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Targeting STAT3 and oxidative phosphorylation in oncogene-addicted tumors

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ABSTRACT

Drug resistance invariably limits the response of oncogene-addicted cancer cells to targeted therapy. The up-regulation of signal transducer and activator of transcription 3 (STAT3) has been implicated as a mechanism of drug resistance in a range of oncogene-addicted cancers. However, the development of inhibitors against STAT3 has been fraught with challenges such as poor delivery or lack of specificity. Clinical experience with small molecule STAT3 inhibitors has seen efficacy signals, but this success has been tempered by drug limiting toxicities from off-target adverse events.

It has emerged in recent years that, contrary to the Warburg theory, certain tumor types undergo metabolic reprogramming towards oxidative phosphorylation (OXPHOS) to satisfy their energy production. In particular, certain drug-resistant oncogene-addicted tumors have been found to rely on OXPHOS as a mechanism of survival. Multiple cellular signaling pathways converge on STAT3, hence the localization of STAT3 to the mitochondria may provide the link between oncogene-induced signaling pathways and cancer cell metabolism.

In this article, we review the role of STAT3 and OXPHOS as targets of novel therapeutic strategies aimed at restoring drug sensitivity in treatment-resistant oncogene-addicted tumor types. Apart from drugs which have been re-purposed as OXPHOS inhibitors for-anti-cancer therapy (e.g., metformin and phenformin), several novel compounds in the drug-development pipeline have demonstrated promising pre-clinical and clinical activity. However, the clinical development of OXPHOS inhibitors remains in its infancy. The further identification of compounds with acceptable toxicity profiles, alongside the discovery of robust companion biomarkers of OXPHOS inhibition, would represent tangible early steps in transforming the therapeutic landscape of cancer cell metabolism.

1. STAT3 signaling in cancers and STAT3 as a therapeutic target

Complex cell signaling pathways have been described to be interconnected, and integrate information which regulate processes such as protein synthesis, cell growth, differentiation, and cell death. Signal transducer and activator of transcription 3 (STAT3) is a key element in multiple oncogenic signaling pathways, and is the most strongly associated with tumorigenesis of the seven members of the STAT protein family. Distinct from the transient physiological STAT3 signaling observed in normal cells, STAT3 is persistently activated in many cancers and in many human cancer models, in-vitro STAT3 inhibition leads to growth inhibition and apoptosis [1]. STAT3 Tyrosine^{705} (Y705) phosphorylation is responsible for the activation of this canonical pathway, and results in malignant transformation by promoting cell proliferation, angiogenesis, and immune evasion [2]. Aside from this, an additional role of STAT3 as a modulator of mitochondrial respiration has been described, which is activated by non-canonical signaling through the phosphorylation of the Serine^{727} (Ser727) residue of STAT3 [2–4]. The major pathways contributing to STAT3 activation are the cytokine and growth factor rich tumor microenvironment, overexpression of tyrosine kinases, and epigenetic modulation of negative regulators of STAT3.

STAT3 has been extensively studied as a therapeutic target for several reasons: it is frequently activated in a variety of malignancies

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Table 1
Preclinical and clinical development of STAT3 inhibitors.

Site of action	Class	Preclinical data	Clinical Data	Challenges
SH2 domain dimerization inhibitors	**Peptides**	Src-transformed fibroblasts	–	Poor in-vivo stability, and cellular permeability
	XpYL [65]			
	Peptidomimetics	Breast, NSCLC, Src-transformed fibroblasts	–	
	ISS610 [66]			
	Small molecule inhibitors			
	STA-21 and analogues	Breast, sarcoma, GBM	–	Lack of potency and specificity
	LLL-3	GBM	–	
	S3I-201	Breast	–	
	STATTIC [67]	Breast, HCC, GI	–	
	OPB-31121 [68]	HCC, Leukemia [69]	Phase I	Toxicities
	OPB-51602 [9]	CRC, Liver, Lung	Phase I	
	Natural compounds	RCC, Breast, Pancreas, HCC, GI, NSCLC	–	Lack of potency and specificity
	Cucurmin and analogues [70]			
Upstream TKI	**Small molecule inhibitors**			
	AZD1480 [10]	EGFR NSCLC	Phase I	Toxicities
	Dasatinib [71]	NSCLC, HNSCC	Phase II	Lack of efficacy
Oligonucleotides	**Antisense oligonucleotide** (mRNA)	Lymphoma, NSCLC	Phase I/II	Rapid degradation
	STAT3 decoy oligonucleotide(DNA-binding) [72,73]	NSCLC, colorectal, HNSCC	Phase 0	
	STAT3 post-transcriptional(siRNA)	Breast, Brain, SCC		
STAT3 DNA-binding domain	**Platinum IV compounds** [74]		–	Lack of specificity
	IS3295	Breast		
	CPA-1	Colon		
	CPA-7	NSCLC		

NSCLC – non small cell lung cancer, GBM – glioblastoma, HCC – hepatocellular carcinoma, GI – gastrointestinal, RCC – renal cell carcinoma, HNSCC – head and neck squamous cell carcinoma, SCC – squamous cell carcinoma, TKI – tyrosine kinase inhibitor.

and the inhibition of STAT3 signaling results in selective apoptosis of STAT3 dependent tumor cells, but not normal cells [5]. Several oncogenic pathways converge on STAT3, hence its inhibition has the potential to simultaneously block several upstream pathways. Alvarez et al. made the observation that STAT3 is a critical mediator of the oncogenic effects of epidermal growth factor receptor (EGFR) mutations and postulated that targeting it might be an effective strategy in the treatment of EGFR oncogene-addicted non-small cell lung cancer (NSCLC) [2].

Several strategies have been studied to target the STAT3 signaling pathway. These include direct inhibition of the STAT3 protein, inhibiting upstream tyrosine kinases, and the DNA binding complex. However, in contrast to the “druggable” classic binding pockets found in tyrosine kinase receptors or other enzymatic targets, targeting the STAT3 protein-protein interaction with a large and diffuse surface area has been technically challenging [6]. Upstream regulators of STAT3 and platinum complexes lack specificity, while decoy oligonucleotides and antisense oligonucleotide inhibitors pose a challenge in delivery [7,8]. While direct STAT3 small molecular inhibitors appear to be the most favorable candidates, those evaluated to date have suboptimal potency, unfavorable PK properties, and even potentially life threatening toxicities [9,10]. Table 1 summarizes the development of STAT3 inhibitors to date. Overall, single-pathway STAT3 inhibition has not demonstrated a high potential for success, due to extensive cross talk and alternative signaling pathways found in STAT3 activated malignancies, in addition to its activation by non-canonical pathways, including epigenetic mechanisms [8].

2. The upregulation of STAT3 as a mechanism of drug resistance

More recently, STAT3 activation has surfaced as a mechanism of resistance in oncogene-addicted tumor types which have been treated with their respective oncogene-pathway inhibitors. The phosphorylation of STAT3 has been shown to be activated by various forms of mutant EGFR [L858R, E746_A750 deletion] and BRAF V600E signaling [11,12]. The BRAF/STAT3/Mcl1 signaling cascade has been described to be crucial for melanocyte and melanoma cell survival, and increased levels of phosphorylated STAT3 occur frequently during the progression of melanoma from local to metastatic disease [13]. Lee et al. demonstrated that STAT3 upregulation was a generalizable mechanism of drug resistance across a range of oncogene-addicted phenotypes treated with their primary pathway inhibitors, including EGFR, HER2, ALK, MET and KRAS mutant cell lines [11]. Interestingly, the co-targeting of the MEK/ERK pathway and STAT3 through FGFR/JAK1 inhibition restored sensitivity of the oncogene-addicted cells to their respective pathway inhibitors. The clinical significance of this finding was confirmed in a phase I clinical trial of a small molecular inhibitor, OPB-51602, where partial responses were observed in two patients with EGFR TKI-resistant NSCLC. However, the further development of this compound was curtailed by a toxicity profile suggestive of mitochondrial dysfunction, namely peripheral neuropathy and elevated serum lactate [9]. These toxicities were eventually explained when the mechanism of action of OPB-51602 was elucidated; the compound binds with high affinity to the STAT3-SH2 domain, triggering a downstream cascade by interfering with mitochondrial STAT3 (mSTAT3). This induces mitochondrial dysfunction and the accumulation of proteotoxic STAT3 aggregates, leading to cell death. Its efficacy is enhanced under conditions which increase reliance on mitochondrial respiration (*e.g.* glucose starvation), suggesting that the inhibition of STAT3 in metabolically stressed cells results in metabolic synthetic lethality [14].

3. The link between STAT3 signaling and mitochondrial respiration

The discovery of mSTAT3 revealed its novel role and provided a link between oncogene-induced cellular signaling pathways and cancer cell metabolism, which is now recognized as a hallmark of cancer [15]. STAT3 has been reported to reside in the mitochondria and a

mitochondrial protein, gene associated with retinoid interferon-induced mortality 19 (GRIM-19). Specifically, it is associated with the GRIM-19-containing mitochondrial complexes I and II, which are components of the electron transport chain (ETC) that generate energy by oxidative phosphorylation (OXPHOS) [4]. Functionally, mSTAT3 augments ETC activity; cells which are deficient in mSTAT3 display markedly reduced cellular ATP production [16].

In addition, mSTAT3 confers a protective role during cellular stress, by the reduction of reactive oxygen species (ROS) production and retention of cytochrome C in the mitochondria of cardiomyocytes exposed to ischemic damage [17]. Targeting STAT3 results in the dysregulation of mitochondrial activity, which is associated with excessive ROS formation, reduced mitochondrial membrane potential and enhanced apoptosis [17–19]. In cells under oxidative stress, functional mSTAT3 has a crucial role in preventing ROS-induced ASK1/$p38^{MAPK}$-mediated apoptosis [19]. Excessive or prolonged endoplasmic reticulum (ER) stress response results in a progressive reduction in mSTAT3 and resultant endothelial cell death via the loss of focal adhesion kinase-mSTAT3 signaling, leading investigators to conclude that mSTAT3 also has a protective effect on endothelial cells [20]. Furthermore, functional mSTAT3 is required for malignant transformation by protein tyrosine kinase oncoproteins, such as anaplastic lymphoma kinase (ALK) and v-Src, as well as oncogenes that lack tyrosine kinase activity, *e.g* the RAS oncogene [11,12,16,21]. A preclinical study demonstrated that in certain circumstances, mSTAT3 plays a more critical role in malignant transformation than canonical STAT3 activation, as in the case of Barrett's cells possessing oncogenic H-RasG12V [22].

4. Cancer cell metabolism

Half a century ago, Otto Warburg described the metabolic switch from OXPHOS to glycolysis in cancer cells, even in conditions of high oxygen tension ("aerobic glycolysis") [23]. It is now evident that tumor mitochondrial metabolism is not defective, but rather, reprogrammed to meet the challenges of macromolecular synthesis in proliferating cells [24]. Metabolic reprogramming of cancer cells leading to OXPHOS upregulation is now well-described, representing a paradigm shift from Warburg's classic hypothesis. It has been proposed that the cancer cell progresses through four waves of metabolic regulation. Oncogene mediated signaling leads to cancer stem cell transformation in the first wave. The second wave is prompted by hypoxia, inducing hypoxia-inducible factor (HIF) pathway signaling and a glycolytic switch. These first two waves provide gene reprogramming towards the glycolytic Warburg phenotype. From aglycemia secondary to high proliferation rates, arises the third wave, wherein the AMP-activated protein kinase (AMPK)-liver kinase B1 (LKB1) pathway is upregulated. This functions as a metabolic checkpoint, driving cells back towards oxidative metabolism. AMPK enhances sirtuin-1 (SIRT1) activity by increasing cellular NAD + levels, leading to deacetylation and modulation of the activity of downstream targets, such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). This causes expression of genes controlling mitochondrial biogenesis and activity. Retrograde signaling from revitalized mitochondria constitutes the fourth wave [25]. The bioenergetic mode of a tumor switches between glycolytic and oxidative depending on tumor microenvironment and activated oncogenes [26]. Cell lines of various tumor types, including breast, cervical, pancreatic and liver cancers, have demonstrated flexibility in switching from aerobic glycolysis to OXPHOS for derivation of energy in glucose limited conditions [25]. Despite these attempts to describe cancer cell metabolism, it is likely a more complex entity with different states occurring simultaneously within heterogenous tumor populations. Apart from aerobic glycolysis and OXPHOS, it is recognized that cancer cells adapt to their microenvironment and the availability of nutrients, then utilize alternate metabolic fuel such as glutamine via reductive carboxylation, and fatty acids via lipid metabolism [27,28].

5. The upregulation of oxidative phosphorylation as a mechanism of drug resistance

The metabolic switch towards OXPHOS as a mechanism of drug resistance is best described in relation to oncogene-addicted tumors. Many patients with oncogene-addicted tumors are treated with tyrosine kinase inhibitors (TKIs) with excellent response rates and limited toxicities. Examples are NSCLC with activating EGFR mutations or which contain EML4-ALK fusions, malignant melanoma with BRAF mutations, chronic myelogenous leukemia (CML) harboring the BCR-ABL fusion oncogene, myelodysplastic syndrome with JAK2 mutations [29–32]. These can be treated with EGFR kinase, ALK kinase, ABL kinase and BRAF and JAK2 kinase inhibitors respectively [33]. However, the duration of benefit from these TKIs are finite and drug resistance eventually sets in [34]. Small molecule inhibitors that target driver oncogenes can potentially inhibit the glycolytic pathway [35]. Therefore, cancer cells which have survived TKI therapy are critically reliant on OXPHOS for efficient production of ATP [36]. This highlights the role of metabolic plasticity in cancer cell survival [34]. To date, this has been shown in oncogene-addicted malignant melanoma and NSCLC. BRAF mutant melanomas treated with BRAF inhibitors became addicted to oxidative metabolism via increased expression of the mitochondrial master regulator, PGC1α. This adaptive induction of OXPHOS resulted in the limited the efficacy of BRAF inhibition [37]. The EGFR T790M mutation, commonly associated with secondary drug resistance in EGFR mutant NSCLC, may be effectively targeted by osimertinib, a third generation EGFR TKI [38,39]. In osimertinib-sensitive EGFR-mutant NSCLC cell lines, osimertinib treatment suppressed glycolysis and induced a metabolic switch towards OXPHOS, making the cells critically reliant on OXPHOS for survival. The combination of osimertinib with OXPHOS inhibition significantly delayed the time to osimertinib drug resistance, representing a novel therapeutic strategy to overcome resistance [35].

Cancer stem cells (CSCs) are a sub-population of cells within a tumor, which have the ability to self-renew and simultaneously generate progenitors that lose their stemness [40]. CSCs are notoriously resistant to standard anti-cancer therapies, and are critical drivers of relapse and disease progression. Previously, CSC metabolism was thought to be glycolytic, however there is growing evidence that show CSCs possess metabolic plasticity and have a preference for mitochondrial oxidative metabolism [41,42]. This metabolic plasticity enables CSCs to survive in the unfavorable environments related to tumor progression and metastatic sites. Newly emergent data from a BCR-ABL oncogene-addicted CML model revealed that although TKIs successfully targeted differentiated cells, acquired resistance arose due to the persistence of leukemic stem cells (LSCs). These stem cells were found to be critically dependent on OXPHOS for their survival and the combined targeting of OXPHOS and BCR-ABL successfully led to their eradication [43]. Surviving tumor cells of oncogene ablation in pancreatic adenocarcinoma, responsible for tumor relapse, have also demonstrated reliance on mitochondrial respiration for energy. Though resistant to nutrient deprivation and environmental stressors, they were unable to compensate for fluxes induced by OXPHOS inhibition. This high sensitivity to OXPHOS inhibitors impacted their tumorigenic potential, implying that targeting OXPHOS may eliminate these surviving cells and prevent tumor relapse [44]. Overall, we conclude that a subset of oncogene-addicted cancers acquire a metabolic switch towards the OXPHOS pathway as a mechanism of secondary resistance, mediated by the metabolic reprogramming of stem cell populations. This represents a therapeutic niche for OXPHOS inhibition, either alone or in combination with inhibitors of activated co-operative signaling pathways (Fig. 1).

6. Oxidative phosphorylation as a therapeutic target

There are presently very few agents targeting mitochondrial

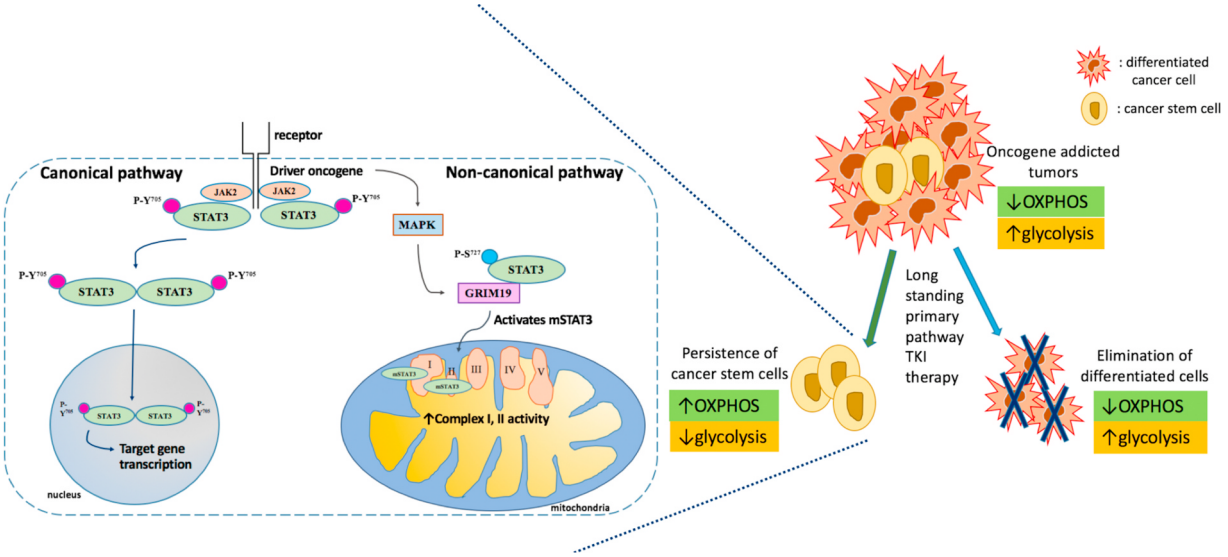

Fig. 1. Warburg's theory that cancer cells depend on aerobic glycolysis led to the assumption that OXPHOS is downregulated in cancer. Increasing evidence demonstrates that certain cancers are reliant on OXPHOS for energy production. Oncogene addicted tumors are typically sensitive to primary pathway tyrosine kinase inhibitor therapy. After prolonged treatment, differentiated cancer cells are eliminated, however a subpopulation of malignant cells, the cancer stem cells, persist. Upregulation of STAT3 occurs via both canonical and non-canonical pathways. In the non-canonical pathway, serine^{727}-phosphorylation (P-S^{727}) of STAT3 is signaled via the MAP kinase (MAPK). P-S^{727}-STAT3 activates mitochondrial STAT3 (mSTAT3), while GRIM-19 imports STAT3 into the mitochondria. The upregulation of mitochondrial STAT3 increases mitochondrial complex I and II activity, and therefore OXPHOS. The upregulation of mitochondrial STAT3 and OXPHOS are resistance mechanisms to TKI therapy, evident in work studying the metabolic reprogramming of persistent cancer stem cells. Treating oncogene addicted tumors with both primary pathway TKIs and OXPHOS inhibitors may therefore reverse resistance. P-Y^{705}: Tyrosine^{705}-phosphorylation.

function in clinical use as anti-cancer therapy or in the developmental pipeline for solid tumors. Efforts have been made to repurpose metformin, widely used in the treatment of type II diabetes mellitus, into an anti-neoplastic agent. The antitumor mechanism of metformin involves the activation of AMPK which inhibits the phosphoinositide 3 kinase (PI3K)/ Akt/ mTOR signal transduction pathway, thus reducing cell growth [45]. A recent phase II study of metformin in the pre- and post-operative management of ovarian cancer has revealed tolerability and favorable results. Tumors treated with metformin were found to have an improved sensitivity to cisplatin in-vitro, associated with a reduction in CSC populations [46]. Despite these promising results, trials have yet to determine if the administration at clinically tolerable doses will achieve sufficient drug levels in neoplastic tissue, and have yet to establish a definitive role as anti-cancer therapy [47]. Another biguanide, phenformin, similarly decreases ATP levels by inhibiting mitochondrial complex I, and is more potent and bioavailable than metformin due to more efficient delivery to the mitochondria [36]. It was developed as an anti-diabetic drug but withdrawn in the 1970s due to risks of fatal lactic acidosis. It is currently being re-purposed for use as cancer therapy. Phenformin has been shown to selectively trigger apoptosis in NSCLC cell lines lacking functional LKB1 pathways [48]. Synergy of phenformin in combination with selumetinib, a MEK inhibitor, was demonstrated in-vitro, and confirmed in-vivo using xenograft models. The loss of LKB1 in KRAS-mutant NSCLC conferred resistance to selumetinib, while sensitizing cells to phenformin. Phenformin enhanced the therapeutic effect of selumetinib, regardless of LKB1 status. Thus, targeting both MEK and cancer metabolism is a potential strategy to treat this subset of NSCLC [49]. An ongoing phase I trial is evaluating the safety and efficacy signals of administering phenformin in combination with dabrafenib and trametinib in patients with metastatic BRAFV600E/K mutated melanoma. (ClinicalTrials.gov Identifier: NCT03026517).

IACS-10759 is a novel and potent small molecular inhibitor of mitochondrial complex I, which possesses favorable physicochemical properties in-vivo. It demonstrated robust activity in specific biologic contexts, including acute myeloid leukemia (AML) and glycolysis-deficient [Enolase-1 (ENO1) - and phosphoglycerate dehydrogenases (PGD)-null] glioblastoma multiforme and neuroblastoma models. There is an ongoing phase I trial of this agent in AML and other solid tumors [50,51]. (ClinicalTrials.gov Identifier: NCT03291938, NCT02882321). Other inhibitors of mitochondrial complex I include carboxyamidotriazole (CAI) and BAY 87–2243. CAI inhibits angiogenesis and tumor growth in a range of cell lines in-vitro and in-vivo [52]. Despite positive outcomes in preclinical studies, CAI did not demonstrate clinical benefit in NSCLC as maintenance therapy against placebo in a phase III randomized trial [53]. The novel complex I inhibitor, BAY 87–2243, reduces HIF protein levels under hypoxic conditions. *In-vivo*, BAY 87–2243 suppressed HIFα protein levels and reduced target gene expression in a H460 NSCLC xenograft model. Under glucose deprived conditions, which shifts cells towards reliance on OXPHOS for energy production, BAY 87–2243 potently inhibited cell proliferation [54]. It significantly reduced tumor growth in BRAF mutant melanoma mouse xenografts, especially in more OXPHOS-dependent slowly proliferating tumors. BAY 87–2243 induced cell death by stimulating ROS generation and inducing oxidative stress [55]. Unfortunately, a phase I trial of the compound has been terminated early due to toxicities [56]. (ClinicalTrials.gov Identifier: NCT01297530).

Inhibiting STAT3 has been found to inhibit OXPHOS. The OPB compounds have been found to indirectly inhibit mitochondrial complex I and II via their action on mSTAT3. A second generation OPB compound (OPB-111077) with an improved safety profile has completed evaluation in a phase I study of advanced, treatment-refractory solid tumors, and is currently being studied in drug resistant oncogene-addicted tumors [57]. (ClinicalTrials.gov Identifier: NCT03158324). The feasibility of inhibiting OXPHOS by targeting mSTAT3 has also been demonstrated in preclinical studies of compounds developed as STAT3 inhibitors. MDC-1112 inhibited STAT3 mitochondrial localization and selectively induced mitochondrial ROS in pancreatic cancer cells. By blocking the mitochondrial membrane potential, MDC-1112 was able to induce mitochondrial cell death [18]. Mitocur-1 and Mitocur-3 are STAT3 inhibitors which have been shown to reduce mitochondrial oxygen consumption and levels of Ser727 STAT3 phosphorylation in a dose dependent manner, but have yet to be evaluated in cancer [58]. Similarly, the treatment of cardiomyocytes with Stattic,

Table 2
Preclinical and clinical development of some OXPHOS inhibitors and other mitochondrial inhibitors.

Site of action	Agent	Preclinical data	Clinical Data	Challenges
OXPHOS inhibitors	Metformin	Pancreas [75], Breast [76], Colon [76]	Phase I – advanced/ refractory cancers [77] Phase II Ovarian [46], Breast [78,79], NSCLC [80,81] Several other trials are currently ongoing [56]	Difficulty in achieving sufficient drug levels in neoplastic tissue; accumulates in small intestine [82].
	Phenformin	KRAS mutant NSCLC [48,49], melanoma [83–85] GBM [86]	Ongoing Phase I trial in combination with dabrafenib and trametinib in melanoma (NCT03026517)	Withdrawn from the market in the 1970s due to the elevated risk of lactic acidosis [87]
	CAI	LLC tumors [52]	Phase III - NSCLC [53]	
	BAY 87–2243	HNSCC [54]	Phase I – terminated	Toxicities
	IACS-010759	AML [50]	-	
	IACS-1131	AML [88]	-	
	OPB-51602	Prostate [14], other cell lines (colon, liver, lung) in unpublished data	Phase I – advanced/ refractory cancers [9]. Phase I – hematological malignancies (terminated) [89]	Toxicities including peripheral neuropathy and hyperlactatemia
	OPB-111077	DLBCL [57]	Phase I - advanced/ refractory cancers [57]	
	VLX600	Colon [90]	Phase I – terminated	Lack of efficacy
Other mitochondrial complex inhibitors	Lonidamine	Melanoma [91]	Phase III - Breast [92]	Lack of efficacy
	Atovaquone	Breast CSCs [93]	-	
	Arsenic trioxide	TLT model, LLC tumor [94]	-	
	Tigecycline	AML [95], CML [96], NSCLC [97], breast [98], ovarian [98], pancreatic [98], melanoma [98], GBM [98], prostate [98]	Phase I - AML [99]	
	Menadione (Vitamine K3)	Breast [100], ALL [101], colon [102]	-	
	Gamitrinib	Prostate [103,104]	-	

NSCLC – non small cell lung cancer, GBM – glioblastoma, AML – acute myeloid leukemia, DLBCL – diffuse large B cell lymphoma, CSC – cancer stem cell, HNSCC – head and neck squamous cell carcinoma, LLC – Lewis lung carcinoma, TLT – transplantable mouse liver tumor, CML – chronic myeloid leukemia, ALL – acute lymphoblastic leukemia

a small molecule STAT3 inhibitor, led to lower mitochondrial oxygen consumption and ATP production. Oxidative stress and therefore ROS formation was enhanced, resulting in cell death via mitochondrial permeability transition pore (MPTP) opening [59].

Aside from targeting OXPHOS and the electron transport chain, there are other ways to target the mitochondria. Several agents have shown promising preclinical data but few have advanced to clinical trials. Table 2 summarizes the development of OXPHOS inhibitors and other mitochondrial targeted therapy to date.

7. Biomarkers of OXPHOS inhibition

Patient selection is pivotal to the successful development of OXPHOS inhibitors. A major hindrance to the development of this class of compounds is the lack of robust biomarker strategies and the evaluation of these compounds in unenriched patient populations. Rigorous clinical evaluation of novel OXPHOS inhibitors in the relevant molecular contexts has the potential to result in the clinical application as single-agent therapy and in synthetic lethality with inhibitors of cooperative signaling pathways in oncogene-addicted tumor types. If a therapeutic niche for OXPHOS inhibition in drug-resistant oncogene-addicted tumors can be established, the practical implications of this will be immense.

In terms of identifying molecular contexts of susceptibility to OXPHOS inhibition, mitochondrial genomic sequencing analysis by The Cancer Genome Atlas (TCGA) has revealed deleterious tumor-specific somatic mitochondrial DNA (mtDNA) mutations in significant proportions of colorectal adenocarcinomas, ovarian serous cyst adenocarcinomas, and AMLs. These mutations conferred greater sensitivity to mitochondrial complex I inhibitors, metformin and phenformin, indicating their potential to be pursued as predictive biomarkers. However, analysis of mtDNA or cancer cell metabolism gene expression profiling may not directly correlate with functional oxidative metabolism, and other tools of characterizing OXPHOS activity should also be considered. These include metabolomics profiling and the functional evaluation of oxygen consumption and lactate production [56,60].

GRIM-19 was originally identified as a tumor suppressor, but subsequently was found to be an essential subunit of mitochondrial complex I, with STAT3-suppressive effects. Downregulation or loss of GRIM-19 is associated with a more aggressive phenotype of gastric cancer. The progressive depression of GRIM-19 is parallel with malignant transformation of chronic atrophic gastritis [61]. Complex I catalyzes the first step of electron transfer in the OXPHOS system, therefore being able to measure its function via a surrogate entity such as GRIM-19 may help discern which tumors are more susceptible to OXPHOS inhibition [62]. PGC1α-dependent signaling, indicative of OXPHOS upregulation, has been observed in several cancers [63]. Cells with high protein expression of PGC1α have a survival advantage when exposed to metabolic stresses. Although specific inhibitors of PGC1α are not yet available, OXPHOS inhibitors have been observed to reduce cell growth in PGC1α immunohistochemistry (IHC)-positive cancer cells [64]. Moreover, investigators of IACS-10759 have attempted to define a glycolysis-deficient context. ENO1 and PGD IHC was performed on 92 brain tumors, with 8.6% found to be unambiguously ENO1-null or showing very low ENO1 staining, while none were PGD-null [50].

8. Conclusion

Despite advances in targeting oncogene-addicted cancers with TKIs, drug resistance inevitably occurs. Novel strategies to overcome drug resistance are crucial to successful anti-cancer therapy. Aside from targeting alternative pathways of cellular signaling and the tumor immune landscape, cancer cell metabolism has emerged as an important mechanism of resistance, and hence is an attractive therapeutic target. STAT3, found to be constitutively activated in several malignancies, has long been regarded as a valuable target and well-studied over the last two decades. Unfortunately, efforts to develop STAT3 inhibitors have not been fruitful due to the lack of a reliable biomarker, unfavorable PK properties and off-target adverse events. The discovery of mSTAT3 has provided a critical link between cellular signaling pathways and cancer cell metabolism, and emerging evidence of the metabolic reprogramming of cancer cells towards OXPHOS as a mechanism of drug resistance has validated OXPHOS as a therapeutic target. This is made even more promising by the discovery of OXPHOS as a mechanism of survival in cancer stem cells, and has given rise to the prospect of eradicating traditionally therapy-resistant CSCs through OXPHOS inhibition. This provides strong rationale for the further investigation of this class of compounds in combination with primary pathways inhibitors, in a synthetic lethality approach aimed at restoring sensitivity to treatment. One of the main challenges undermining the development of OXPHOS inhibitors for clinical use is their narrow therapeutic index and potential for life-threatening toxicities, e.g. lactic acidosis. The addition of OXPHOS inhibitors to TKI therapy in oncogene-addicted tumors has the added advantage of limiting exposure to individual agents, therefore minimizing toxicities overall.

The further identification of compounds with acceptable toxicity profiles alongside the discovery of companion biomarkers of OXPHOS inhibition in upcoming clinical trials, will be crucial in establishing the role of this novel class of agents in the pharmaceutical pipeline. With a deeper understanding of the PK and safety profile of these compounds, the potential to effectively reverse drug resistance in oncogene-addicted cancers is not far off on the horizon.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.redox.2018.101073.

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