Review

Clinical Cancer Research

Oxidative Phosphorylation as an Emerging Target in Cancer Therapy

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Abstract

Cancer cells have upregulated glycolysis compared with normal cells, which has led many to the assumption that oxidative phosphorylation (OXPHOS) is downregulated in all cancers. However, recent studies have shown that OXPHOS can be also upregulated in certain cancers, including leukemias, lymphomas, pancreatic ductal adenocarcinoma, high OXPHOS subtype melanoma, and endometrial carcinoma, and that this can occur even in the face of active glycolysis. OXPHOS inhibitors could therefore be used to target cancer subtypes in which OXPHOS is upregulated and to alleviate therapeutically adverse tumor hypoxia. Several drugs including metformin, atovaquone, and arsenic trioxide are used clinically for non-oncologic indications, but emerging data demonstrate their potential use as OXPHOS inhibitors. We highlight novel applications of OXPHOS inhibitors with a suitable therapeutic index to target cancer cell metabolism. *Clin Cancer Res; 24(11); 2482–90. ©2018 AACR.*

Introduction

In the 1920s, Otto Warburg discovered that even well-oxygenated cancer cells have high glucose consumption and high lactate production, indicating that glycolysis is upregulated. The observation that cancer cells have upregulated glycolysis compared with normal cells leads to the assumption that oxidative phosphorylation (OXPHOS) is universally downregulated in cancer. This is indeed the case for many cancers, but in some cancers, this assumption is being challenged by an increasing body of evidence to suggest that mitochondrial metabolism is not impaired, including leukemias, lymphomas, pancreatic ductal adenocarcinoma, high OXPHOS subtype melanoma, and endometrial carcinoma (1, 2).

The OXPHOS metabolic pathway generates ATP by transport of electrons to a series of transmembrane protein complexes in the mitochondrial inner membrane, known as the electron transport chain (ETC). NADH, $FADH_2$ and succinate act as electron donors. As the electrons pass through the multiprotein ETC complexes I to IV, protons are pumped from the mitochondrial matrix into the intermembrane space by complexes I, III, and IV (Fig. 1). When OXPHOS is active, there is a high proton gradient across the membrane, and protons flow from the inner intermembrane space back into the mitochondrial matrix through complex V, ATP synthase, driving the synthesis of ATP. Oxygen acts as the terminal electron acceptor.

The last 5 years have heralded novel uses for OXPHOS inhibitors either to treat cancers in which OXPHOS is upregulated or to alleviate tumor hypoxia to improve treatment outcomes. Alleviation of tumor hypoxia may be achieved in cancers in which OXPHOS is not upregulated, so this approach could be widely applicable. Several recent reviews have highlighted mitochondrial metabolism as a target for anticancer therapy, with a particular focus on metformin as an OXPHOS inhibitor (1, 3–9). This review discusses novel applications of a wide range of OXPHOS inhibitors that have a suitable therapeutic index to target cancer cell metabolism.

OXPHOS as an Anticancer Target

Reduced OXPHOS activity in cancer

There is a group of cancers in which OXPHOS is downregulated, and in those cancers, decreased OXPHOS activity may be related to mitochondrial DNA (mtDNA) mutations, or reduced mtDNA content, as mtDNA codes for 13 subunits of OXPHOS protein complexes I to V (10). OXPHOS downregulation is associated with poor clinical outcome across all cancer types and correlates with a gene signature characteristic of invasive and metastatic tumors (11). Decreases in mtDNA content have been observed in a range of cancers, including breast cancer, gastric cancer, hepatocellular carcinoma, and non–small cell lung cancer (NSCLC). However, in some cancers, mtDNA is a requirement for tumorigenesis for cancer cells to grow in an anchorage-dependent manner and to mediate resistance to cytotoxic drugs (9, 12). For example, Weinberg and colleagues demonstrated that mitochondrial metabolism and reactive oxygen species (ROS) generation are essential for Kras-mediated tumorigenicity (13).

Mitochondrial genome sequence analysis of 226 paired tumor and normal tissue samples from The Cancer Genome Atlas

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doi: 10.1158/1078-0432.CCR-17-3070

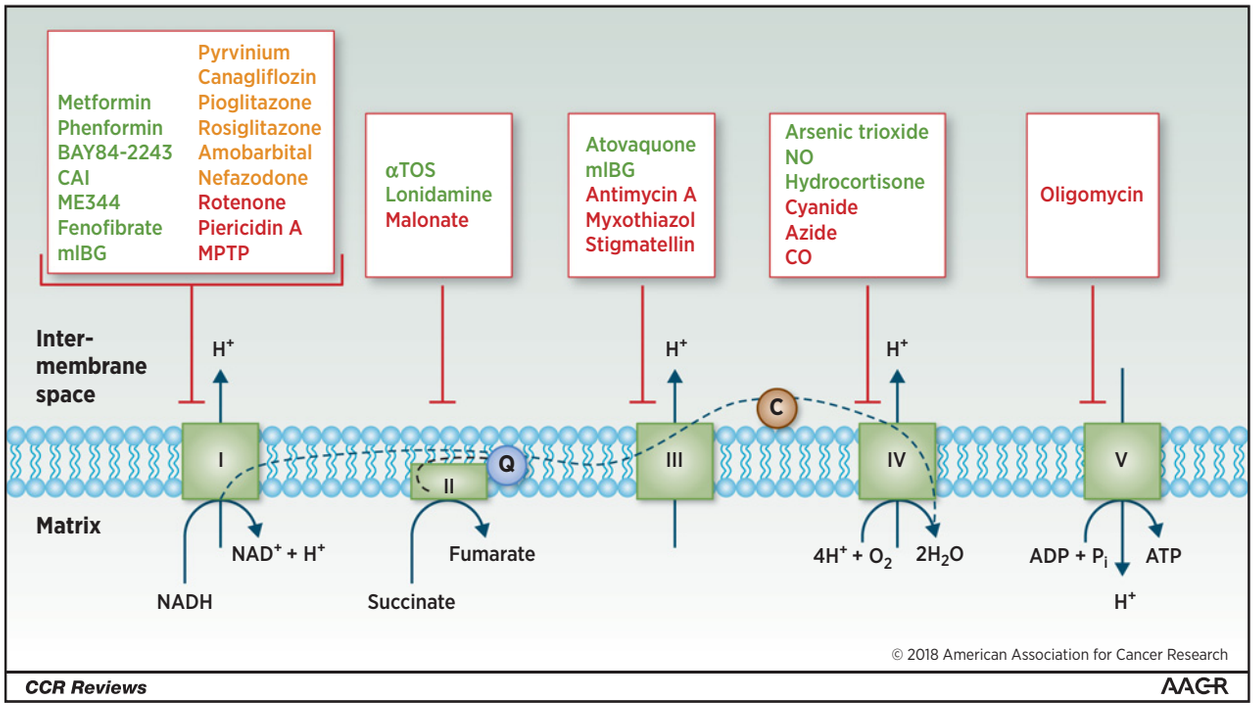

Figure 1.
Inhibitors of OXPHOS. The OXPHOS metabolic pathway generates ATP by transport of electrons to a series of transmembrane protein complexes in the mitochondrial inner membrane, known as the ETC. The dotted line indicates the flow of electrons through complex I, complex II, Coenzyme Q10 (Q), complex III, cytochrome c (C), and complex IV, with O_2 acting as the terminal electron acceptor. Compounds of therapeutic potential being studied as OXPHOS inhibitors *in vivo* or in the clinic are shown in green, those being studied *in vitro* are shown in orange, and classical mitochondrial poisons are shown in red. αTOS, α-tocopheryl succinate; CAI, carboxyamidotriazole; CO, carbon monoxide; mIBG, meta-iodobenzylguanidine; MPTP, 1-methyl 4-phenyl 1,2,3,6 tetrahydropyridine; NO, nitric oxide.

(TCGA) revealed deleterious tumor-specific somatic mtDNA mutations in 63% of rectal adenocarcinomas, 53% of colon adenocarcinomas, 36% of ovarian serous cyst adenocarcinomas, and 30% of acute myeloid leukemias (14). Mutations were identified in all mitochondrially encoded genes and are predicted to impact protein function, potentially affecting OXPHOS levels. Interestingly, however, cancer cells harboring mtDNA mutations in complex I subunits were 5- to 20-fold more sensitive to the complex I inhibitors, metformin and phenformin, compared with cell lines lacking such mutations (15). Metformin is a biguanide widely used to treat type II diabetes, and phenformin is a precursor of metformin not currently in clinical use. Phenformin also inhibited the growth of xenografts derived from two independent cell lines (Cal-62 and U-937) harboring mtDNA mutations. This study thus demonstrated that complex I inhibition causes decreased growth in cells with mtDNA mutations in complex I subunits. It is also important to note that many mtDNA mutations do not simply cause a decrease in OXPHOS but may facilitate adaptation to the bioenergetic demands of the tumor microenvironment without altering OXPHOS (6).

OXPHOS is upregulated in some cancers

An increasing body of evidence demonstrates that certain cancers are heavily reliant on OXPHOS, and many recent studies have revealed that OXPHOS inhibition is effective in targeting these cancer subtypes (Table 1). A meta-analysis of 16 normal cell types and 31 cancer cell lines indicated that the relative contribution of glycolysis and OXPHOS to ATP production is highly variable between cell types, but that the average contribution of OXPHOS to ATP production is 80% in normal cells and 83% in cancer cells (16). This is in accordance with the *in vivo* data from Vaupel's group demonstrating that the availability of O_2 in solid tumors is the key determinant of the oxygen consumption rate (OCR), suggesting that mitochondrial respiratory capacity is not always functionally impaired (17). One cause of the variability in the contribution of OXPHOS between cancer types may be mtDNA content. Many cancers have increased mtDNA content relative to normal tissue, including acute lymphoblastic leukemia (ALL), non-Hodgkin lymphoma, endometrial cancer, colorectal cancer, ovarian cancer, prostate cancer, head and neck cancer, lung adenocarcinoma, esophageal squamous cell carcinoma, and thyroid cancer (10, 18). To add further complexity, recent studies suggest that tumors may be metabolically heterogeneous, and that cancer stem cells with high metastatic and tumorigenic potential are more reliant upon OXPHOS than the bulk, and putatively nonstem, component of pancreatic tumors (9, 19). Metabolic heterogeneity has also been demonstrated in NSCLC tumors (20, 21). As analysis of mtDNA content or the expression of OXPHOS genes may not reflect the level of functional OXPHOS, it is important to pursue a multiexperimental approach to fully characterize OXPHOS activity. Therefore, examples are provided of tumor types in which high OXPHOS gene expression correlates with high OXPHOS protein levels, as determined by IHC or proteomics, and high OXPHOS activity, as determined by metabolomics, oxygen consumption, or sensitivity to well-characterized OXPHOS inhibitors.

Table 1. Potential clinical applications of OXPHOS inhibitors

Cancer	Subtype	Associated gene expression	Ref.
Acute myelogenous leukemia (AML)	AML stem cells	↑*BCL-2*	(27)
Chronic lymphocytic leukemia (CLL)	Src-sensitive CLL after Src inhibition	↓*AKT*	(74)
Classical Hodgkin lymphoma		↑*NF-κB*	(25)
Diffuse large B-cell lymphoma	High OXPHOS expression		(26)
Breast		↓*RB1*	(22, 24)
Pancreatic ductal adenocarcinoma (PDAC)	PDAC (stem-like) cells	↑Ras	(29)
Lung adenocarcinoma			(18)
NSCLC	NSCLC after EGFR inhibition	↓*EGFR*	(75)
NSCLC	Oncogenic Kras and loss of *LKB1*	↓*LKB1*, oncogenic Kras	(35)
Endometrial carcinoma	Serous-like endometrial	mtDNA copy number alteration	(18)
Melanoma	High OXPHOS expression	↑ *PPARGC1A* (PGC1α)	(34)
Melanoma	BRAF mutant after BRAF inhibition	↑ *PPARGC1A* (PGC1α), BRAF activating mutation	(32, 33)
Glioma	Low-grade glioma	IDH1 activating mutation	(18)
Head and neck, cervix, lung, brain, bowel, prostate, pancreas	Hypoxic solid tumors		(36)

Several studies indicate that OXPHOS may be upregulated in breast cancer and classical Hodgkin lymphoma. Complex I, II, and IV activity respectively assayed by NADH, succinate dehydrogenase, and cytochrome oxidase histochemical staining of breast cancer tissue reveals that ETC proteins are upregulated in breast cancer cells relative to adjacent stromal and normal epithelial cells (22). The activity of these complexes could be overcome by treatment of the tissue sections with metformin or sodium azide, an inhibitor of complex IV. Analysis of gene expression data from 2,000 patients with breast cancer revealed significant transcriptional upregulation of OXPHOS, suggesting that OXPHOS is a possible target in breast cancer (22). Transcriptomic data and Western blotting demonstrated that OXPHOS is highly upregulated in breast cancers deficient in RB1, a protein lost in 20% to 30% of basal-like breast cancers (23, 24). The mitochondrial translation inhibitor, tigecycline, strongly attenuated growth of *RB1*-deficient MDA-MB-436 breast xenografts (24). OXPHOS is also globally upregulated in classical Hodgkin lymphoma, with an increase in expression of OXPHOS genes, increase in mitochondrial mass, increase in ETC protein expression, increase in the OCR, and decrease in lactate production promoted by NF-κB (25). In many cancers, however, OXPHOS upregulation is limited to particular cancer subtypes, as exemplified below.

Diffuse large B-cell lymphomas (DLBCL) can be divided into OXPHOS-high and -low subsets (26). Mitochondrial proteomics and gene expression analysis revealed that ETC components are upregulated in the OXPHOS-high subset, particularly subunits of complexes I and IV. OXPHOS is also enhanced in acute myelogenous leukemia (AML) stem cells, dependent upon expression of the *BCL-2* oncogene (27). Inhibition of BCL-2 reduces OXPHOS and selectively eradicates quiescent chemotherapy-resistant AML stem cells. Expression of genes other than *BCL-2* also alters the reliance on OXPHOS, as AML cells with low basal phosphorylation of AKT or low basal glycolysis have increased OXPHOS and greater sensitivity to the complex I inhibitor metformin, reducing leukemia growth *in vivo* (28).

Transcriptomic and metabolic analyses of Ras-driven pancreatic ductal adenocarcinoma (PDAC) stem-like cells reveal a strong reliance on OXPHOS and decreased glycolysis (19). These cells are highly resistant to conventional chemotherapies and are able to repopulate heterogeneous cancer cell populations (29). Treatment with metformin or the complex V inhibitor oligomycin retards growth of these cells *in vitro* and causes growth delay of PDAC-215 and PDAC-A6L xenografts (29). Furthermore, immortalization and transformation of bronchial epithelial cells with the H-Ras^{V12} oncogenic Ras allele cause an increase in the OCR, and expression of H-Ras^{V12} increases sensitivity to the complex I inhibitor rotenone (30). Therapy-resistant chronic myeloid leukemia stem cells also have upregulated OXPHOS, as determined by metabolomics and functional assays (31).

It is important to note that tumors can display metabolic flexibility (5, 7), so a high reliance on OXPHOS does not necessarily confer dependence. Tumors with a high reliance on OXPHOS that are able to switch to glycolysis for ATP production may still be susceptible to OXPHOS inhibition, but this remains to be determined experimentally.

Molecularly targeted therapy can cause OXPHOS upregulation

Several cases have been described in which cancer cells become more dependent upon OXPHOS following treatment with targeted therapies, including inhibition of the protein kinase BRAF in melanomas with an activating mutation in the *BRAF* gene. Roughly 50% of melanomas carry activating BRAF mutations, such as BRAF V600E, and are therefore initially susceptible to BRAF inhibitors. BRAF inhibitors induce PGC1α, a regulator of mitochondrial biogenesis, which in turn causes OXPHOS dependence (32). Consequently, BRAF inhibitors synergize with the complex I inhibitor phenformin to reduce the viability of BRAF V600E–mutant melanoma cells and to induce tumor regression in a BRAF^{V600E}/PTEN^{null}-driven mouse melanoma model (33). In addition, a subset of melanomas have high PGC1α expression and high levels of OXPHOS that do not appear to correlate with BRAF or p53 mutational status (34). OXPHOS inhibitors may thus be useful as stand-alone agents for the treatment of melanomas with high PGC1α expression, and in combination with BRAF inhibitors for targeting BRAF-mutant melanomas.

OXPHOS upregulation can be driven by gene mutation

The characterization of cancer cells with an OXPHOS phenotype and gene mutations driving OXPHOS upregulation is ongoing. For example, NSCLC tumors with oncogenic Kras and loss of the LKB1 tumor suppressor are selectively sensitive to the complex I inhibitor phenformin (35). Phenformin and rotenone caused complete inhibition of oxygen consumption in these cells, demonstrating OXPHOS functionality. About 20% of all NSCLCs have mutated LKB1. LKB1 is the primary kinase responsible for activation of AMPK, which is required to enhance glycolysis to compensate for the reduction in ATP under reduced OXPHOS. NSCLC tumors that are unable to sufficiently upregulate glycolysis are thus particularly sensitive

to OXPHOS inhibition. AMPK-independent activation of stress signaling pathways is also considered to contribute to the sensitivity of these cells to phenformin.

OCR Inhibition to Alleviate Tumor Hypoxia

Hypoxia is associated with poor clinical outcomes

It has been known since the work of Gray and his colleagues in the 1950s that solid tumors frequently have regions of low oxygen known as hypoxia, which results from an imbalance between oxygen demand and poor oxygen supply due to abnormal vasculature (36, 37). As Gray predicted, and has been frequently subsequently demonstrated, tumor hypoxia results in worse clinical outcomes because hypoxic cells are resistant to cancer therapy, leading to local recurrence and an increased propensity toward metastasis (38). Tumor hypoxia is known to be associated with poor clinical outcomes in many cancers, including head and neck, cervix, lung, brain, bowel, prostate, and pancreas (36). Hypoxic tumor cells are also up to three times more resistant to radiotherapy than normoxic tumor cells due to the absence of the oxygen enhancement effect (37). This effect is a result of the ROS generated by the radiolysis of water that attack DNA, forming readily reversible DNA radicals. These radicals are converted into DNA peroxides in the presence of oxygen, which must be physically present within microseconds of the damage, forming more stable intermediates that are more difficult to repair (39). Even very low levels of oxygen, around 2%, are sufficient to yield oxygen enhancement.

Strategies for the modification of tumor hypoxia

Previous attempts to overcome tumor hypoxia have included the use of nitroimidazoles such as misonidazole and nimorazole, inhalation of hyperbaric oxygen, and the use of carbogen (95% O_2, 5% CO_2) in combination with the vasodilator nicotinamide (ARCON). The reasons why these attempts at increasing oxygen "supply" have had limited clinical success are multifactorial. However, the use of drugs that were poorly tolerated, practical challenges associated with delivering some of these treatments, and the absence of predictive biomarkers all contributed to the failure of these treatments to enter widespread clinical use. An additional drawback to approaches that require a drug to be delivered to hypoxic tumor regions is that these regions are usually poorly vascularized, so high doses may be required to achieve the local drug concentrations required to elicit an effect. A more novel approach is to reduce the OCR, increasing the retention of oxygen throughout the tumor and subsequently decreasing tumor hypoxia. This could be achieved with OXPHOS inhibition (Fig. 2), as shall be further highlighted (39–42).

Reduction of oxygen consumption alleviates hypoxia

The low oxygen concentrations in hypoxic regions of tumors may not be limiting for OXPHOS (2), and ATP is generated by OXPHOS in tumors even at very low oxygen tensions (13, 43). Therefore, OXPHOS inhibition could be an effective way to reduce the consumption of oxygen (the terminal electron acceptor in the ETC) and to consequently increase oxygen availability in the tissue. As a result, oxygen could diffuse into initially hypoxic tumor regions, reducing or eradicating tumor hypoxia. Furthermore, this could be a potential strategy for all hypoxic tumors, not simply those in which OXPHOS is upregulated. Studies in 3D multicellular spheroids indicate that reducing the OCR can alleviate the central region of hypoxia by increasing the availability of free oxygen (44–46). Mathematical modeling suggests that complete inhibition of oxygen consumption is not required for alleviation of tumor hypoxia, and that even a 30% decrease in consumption would abolish severe hypoxia (44, 45).

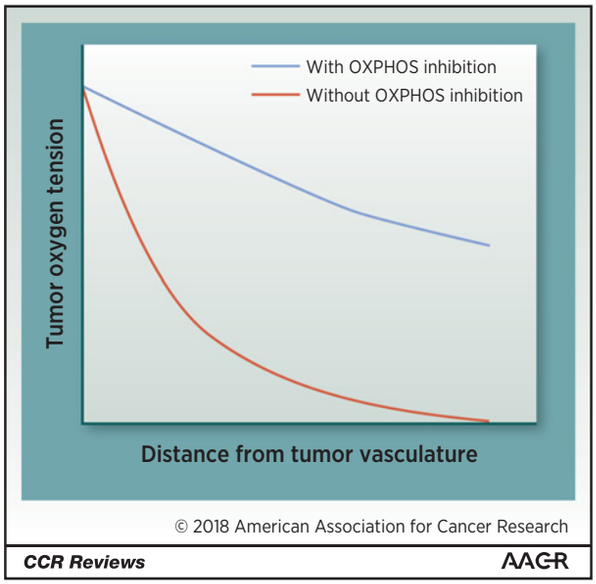

Figure 2.
The hypothetical effect of OXPHOS inhibition on tumor oxygen tension. In the absence of OXPHOS inhibition, tumor oxygen tension decreases steadily with increasing distance from tumor vasculature (44). Both tumor areas with limited oxygen diffusion due to the pathologic tumor vasculature, and microregions distant from perfused vessels, are therefore chronically hypoxic. Under OXPHOS inhibition, we hypothesize that OXPHOS activity is greatly reduced throughout the tumor, and that the decreased cellular oxygen consumption lowers the slope of the oxygen gradient from the vessels into the tumor tissue.

There are several possible benefits of modifying hypoxia by reducing the OCR compared with other methods of reducing hypoxia. First, targeting OXPHOS appears to reduce the OCR in a wide range of cancer types, suggesting broad applicability for this approach (40, 42, 47). Second, diffusion of the inhibitor to poorly vascularized hypoxic regions may not be required, as OXPHOS inhibitors acting primarily on the normoxic regions to reduce the OCR may indirectly lead to higher oxygen levels in regions that are chronically hypoxic prior to treatment by allowing molecular oxygen, which very readily diffuses, to reach formerly hypoxic regions. In contrast, nitroimidazoles must reach all hypoxic regions.

OXPHOS Inhibitors with Therapeutic Potential

OXPHOS inhibitors with a suitable therapeutic index

Therapeutically viable OXPHOS inhibitors must be efficacious *in vitro* and *in vivo* at concentrations that are achievable in the tumors of patients. Drug plasma concentrations determined in previous pharmacokinetic studies for FDA-approved drugs may be used as a surrogate, although the concentration in the tumors may be lower. Furthermore, the metabolism of the inhibitors and the effects of any secondary metabolites have to be considered. A

partial list of OXPHOS inhibitors that meet these criteria is shown in Table 2 and Fig. 1, and these inhibitors are discussed below.

Epidemiologic and retrospective studies have revealed a lower incidence of cancer and better outcomes in patients with diabetes taking the antidiabetic metformin compared with patients with or without diabetes taking alternative medications (3, 48). *In vitro* studies have demonstrated that metformin reduces the OCR in many cancer cell lines, a response that is not correlated with its antiproliferative effect (40, 42, 47). Many subsequent *in vivo* studies have revealed that metformin inhibits tumor growth in a variety of different models (28, 29, 48, 49). It also reduces hypoxia in spheroids and xenografted tumors, with a corresponding improvement in radiation sensitivity (40, 42). As a consequence of these findings, metformin is already in several hundred ongoing clinical trials to assess its efficacy as an anticancer therapeutic. A key mechanism of action of metformin in cancer cells *in vitro* is complex I inhibition (48, 49). This results in a decrease in ATP production and thus activation of AMPK and inhibition of mTORC1. The growth inhibition of HCT116 xenograft tumors by metformin is complex I dependent, suggesting that complex I inhibition is the mechanism underlying the growth inhibitory effect at least in this model (49). The antitumorigenic properties of metformin may also be partly due to systemically lowered insulin levels, resulting in reduced activation of insulin-like receptor tyrosine kinases such as IGF1 in cancer cells (48). However, there is concern that the concentrations of metformin reached in tumors are not sufficient to inhibit complex I. This has led some groups to develop particular mitochondria-targeting metformin analogues with enhanced efficacy in a physiologic environment (50) and to study other biguanides with higher potency, such as phenformin. Although phenformin was withdrawn from clinical use in the 1970s due to a high risk of fatal lactic acidosis, it may have application as an anticancer therapeutic at lower doses. Indeed, recent work has shown that phenformin causes growth delay of xenograft tumors, and that this is also likely mediated by complex I inhibition (15, 33, 35, 49, 51).

Atovaquone is FDA approved to treat pneumocystis pneumonia and malaria, caused by the parasites *Pneumocystis jirovecii* and *Plasmodium falciparum*, respectively (52). It has an excellent safety profile and has been used in the clinic for over 30 years with approximately 3.7 million prescriptions being issued in the United States every year. It is a ubiquinone analogue that acts as a complex III inhibitor in parasites, cancer cell lines, and breast cancer stem cells, causing a reduction in the OCR and alleviating tumor hypoxia at pharmacologically achievable concentrations (40, 53–56). Correspondingly, there is an improvement in radiation response in spheroids and in xenografted tumors following atovaquone treatment (40). Atovaquone also has antitumor activity in U266 multiple myeloma xenografts, although this could be due to inhibition of STAT3 rather than complex III (57).

Arsenic trioxide is a complex IV inhibitor that is FDA approved for the treatment of acute promyelocytic leukemia (APL) and is being investigated in other cancer types. It reduces hypoxia in Lewis lung carcinoma (LLC) and transplantable mouse liver (TLT) tumors, leading to an improvement in radiation response (41). Nitric oxide (NO) is a vasodilator but also inhibits complex IV (58). NO is released from compounds such as isosorbide dinitrate, xanthinol nicotinate, and *S*-nitrosocaptopril, and endogenous NO can be stimulated by administration of insulin (39). NO delivered by these methods causes a decrease in tumor hypoxia and corresponding enhancement of radiation response, an effect that may be mediated both by improved blood flow and OXPHOS inhibition (39). Hydrocortisone is another compound that inhibits complex IV in isolated mitochondria and is able to alleviate hypoxia in TLT and FSaII fibrosarcoma tumors, ameliorating radiation response (59, 60).

There are comparatively few well-characterized complex II inhibitors, but lonidamine and the vitamin E analogue α-tocopheryl succinate (α-TOS) may have suitable therapeutic indices. Lonidamine is classically described as an inhibitor of glycolytic hexokinases but has recently been shown to inhibit complex II in isolated mitochondria and in DB-1 melanoma cells (5, 61). Despite early promise, it was not beneficial in two randomized, phase III trials in combination with chemotherapy, so is no longer being developed clinically (5). α-TOS has not yet been studied clinically but causes growth reduction in H-Ras–transformed Chinese Hamster fibroblast tumors via complex II inhibition, an effect reversed in tumors with dysfunctional complex II and rescued by reconstitution of complex II activity (62).

Aside from the biguanides, several other compounds targeting complex I may have a suitable therapeutic index. Carboxyamidotriazole (CAI) is a putative complex I inhibitor that was initially characterized as an agonist of non–voltage-gated calcium channels and inhibits angiogenesis, tumor growth, and metastatic potential (63, 64). CAI inhibits growth of a wide range of cell lines *in vitro* and *in vivo* and has an additive effect in LLC xenografts in combination with the glycolytic inhibitor 2-deoxyglucose (63). Despite these promising preclinical studies, CAI failed to demonstrate clinical benefits in NSCLC, glioblastoma, or metastatic renal cell carcinoma (64). CAI might be more successful if used to treat cancers with upregulated OXPHOS. ME344 is a complex I inhibitor that synergizes with TKIs to induce tumor control in a spontaneous breast cancer model and is currently being combined with bevacizumab in a clinical trial in patients with early HER2-negative breast cancer (65, 66). Fenofibrate is a peroxisome proliferator-activated receptor α (PPARα) agonist approved to treat hyperlipidemia but also inhibits complex I in isolated mitochondria and in glioblastoma cell lines, causing a significant growth decrease in an orthotopic U87 intracranial glioblastoma model (67, 68). However, fenofibrate is hydrolyzed in the blood to fenofibric acid, which does not inhibit complex I, so the effect was only observed following direct intracranial delivery of fenofibrate. Meta-iodobenzylguanidine (mIBG) is a tumor-targeted radiopharmaceutical that inhibits both complexes I and III, reducing hypoxia in melanoma xenografts (69, 70).

In summary, the studies of biguanides and other OXPHOS modulators demonstrate that complex I is a particularly attractive target. Caution is required, however, as the novel BAY87-2243 complex I inhibitor alleviated hypoxia and improved radiation response without toxicity in mice, but the initial phase I trial had to be terminated due to unexpected toxicity (71, 72). Therefore, the pharmacokinetic properties and potency of OXPHOS inhibitors must be carefully tailored.

OXPHOS inhibitors studied *in vitro* with potential as therapeutics

At first glance, it would not appear fruitful to study an OXPHOS inhibitor if the plasma concentration achievable in patients is reported to be lower than the concentration required to cause a significant decrease in the OCR of cancer cells. However, *in vivo* studies with inhibitors that show promise *in vitro* may be

Table 2. A nonexhaustive list of OXPHOS inhibitors under study *in vivo* or in the clinic as anticancer therapeutics

Compound	Clinical use	Complex	*In vivo* results	Selected oncology clinical trials	Ref.
Metformin	Diabetes	I	Inhibits tumor growth in many tumor types, alleviates hypoxia, and improves IR response	Several hundred trials in progress	(3, 42, 49)
Phenformin	Diabetes	I	Tumor growth delay, NSCLC with oncogenic Kras and *LKB1* loss, and in MCF7/MDA-MB-231	Preclinical	(35, 49, 51)
BAY87-2243	Experimental	I	Alleviates hypoxia and improves IR response, UT-SCC5 tumors	Phase I, solid tumors, terminated NCT01297530, dose escalation	(71, 72)
CAI	Experimental	I	Growth delay, LLC tumors	Phase III, NSCLC, completed NCT00003869	(63, 64)
ME344	Experimental	I	Growth delay, PyMT spontaneous breast	Phase 0, HER2-negative breast, recruiting NCT02806817	(65, 66)
Fenofibrate	Hyperlipidemia	I	Growth delay, U87-MG tumors after intercranial delivery	Phase II, myeloma, not recruiting NCT01965834, dose response	(67, 68)
Lonidamine	Experimental	II	Growth delay in many tumor types	Phase III, breast, completed	(61, 76)
α-TOS	Vitamin E analogue	II	Inhibits complex II in Chinese Hamster fibroblast tumors	Preclinical	(62)
Atovaquone	Malaria	III	Alleviates hypoxia and improves IR response, FaDu tumors	Phase 0, NSCLC, recruiting NCT02628080, 18F-MISO-PET	(40, 56, 57)
Arsenic trioxide	APL	IV	Improves IR response, TLT tumors	Clinical use for APL	(41, 58)
Hydrocortisone	Eczema	IV	Alleviates hypoxia in FSaII tumors, improves IR response	Preclinical	(59, 60)
NO	Experimental	IV	Alleviates hypoxia in many tumors, improves IR response	Phase II, NSCLC, not yet recruiting NCT01210378	(39)
mIBG	Radioactive tracer	I and III	Alleviates hypoxia in melanoma, improves IR response under hyperglycemia, R3230 Ac tumors	Preclinical	(69, 70)
VLX600	Experimental	I, II, and IV	Growth delay, HT29 tumors	Phase I, solid tumors, recruiting NCT02222363, dose escalation	(77)

Abbreviations: APL, acute promyelocytic leukemia; αTOS, α-tocopheryl succinate; CAI, carboxyamidotriazole; IR, ionizing radiation; mIBG, meta-iodobenzylguanidine; NO, nitric oxide; TLT, transplantable mouse liver.

Table 3. OXPHOS inhibitors studied*in vitro* with potential as anticancer therapeutics

Compound	Primary clinical use	Target complex	*In vitro* results	Plasma concentration in patients	Ref.
Pyrvinium	Antihelminth	I	Reduces OCR and spheroid hypoxia at 1 μmol/L	Poor bioavailability but has been safely delivered i.p. in mice	(78, 79)
Canagliflozin	Antidiabetic	I	Reduces OCR and clonogenic survival at 10–30 μmol/L in cancer cell lines	5–30 μmol/L, 50–300 mg/day	(80)
Pioglitazone	Antidiabetic	I	Complex I inhibition in liver of pioglitazone-treated mice	4.5 μmol/L, 15–30 mg/day	(81, 82)
Rosiglitazone	Antidiabetic	I	Inhibits at 100 μmol/L in isolated mitochondria	1.04 μmol/L, 4–8 mg/day	(83)
Amobarbital	Sedative	I	Suppresses drug-resistant cancer spheroid subpopulation at 1 mmol/L	17.7 μmol/L, 200 mg/day	(84, 85)
Nefazodone	Antidepressant	I	4 μmol/L IC_{50} in isolated mitochondria	0.92 μmol/L, 100 mg twice/day	(83)
Simvastin	Antilipidemic	II	30 μmol/L IC_{50} in isolated mitochondria	0.02 μmol/L, 5–40 mg/day	(83)
Paroxetine	Antidepressant	V	1.6 μmol/L IC_{50} in isolated mitochondria	0.06 μmol/L, 10–60 mg/day	(83)
Chlorpromazine	Antipsychotic	V	26 μmol/L IC_{50} in isolated mitochondria	0.9 μmol/L, 25–75 mg/day	(83)
Tamoxifen	Anticancer	III–V	8.8–26.6 μmol/L IC_{50} in isolated mitochondria	0.16 μmol/L, 20–40 mg/day	(83)

warranted, as it is possible that higher dose regimens could be effective, that the compound could accumulate in the tumor, or that even a mild reduction in the OCR by these compounds could translate to a significant antitumor effect or elevated free oxygen levels. For example, metformin reaches concentrations of up to 184 μmol/L in mouse xenograft tumors, which is sufficient to activate AMPK (73). However, a more than 300-fold excess of metformin is required to achieve a comparable effect *in vitro*, suggesting that the complex metabolic flux of the tumor microenvironment is poorly modeled *in vitro* (73). It may also be of interest to attempt novel routes of administration or chemical modification of compounds with poor bioavailability in order to improve their bioavailability. Selected examples of OXPHOS inhibitors studied *in vitro* are shown in Table 3, but future *in vivo* experiments are required to determine the suitability of these compounds as anticancer therapeutics.

Conclusions

Many recent studies have demonstrated that OXPHOS is upregulated in a variety of cancers, potentially rendering them sensitive to OXPHOS inhibition. Furthermore, OXPHOS inhibition has been shown to reduce the OCR, alleviating tumor hypoxia, and even to be effective in some cancers with mtDNA mutations. Repurposing of FDA-approved drugs has revealed that many well-tolerated, widely prescribed drugs such as metformin, arsenic trioxide, and atovaquone act as OXPHOS inhibitors, and have potential as anticancer therapeutics. High-throughput screening approaches could be used to reveal similar compounds with therapeutic potential.

Overall, there is increasing interest in the use of OXPHOS inhibitors against malignant cells, but careful evaluation of potency, pharmacokinetics, and dose regimes will be required, as classical mitochondrial poisons and potent novel inhibitors such as BAY87-2243 can cause unacceptable side effects. Indeed, some inhibitors may be best suited to treat cancers in which OXPHOS is upregulated but may need to be avoided by some patient groups, such as those with preexisting mitochondrial disorders. Ultimately, clinical trials with clear patient stratification will be required to determine whether OXPHOS inhibitors have a suitable therapeutic index. Although the example of thalidomide proves that drug repurposing can be successful, funding expensive, late-phase clinical trials for such drugs may be challenging, and pharmaceutical-driven development of novel inhibitors may be required to overcome this issue. There is also potential for synergy of OXPHOS inhibitors with conventional chemotherapeutics, targeted therapies such as Src, EGFR, and BRAF inhibitors, vascular modifiers, inhibitors of other metabolic pathways such as glycolysis, and radiation in hypoxic tumors.

Therefore, cancers intrinsically sensitive to OXPHOS inhibition should continue to be characterized, environmental and epigenetic drivers of cancer cell susceptibility to OXPHOS inhibitors must be fully recognized, and combinations with other therapies explored.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The funding sources were Cancer Research UK (C34326/A13092; to G.S. Higgins and C5255/A23755; to W.G. McKenna), Medical Research Council (MC_PC_12004; to W.G. McKenna), and National Institute for Health Research Biomedical Research Centre, Oxford, UK. G.S. Higgins is supported by a Cancer Research UK Clinician Scientist Award (grant number C34326/A13092). The authors thank James Coates for helpful discussions.

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Received October 17, 2017; revised January 7, 2018; accepted January 30, 2018; published first February 2, 2018.

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