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Abstract

Precision oncology is the practice of matching one therapy to one specific patient, based on particular genetic tumor alterations, in order to achieve the best clinical response. Despite an expanding arsenal of targeted therapies, many patients still have a poor outcome because tumor cells show a remarkable capacity to develop drug resistance, thereby leading to tumor relapse. Besides genotype-driven resistance mechanisms, tumor microenvironment (TME) peculiarities strongly contribute to generate an intratumoral phenotypic heterogeneity that affects disease progression and treatment outcome. In this Review, we describe how TME-mediated metabolic heterogeneities actively participate to therapeutic failure. We report how a lactate-based metabolic symbiosis acts as a mechanism of adaptive resistance to targeted therapies and we describe the role of mitochondrial metabolism, in particular oxidative phosphorylation (OXPHOS), to support the growth and survival of therapy-resistant tumor cells in a variety of cancers. Finally, we detail potential metabolism-interfering therapeutic strategies aiming to eradicate OXPHOS-dependent relapse-sustaining malignant cells and we discuss relevant (pre)clinical models that may help integrate TME-driven metabolic heterogeneity in precision oncology.

Keywords	precision oncology; metabolism; cancer, targeted therapy; drug resistance; oxidative phosphorylation; organoid

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May 15th, 2019

Dear Editor,

I am pleased to submit a revised version of my manuscript entitled: *«Reconciling environment-mediated metabolic heterogeneity with the oncogene-driven cancer paradigm in precision oncology»* by C. Vander Linden and C. Corbet, for publication in *Seminars in Cell and Developmental Biology.*

In the revised manuscript and figures, we have taken into account all reviewer's comments, as detailed in the rebuttal provided with the article.

We hope that you will find the revised version of our review suitable for publication in *Seminars in Cell and Developmental Biology.*

Yours sincerely,

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Comments from the editors and reviewers:
-**Reviewer 1**

In the review entitled "*Reconciling environment-mediated metabolic heterogeneity with the oncogene driven cancer paradigm in precision oncology*" Vander Linden and Corbet describe how tumor microenvironment-mediated metabolic heterogeneities actively participate to therapeutic chemoresistance. In particular, authors report how a lactate-based metabolic symbiosis acts as a mechanism of adaptive resistance to targeted therapies and the role of oxidative phosphorylation (OXPHOS) to support the growth and survival of therapy-resistant tumor cells in a variety of cancers. Finally, potential metabolism-interfering therapeutic strategies aiming to eradicate OXPHOS-dependent relapse-sustaining malignant cells and relevant (pre)clinical models are discussed. Overall, this paper is a timely, highly detailed and informative review. The text is clearly written and only minimal revisions are required:

1) In the section “2.3 Cancer cell oxidative metabolism and therapy resistance” authors make the following statement “*there is now increasing experimental evidence in the literature for a direct contribution of oxidative mitochondrial metabolism in cancer progression*”. It would be helpful and more informative for the reader to include a piece of literature concerning the role of mitochondrial DNA mutations and OXPHOS enzymes as modulators of both tumorigenesis and response to therapy.

We thank the reviewer for this comment. A new paragraph has been added in the section 2.3 to report the role of mtDNA mutations in tumorigenesis and response to therapy. The role of OXPHOS in these processes has been also more illustrated by adding several references on different cancer subptypes relying on OXPHOS upon genetic or microenvironmental selection pressures. The role for OXPHOS enzymes (*i.e.* electron transport chain complexes) in tumorigenesis has been recently by Ashton et al Clin Cancer Res 2018. In our review, for a sake of clarity and because of length restrictions, we focused on the emerging evidence that, in many clinical contexts, therapy-resistant cancer cells rely on OXPHOS. We described the OXPHOS-targeting therapeutic interventions that may help to overcome this resistance.

2) Figure 1 would be more complete and informative modifying this sentence “intratumoral phenotypic (and genotypic) heterogeneity” as “intratumoral phenotypic (genotypic and metabolic) heterogeneity”. The related legend should be changed.

The figure 1 and the related legend have been edited according to the reviewer’s comments.

3) The information of Figure 2 would be more direct for the reader if the following changes are made: i) to add “Tumor metabolic profile” as output upon infusion of 13C tracers (as reported in the figure legend); ii) to change the sentence “genotypic and phenotypic heterogeneity analysis” as “genotypic and metabolic heterogeneity analysis.

Figure 2 has been edited according to the reviewer’s comments.

Reconciling environment-mediated metabolic heterogeneity with the oncogene-driven cancer paradigm in precision oncology

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Invited review for the Special Issue on "**Mitochondrial metabolic alterations in cancer cells and related therapeutic targets**" in Seminars in Cell and Developmental Biology

ABSTRACT

Precision oncology is the practice of matching one therapy to one specific patient, based on particular genetic tumor alterations, in order to achieve the best clinical response. Despite an expanding arsenal of targeted therapies, many patients still have a poor outcome because tumor cells show a remarkable capacity to develop drug resistance, thereby leading to tumor relapse. Besides genotype-driven resistance mechanisms, tumor microenvironment (TME) peculiarities strongly contribute to generate an intratumoral phenotypic heterogeneity that affects disease progression and treatment outcome. In this Review, we describe how TME-mediated metabolic heterogeneities actively participate to therapeutic failure. We report how a lactate-based metabolic symbiosis acts as a mechanism of adaptive resistance to targeted therapies and we describe the role of mitochondrial metabolism, in particular oxidative phosphorylation (OXPHOS), to support the growth and survival of therapy-resistant tumor cells in a variety of cancers. Finally, we detail potential metabolism-interfering therapeutic strategies aiming to eradicate OXPHOS-dependent relapse-sustaining malignant cells and we discuss relevant (pre)clinical models that may help integrate TME-driven metabolic heterogeneity in precision oncology.

Running title: Targeting tumor metabolism in precision oncology

Keywords: precision oncology; metabolism; cancer, targeted therapy; drug resistance; oxidative phosphorylation; organoid

INTRODUCTION

Despite the early responses generally observed with personalized medicine (precision oncology), prognosis for cancer patients remains very poor due to frequent tumor relapses. Indeed, the clinical efficacy of anti-cancer targeted therapies is strongly limited by the development of acquired drug resistance. Moreover, besides genotype-mediated resistance mechanisms, tumor microenvironment (TME) conditions are increasingly recognized to generate intratumoral phenotypic heterogeneity that may select for pre-resistant cell populations and actively contribute to therapeutic failure and minimal residual disease. Tumor metabolism is the perfect example to illustrate how TME strongly influences cancer cell phenotype and from there, treatment outcome. In this review, we describe the current knowledge about metabolism-based mechanisms of adaptive resistance to targeted therapies. More precisely, we report the role of lactate-based metabolic symbiosis, as an example of non-cell autonomous mechanism of resistance, and the reliance of many therapy-resistant cancer types and/or subpopulations on mitochondrial metabolism, in particular oxidative phosphorylation (OXPHOS). We also discuss potential therapeutic strategies that may enable eradicate OXPHOS-dependent relapse-sustaining cancer cells. This review finally explores relevant (pre)clinical models that may help integrate TME-driven metabolic heterogeneity in precision oncology.

1. Precision oncology, more than a clinical illusion?

In the current era of precision oncology, clinicians aim to exploit patient-specific molecular alterations (*e.g.* gene mutations or amplifications, genomic rearrangements) to identify treatments (the so-called targeted therapies) with the greatest probability of clinical benefit. Next-generation sequencing technology has indeed emerged to allow low-cost, quick and sensitive high-throughput analysis of individual genetic variability and it is now adopted for routine clinical use to diagnose and orientate selection of tailored therapeutic options [1].

Such genomic testing enabled to highlight the importance of "driver" somatic alterations that activate crucial oncogenes (*e.g*. *RAS*, *EGFR*, *BRAF*, *BCR-ABL1*), giving rise to a pivotal tumor dependency (*i.e.* oncogene addiction) [2]. Over the years, the number of cancer-associated genetic alterations has rapidly increased, leading to an expansion in the oncology pipeline for drugs targeting these specific molecular characteristics. From November 2016 to October 2017, the US Food and Drug Administration (FDA) approved more than 30 new cancer therapies, with nearly half of them for the treatment of patients with cancers harboring particular sequence alterations [3]. Among them, pembrolizumab (Keytruda®), a monoclonal antibody targeting programmed death-1 (PD-1), became the first therapy being FDA-approved for cancer patients based on a common biomarker (*i.e.* deficient DNA mismatch repair or high degree of microsatellite instability) rather than tumor site or histology (the so-called tumor-agnostic therapy, an approach tested in "basket" trials) [4]. Still, the number of targetable alterations keeps expanding, making very challenging the application of personalized medicine in daily oncology practices. The broader arsenal of (targeted) therapies constantly requires new approaches to provide clinical interpretations of data from large-scale genetic testing [5]. Even more concerning is the large number of patients (>75%) that still do not benefit from precision oncology because genetic profiling fails to pair them with a targeted therapy [6-9]. The early success of imatinib in patients with *BCR-ABL1*-driven chronic myeloid leukemia (CML) generated a great deal of optimism for both patients and clinicians but in reality, the efficacy of this agent must be seen as an exception rather than a rule [10]. Indeed, when considering all advanced cancer types, the clinical benefit of being assigned a targeted therapy is still limited to about 30% of patients and the median progression-free survival (PFS) does not exceed 6 months [11].

Genetic alterations, associated with primary and acquired resistance to targeted therapies, are very diverse and heterogeneous within a single tumor, thereby limiting the clinical

applicability of such therapies. In this case, resistance largely arises from cell-autonomous mechanisms as the treatment often selects for pre-existing clones bearing genetic alterations either in the target itself or in downstream/parallel signaling pathways that may compensate for the drug-induced inhibitory effects. For example, although epidermal growth factor receptor (EGFR) is (over)expressed in about 85% of patients with metastatic colorectal cancers (mCRC), the clinical efficacy of EGFR-targeting monoclonal antibodies, cetuximab and panitumumab, is restricted to a subset of patients (<25%) [12]. Indeed, activating mutations within *KRAS*, *NRAS* and *BRAF* genes have been significantly associated with lack of response to cetuximab or panitumumab therapy in patients with mCRC [13-18]. More recently, many other gene alterations (*e.g*. *MET* or *ERBB2* amplification, *PIK3CA* activating mutations, *PTEN* loss-of-function mutations) have been reported as potential mechanisms of primary resistance to anti-EGFR antibody therapy [19, 20].

Altogether, these observations point to a high degree of genetic heterogeneity as a major contributor to therapy resistance and underscore the complexity of genomic profiling for clinical decision-making. Moreover, current limitations in precision oncology largely arise from the strictly genome-based tumor characterization, while ignoring the adaptive landscape. For instance, the maximum-tolerated dose-based therapy aims to kill the greatest number of cancer cells but, at the same time, is thought to select for resistant phenotypes and to eliminate all potentially competing populations (the so-called “competitive release”) [21, 22]. Exploiting environmental selection forces and/or targeting associated phenotypic changes appear therefore as promising strategies to help prevent outgrowth of resistant cancer cell populations, and by doing so, improve patient outcome.

2. TME-driven metabolic heterogeneity and therapy resistance

Therapy resistance is associated with the existence of specific local microenvironments (the so-called niches) that can act as selection forces to promote intrinsic diversity and phenotypic plasticity and help tumor cells adapt and survive anti-cancer therapies [22-24]. Along cancer progression, both at primary and distant sites, specific TME conditions, such as hypoxia (low pO_2), acidosis (low pH) or nutrient deprivation, are indeed high selection barriers and impose profound constraints that cancer cells must face in order to survive and grow [25-28]. They can promote the emergence of a "mutator phenotype" [29], as reported, for instance, with glucose deprivation acting as a strong selection pressure for mutated *KRAS* in CRC cells [30]. Nevertheless, TME peculiarities can also drive the emergence and/or acquisition of phenotypic traits (*e.g*. resistance to apoptosis, low proliferation/quiescence, immune surveillance escape, invasion and metabolic reprogramming), that contribute to therapy resistance, minimal residual disease and poor outcome in cancer patients, irrespectively of the occurrence of genetic alterations.

2.1 TME peculiarities and associated metabolic preferences

Within the tumors, the limited access to nutrients and/or oxygen, the local accumulation of protons, together with the presence of tumor-associated stroma cells generate a metabolic heterogeneity [31-33]. Thus, cancer cells can use a variety of substrates to fulfill their need in energy and/or biosynthetic precursors according to the local TME [34-36]. Beyond the well-known Warburg effect (*i.e*. glucose-to-lactate conversion regardless oxygen levels), changes in amino acid metabolism have been observed to support cancer cell growth. Some highly proliferating cancer types for instance rely more on the use of non-essential amino acids (*e.g*. aspartate, glycine, glutamine, and serine) to support the bioenergetics and macromolecular synthesis [37-42]. A variety of cancers is also capable of capturing and metabolizing

exogenous acetate to maintain cancer cell growth under low oxygen and lipid-depleted conditions [43-45]. In addition, exogenous fatty acids also represent a valuable source of carbon, via acetyl-CoA generation, for energy production but also biosynthesis of membranes and signaling molecules that help sustain cancer cell proliferation [46].

Such metabolic heterogeneity offers cancer cells with multiple adaptation and escape options when facing hostile conditions including drug-induced stress, through both cell-autonomous and non-cell-autonomous mechanisms. However, although there are numerous examples of intimate relationship between microenvironmental niches and cancer cell metabolic preferences [47, 48], evidence for a straightforward contribution of this interplay to therapy resistance only begins to be explored [49, 50]. TME is indeed most often associated to drug resistance through its physico-chemical components. Hypoxia, for instance, is well known to be associated with multiple pathways accounting for drug resistance (*e.g*. genomic instability, cell cycle arrest or apoptosis inhibition) [51]. Acidosis also participates to drug resistance by affecting the permeability of charged compounds through the cellular membrane (ion trapping phenomenon) [31, 52]. Nevertheless, some insights on a role of metabolism in TME-dependent dynamic regulation of therapy resistance can be found in the literature.

2.2 Lactate-based metabolic symbiosis as an adaptive mechanism of resistance to anticancer targeted therapies

Microenvironment-driven metabolic heterogeneity, within a tumor, is well exemplified by the occurrence of a lactate shuttle between hypoxic and oxidative cancer cells [53, 54]. Indeed, cancer cells, that are distant of blood vessels, are poorly oxygenated and hence forced to fulfill their energy needs through glycolysis resulting in the production of lactate. Rather than being a waste metabolite, lactate is secreted out of the cytoplasm and taken up by other tumor cells that are closer to blood vessels and well oxygenated. Instead of glucose, oxidative cancer

cells can utilize lactate as an alternative energy source by conversion into pyruvate which then fuels the tricarboxylic acid (TCA) cycle. The spared glucose can thus be used for anaerobic glycolysis by the poorly oxygenated cancer cells. This lactate-based metabolic symbiosis between lactate-generating and lactate-consuming tumor cells has been reported in various cancer types [55, 56], and shown to involve also cancer-associated fibroblasts (CAFs) and angiogenic endothelial cells [57-60]. More recently, *in vivo* ^{13}C-lactate tracing experiments revealed extensive labelling of TCA cycle intermediates in human patients and genetically engineered mouse models with non-small-cell lung cancers (NSCLC) [48, 61, 62]. These studies have documented that circulating (and not only tumor-derived) lactate may be captured and oxidized in cancer cells and importantly that the contribution of lactate to TCA cycle intermediates could be greater than that of glucose [61, 62], thereby corroborating a straightforward link between lactate metabolism and mitochondrial activity in high-proliferating cancer cells [63].

Lactate transport across the plasma membrane is facilitated by monocarboxylate transporters (MCT) and is coupled to the symport of protons. The high-affinity transporter MCT1 is the main entry path for lactate in oxidative cancer cells and tumor-associated endothelial cells while hypoxia-induced MCT4 has a low affinity for lactate, making it the *bona fide* transporter for lactate export in glycolytic tumor cells and tumor-associated cells [53, 64].

In recent studies, a lactate-based metabolic symbiosis has been described to strongly support adaptive resistance to anti-angiogenic therapies in pancreatic neuroendocrine tumors [65], murine breast cancer models [66], patient-derived renal cell carcinoma (RCC) orthoxenograft (PDX) models and in clinical human RCC samples [67]. Indeed, after an initial regression, murine and human tumors, treated with antiangiogenics, resume growth in the absence of active neo-vascularization by exhibiting a compartmentalized expression of MCT1 and MCT4 whereby hypoxic cancer cells import glucose and export lactate, while normoxic cells import

and catabolize lactate. Those studies have also reported that inhibition of glycolysis upon 3-PO treatment or genetic ablation of MCT4 expression [66], as well as mTOR signaling blockade [65, 67] can disrupt this metabolic symbiosis and overcome the resistance development to anti-angiogenic therapy in patients (Table 1).

Metabolic symbiosis has also been reported in breast cancer, with lactate acting as an energetic fuel for tumor cells in a MCT1-dependent manner [68, 69]. More precisely, triple-negative breast cancer (TNBC) cells have been found to use lactate as a primary source of energy, allowing them to survive glucose deprivation for extended periods [69]. This metabolic adaptation is mediated by the estrogen-related receptor alpha (ERRα) nuclear receptor and helps cancer cells resist to anti-PI3K/mTOR targeted therapies. Blockade of lactate oxidation capacities upon genetic or pharmacological inhibition of ERRα activity sensitizes breast cancer cells to PI3K/mTOR inhibitors both *in vitro* and *in vivo* [69]. A recent study has also shown a role for lactate metabolism in the resistance to MET and EGFR tyrosine kinase inhibitors (TKI) (JNJ-605, crizotinib and erlotinib, respectively) [70]. The authors have actually observed an exacerbated glycolytic metabolism, with an increased lactate release, in patient-derived NSCLC and gastric cancer cells upon continuous and prolonged treatment with MET or EGFR TKI. Secreted lactate can act as a signalling molecule instructing CAFs to overproduce hepatocyte growth factor (HGF) which, in turn, activates MET in cancer cells, thereby overcoming TKI inhibitory effects. Importantly, pharmacological inhibition or genetic knockdown of lactate metabolism-related enzymes/transporters (*e.g.* lactate dehydrogenases, MCT1, MCT4) is sufficient to overcome resistance. Altogether, these observations support the relevance of such metabolic symbiosis in therapy failure and the potential clinical utility of symbiosis-interfering therapeutic modalities [71, 72]. More generally, these studies pinpoint the metabolic plasticity as an effective strategy exploited by cancer cells to counteract environmental- or drug-induced

stresses [72], and open new therapeutic perspectives to prevent/overcome resistance onset, with for instance novel combinatorial treatments, simultaneously targeting (lactate) metabolism and driver oncogene.

2.3 Cancer cell oxidative metabolism and therapy resistance

Beyond the "Warburg effect" dogma, there is now increasing experimental evidence in the literature for a direct contribution of oxidative mitochondrial metabolism in cancer progression and response to (targeted) therapies. For instance, cancer cells deficient in OXPHOS due to mitochondrial DNA (mtDNA) depletion (ρ0 cells) do not form tumors *in vivo* [73-75], while a low mtDNA content correlates with a better outcome in breast cancer patients treated with anthracycline-based chemotherapy [76]. Moreover, mtDNA mutations have been reported to increase the tumorigenic potential of prostate cancer cells [77] and to alter the response to treatments in a variety of tumor types [78-80]. Indeed, mutations in the non-coding mtDNA controlling region (*e.g.* displacement loop) have been associated with chemoresistance in colorectal cancer patients [81] and a somatic mutation in ND4 complex I subunit correlates with resistance to chemotherapy in serous ovarian cancers [82].

Genetic, cell lineage and microenvironment factors determine the relative contribution of, and dependence on OXPHOS (*vs* glycolysis) in tumorigenesis. Indeed, lung cancer cells harboring mutations in the SWI/SNF complex (*e.g. SMARCA4* and *ARID1A* gene mutations) [83] or in *KRAS* oncogene [47] depend on OXPHOS for their growth and survival. A similar OXPHOS dependence has been reported, among others, in a subpopulation of glioblastomas with homozygous deletion of enolase 1 [84, 85], in TNBC patients with low expression of the BACH transcription factor [86] as well as in cancer cells exposed to an acidic microenvironment [87, 88].

~~Furthermore,~~ F~~f~~or a variety of cancers, therapy-resistant tumor cells, including the so-called cancer stem cells, have been also found to mostly rely on mitochondrial metabolism, in particular OXPHOS [89-95] (Table 1). Several studies have actually shown that therapy-resistant acute myeloid leukemia (AML) cells exhibit metabolic features and gene signatures consistent with a high OXPHOS status necessary to support their growth [90, 95, 96]. Quiescent human AML stem-like cells, responsible for tumor resistance, can be targeted through their high dependency to OXPHOS, either by inhibiting the expression of *BCL2* oncogene [96] or by interfering with the oxidation of adipose tissue-derived fatty acids [95]. Recently, a study has shown that therapy-resistant CML stem cells mostly rely on upregulated oxidative metabolism for their survival [91]. Combinatory treatment with imatinib and tigecycline, a FDA-approved mitochondrial translation inhibitor, is able to overcome resistance by eradicating the stem cell-enriched populations ($CD34^+$ and $CD34^+CD38^-$). In *KRAS*-driven pancreatic ductal adenocarcinoma (PDAC), genetic deletion of the $KRAS^{G12D}$ allele leads to a dramatic tumor regression, but a small subpopulation of cells resists and eventually contributes to tumor relapse [97]. Importantly, these resilient cells rely on OXPHOS for survival (unlike the tumor bulk that is mainly glycolytic) and inhibitors of oxidative metabolism can impair tumor relapse when $KRAS^{G12D}$ is re-expressed [97]. In $BRAF^{V600E}$-mutant melanomas, BRAF inhibitors have been reported to induce PGC1α, a regulator of mitochondrial biogenesis, thereby causing OXPHOS dependence [98]. Consequently, BRAF inhibitors synergize with phenformin, a complex I inhibitor, 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 [99]. Of note, cancer stem cells of human pancreatic cancer-derived xenografts, responsible for tumor resistance, have been also found to rely on OXPHOS [100] (Table 1).

Besides a direct role in tumor cells, compelling evidence in the literature suggests that OXPHOS inhibition may also reduce tumor growth and improve drug response by enhancing anti-tumor immunity. Indeed, while anti-tumor cytotoxic and effector T cells require glycolysis to proliferate and produce cytokines [101-104], immune-suppressive cell types such as regulatory T (Treg) cells in the TME have been reported to heavily rely on OXPHOS for their energy production [105-107]. Biguanides (*e.g.* metformin, phenformin) are known (albeit weakly potent) complex I inhibitors, and are safely used to treat diabetes. IM188 and IM156 (ImmunoMet) are novel biguanides in late stage lead optimization and have been selected, on the basis of the immunomodulatory effect of OXPHOS inhibition, to increase T effector cells while decreasing myeloid derived suppressor cells (MDSC) and Treg cell pools in the TME.

Recent studies have also identified two OXPHOS inhibitors, namely IACS-010759 [93] and gboxin [108], as potent anticancer drugs in various tumor subtypes. IACS-010759 has been reported as a selective inhibitor of the ND1 subunit of complex I of the electron transport chain (ETC) and shown to preferentially inhibit tumor growth, both *in vitro* and *in vivo*, of certain "OXPHOS-dependent" tumor types such as chemo-resistant AML, glycolysis-deficient glioma, SWI/SNF mutant lung cancer and brain metastasis in melanoma [83, 93, 109]. This compound is currently being evaluated in phase 1 clinical trials in relapsed/refractory AML and solid tumors (NCT02882321 and NCT03291938, respectively). In another study, gboxin has been found to specifically inhibit the growth of primary mouse and human glioblastoma stem-like cells while not affecting the viability of mouse embryonic fibroblasts or neonatal astrocytes. Gboxin reduces OXPHOS by interacting with respiratory chain proteins and inhibiting the ATP synthase activity [108].

Importantly, OXPHOS-interfering compounds have shown yet major caveats for their clinical application, including low efficiency (*e.g.* biguanides), detrimental "off-target" effects (*e.g.*

290 rotenone) or a limited pharmacokinetic profile (*e.g*. oligomycin), thereby limiting their use as
291 anticancer drugs [110]. Furthermore, BAY87-2243, a complex I inhibitor, has been reported
292 to alleviate hypoxia and improved radiation response without toxicity in mice, but the initial
293 phase I trial had to be terminated due to unexpected toxicity issues [111]. Nevertheless, the
294 recent development and characterization of IACS-010759 and gboxin provide rationale that
295 targeting OXPHOS may be done safely (*i.e.* with compounds showing a high therapeutic
296 index) and thus reconsiders it as a very attractive anti-cancer strategy, in particular for
297 therapy-refractory cancer patients. It is also noteworthy that, besides triggering direct anti-
298 tumor effects, inhibition of mitochondrial metabolism may sensitize tumors to radiation
299 therapy (via tumor reoxygenation), as recently reported with 7ACC2, an inhibitor of the
300 mitochondrial pyruvate complex that blocks both lactate- and glucose-fueled TCA cycle [71].
301
302 **3. Relevant (pre)clinical models to integrate TME-driven metabolic heterogeneity in**
303 **precision oncology**
304 Environment-mediated therapy-resistant phenotype of cancer cells evolves *de facto* with time
305 and tumor development. Relevant (pre)clinical models are therefore needed to integrate the
306 influence of not only the driver oncogene(s), cell lineage, tissue of origin and/or the type of
307 therapy applied but also TME peculiarities and to further explore the intimate relationship
308 between TME, cancer cell metabolic preferences and drug response (Figure 1). Although the
309 most accurate way to study environment-mediated metabolic alterations is by using orthotopic
310 *in vivo* models, the complexity of such systems limits experimental control and potential use
311 for high-throughput drug screening [112]. To date, the most prevalent human-derived cancer
312 models used in drug-testing studies are cell lines and patient-derived xenografts (PDX). These
313 two approaches have however intrinsic limitations. For cell lines, culture conditions (*e.g*.
314 nutrient, oxygen and pH levels) are usually far from those existing *in vivo* and when TME is

part of the equation, all cancer cells usually undergo exposure to the same microenvironmental tumor peculiarities in 2D culture conditions (*i.e*. cancer cell monolayers), still making the model poorly representative of tumor heterogeneity. Also, long-term interactions between different cell populations growing together (as observed with most tumors) cannot be easily evaluated. Here, we describe the *in vivo* infusion of ^{13}C tracers and the generation of tumor organoids as two experimental patient-based approaches that can represent the physiological complexity and heterogeneity of cells observed in human cancers, and integrate the influence of both genetic pattern and TME on cancer cell metabolic preferences and response to therapies (Figure 1).

3.1 *In vivo* infusion of ^{13}C tracers

Stable isotope-based metabolic analysis can be used to evaluate how cellular metabolism, in particular the fate of biosynthetic fuels (*e.g*. glucose, glutamine, lactate), is altered during cancer progression. In the last decade, this approach has been increasingly used to analyze metabolism *in vivo*, in various mouse tumor models [47, 62, 113, 114] but also in human cancer patients [48, 61, 115-118]. Stable tracers (*i.e.* ^{13}C-glucose and other isotopically labeled nutrients) are administered as a bolus followed by an intra-operative infusion, through a peripheral intravenous line, before surgical tumor resection and analysis of isotope enrichment downstream of labeled nutrients via ^{13}C NMR spectroscopy [119]. By infusing [U-^{13}C]glucose in patients, several recent studies have actually revealed a metabolic heterogeneity depending on the tumor type. Indeed, some tumors, such as clear cell renal cell carcinoma (ccRCC), have been found to be highly glycolytic, with pyruvate being converted into lactate rather than entering the mitochondrial TCA cycle, even in the presence of oxygen (the so-called Warburg effect) [115]. On the other hand, a clear demonstration of glucose consumption through mitochondrial oxidative metabolism has been reported over the last

340 years in multiple cancer types, including glioma, brain metastases and NSCLC [47, 48, 116-
341 118]. Indeed, infusion of [U-^{13}C]glucose in patients with FDG-PET-positive tumors in the
342 brain and lungs reveals robust glucose oxidation, even in excess of adjacent tissue [48, 118].
343 These reports indicate an uncoupling between high rates of glucose uptake and suppressed
344 glucose oxidation, suggesting that the TCA cycle retains its ability to produce energy via
345 oxidative metabolism in aggressive tumors. Moreover, less than 50% of the acetyl-CoA pool
346 has been found to derive from blood-borne glucose in human brain tumors [117], suggesting
347 that additional substrates contribute to tumor bioenergetics. Other studies have indeed
348 highlighted the role of alternative respiratory fuels, including acetate and lactate, in
349 complementing glucose oxidation to support biomass and energy production in living tumors
350 [44, 48, 61, 62]. These authors have also reported that contribution of lactate and glutamine to
351 the TCA cycle was even greater than that of glucose in lung and pancreatic cancers,
352 respectively [62]. Interestingly, the metabolic profile of tumors has been found to be
353 dependent on both the genotype and tissue of origin. For instance, *MYC*-driven liver and
354 triple-negative breast cancers are more glutamine-dependent than *MET*-driven liver and *MYC*-
355 driven lung tumors [114, 120, 121]. *In vivo* ^{13}C-based metabolic profiling also allowed to
356 evidence the important role of pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC)
357 to support tumor growth in human lung cancer patients [47, 116, 118], making them attractive
358 therapeutic targets in this specific tumor type.
359 However, interpretation of such stable isotope-based metabolic analysis is complicated, since
360 stromal cells present in the collected tumor fragments may contribute to the labelling patterns.
361 Moreover, occlusion of afferent arteries, prior to surgical resection, could temporarily impact
362 metabolism and ^{13}C labeling and lead to a misinterpretation of the preferential use of ^{13}C-
363 labeled nutrients in tumors of cancer patients. Besides these limitations, such *in vivo*
364 metabolomic studies underscore the important contribution of mitochondrial oxidative

365 metabolism in tumors and position it as an attractive target for the design of further
366 therapeutic strategies aiming to interfere with cancer progression and overcome therapy
367 resistance.

368

369 3.2 Patient-derived tumor organoids

370 To better integrate the influence of TME conditions on cancer cell phenotype, including
371 response to therapies, tumor cells can be cultured in a matrix that allows 3D growth as
372 organoids. In the last decade, patient-derived tumor organoids (PDTO) have been established
373 for a variety of cancers, including breast [122], bladder [123], colorectal [124], liver [125],
374 pancreatic [126], gastrointestinal [127, 128], glioblastoma [129], retinoblastoma [130] and
375 prostate cancers [131]. In this method, tumor cells are commonly embedded in an
376 extracellular matrix (*e.g*. Matrigel) and cultured in a specific medium containing a defined
377 combination of stem cell niche factors (*e.g*. R-spondin-1, EGF, Wnt-3a and noggin). These
378 adequate culturing conditions influence the spatial organization of cell surface receptors and
379 induce physical constraints on tumor cells, thereby modifying both their morphology and their
380 phenotype (*vs* 2D monolayer cultures) through changes in gene expression [132]. Importantly,
381 PDTO recapitulate the histological and genetic features of the primary tumor and even retain
382 physico-chemical characteristics of TME, such as hypoxic gradients [133, 134]. In some
383 cases, they have even been able to predict tumor drug responses *in vivo* [123, 127], making
384 them amenable tools for high-throughput drug screening [135].
385 Although the majority of PDTO models reported so far still lack the cell and matrix diversity
386 found within the TME, recent studies have reported new modalities of PDTO generation (*e.g*.
387 air-liquid-interface; ALI) to preserve stromal cells such as CAFs and even functional immune
388 cells [136-138], together with cancer cells (instead of the more usual strictly cancer stem cell-
389 based organotypic structures). Another limitation of such 3D culture models is their reliance

on defined medium conditions that might not represent nutrient levels experienced by cells in tumors. Finally, PDTO are tractable and can be studied through both pharmacological and genetic screening approaches [139], thereby offering exquisite models to finely dissect how TME peculiarities and therapies influence the metabolic phenotype of cancer cells in a relevant physical environment (Figure 2).

Concluding remarks

While it would be unfair to condemn precision oncology because of the limited success of a number of clinical trials (largely due to the amazing diversity of resistance-associated genomic landscapes cancer patients), the current record of partial failure also highlights the urgent need to implement fundamental changes in the tumor treatment paradigm. Focusing on phenotype-driven rather than genotype-mediated drug resistance mechanisms may help break with the usual concept of targeted therapies in order to provide cancer patients with a new mode of precision medicine in which the identification of biological (metabolic) pathways, shared by tumors exhibiting multidrug resistance, could offer a "one-size-fits-all" approach (Figure 3).

Importantly, among the handful of metabolism-modulating drug candidates that have reached late clinical phases, ivosidenib (Tibsovo) and enasidenib (Idhifa) have become recently FDA-approved for patients with relapsed/refractory or recurrent acute myeloid leukemia (AML) with a mutation in isocitrate dehydrogenases 1 (*IDH1*) and 2 (*IDH2*) metabolic genes, respectively. Mutated forms of these metabolic enzymes actually act as oncogenic drivers in AML [140]. Almost ironically, these strategies can thus be classified as targeted therapies and although they represent for some patients a real hope of cure, such metabolism-related precision medicine will face the same obstacles as those enumerated in the introduction of this review such as high cost and limited applicability to a small subset of patients but also

importantly, an intrinsic high risk of acquired resistance and relapse. Indeed, the enthusiasm of the oncology community has been recently dampened by the identification of second-site mutations, in the *IDH2* gene, that confer acquired resistance to enasidenib [141].

Finally, relevant patient-based (pre)clinical models are absolutely required to integrate the genomic landscape of the tumor and the influence of TME in order to better appreciate intratumoral phenotypic heterogeneity and to identify metabolism-related synthetic lethality that may help overcome therapy resistance in cancer patients.

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FIGURE LEGENDS

Figure 1: Intratumoral heterogeneity and therapy resistance. Besides the contribution of multi-lineage differentiation potential and genetic instability of cancer cells, local TME peculiarities such as hypoxia, acidosis and nutrient deprivation actively contribute to intratumoral phenotypic (genotypic and metabolic) heterogeneity, thereby supporting therapy resistance, minimal residual disease, and long-term clinical relapse.

Figure 2: Strategies to integrate tumor metabolic profiling in precision oncology. Tumor specimens obtained by biopsy are subjected to clinical and histological examination as well as NGS-based genomic analysis in order to identify potential targetable genomic alterations. *In vivo* metabolic profiling can also be carried out upon infusion of ^{13}C tracers and subsequent NMR spectroscopy analysis. Patient-derived tumor organoids are also established in order to perform high-throughput screening for a variety of anticancer drugs, including metabolism-interfering compounds.

Figure 3: Metabolism inhibition to overcome resistance to targeted therapies. (**A**) Anti-EGFR therapies (*e.g*. cetuximab, panitumumab) block the activation of the receptor and associated downstream signaling pathways (*e.g.* Ras and PI3K pathways), resulting in tumor growth inhibitory effects in mCRC patients. (**B**) Intrinsic activating mutations in key effectors of EGFR signaling (*e.g*. mutated Ras denoted as Ras*) bypass anti-EGFR therapy and lead to *de novo* drug resistance. (**C**) Metabolism inhibition may oppose Ras*-driven resistance and restore anti-tumor effects. (**D**) One given metabolism-targeting therapeutic strategy has the potential to increase the efficacy of various targeted therapies, regardless of the nature of genetic alterations that contribute to resistance.

Table 1. OXPHOS dependence in therapy-resistant cancers and associated metabolism-interfering therapeutic modalities. For different therapy-resistant cancers, are indicated the biological model(s) used in the study, the metabolic phenotype of resistant cancer cells and therapeutic strategies used to interfere with the OXPHOS dependence; inhibitors are indicated between brackets. ETC; Electron transport chain; MPC: Mitochondrial pyruvate carrier; PC: Pyruvate carboxylase; PDX: Patient-derived xenograft; TKI: Tyrosine kinase inhibitor.

Cancer	Subtype	Biological model	Therapy resistance	Metabolic rewiring	Metabolism-interfering therapeutic modalities	Ref
AML	/	PDX + human AML cell lines	cytarabine	-OXPHOS gene signature -increased FAO, ROS levels, mito mass	-mito protein synthesis inhib. (tigecycline, ethidium bromide) -ETC complex activity inhib. (rotenone, phenformin and metformin for complex I; antimycin A and atovaquone for complex III) -CPT1 inhib. (etomoxir)	[90]
AML	Relapsed/ refractory AML	Human primary AML samples; PDX and cell lines	standard-of-care treatment in AML	-High OXPHOS	-complex I inhib. (IACS-010759)	[93]
AML/ CML	Sca-1$^+$/Lin$^-$ stem cells	murine model of blast crisis CML; human primary bcCML and AML samples; xenografts	cytarabine, doxorubicin, etoposide, SN-38, irinotecan, dasatinib	-Increased FAO and CD36 expression	-CPT1 inhib. (etomoxir) -CD36 inhib. (SSO) -Glycolysis inhib. (2-DG) -complex I inhib. (rotenone)	[95]
CML	Stem cell–enriched CD34$^+$ cells	Human primary CML samples	imatinib (TKI)	-Increased FAO, PC activity and mito respiration	-inhib of mito protein synthesis (tigecycline) -complex I inhib (phenformin)	[91]
Breast cancer	/	Py2T cell line from *MMTV-PyMT* transgenic mouse breast cancer model	anti-angiogenics (nintedanib)	-Lactate-based metabolic symbiosis (increased GLUT1 and MCT4 expression in hypoxic tumor areas)	-glycolysis inhib. (PFKFB3 inhib. 3PO) -MCT4 genetic knockout	[66]
Breast cancer	Triple negative breast cancers (TNBC)	Human cell lines and xenografts	PI3K/mTOR targeted therapies	-ERRα-dependent lactate metabolism (+ glutamine metabo)	-ERRα antagonists (cpd 29 + XCT790) -complex I inhib. (metformin) -MPC inhibition (UK5099)	[69]

Breast cancer	$CD44^+/CD24^-$ stem cells (TNBC)	Human cell lines + *MMTV-PyMT* mice	paclitaxel	-JAK/STAT3-induced FAO	CPT1 inhib. (etomoxir, perhexiline)	[94]
Breast cancer	$ALDH^+$ stem cells (TNBC)	Human primary tumor samples + cell lines	paclitaxel	-co-amplification of *MYC* and *MCL1* genes -MYC-dependent mito biogenesis -increased mito respiration (FAO) and ROS levels	-complex I inhib. (metformin) -CPT1 inhib. (etomoxir) -HIF1α translation inhib. (digoxin)	[92]
Pancreatic cancer	Pancreatic neuroendocrine tumors (PanNET)	RIP1Tag2 transgenic mice + mouse PanNET cell lines	anti-angiogenics (sunitinib, axitinib)	Lactate-based metabolic symbiosis: -compartmentalized expression of MCT1 in normoxic tumor cells and MCT4 and GLUT1 in hypoxic tumor cells -Increased mTOR signaling	-lactate uptake inhib. (CHC, 7ACC2) -glutaminase and alanine aminotransferase inhib. (DON, AOA) -mTOR signaling inhib. (rapamycin)	[65]
Pancreatic cancer	$KRAS^{G12D}$-driven PDAC; $CD133^+/CD44^{high}$ stem cells	inducible mouse model of mutated $KRAS^{G12D}$; PDX	MEK inhib. (AZD8330); PI3K/mTOR inhib (BEZ235)	-Increased OXPHOS gene signature -Increased mito activity (FAO)	ATP synthase inhib. (oligomycin) CPT1 inhib. (etomoxir)	[97]
Pancreatic cancer	$CD133^+$ cancer stem cells	PDX	/	High mitochondrial respiration and biogenesis in a MYC/PGC1alpha-dependent manner	-complex I inhib. (metformin, rotenone) -mito ROS induction (menadione) -ATP synthase inhib. (resveratrol)	[100]
Renal cell carcinoma (RCC)	/	PDX	anti-angiogenics (sunitinib)	Lactate-based metabolic symbiosis: -compartmentalized expression of MCT1 in normoxic tumor cells and MCT4 and GLUT1 in hypoxic tumor cells -Increased mTOR signaling	- mTOR signaling inhib. (everolimus)	[67]
NSCLC	MET- and EGFR-addicted tumors	Cell lines and xenografts + patient tumor samples	MET inhib. (JNJ-605, crizotinib); EGFR inhib. (erlotinib)	Lactate-based symbiosis between tumor cells (increased GLUT1 and MCT4) and CAFs (HGF secretion)	-pharmacological and genetic inhibition of MCT1 (AZD3965) and LDH (NHI-Glc-2)	[70]
Melanoma	$BRAF^{V600E}$-driven melanoma	Human cell lines + primary human melanoma	BRAF inhib. (vemurafenib and PLX4720)	-Increased OXPHOS gene signature -Increased mito number (mito biogenesis)	-Mitochondrial uncoupling (DNP and CCCP) -OXPHOS inhib. (oligomycin A, rotenone, TTFA)	[98]

		specimens +xenografts				
Melanoma	*BRAF*^{V600E}-driven melanoma	Human cell lines, xenografts and *BRAF*^{V600E}/*PTEN*^{nul}]-driven mouse melanoma model	BRAF inhib. (PLX4720)	Reliance on OXPHOS for cell growth	phenformin	[99]
Melanoma	*BRAF*^{V600E}-driven melanoma	Human cell lines, human primary tumor specimens	BRAF inhib. (RAF265, PLX4720) MEK inhib. (PD0325901; AZD6244)	-High "MitoBiogenesis" gene signature -Increase in mtDNA, mito mass and ROS levels	-mito biogenesis inhib. (gamitrinib) -complex I inhib. (phenformin)	[142]

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

CVL and CC contributed to conception and design of the figures and the manuscript. CC developed the study concept, obtained funding and wrote the final version of the manuscript. All authors wrote sections, revised, read and approved the submitted version.

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Reconciling environment-mediated metabolic heterogeneity with the oncogene-driven cancer paradigm in precision oncology

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Invited review for the Special Issue on "**Mitochondrial metabolic alterations in cancer cells and related therapeutic targets**" in Seminars in Cell and Developmental Biology

ABSTRACT

Precision oncology is the practice of matching one therapy to one specific patient, based on particular genetic tumor alterations, in order to achieve the best clinical response. Despite an expanding arsenal of targeted therapies, many patients still have a poor outcome because tumor cells show a remarkable capacity to develop drug resistance, thereby leading to tumor relapse. Besides genotype-driven resistance mechanisms, tumor microenvironment (TME) peculiarities strongly contribute to generate an intratumoral phenotypic heterogeneity that affects disease progression and treatment outcome. In this Review, we describe how TME-mediated metabolic heterogeneities actively participate to therapeutic failure. We report how a lactate-based metabolic symbiosis acts as a mechanism of adaptive resistance to targeted therapies and we describe the role of mitochondrial metabolism, in particular oxidative phosphorylation (OXPHOS), to support the growth and survival of therapy-resistant tumor cells in a variety of cancers. Finally, we detail potential metabolism-interfering therapeutic strategies aiming to eradicate OXPHOS-dependent relapse-sustaining malignant cells and we discuss relevant (pre)clinical models that may help integrate TME-driven metabolic heterogeneity in precision oncology.

Running title: Targeting tumor metabolism in precision oncology

Keywords: precision oncology; metabolism; cancer, targeted therapy; drug resistance; oxidative phosphorylation; organoid

INTRODUCTION

Despite the early responses generally observed with personalized medicine (precision oncology), prognosis for cancer patients remains very poor due to frequent tumor relapses. Indeed, the clinical efficacy of anti-cancer targeted therapies is strongly limited by the development of acquired drug resistance. Moreover, besides genotype-mediated resistance mechanisms, tumor microenvironment (TME) conditions are increasingly recognized to generate intratumoral phenotypic heterogeneity that may select for pre-resistant cell populations and actively contribute to therapeutic failure and minimal residual disease. Tumor metabolism is the perfect example to illustrate how TME strongly influences cancer cell phenotype and from there, treatment outcome. In this review, we describe the current knowledge about metabolism-based mechanisms of adaptive resistance to targeted therapies. More precisely, we report the role of lactate-based metabolic symbiosis, as an example of non-cell autonomous mechanism of resistance, and the reliance of many therapy-resistant cancer types and/or subpopulations on mitochondrial metabolism, in particular oxidative phosphorylation (OXPHOS). We also discuss potential therapeutic strategies that may enable eradicate OXPHOS-dependent relapse-sustaining cancer cells. This review finally explores relevant (pre)clinical models that may help integrate TME-driven metabolic heterogeneity in precision oncology.

1. Precision oncology, more than a clinical illusion?

In the current era of precision oncology, clinicians aim to exploit patient-specific molecular alterations (*e.g.* gene mutations or amplifications, genomic rearrangements) to identify treatments (the so-called targeted therapies) with the greatest probability of clinical benefit. Next-generation sequencing technology has indeed emerged to allow low-cost, quick and sensitive high-throughput analysis of individual genetic variability and it is now adopted for routine clinical use to diagnose and orientate selection of tailored therapeutic options [1].

Such genomic testing enabled to highlight the importance of "driver" somatic alterations that activate crucial oncogenes (*e.g*. *RAS*, *EGFR*, *BRAF*, *BCR-ABL1*), giving rise to a pivotal tumor dependency (*i.e.* oncogene addiction) [2]. Over the years, the number of cancer-associated genetic alterations has rapidly increased, leading to an expansion in the oncology pipeline for drugs targeting these specific molecular characteristics. From November 2016 to October 2017, the US Food and Drug Administration (FDA) approved more than 30 new cancer therapies, with nearly half of them for the treatment of patients with cancers harboring particular sequence alterations [3]. Among them, pembrolizumab (Keytruda®), a monoclonal antibody targeting programmed death-1 (PD-1), became the first therapy being FDA-approved for cancer patients based on a common biomarker (*i.e.* deficient DNA mismatch repair or high degree of microsatellite instability) rather than tumor site or histology (the so-called tumor-agnostic therapy, an approach tested in "basket" trials) [4]. Still, the number of targetable alterations keeps expanding, making very challenging the application of personalized medicine in daily oncology practices. The broader arsenal of (targeted) therapies constantly requires new approaches to provide clinical interpretations of data from large-scale genetic testing [5]. Even more concerning is the large number of patients (>75%) that still do not benefit from precision oncology because genetic profiling fails to pair them with a targeted therapy [6-9]. The early success of imatinib in patients with *BCR-ABL1*-driven chronic myeloid leukemia (CML) generated a great deal of optimism for both patients and clinicians but in reality, the efficacy of this agent must be seen as an exception rather than a rule [10]. Indeed, when considering all advanced cancer types, the clinical benefit of being assigned a targeted therapy is still limited to about 30% of patients and the median progression-free survival (PFS) does not exceed 6 months [11].

Genetic alterations, associated with primary and acquired resistance to targeted therapies, are very diverse and heterogeneous within a single tumor, thereby limiting the clinical

applicability of such therapies. In this case, resistance largely arises from cell-autonomous mechanisms as the treatment often selects for pre-existing clones bearing genetic alterations either in the target itself or in downstream/parallel signaling pathways that may compensate for the drug-induced inhibitory effects. For example, although epidermal growth factor receptor (EGFR) is (over)expressed in about 85% of patients with metastatic colorectal cancers (mCRC), the clinical efficacy of EGFR-targeting monoclonal antibodies, cetuximab and panitumumab, is restricted to a subset of patients (<25%) [12]. Indeed, activating mutations within *KRAS*, *NRAS* and *BRAF* genes have been significantly associated with lack of response to cetuximab or panitumumab therapy in patients with mCRC [13-18]. More recently, many other gene alterations (*e.g*. *MET* or *ERBB2* amplification, *PIK3CA* activating mutations, *PTEN* loss-of-function mutations) have been reported as potential mechanisms of primary resistance to anti-EGFR antibody therapy [19, 20].

Altogether, these observations point to a high degree of genetic heterogeneity as a major contributor to therapy resistance and underscore the complexity of genomic profiling for clinical decision-making. Moreover, current limitations in precision oncology largely arise from the strictly genome-based tumor characterization, while ignoring the adaptive landscape. For instance, the maximum-tolerated dose-based therapy aims to kill the greatest number of cancer cells but, at the same time, is thought to select for resistant phenotypes and to eliminate all potentially competing populations (the so-called “competitive release”) [21, 22]. Exploiting environmental selection forces and/or targeting associated phenotypic changes appear therefore as promising strategies to help prevent outgrowth of resistant cancer cell populations, and by doing so, improve patient outcome.

2. TME-driven metabolic heterogeneity and therapy resistance

Therapy resistance is associated with the existence of specific local microenvironments (the so-called niches) that can act as selection forces to promote intrinsic diversity and phenotypic plasticity and help tumor cells adapt and survive anti-cancer therapies [22-24]. Along cancer progression, both at primary and distant sites, specific TME conditions, such as hypoxia (low pO_2), acidosis (low pH) or nutrient deprivation, are indeed high selection barriers and impose profound constraints that cancer cells must face in order to survive and grow [25-28]. They can promote the emergence of a "mutator phenotype" [29], as reported, for instance, with glucose deprivation acting as a strong selection pressure for mutated *KRAS* in CRC cells [30]. Nevertheless, TME peculiarities can also drive the emergence and/or acquisition of phenotypic traits (*e.g*. resistance to apoptosis, low proliferation/quiescence, immune surveillance escape, invasion and metabolic reprogramming), that contribute to therapy resistance, minimal residual disease and poor outcome in cancer patients, irrespectively of the occurrence of genetic alterations.

2.1 TME peculiarities and associated metabolic preferences

Within the tumors, the limited access to nutrients and/or oxygen, the local accumulation of protons, together with the presence of tumor-associated stroma cells generate a metabolic heterogeneity [31-33]. Thus, cancer cells can use a variety of substrates to fulfill their need in energy and/or biosynthetic precursors according to the local TME [34-36]. Beyond the well-known Warburg effect (*i.e*. glucose-to-lactate conversion regardless oxygen levels), changes in amino acid metabolism have been observed to support cancer cell growth. Some highly proliferating cancer types for instance rely more on the use of non-essential amino acids (*e.g*. aspartate, glycine, glutamine, and serine) to support the bioenergetics and macromolecular synthesis [37-42]. A variety of cancers is also capable of capturing and metabolizing

exogenous acetate to maintain cancer cell growth under low oxygen and lipid-depleted conditions [43-45]. In addition, exogenous fatty acids also represent a valuable source of carbon, via acetyl-CoA generation, for energy production but also biosynthesis of membranes and signaling molecules that help sustain cancer cell proliferation [46].

Such metabolic heterogeneity offers cancer cells with multiple adaptation and escape options when facing hostile conditions including drug-induced stress, through both cell-autonomous and non-cell-autonomous mechanisms. However, although there are numerous examples of intimate relationship between microenvironmental niches and cancer cell metabolic preferences [47, 48], evidence for a straightforward contribution of this interplay to therapy resistance only begins to be explored [49, 50]. TME is indeed most often associated to drug resistance through its physico-chemical components. Hypoxia, for instance, is well known to be associated with multiple pathways accounting for drug resistance (*e.g*. genomic instability, cell cycle arrest or apoptosis inhibition) [51]. Acidosis also participates to drug resistance by affecting the permeability of charged compounds through the cellular membrane (ion trapping phenomenon) [31, 52]. Nevertheless, some insights on a role of metabolism in TME-dependent dynamic regulation of therapy resistance can be found in the literature.

2.2 Lactate-based metabolic symbiosis as an adaptive mechanism of resistance to anticancer targeted therapies

Microenvironment-driven metabolic heterogeneity, within a tumor, is well exemplified by the occurrence of a lactate shuttle between hypoxic and oxidative cancer cells [53, 54]. Indeed, cancer cells, that are distant of blood vessels, are poorly oxygenated and hence forced to fulfill their energy needs through glycolysis resulting in the production of lactate. Rather than being a waste metabolite, lactate is secreted out of the cytoplasm and taken up by other tumor cells that are closer to blood vessels and well oxygenated. Instead of glucose, oxidative cancer

cells can utilize lactate as an alternative energy source by conversion into pyruvate which then fuels the tricarboxylic acid (TCA) cycle. The spared glucose can thus be used for anaerobic glycolysis by the poorly oxygenated cancer cells. This lactate-based metabolic symbiosis between lactate-generating and lactate-consuming tumor cells has been reported in various cancer types [55, 56], and shown to involve also cancer-associated fibroblasts (CAFs) and angiogenic endothelial cells [57-60]. More recently, *in vivo* ^{13}C-lactate tracing experiments revealed extensive labelling of TCA cycle intermediates in human patients and genetically engineered mouse models with non-small-cell lung cancers (NSCLC) [48, 61, 62]. These studies have documented that circulating (and not only tumor-derived) lactate may be captured and oxidized in cancer cells and importantly that the contribution of lactate to TCA cycle intermediates could be greater than that of glucose [61, 62], thereby corroborating a straightforward link between lactate metabolism and mitochondrial activity in high-proliferating cancer cells [63].

Lactate transport across the plasma membrane is facilitated by monocarboxylate transporters (MCT) and is coupled to the symport of protons. The high-affinity transporter MCT1 is the main entry path for lactate in oxidative cancer cells and tumor-associated endothelial cells while hypoxia-induced MCT4 has a low affinity for lactate, making it the *bona fide* transporter for lactate export in glycolytic tumor cells and tumor-associated cells [53, 64].

In recent studies, a lactate-based metabolic symbiosis has been described to strongly support adaptive resistance to anti-angiogenic therapies in pancreatic neuroendocrine tumors [65], murine breast cancer models [66], patient-derived renal cell carcinoma (RCC) orthoxenograft (PDX) models and in clinical human RCC samples [67]. Indeed, after an initial regression, murine and human tumors, treated with antiangiogenics, resume growth in the absence of active neo-vascularization by exhibiting a compartmentalized expression of MCT1 and MCT4 whereby hypoxic cancer cells import glucose and export lactate, while normoxic cells import

and catabolize lactate. Those studies have also reported that inhibition of glycolysis upon 3-PO treatment or genetic ablation of MCT4 expression [66], as well as mTOR signaling blockade [65, 67] can disrupt this metabolic symbiosis and overcome the resistance development to anti-angiogenic therapy in patients (Table 1).

Metabolic symbiosis has also been reported in breast cancer, with lactate acting as an energetic fuel for tumor cells in a MCT1-dependent manner [68, 69]. More precisely, triple-negative breast cancer (TNBC) cells have been found to use lactate as a primary source of energy, allowing them to survive glucose deprivation for extended periods [69]. This metabolic adaptation is mediated by the estrogen-related receptor alpha (ERRα) nuclear receptor and helps cancer cells resist to anti-PI3K/mTOR targeted therapies. Blockade of lactate oxidation capacities upon genetic or pharmacological inhibition of ERRα activity sensitizes breast cancer cells to PI3K/mTOR inhibitors both *in vitro* and *in vivo* [69]. A recent study has also shown a role for lactate metabolism in the resistance to MET and EGFR tyrosine kinase inhibitors (TKI) (JNJ-605, crizotinib and erlotinib, respectively) [70]. The authors have actually observed an exacerbated glycolytic metabolism, with an increased lactate release, in patient-derived NSCLC and gastric cancer cells upon continuous and prolonged treatment with MET or EGFR TKI. Secreted lactate can act as a signalling molecule instructing CAFs to overproduce hepatocyte growth factor (HGF) which, in turn, activates MET in cancer cells, thereby overcoming TKI inhibitory effects. Importantly, pharmacological inhibition or genetic knockdown of lactate metabolism-related enzymes/transporters (*e.g.* lactate dehydrogenases, MCT1, MCT4) is sufficient to overcome resistance. Altogether, these observations support the relevance of such metabolic symbiosis in therapy failure and the potential clinical utility of symbiosis-interfering therapeutic modalities [71, 72]. More generally, these studies pinpoint the metabolic plasticity as an effective strategy exploited by cancer cells to counteract environmental- or drug-induced

stresses [72], and open new therapeutic perspectives to prevent/overcome resistance onset, with for instance novel combinatorial treatments, simultaneously targeting (lactate) metabolism and driver oncogene.

2.3 Cancer cell oxidative metabolism and therapy resistance

Beyond the "Warburg effect" dogma, there is now increasing experimental evidence in the literature for a direct contribution of oxidative mitochondrial metabolism in cancer progression and response to (targeted) therapies. For instance, cancer cells deficient in OXPHOS due to mitochondrial DNA (mtDNA) depletion (ρ0 cells) do not form tumors *in vivo* [73-75], while a low mtDNA content correlates with a better outcome in breast cancer patients treated with anthracycline-based chemotherapy [76]. Moreover, mtDNA mutations have been reported to increase the tumorigenic potential of prostate cancer cells [77] and to alter the response to treatments in a variety of tumor types [78-80]. Indeed, mutations in the non-coding mtDNA controlling region (*e.g.* displacement loop) have been associated with chemoresistance in colorectal cancer patients [81] and a somatic mutation in ND4 complex I subunit correlates with resistance to chemotherapy in serous ovarian cancers [82].

Genetic, cell lineage and microenvironment factors determine the relative contribution of, and dependence on OXPHOS (*vs* glycolysis) in tumorigenesis. Indeed, lung cancer cells harboring mutations in the SWI/SNF complex (*e.g. SMARCA4* and *ARID1A* gene mutations) [83] or in *KRAS* oncogene [47] depend on OXPHOS for their growth and survival. A similar OXPHOS dependence has been reported, among others, in a subpopulation of glioblastomas with homozygous deletion of enolase 1 [84, 85], in TNBC patients with low expression of the BACH transcription factor [86] as well as in cancer cells exposed to an acidic microenvironment [87, 88].

For a variety of cancers, therapy-resistant tumor cells, including the so-called cancer stem cells, have been also found to mostly rely on mitochondrial metabolism, in particular OXPHOS [89-95] (Table 1). Several studies have actually shown that therapy-resistant acute myeloid leukemia (AML) cells exhibit metabolic features and gene signatures consistent with a high OXPHOS status necessary to support their growth [90, 95, 96]. Quiescent human AML stem-like cells, responsible for tumor resistance, can be targeted through their high dependency to OXPHOS, either by inhibiting the expression of *BCL2* oncogene [96] or by interfering with the oxidation of adipose tissue-derived fatty acids [95]. Recently, a study has shown that therapy-resistant CML stem cells mostly rely on upregulated oxidative metabolism for their survival [91]. Combinatory treatment with imatinib and tigecycline, a FDA-approved mitochondrial translation inhibitor, is able to overcome resistance by eradicating the stem cell-enriched populations ($CD34^+$ and $CD34^+CD38^-$). In *KRAS*-driven pancreatic ductal adenocarcinoma (PDAC), genetic deletion of the $KRAS^{G12D}$ allele leads to a dramatic tumor regression, but a small subpopulation of cells resists and eventually contributes to tumor relapse [97]. Importantly, these resilient cells rely on OXPHOS for survival (unlike the tumor bulk that is mainly glycolytic) and inhibitors of oxidative metabolism can impair tumor relapse when $KRAS^{G12D}$ is re-expressed [97]. In $BRAF^{V600E}$-mutant melanomas, BRAF inhibitors have been reported to induce PGC1α, a regulator of mitochondrial biogenesis, thereby causing OXPHOS dependence [98]. Consequently, BRAF inhibitors synergize with phenformin, a complex I inhibitor, 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 [99]. Of note, cancer stem cells of human pancreatic cancer-derived xenografts, responsible for tumor resistance, have been also found to rely on OXPHOS [100] (Table 1).

Besides a direct role in tumor cells, compelling evidence in the literature suggests that OXPHOS inhibition may also reduce tumor growth and improve drug response by enhancing

anti-tumor immunity. Indeed, while anti-tumor cytotoxic and effector T cells require glycolysis to proliferate and produce cytokines [101-104], immune-suppressive cell types such as regulatory T (Treg) cells in the TME have been reported to heavily rely on OXPHOS for their energy production [105-107]. Biguanides (*e.g*. metformin, phenformin) are known (albeit weakly potent) complex I inhibitors, and are safely used to treat diabetes. IM188 and IM156 (ImmunoMet) are novel biguanides in late stage lead optimization and have been selected, on the basis of the immunomodulatory effect of OXPHOS inhibition, to increase T effector cells while decreasing myeloid derived suppressor cells (MDSC) and Treg cell pools in the TME.

Recent studies have also identified two OXPHOS inhibitors, namely IACS-010759 [93] and gboxin [108], as potent anticancer drugs in various tumor subtypes. IACS-010759 has been reported as a selective inhibitor of the ND1 subunit of complex I of the electron transport chain (ETC) and shown to preferentially inhibit tumor growth, both *in vitro* and *in vivo*, of certain "OXPHOS-dependent" tumor types such as chemo-resistant AML, glycolysis-deficient glioma, SWI/SNF mutant lung cancer and brain metastasis in melanoma [83, 93, 109]. This compound is currently being evaluated in phase 1 clinical trials in relapsed/refractory AML and solid tumors (NCT02882321 and NCT03291938, respectively). In another study, gboxin has been found to specifically inhibit the growth of primary mouse and human glioblastoma stem-like cells while not affecting the viability of mouse embryonic fibroblasts or neonatal astrocytes. Gboxin reduces OXPHOS by interacting with respiratory chain proteins and inhibiting the ATP synthase activity [108].

Importantly, OXPHOS-interfering compounds have shown yet major caveats for their clinical application, including low efficiency (*e.g*. biguanides), detrimental "off-target" effects (*e.g*. rotenone) or a limited pharmacokinetic profile (*e.g*. oligomycin), thereby limiting their use as anticancer drugs [110]. Furthermore, BAY87-2243, a complex I inhibitor, has been reported

to alleviate hypoxia and improved radiation response without toxicity in mice, but the initial phase I trial had to be terminated due to unexpected toxicity issues [111]. Nevertheless, the recent development and characterization of IACS-010759 and gboxin provide rationale that targeting OXPHOS may be done safely (*i.e.* with compounds showing a high therapeutic index) and thus reconsiders it as a very attractive anti-cancer strategy, in particular for therapy-refractory cancer patients. It is also noteworthy that, besides triggering direct anti-tumor effects, inhibition of mitochondrial metabolism may sensitize tumors to radiation therapy (via tumor reoxygenation), as recently reported with 7ACC2, an inhibitor of the mitochondrial pyruvate complex that blocks both lactate- and glucose-fueled TCA cycle [71].

3. Relevant (pre)clinical models to integrate TME-driven metabolic heterogeneity in precision oncology

Environment-mediated therapy-resistant phenotype of cancer cells evolves *de facto* with time and tumor development. Relevant (pre)clinical models are therefore needed to integrate the influence of not only the driver oncogene(s), cell lineage, tissue of origin and/or the type of therapy applied but also TME peculiarities and to further explore the intimate relationship between TME, cancer cell metabolic preferences and drug response (Figure 1). Although the most accurate way to study environment-mediated metabolic alterations is by using orthotopic *in vivo* models, the complexity of such systems limits experimental control and potential use for high-throughput drug screening [112]. To date, the most prevalent human-derived cancer models used in drug-testing studies are cell lines and patient-derived xenografts (PDX). These two approaches have however intrinsic limitations. For cell lines, culture conditions (*e.g.* nutrient, oxygen and pH levels) are usually far from those existing *in vivo* and when TME is part of the equation, all cancer cells usually undergo exposure to the same microenvironmental tumor peculiarities in 2D culture conditions (*i.e.* cancer cell monolayers),

still making the model poorly representative of tumor heterogeneity. Also, long-term interactions between different cell populations growing together (as observed with most tumors) cannot be easily evaluated. Here, we describe the *in vivo* infusion of ^{13}C tracers and the generation of tumor organoids as two experimental patient-based approaches that can represent the physiological complexity and heterogeneity of cells observed in human cancers, and integrate the influence of both genetic pattern and TME on cancer cell metabolic preferences and response to therapies (Figure 1).

3.1 *In vivo* infusion of ^{13}C tracers

Stable isotope-based metabolic analysis can be used to evaluate how cellular metabolism, in particular the fate of biosynthetic fuels (*e.g.* glucose, glutamine, lactate), is altered during cancer progression. In the last decade, this approach has been increasingly used to analyze metabolism *in vivo*, in various mouse tumor models [47, 62, 113, 114] but also in human cancer patients [48, 61, 115-118]. Stable tracers (*i.e.* ^{13}C-glucose and other isotopically labeled nutrients) are administered as a bolus followed by an intra-operative infusion, through a peripheral intravenous line, before surgical tumor resection and analysis of isotope enrichment downstream of labeled nutrients via ^{13}C NMR spectroscopy [119]. By infusing [U-^{13}C]glucose in patients, several recent studies have actually revealed a metabolic heterogeneity depending on the tumor type. Indeed, some tumors, such as clear cell renal cell carcinoma (ccRCC), have been found to be highly glycolytic, with pyruvate being converted into lactate rather than entering the mitochondrial TCA cycle, even in the presence of oxygen (the so-called Warburg effect) [115]. On the other hand, a clear demonstration of glucose consumption through mitochondrial oxidative metabolism has been reported over the last years in multiple cancer types, including glioma, brain metastases and NSCLC [47, 48, 116-118]. Indeed, infusion of [U-^{13}C]glucose in patients with FDG-PET-positive tumors in the

brain and lungs reveals robust glucose oxidation, even in excess of adjacent tissue [48, 118]. These reports indicate an uncoupling between high rates of glucose uptake and suppressed glucose oxidation, suggesting that the TCA cycle retains its ability to produce energy via oxidative metabolism in aggressive tumors. Moreover, less than 50% of the acetyl-CoA pool has been found to derive from blood-borne glucose in human brain tumors [117], suggesting that additional substrates contribute to tumor bioenergetics. Other studies have indeed highlighted the role of alternative respiratory fuels, including acetate and lactate, in complementing glucose oxidation to support biomass and energy production in living tumors [44, 48, 61, 62]. These authors have also reported that contribution of lactate and glutamine to the TCA cycle was even greater than that of glucose in lung and pancreatic cancers, respectively [62]. Interestingly, the metabolic profile of tumors has been found to be dependent on both the genotype and tissue of origin. For instance, *MYC*-driven liver and triple-negative breast cancers are more glutamine-dependent than *MET*-driven liver and *MYC*-driven lung tumors [114, 120, 121]. *In vivo* ^{13}C-based metabolic profiling also allowed to evidence the important role of pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) to support tumor growth in human lung cancer patients [47, 116, 118], making them attractive therapeutic targets in this specific tumor type.

However, interpretation of such stable isotope-based metabolic analysis is complicated, since stromal cells present in the collected tumor fragments may contribute to the labelling patterns. Moreover, occlusion of afferent arteries, prior to surgical resection, could temporarily impact metabolism and ^{13}C labeling and lead to a misinterpretation of the preferential use of ^{13}C-labeled nutrients in tumors of cancer patients. Besides these limitations, such *in vivo* metabolomic studies underscore the important contribution of mitochondrial oxidative metabolism in tumors and position it as an attractive target for the design of further

therapeutic strategies aiming to interfere with cancer progression and overcome therapy resistance.

3.2 Patient-derived tumor organoids

To better integrate the influence of TME conditions on cancer cell phenotype, including response to therapies, tumor cells can be cultured in a matrix that allows 3D growth as organoids. In the last decade, patient-derived tumor organoids (PDTO) have been established for a variety of cancers, including breast [122], bladder [123], colorectal [124], liver [125], pancreatic [126], gastrointestinal [127, 128], glioblastoma [129], retinoblastoma [130] and prostate cancers [131]. In this method, tumor cells are commonly embedded in an extracellular matrix (*e.g.* Matrigel) and cultured in a specific medium containing a defined combination of stem cell niche factors (*e.g.* R-spondin-1, EGF, Wnt-3a and noggin). These adequate culturing conditions influence the spatial organization of cell surface receptors and induce physical constraints on tumor cells, thereby modifying both their morphology and their phenotype (*vs* 2D monolayer cultures) through changes in gene expression [132]. Importantly, PDTO recapitulate the histological and genetic features of the primary tumor and even retain physico-chemical characteristics of TME, such as hypoxic gradients [133, 134]. In some cases, they have even been able to predict tumor drug responses *in vivo* [123, 127], making them amenable tools for high-throughput drug screening [135].

Although the majority of PDTO models reported so far still lack the cell and matrix diversity found within the TME, recent studies have reported new modalities of PDTO generation (*e.g.* air-liquid-interface; ALI) to preserve stromal cells such as CAFs and even functional immune cells [136-138], together with cancer cells (instead of the more usual strictly cancer stem cell-based organotypic structures). Another limitation of such 3D culture models is their reliance on defined medium conditions that might not represent nutrient levels experienced by cells in

tumors. Finally, PDTO are tractable and can be studied through both pharmacological and genetic screening approaches [139], thereby offering exquisite models to finely dissect how TME peculiarities and therapies influence the metabolic phenotype of cancer cells in a relevant physical environment (Figure 2).

Concluding remarks

While it would be unfair to condemn precision oncology because of the limited success of a number of clinical trials (largely due to the amazing diversity of resistance-associated genomic landscapes cancer patients), the current record of partial failure also highlights the urgent need to implement fundamental changes in the tumor treatment paradigm. Focusing on phenotype-driven rather than genotype-mediated drug resistance mechanisms may help break with the usual concept of targeted therapies in order to provide cancer patients with a new mode of precision medicine in which the identification of biological (metabolic) pathways, shared by tumors exhibiting multidrug resistance, could offer a "one-size-fits-all" approach (Figure 3).

Importantly, among the handful of metabolism-modulating drug candidates that have reached late clinical phases, ivosidenib (Tibsovo) and enasidenib (Idhifa) have become recently FDA-approved for patients with relapsed/refractory or recurrent acute myeloid leukemia (AML) with a mutation in isocitrate dehydrogenases 1 (*IDH1*) and 2 (*IDH2*) metabolic genes, respectively. Mutated forms of these metabolic enzymes actually act as oncogenic drivers in AML [140]. Almost ironically, these strategies can thus be classified as targeted therapies and although they represent for some patients a real hope of cure, such metabolism-related precision medicine will face the same obstacles as those enumerated in the introduction of this review such as high cost and limited applicability to a small subset of patients but also importantly, an intrinsic high risk of acquired resistance and relapse. Indeed, the enthusiasm

414 of the oncology community has been recently dampened by the identification of second-site
415 mutations, in the *IDH2* gene, that confer acquired resistance to enasidenib [141].
416 Finally, relevant patient-based (pre)clinical models are absolutely required to integrate the
417 genomic landscape of the tumor and the influence of TME in order to better appreciate
418 intratumoral phenotypic heterogeneity and to identify metabolism-related synthetic lethality
419 that may help overcome therapy resistance in cancer patients.
420

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FIGURE LEGENDS

Figure 1: Intratumoral heterogeneity and therapy resistance. Besides the contribution of multi-lineage differentiation potential and genetic instability of cancer cells, local TME peculiarities such as hypoxia, acidosis and nutrient deprivation actively contribute to intratumoral phenotypic (genotypic and metabolic) heterogeneity, thereby supporting therapy resistance, minimal residual disease, and long-term clinical relapse.

Figure 2: Strategies to integrate tumor metabolic profiling in precision oncology. Tumor specimens obtained by biopsy are subjected to clinical and histological examination as well as NGS-based genomic analysis in order to identify potential targetable genomic alterations. *In vivo* metabolic profiling can also be carried out upon infusion of ^{13}C tracers and subsequent NMR spectroscopy analysis. Patient-derived tumor organoids are also established in order to perform high-throughput screening for a variety of anticancer drugs, including metabolism-interfering compounds.

Figure 3: Metabolism inhibition to overcome resistance to targeted therapies. (**A**) Anti-EGFR therapies (*e.g*. cetuximab, panitumumab) block the activation of the receptor and associated downstream signaling pathways (*e.g.* Ras and PI3K pathways), resulting in tumor growth inhibitory effects in mCRC patients. (**B**) Intrinsic activating mutations in key effectors of EGFR signaling (*e.g*. mutated Ras denoted as Ras*) bypass anti-EGFR therapy and lead to *de novo* drug resistance. (**C**) Metabolism inhibition may oppose Ras*-driven resistance and restore anti-tumor effects. (**D**) One given metabolism-targeting therapeutic strategy has the potential to increase the efficacy of various targeted therapies, regardless of the nature of genetic alterations that contribute to resistance.

Table 1. OXPHOS dependence in therapy-resistant cancers and associated metabolism-interfering therapeutic modalities. For different therapy-resistant cancers, are indicated the biological model(s) used in the study, the metabolic phenotype of resistant cancer cells and therapeutic strategies used to interfere with the OXPHOS dependence; inhibitors are indicated between brackets. ETC; Electron transport chain; MPC: Mitochondrial pyruvate carrier; PC: Pyruvate carboxylase; PDX: Patient-derived xenograft; TKI: Tyrosine kinase inhibitor.

Cancer	Subtype	Biological model	Therapy resistance	Metabolic rewiring	Metabolism-interfering therapeutic modalities	Ref
AML	/	PDX + human AML cell lines	cytarabine	-OXPHOS gene signature -increased FAO, ROS levels, mito mass	-mito protein synthesis inhib. (tigecycline, ethidium bromide) -ETC complex activity inhib. (rotenone, phenformin and metformin for complex I; antimycin A and atovaquone for complex III) -CPT1 inhib. (etomoxir)	[90]
AML	Relapsed/ refractory AML	Human primary AML samples; PDX and cell lines	standard-of-care treatment in AML	-High OXPHOS	-complex I inhib. (IACS-010759)	[93]
AML/ CML	Sca-1$^+$/Lin$^-$ stem cells	murine model of blast crisis CML; human primary bcCML and AML samples; xenografts	cytarabine, doxorubicin, etoposide, SN-38, irinotecan, dasatinib	-Increased FAO and CD36 expression	-CPT1 inhib. (etomoxir) -CD36 inhib. (SSO) -Glycolysis inhib. (2-DG) -complex I inhib. (rotenone)	[95]
CML	Stem cell–enriched CD34$^+$ cells	Human primary CML samples	imatinib (TKI)	-Increased FAO, PC activity and mito respiration	-inhib of mito protein synthesis (tigecycline) -complex I inhib (phenformin)	[91]
Breast cancer	/	Py2T cell line from *MMTV-PyMT* transgenic mouse breast cancer model	anti-angiogenics (nintedanib)	-Lactate-based metabolic symbiosis (increased GLUT1 and MCT4 expression in hypoxic tumor areas)	-glycolysis inhib. (PFKFB3 inhib. 3PO) -MCT4 genetic knockout	[66]
Breast cancer	Triple negative breast cancers (TNBC)	Human cell lines and xenografts	PI3K/mTOR targeted therapies	-ERRα-dependent lactate metabolism (+ glutamine metabo)	-ERRα antagonists (cpd 29 + XCT790) -complex I inhib. (metformin) -MPC inhibition (UK5099)	[69]

Breast cancer	$CD44^+/CD24^-$ stem cells (TNBC)	Human cell lines + *MMTV-PyMT* mice	paclitaxel	-JAK/STAT3-induced FAO	CPT1 inhib. (etomoxir, perhexiline)	[94]
Breast cancer	$ALDH^+$ stem cells (TNBC)	Human primary tumor samples + cell lines	paclitaxel	-co-amplification of *MYC* and *MCL1* genes -MYC-dependent mito biogenesis -increased mito respiration (FAO) and ROS levels	-complex I inhib. (metformin) -CPT1 inhib. (etomoxir) -HIF1α translation inhib. (digoxin)	[92]
Pancreatic cancer	Pancreatic neuroendocrine tumors (PanNET)	RIP1Tag2 transgenic mice + mouse PanNET cell lines	anti-angiogenics (sunitinib, axitinib)	Lactate-based metabolic symbiosis: -compartmentalized expression of MCT1 in normoxic tumor cells and MCT4 and GLUT1 in hypoxic tumor cells -Increased mTOR signaling	-lactate uptake inhib. (CHC, 7ACC2) -glutaminase and alanine aminotransferase inhib. (DON, AOA) -mTOR signaling inhib. (rapamycin)	[65]
Pancreatic cancer	$KRAS^{G12D}$-driven PDAC; $CD133^+/CD44^{high}$ stem cells	inducible mouse model of mutated $KRAS^{G12D}$; PDX	MEK inhib. (AZD8330); PI3K/mTOR inhib (BEZ235)	-Increased OXPHOS gene signature -Increased mito activity (FAO)	ATP synthase inhib. (oligomycin) CPT1 inhib. (etomoxir)	[97]
Pancreatic cancer	$CD133^+$ cancer stem cells	PDX	/	High mitochondrial respiration and biogenesis in a MYC/PGC1alpha-dependent manner	-complex I inhib. (metformin, rotenone) -mito ROS induction (menadione) -ATP synthase inhib. (resveratrol)	[100]
Renal cell carcinoma (RCC)	/	PDX	anti-angiogenics (sunitinib)	Lactate-based metabolic symbiosis: -compartmentalized expression of MCT1 in normoxic tumor cells and MCT4 and GLUT1 in hypoxic tumor cells -Increased mTOR signaling	- mTOR signaling inhib. (everolimus)	[67]
NSCLC	MET- and EGFR-addicted tumors	Cell lines and xenografts + patient tumor samples	MET inhib. (JNJ-605, crizotinib); EGFR inhib. (erlotinib)	Lactate-based symbiosis between tumor cells (increased GLUT1 and MCT4) and CAFs (HGF secretion)	-pharmacological and genetic inhibition of MCT1 (AZD3965) and LDH (NHI-Glc-2)	[70]
Melanoma	$BRAF^{V600E}$-driven melanoma	Human cell lines + primary human melanoma	BRAF inhib. (vemurafenib and PLX4720)	-Increased OXPHOS gene signature -Increased mito number (mito biogenesis)	-Mitochondrial uncoupling (DNP and CCCP) -OXPHOS inhib. (oligomycin A, rotenone, TTFA)	[98]

		specimens +xenografts				
Melanoma	*BRAF*^{V600E}-driven melanoma	Human cell lines, xenografts and *BRAF*^{V600E}/*PTEN*^{nul}]-driven mouse melanoma model	BRAF inhib. (PLX4720)	Reliance on OXPHOS for cell growth	phenformin	[99]
Melanoma	*BRAF*^{V600E}-driven melanoma	Human cell lines, human primary tumor specimens	BRAF inhib. (RAF265, PLX4720) MEK inhib. (PD0325901; AZD6244)	-High "MitoBiogenesis" gene signature -Increase in mtDNA, mito mass and ROS levels	-mito biogenesis inhib. (gamitrinib) -complex I inhib. (phenformin)	[142]

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

CVL and CC contributed to conception and design of the figures and the manuscript. CC developed the study concept, obtained funding and wrote the final version of the manuscript. All authors wrote sections, revised, read and approved the submitted version.

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Figure 1

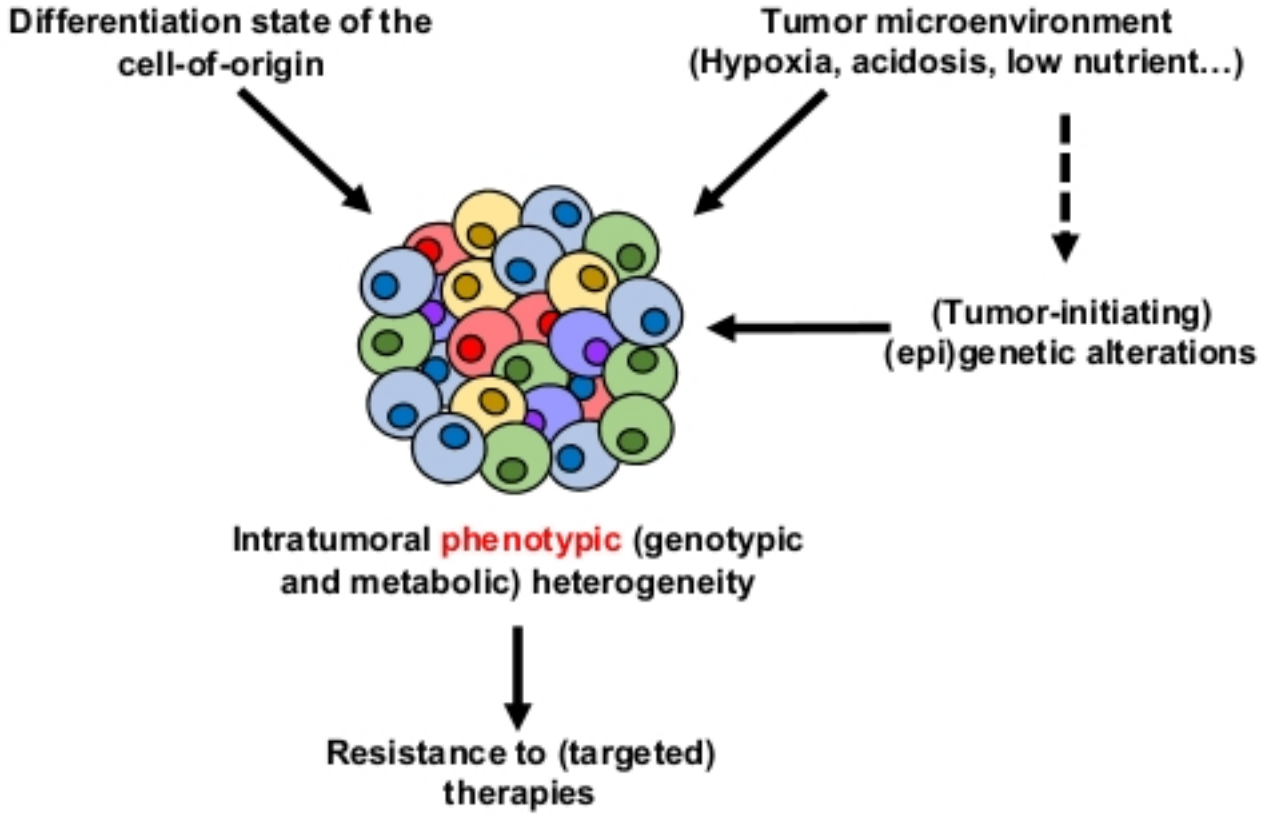

Differentiation state of the cell-of-origin
Tumor microenvironment (Hypoxia, acidosis, low nutrient…)
(Tumor-initiating) (epi)genetic alterations
Intratumoral phenotypic (genotypic and metabolic) heterogeneity
Resistance to (targeted) therapies

Figure 2

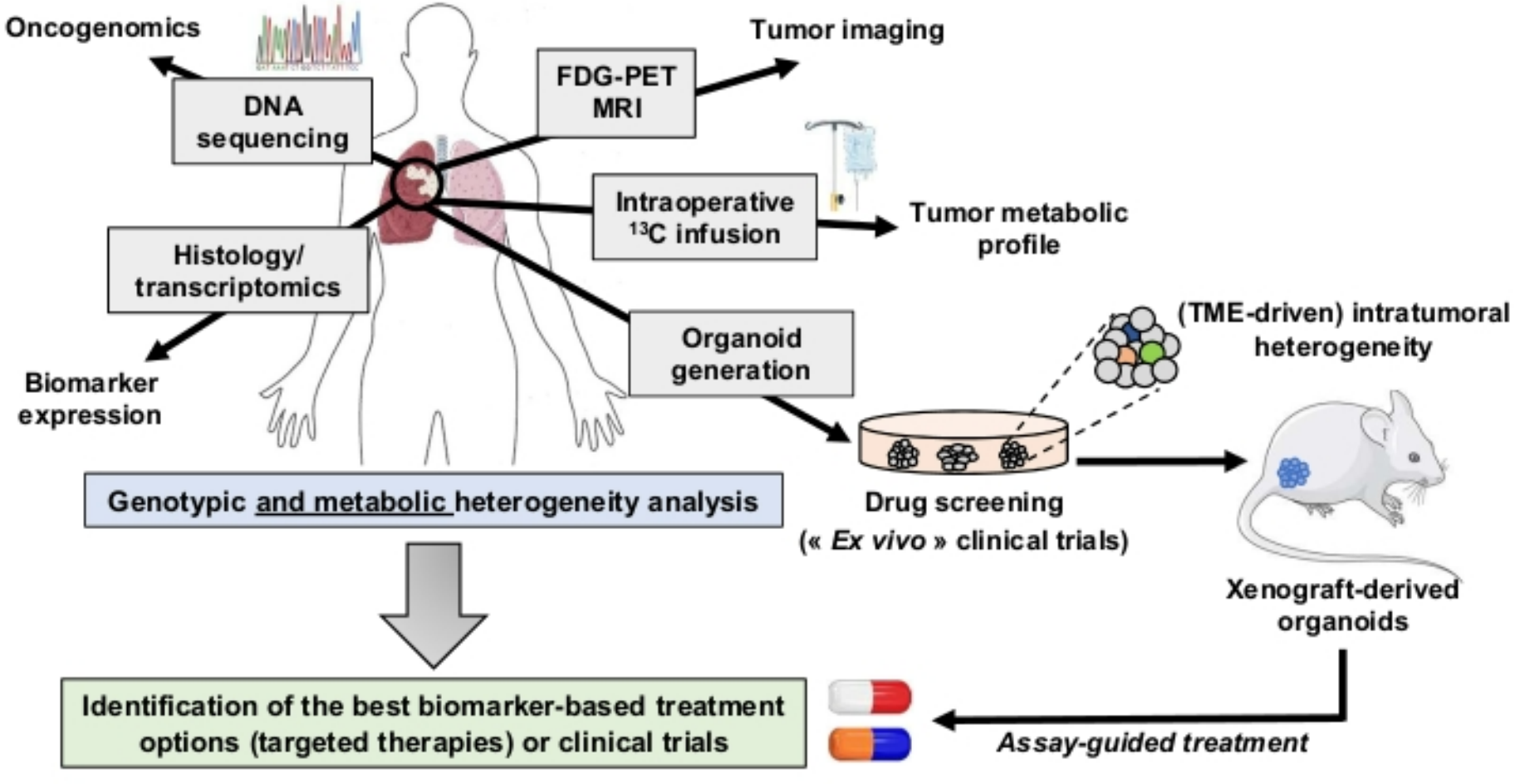
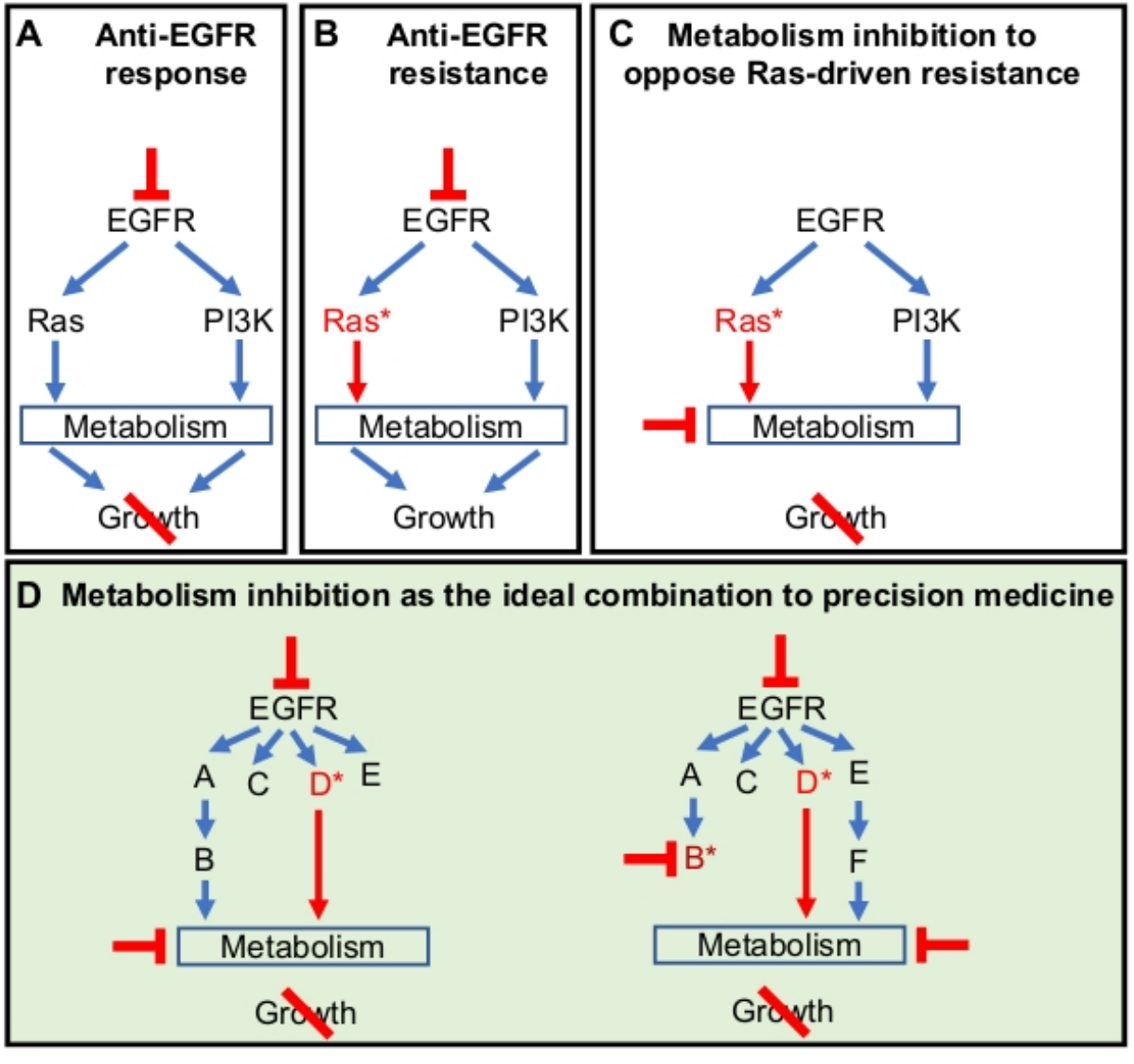

Oncogenomics
DNA sequencing
Tumor imaging
FDG-PET MRI
Intraoperative ^{13}C infusion
Tumor metabolic profile
Histology/ transcriptomics
Biomarker expression
Organoid generation
(TME-driven) intratumoral heterogeneity
Genotypic and metabolic heterogeneity analysis
Drug screening
(« Ex vivo » clinical trials)
Xenograft-derived organoids
Identification of the best biomarker-based treatment options (targeted therapies) or clinical trials
Assay-guided treatment

Figure 3


A Anti-EGFR response
EGFR
Ras
PI3K
Metabolism
Growth
B Anti-EGFR resistance
EGFR
Ras*
PI3K
Metabolism
Growth
C Metabolism inhibition to oppose Ras-driven resistance
EGFR
Ras*
PI3K
Metabolism
Growth
D Metabolism inhibition as the ideal combination to precision medicine
EGFR
A
C
D*
E
B
Metabolism
Growth
EGFR
A
C
D*
E
B*
F
Metabolism
Growth