabolic vulnerability in cancer cells [8]. The enzyme glutaminase is a key regulator of the glutaminolysis pathway. CB-839 is a first in class small molecule glutaminase 1 inhibitor (see Fig. 2) that is currently in clinical trials and preclinical data has demonstrated potential in the combination with metformin in a 'glutamine addicted' squamous oesophageal cancer model [114].

As well as modulation of the hypoxic tumour microenvironment as described above, direct effects on immune cells have the potential to augment the effects of immunotherapy. In patients with melanoma receiving checkpoint immunotherapy, a high OXPHOS CD8 T cell subset measured using single-cell transcriptomics of blood and tumour, was associated with resistance to immunotherapy [115]. Conversely, another study suggested that promotion of OXPHOS by interleukin-10-Fc enhanced the response of exhausted CD8 + T cells and hence response to immunotherapy [116].

5. Biomarkers for drugs that target OXPHOS

There are several putative genetic markers for OXPHOS susceptibility. The oncogenic transcription factor, Myc regulates a number of bioenergetic processes including mitochondrial gene expression programmes [117]. Overexpression of Myc has been considered as a cancer biomarker for metformin, as it has been shown that metformin suppresses Myc expression in preclinical models in an AMPK dependent manner [118]. Complex crosstalk exists between AMPK signalling and TP53 and under conditions of energy stress AMPK activates TP53 to promote cell survival. A number of preclinical studies have suggested that TP53 mutant cells may be more susceptible to biguanide therapy and that this metabolic vulnerability may define sensitivity to metformin in an AMPK dependent manner [119,120]. Other markers of AMPK activation have also been postulated as potential biomarkers. LKB1 is a kinase upstream of AMPK and an inactivating mutation is common in NSCLC. LKB1-deficient cells are unable to appropriately sense metabolic stress and preclinical data suggest this may be a marker for biguanide sensitivity [3]. Recent work has shown that mutations in the SMARCA4 gene which encodes for a component of the chromatic remodelling SWI/SNF complex associates with an increased OXPHOS. *in vitro* and *in vivo* lung cancer models with SMARCA4 mutations were more susceptible to the complex 1 inhibitor IACS-010,759 [9].

Drug uptake may define response to treatment and cell line studies and clinical studies of hepatic expression in diabetes have suggested that expression of the organic cation transporter may define response to metformin [121]. The expression of another drug transporter, MATE2 has also been implicated in cancer cell response to metformin [122]. Dynamic change in transcriptomic gene signatures may define resistance to OXPHOS targeted drugs. In breast cancer, an increase in OXPHOS gene transcription was found to link to lack of change in a proliferation signature suggesting that these patients may be resistant to metformin [27].

OXPHOS activity has been previously shown to be associated with the level of mitochondrial DNA (mtDNA) mutations present within the tumour [123]. Somatic mtDNA mutations are believed to be present in up to 60 % of cancers and hence there has been interest in using mtDNA mutations as possible biomarkers [124]. An *in vitro* study showed that specific mtDNA mutations in complex I subunits can define sensitivity to biguanides [35]. However, the heteroplasmy and heterogeneity of the mtDNA genetic landscape may preclude the use of specific mutations in mitochondrial DNA as biomarkers.

MicroRNAs (miRNAs) are a class of non-coding RNAs intimately involved in gene regulation by binding messenger RNAs. They participate in various cellular processes including proliferation, signal transduction, metabolism, apoptosis, and immune responses [125]. A number of miRNAs have been shown to be associated with a lower oxygen consumption rate, changes in lipid and metabolite profiles, increased ROS production and mitochondrial dysfunction [126,127]. For example, one study revealed that reduction of macrophage-derived exosomal miR-503−3p repressed glycolysis and promoted mitochondrial OXPHOS in breast cancer by elevating Dapper homolog 2 (DACT2), one of the Dact gene family members, which are important modulators of the Wnt signalling pathway [128]. Circular RNAs (circRNAs), a novel type of non-coding RNA, have attracted increasing attention from cancer researchers as potential biomarkers due to their stability and resistance to degradation [129]. circRNAs are differentially regulated in human cancers including breast, prostate, brain, bladder, colorectal, ovarian, liver, and kidney suggesting a significant role in oncogenesis and have also been shown to be associated with resistance to anti-cancer treatments [130]. In breast cancer cells, silencing of circNFATC3 alters cellular bioenergetics by downregulating the key genes involved in OXPHOS and mitochondrial dysfunction, suggesting that circNFATC3 is a functionally relevant circRNA in breast cancer [131]. Extracellular vesicles (EVs) or exosomes have been recognized as crucial signalling mediators in regulating the tumour microenvironment and can encapsulate genetic cargo including miRNAs [132]. Growing evidence suggests that exosomes can mediate metabolic reprogramming between cancer cells and cancer-associated fibroblasts (CAFs). For example, EVs play a specific role in promoting the metabolic switch from OXPHOS to glycolysis in CAFs [133,134].

6. Conclusion

It has now been clearly shown that mitochondrial metabolism plays a key role in maintaining tumour growth and survival. In particular, the OXPHOS metabolic pathway provides the bioenergetic requirements and directs macromolecular synthesis to promote cancer cell proliferation. Several drugs are now being repurposed or developed de novo that target OXPHOS, and preliminary data has suggested clinical activity for some of these approaches. There are a number of clinical scenarios in which targeting OXPHOS and the ETC may have a role, including cancer prevention, as monotherapy or in combination for the treatment of tumours, and as a tumour hypoxia modifier to sensitise tumours to radiotherapy and immunotherapy. There is a need to develop biomarkers such as metabolic gene signatures as a measure of OXPHOS

dependence to identify target patient populations and in order to better select patients for future clinical trials. The next decade is likely to reveal whether targeting OXPHOS and the ETC has wider utility for cancer treatment.

Data availability

Data will be made available on request.
Data will be made available on request.

Funding source

None

Declaration of Competing Interest

The authors declare that there are no conflicts of interest

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