Cancer and Metastasis Reviews (2019) 38:205–222
https://doi.org/10.1007/s10555-019-09792-7

Causes, consequences, and therapy of tumors acidosis

Smitha R. Pillai[1] · Mehdi Damaghi[1] · Yoshinori Marunaka[2,3,4] · Enrico Pierluigi Spugnini[5] · Stefano Fais[6] · Robert J. Gillies[1]

Published online: 26 March 2019

Abstract
While cancer is commonly described as "a disease of the genes," it is also associated with massive metabolic reprogramming that is now accepted as a disease "Hallmark." This programming is complex and often involves metabolic cooperativity between cancer cells and their surrounding stroma. Indeed, there is emerging clinical evidence that interrupting a cancer's metabolic program can improve patients' outcomes. The most commonly observed and well-studied metabolic adaptation in cancers is the fermentation of glucose to lactic acid, even in the presence of oxygen, also known as "aerobic glycolysis" or the "Warburg Effect." Much has been written about the mechanisms of the Warburg effect, and this remains a topic of great debate. However, herein, we will focus on an important sequela of this metabolic program: the acidification of the tumor microenvironment. Rather than being an epiphenomenon, it is now appreciated that this acidosis is a key player in cancer somatic evolution and progression to malignancy. Adaptation to acidosis induces and selects for malignant behaviors, such as increased invasion and metastasis, chemoresistance, and inhibition of immune surveillance. However, the metabolic reprogramming that occurs during adaptation to acidosis also introduces therapeutic vulnerabilities. Thus, tumor acidosis is a relevant therapeutic target, and we describe herein four approaches to accomplish this: (1) neutralizing acid directly with buffers, (2) targeting metabolic vulnerabilities revealed by acidosis, (3) developing acid-activatable drugs and nanomedicines, and (4) inhibiting metabolic processes responsible for generating acids in the first place.

Keywords Cancer · Microenvironment acidity · Exosomes · Anti-acidic therapy

1 Introduction

1.1 Acid-base balance

Biochemically, all catabolism is oxidative, resulting in the conversion of fats (hydrocarbons) and carbohydrates (alcohols) into carbonic or keto acids. Fermentation results in the production of non-oxidized acids, such as lactic acid, which can be metabolized oxidatively by other organs (liver, kidney) or nearby cells within tumors. Maintenance of systemic and tissue pH values involves a complex system that includes both passive and active buffering. Passive elements include mobile buffers (bicarbonate, phosphate), alkaline ions (Na^+, K^+), and immobile buffers (proteins, nucleic acids). Active elements include the release of the volatile acid CO_2 in the lungs and the base HCO_3^- or sulfuric acid (from cysteine and methionine) by the kidneys. Urea is excreted by the kidneys, and, as it is uncharged at neutral pH, its formation results in the next generation of one H^+ equivalent and is thus also acidifying.

✉ Stefano Fais
stefano.fais@iss.it

✉ Robert J. Gillies
Robert.gillies@moffitt.org

[1] Department of Cancer Physiology, H. Lee Moffitt Cancer Center and Research Institute, 12902 Magnolia Dr., Tampa, FL 33602, USA

[2] Research Institute for Clinical Physiology, Kyoto 604-8472, Japan

[3] Research Center for Drug Discovery and Pharmaceutical Development Science, Research Organization of Science and Technology, Ritsumeikan University, Kusatsu 525-8577, Japan

[4] Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

[5] SAFU, Regina Elena Cancer Institute, Via delle Messi d' Oro 156, Rome, Italy

[6] Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità (National Institute of Health), Viale Regina Elena, 299, 00161 Rome, Italy

Metabolic acid loads occur intracellularly, and cells have evolved robust and redundant mechanisms to export H^+ and maintain intracellular pH within strict bounds (Fig. 1). Active (ATP-requiring) H^+ equivalent transporters include the following: vacuolar-type H^+-ATPases that are normally found in lysosomes but can be expressed in the plasma membrane [1, 2]; and Na^+-driven H^+ extrusion which can be either direct (Na–H exchange, or NHE) or indirect *via* Na-bicarbonate co-transport, NBC [3–5]. Notably, the bicarbonate is dehydrated intracellularly *via* carbonic anhydrases (usually CA2) into CO_2 (consuming a H^+), which leaves the cell to be rehydrated extracellularly (producing a H^+) *via* membrane-bound, exofacial carbonic anhydrases (CA4, CA9, or CA12) [6]. Of these, CA9 is active at very low pH values [7] and is considered a "pH-stat" responsible for acidifying the extracellular microenvironment [8]. CA9 has long been known to be a negative prognostic indicator in breast and other cancers [9].

Anion exchanger 2 (AE2) participates in the exchange of Cl^- with HCO_3^-, regulating acid-base balance in the intracellular space and microenvironments surrounding cells. A recent study has reported the prognostic value of AE2 expression in esophageal squamous cell carcinoma (ESCC) [10]. AE2 is strongly expressed in ESCC cells and is coupled to matrix metalloproteinases, migration, and invasion.

Although systemic pH is highly regulated, chronic alterations in tissue and systemic pH are associated with many diseases including cancer, diabetes, epilepsy, and MELAS. Measurement of intra- and extracellular pH, and its correlations with the disease, is an extremely active area of research, because there is a compelling need for robust, accurate, and clinically translatable methods of measuring pH *in vivo*. Such technologies that could be used as diagnostic, predictive, and/or response biomarkers have been comprehensively reviewed elsewhere [11].

1.2 Causes of tumor acidity

A hallmark of cancer is that tumors are highly heterogeneous at the genomic, anatomic, physiologic, and metabolic levels. The proximal cause of this heterogeneity is the "chaotic" and abnormal tumor vasculature, which leads to different microenvironments with different perfusion characteristics [12]. These perfusion deficits can lead to profound deprivation of nutrients and substrates, such as amino acids, glucose, and oxygen. In oxygen deprived (hypoxic) environments, cells must rely on fermentative glycolysis, i.e., the non-oxidative conversion of glucose to lactic acid, induced *via* the "Pasteur Effect," to meet their energy demands. It is axiomatic that combined oxygen and glucose deprivation cannot be corrected, and cells will die of necrosis. The necrotic core is commonly observed radiographically in clinical cancers, and this is related to profound perfusion deficits [13, 14].

However, in malignant cancer, fermentative metabolism occurs even under well-oxygenated conditions, known as "aerobic glycolysis" or the "Warburg Effect." This glycolytic switch is the result of hardwiring the stability of hypoxia-inducible transcription factors, HIF-1 (and/or HIF-2), a condition known as "pseudohypoxia." [15] This phenotype is selected early in carcinogenesis and likely provides a selection benefit of increased fitness. [16, 17] The mechanisms driving aerobic glycolysis are not known and remain controversial, as do the fitness advantages of constitutively expressing pseudohypoxic phenotype. However, it is an unequivocal fact that tumors exhibiting a Warburg effect consume significant amounts of glucose and produce copious amounts of non-oxidized (lactic) acids as a result. Indeed, recent evidence has suggested a strong correlation between glucose uptake, measured by FDG PET imaging, and acidosis measured with CEST MRI [18]. Acidosis is also exacerbated by poor

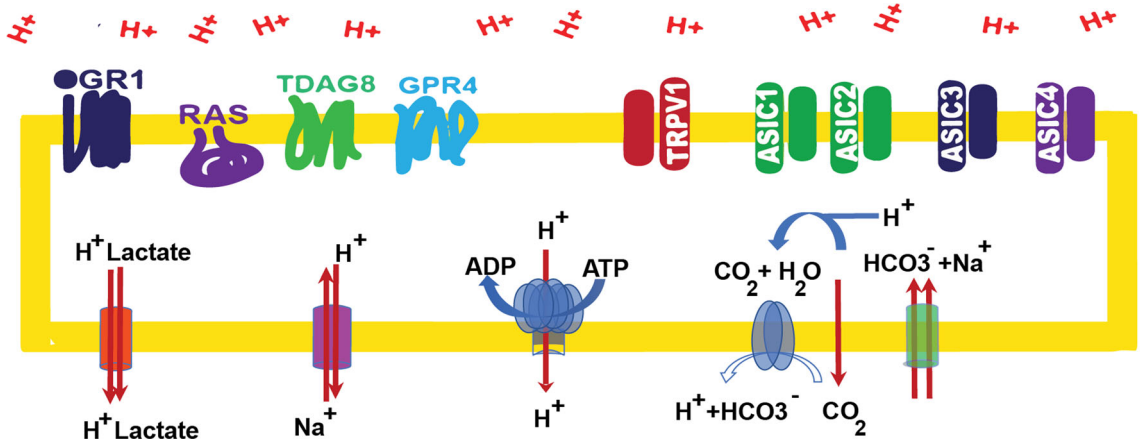

Fig. 1 Mechanisms to export H^+ and maintain intracellular pH. Extracellular pH is sensed with acid receptors, either G protein-coupled receptors OGR1, TDAG8, GPR4, or acid-sensing ion channels, TRPV1 or ASICs. Because metabolism results in acid production, acid equivalents are removed from the cytoplasm by a multitude of mechanisms, each with their own regulation and behavior. These include (from left) monocarboxylate transporters to remove lactic acid, N-hydrogen exchange, vacuolar H-ATPase, and Na-driven uptake of bicarbonate, which is then removed from the cell as CO2 and rehydrated with exofacial carbonic anhydrases

perfusion, as pH decreases with increasing distance from blood vessels [19]. This results in the accumulation of acids in the extracellular environment and an acidic tumor pH, with values as low as pH 6.5 [20–23]. The elevated levels of extracellular lactate can be a nutrient source for other cancer or stromal cells in the tumor. Lactate is taken up by cells through monocarboxylate transporters, primarily MCT-1 and/or MCT-4, and utilized for energy production through oxidative metabolism (Fig. 2).

Although MCT-4 is commonly thought to be responsible for lactate efflux, and MCT-1 for lactate influx, in fact, both of these transporters are non-electrogenic permeases and respond to the immediate concentrations of lactate and H^+ on either side of the membrane [24]. A recent study using ^{13}C-labeled precursors in human tumors has shown unequivocally that lactate produced in one region of a lung tumor can be a major fuel source for other cells in the tumor and that the uptake was mediated *via* MCT1 [25]. Similarly, the conversion of ^{13}C pyruvate to ^{13}C lactate (and *vice versa*) can be monitored in human patients using dynamic nuclear hyperpolarized magnetic resonance imaging [26]. This release of acid by fermentative cells, results in profound acidity in areas that are proximal to the cell membrane. A recent study using the Raman spectroscopy with gold nanoparticles reported an NHE dependent extremely low pH (~ 6.0) within 20 nM from the plasma membrane of tumor cells [27]. This information would be helpful for the designing of pH-dependent drug development.

1.3 Consequences of tumor acidosis

1.3.1 Mechanisms of acid adaptation

It is axiomatic that cancer cells must adapt to living in acidic pH in order to survive and thrive. These adaptations eventually make cancer cells more fit than the normal stromal cells that are their competitors. Because cultured cells are adapted to growth in alkaline media, switching them to a more acidic medium invariably slows their growth. However, cancer cells eventually adapt to growth in acidic media. As discussed below, this adaptation is pleiotropic.

There are significant metabolic adaptations to growth in acidic media (Fig. 3). At the genomic level, acidosis can induce genome instability through chromosome breakages and translocations driving somatic evolution [28]. Acidosis in the microenvironment provides a strong evolutionary selection pressure that contributes to the emergence of aggressive,

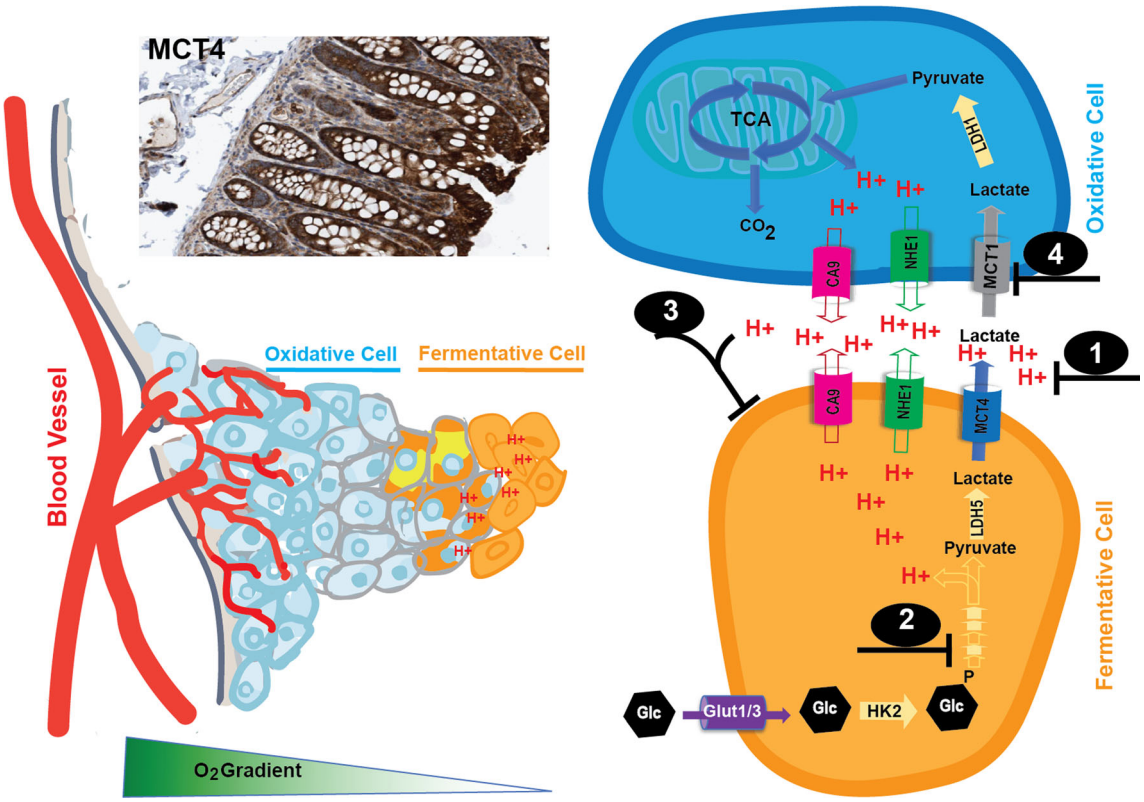

Fig. 2 Acid phenotypic heterogeneity and metabolic cooperativity in tumors (counterclockwise from lower left). The diffusion distance of O_2 in tissues is ca. 0.2 mm, and cells that are further from this from a blood vessel are hypoxic, and rely on fermentative metabolism. Fermentative cells (orange) metabolize glucose and produce lactic acid, which is removed by MCT1 or MCT4. The lactic is taken up by an adjacent oxidative cell and oxidized to CO_2. In tumors (in this case, a colorectal cancer), the expression of MCT4, often associated with fermentation, can be expressed in both cancer cells and adjacent stroma, emphasizing the tumor-stromal metabolic cooperativity

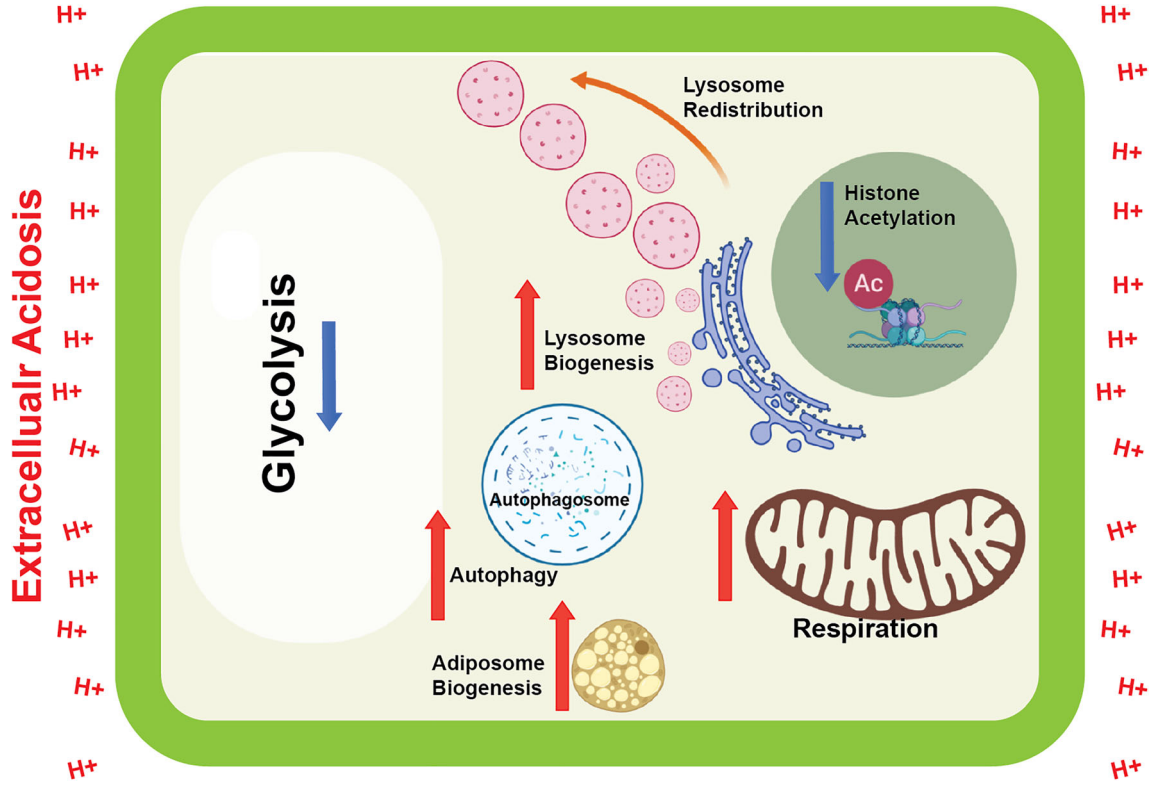

Fig. 3 Metabolic adaptations to growth in an acidic environment. After prolonged growth in acidic conditions, cells are metabolically reprogrammed to increase reliance on respiration, fueled by fatty acid oxidation, coupled to a profound decrease in glycolysis. These are coupled to chronic autophagy, storage of lipids in adiposomes, increased lysosomogenesis, and redistribution of lysosomes to be adjacent to the plasma membrane. All of these are necessary adaptations and reveal metabolic vulnerabilities

therapy-resistant clones [29]. A very common response to acidosis is a cessation of glucose fermentation and an increase in respiration fueled by glutamine consumption or beta-oxidation (β-ox) of fatty acids [30]. Increased oxidative flux increases reactive oxygen species [31], and increased accumulation of the highly reactive acetyl CoA, which has been shown to non-enzymatically acetylate complex I, which restrains β-ox. Acid adaptation also leads to sirtuin-mediated histone deacetylation, which downregulates AcetylCoA carboxylase ACC2, leading to the reversal of FAS inhibition and driving fatty acid synthesis that can paradoxically occur simultaneously with β-ox [32]. This may be related to the significant increase in the abundance of adiposomes, which is a rapid and reversible response to acidosis [30].

One of the most profound adaptations to acidosis is an increase in lysosomogenesis and redistribution of lysosomes from a perinuclear location to the plasma membrane, where they can fuse and release their contents (including proteases and H^+) to the surrounding microenvironment [33, 34]. This is hypothesized to contribute to ECM remodeling and local invasion [35, 36]. This is also associated with the redistribution of the lysosomal protein LAMP2 to the plasma membrane where it protects from acid hydrolysis [34]. LAMP2 is also a key participant in inducing autophagy, and acid-adapted cells are known to be chronically autophagic [37]. The redistribution of lysosomes has also been associated with the separation of mTORC from its regulatory complex, including RHEB [38], and this may contribute to the metabolic changes mentioned above.

Although its importance is not well established, it is also known that acid adaptation is associated with an increase in the release of extracellular vesicles (EVs) by tumor cells [39, 40]. It has been speculated that EVs are mediators of cell-to-cell communication [41] and indeed, acidosis has been shown to stabilize and increase exosomal RNA and protein content [42]. In a recent study, precise quantification of exosome release under different pH conditions was demonstrated in a number of cell lines derived from cancers of colon, breast, prostate, melanoma, and osteosarcoma [43]. The results obtained using a nanoscale flow cytometry or tracking analysis showed that cells grown in acidic pH (6.5) release, on average, 4.6-fold more exosomes than the same cells grown in physiological pH (7.4) medium. pH dependence of exosome release was further demonstrated by progressively increasing the pH from 6.5 or decreasing the pH from 7.4. Cells grown in acidic media showed an increase in the activity of the endosomal compartment as seen by CD63 staining leading, in turn, to increased EV formation and release.

1.3.2 Invasion and metastasis

Acidosis is a potent stimulator of local invasion (Fig. 4). Indeed, it has been shown that invadopodia contain proton-exporting machinery, which can either include NHE1 or a complex of NBCn1 coupled to CA9 [44, 45]. This establishes an alkaline intracellular pH (pHi) at the leading edge, with a more acidic pHi near the tailing end of the cell, leading to directional migration. The acidic extracellular pH at the leading edge can activate proteases, such as lysosome-released cathepsins. In tumors, cells at the invading edge express significantly more CA9 than those in the tumor core, prompting many to hypothesize that this distribution facilitates acidification of the invasion front [46, 47]. Consistent with this, we have observed with intravital microscopy that invading tumors secrete acid into their surrounding stroma [48, 49], which induces ECM remodeling and local invasion.

1.3.3 Immune evasion

Tumor-derived acidosis has also been shown to promote tumor progression *via* inhibition of T cell activation and induction of a macrophage phenotypic switch towards an M2-polarized phenotype [50–52]. While the exact mechanisms by which acid pH inhibits the effector function of tumor-infiltrating lymphocytes, it is well established that acidosis results in reduced secretion of IFN-g and IL-2, upregulation of CD25, and activation of STA5/ERK signaling [53–55]. Harold Dvorak famously characterized tumors as "wounds that do not heal." [56] One component of the wound response is transient ischemia-driven tissue acidification, which resolves as the wound heals [57]. The role of acidification in physiological wound healing is not known with certainty, but recent data suggest that acidification stimulates the production of inflammatory cytokines by the stroma or endothelium [58]. As these induce

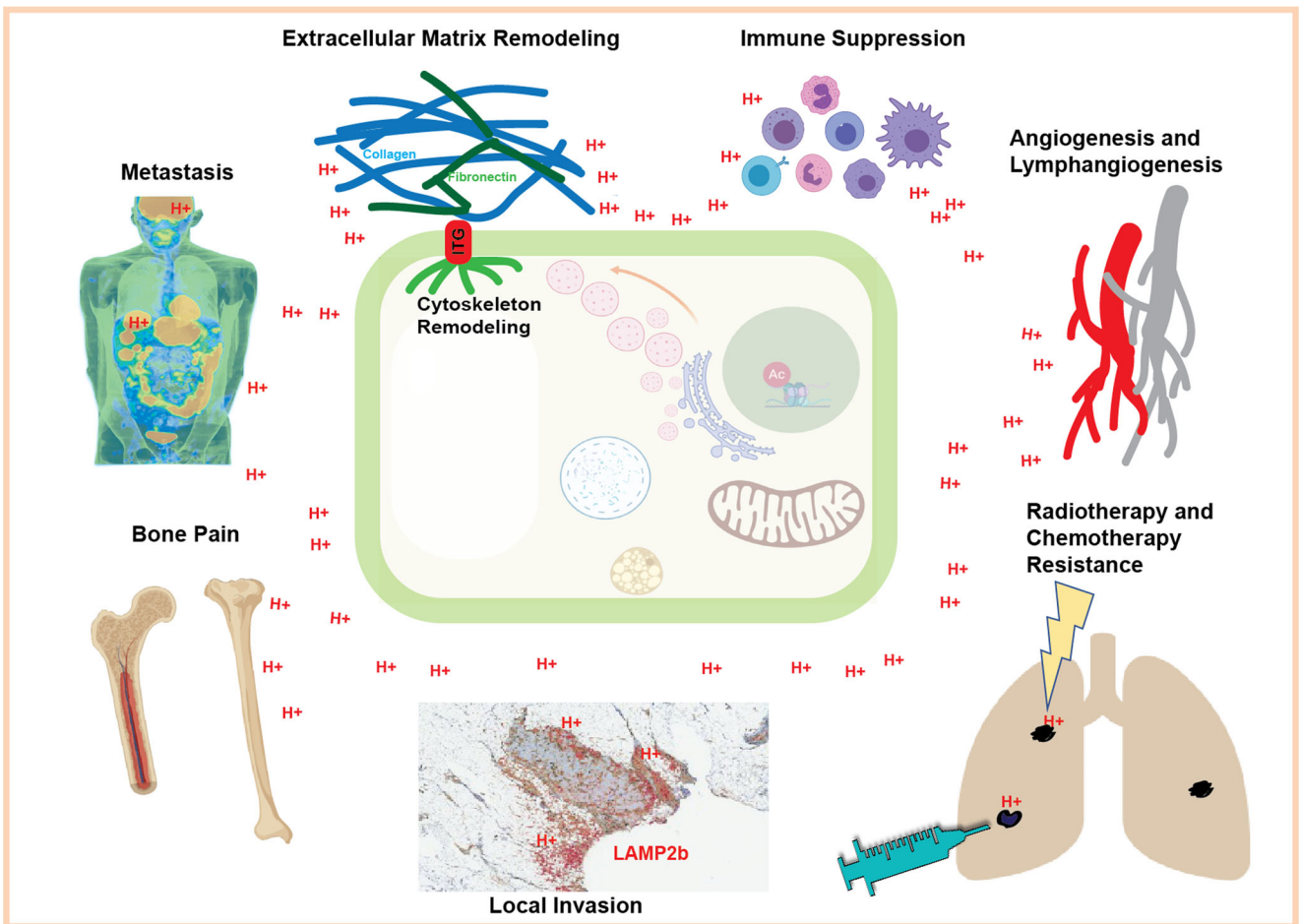

Fig. 4 Consequences of extracellular acidosis. Following cellular adaptations described above, there are a number of systemic consequences to acidosis as well. These include (counterclockwise from top) remodeling of the extracellular matrix, allowing local invasion, leading to increased metastasis to other organs, and bone, where the tumor generated acidity can be a potent effector of bone pain *via* ASICs (Fig. 1). Local invasion is associated also with the expression of acid generating cells at the invading edge. Acidic tumors are resistant to radiation and chemotherapy and, in some systems, can induce angiogenesis and lymphangiogenesis, which paradoxically often lead to poorer perfusion. Finally, acid is a potent inhibitor of effector T cell function, inhibiting immune surveillance

neo-angiogenesis, the acidosis is reduced and inflammation resolves. In tumors, this acidification never resolves.

1.3.4 Drug resistance

Multiple mechanisms have been identified that underlie intrinsic and acquired chemoresistance: these include impaired drug uptake, increased drug efflux, deletion of receptors, altered drug metabolism, quantitative and qualitative alterations in drug targets, increased DNA damage repair, and various anti-apoptotic mechanisms. The rapid efflux of anti-cancer drugs mediated by multidrug transporters and the partial or complete reversibility of chemoresistance combined with the absence of genetic mutations suggests a multifactorial process. However, a growing body of recent evidence suggests that chemoresistance can also be triggered by the highly acidic microenvironment of tumors. A large number of drugs, including conventional chemotherapeutics and more recent biological agents, are weak bases that are quickly protonated and are sequestered through the well-known phenomenon of "ion trapping" in acidic environments such as the extracellular microenvironment and the acidic organelles of tumor cells. It is therefore essential to develop new strategies to overcome the entrapment and neutralization of weak base drugs. As described below, one such strategy is to directly increase the pH of the tumor microenvironment. A deal of preclinical evidence on the ability of both buffers and proton exchange inhibitors to improve the effectiveness of anti-cancer drugs have supported clinical trials in both human patients and animals with spontaneous tumors whose results are discussed below.

1.4 Targeting acidity

Tumor acidity is associated with cancer progression and poor outcomes. Preclinical and some clinical studies have shown that targeting acidity can improve therapy responses. Hence, targeting tumor acidosis is a relevant therapeutic target, and we describe herein four approaches for targeting acidosis: (1) targeting to neutralize tumor acid directly, (2) targeting metabolic vulnerabilities revealed by acidosis, (3) acid-activatable drugs and nanomedicines, and (4) inhibiting metabolic processes responsible for generating acids in the first place (Fig. 4).

1.4.1 Direct targeting

The most direct approach to target tumor acidity is to neutralize it through the administration of oral buffers, such as $NaHCO_3$. It has been shown that mice can thrive on buffered drinking water with, e.g., *ad lib* 200 mM sodium bicarbonate or THAM, and that these treatments specifically increase tumor pH without affecting systemic pH balance. The specificity for tumors can best be understood in light of the fact that tumor pH is acidic and unregulated, whereas systemic pH is alkaline and highly regulated [59]. Hence, buffers act to bring tumor pH to be consistent with that of the rest of the body. In multiple studies, oral buffers have been shown to not affect the growth of primary tumors but to significantly prevent metastases [60, 61] and that these treatments can reduce the aggressiveness of spontaneous genetically engineered mouse cancer models (GEMMs) [62, 63]. However, it has proven difficult to translate these findings to the clinic, as three clinical trials with sodium bicarbonate were pursued and failed to reach their dose targets due to poor compliance (taste) and moderate SAEs [64].

An alternative to directly target tumor acidity is provided by L-DOS47 (Helix Biopharma). L-DOS47 is a jack bean urease targeted with camelid antibodies to CEACAM6 antigen, which is overexpressed in a number of cancers [65]. Once at the target, the urease converts endogenous urea to 2 NH_4^+ and 1 HCO_3^-, producing a net local increase in pH. This has been well tolerated in phase I/II studies in NSCLC (NCT02309892) [66]. Alternatively, TRC101 is an orally available HCl-absorbing and non-digested micron-sized particle (buffer) that has been used to treat patients with chronic kidney disease and shown to induce compensated metabolic alkalosis, which is the target [67]. It has just completed a phase III (NCT03317444) but has not yet been investigated in cancer.

1.4.2 Targeting metabolic vulnerabilities

As described above, adaptation to acidic microenvironment involves significant metabolic reprogramming. The fact that metabolic pathways under acidosis are different than those under neutral pH can be exploited, as these present vulnerabilities that can be targeted (Table 1). This was directly tested by Persi et al. who predicted the pH profiles of all intracellular enzymes through homology modeling, and used this information to identify enzymes whose activities would be crucial for the survival at low, but not neutral, pH [107]. In their study, these vulnerabilities were validated with siRNA knockdowns, but pharmacological agents are available for two of the most vulnerable enzymes: GAPDH and G6PD.

Glyceraldehyde phosphate dehydrogenase, GAPDH, is a rate-limiting enzyme in glycolysis that has been demonstrated to have translational potential in combating tumor growth. Several inhibitors including arsenate, arsenic trioxide, 3-bromopyruvate, iodoacetate, and many natural compounds such as koningic acid (KA) are implicated to have anti-GAPDH activity. Koningic acid (KA) also known as heptelidic acid, is a sesquiterpene lactone isolated from soil fungi that directly dock/bind to the active site of human GAPDH [108]. Using machine learning, pharmacogenomics and metabolomics, a recent study demonstrated that the cytotoxic effect of the KA treatment is heterogeneous and is determined by the quantitative extent of Warburg effect, glucose uptake and lactate secretion [82]. This study extended the

Table 1 Strategies to target acidosis for cancer treatment

Metabolic pathway targeted	Agent	Stage of development	Comments	References
1. Direct pH Targeting				
Oral buffers	Sodium bicarbonate	Preclinical and clinical	Clinical trials failed due to poor patient compliance	[61, 62, 64]
HCl-absorbing particle	TRC101	Clinical	Phase III completed; not in cancer	NCT03317444
Urease	L-DOS47	Clinical	Phase I and II for NSCLC	NCT02309892 [66]
2. Targeting acidogenic metabolism/acid-base balance				
CA9	Indisulam, acetazolamide, and other sulfonamides	Clinical	Some trials terminated due to lack of clinical activity; some ongoing for solid tumors	NCT00060567
CA9/CA12	SLC0111	Clinical	Phase 1	NCT00165594 NCT00165880 [68] NCT02215850 NCT03450018 [69]
NHE1	Cariporide	Preclinical		[70]
Lactate transport				
MCT1	AZD3965	Preclinical and clinical	Ongoing; will be concluded in August 2019	NCT01791595 [71–73]
MCT1 and 2	AR-C155858	Preclinical		[74]
MCT1 and 2	SR13800	Preclinical		[75]
MCT4	AZ93	Preclinical		[76]
	Syrosingopine	Preclinical	Inhibits MCT1 also	[77]
3. Targeting metabolic vulnerabilities under acidosis				
A) Pentose phosphate pathway				
Polydatin		Preclinical and clinical	Phase II; well tolerated as an analgesic in irritable bowel syndrome and endometriosis	[78] [78–80]
PGAM1	PGMI-004A	Preclinical		[81]
B) Glycolysis				
GAPDH	Koningic acid	Preclinical		[82]
C) Lipid synthesis				
ACC (acetylCoA carboxylase)	NDI-010976 (ND-630, GS-0976, or firsocostat	Preclinical and clinical (phase II) for NASH	Allosteric inhibitor; prevents dimerization of ACC, reduce FA synthesis, induce β-ox	[83]
	MK4074	Clinical	Liver-specific ACC1 and 2 inhibitors, for liver steatosis	[84] NCT01431521 Phase1 completed, no data available
Choline kinase	TCD-717	TCD-717 in clinical development, breast, lung, and colon	Promising phase 1 data	[85]
	RSM932A	Preclinical		[86]

Table 1 (continued)

Metabolic pathway targeted	Agent	Stage of development	Comments	References
	MN58b	Preclinical		[87, 88]
Fatty acid synthase(FASN)	TVB-2640 TVB-3664	Clinical phase II Preclinical		NCT03032484 [89]
Mevalonate Pathway				
HMG-CoA reductase	Simvastatin	Phase II clinical trial	Breast cancer stage I–II, preventing liver cancer in patients with liver cirrhosis	NCT00334542
D) Lipid oxidation				
CPT1	Etomoxir, perhexiline	Clinical	Perhexiline is approved for antianginal therapy	NCT02431221
4. Acid-activated agents				
Proton pump inhibitors H^+/K^+-ATPase	Omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole	Clinical, phase II completed	Intermittent high-dose PPI enhance the antitumor effects of chemo-therapy in metastatic breast cancer patients	NCT01069081 [90–92]
Nanoparticles	Liposomes, micelles, polymeric nanoparticles	*In vitro*, preclinical	Drug release under acidic pH.	[93–96]
ADCs	Acid-labile linkers (e.g., hydrazone)	Clinical	Many ADCs have been approved by FDA. ADCs with drug release at acidic pH are under development	[97, 98]
pHLIPS	pHLIP MMAE conjugates, HAuNS-pHLIP-Ce6 particles	Preclinical	Intracellular delivery of therapeutic agents	[99–106]

concept of synthetic lethality to glycolytic/acidic tumors by demonstrating that during the WE, the rate-controlling steps in glycolysis are different than in fully oxidative energy metabolism. Thus, pharmacological interventions using KA have the potential to specifically disrupt metabolic pathways important in neoplastic settings but render healthy tissue largely unaffected. [109, 110]

Glucose-6-phosphate dehydrogenase (G6PDH) is a critical enzyme conferring pH sensitivity. Polydatin is a natural glucoside and a precursor to Resveratrol that has long been used in traditional Chinese medicine for many purposes, including anti-cancer properties [111]. Polydatin has been used as an anti-cancer agent for years, without the knowledge of its biochemical target [112]. Recently, it has been shown to be a potent inhibitor of G6PD, the rate-limiting step to enter the pentose phosphate pathway, PPP [78]. In this study, it was shown that polydatin limited NADPH production *via* the PPP and led to toxic oxidative ER stress and that these effects could be mitigated by the overexpression of G6PD. Further, it is well-tolerated *in vivo*, and a phase II trial (albeit not in cancer) has been completed [79]. Genetic or pharmacologic inhibition of PGM1 (phosphoglycerate mutase enzyme 1) that catalyze 3-phosphoglycerate (3PG) to 2 PG has also shown to inhibit PPP flux and tumor growth in preclinical studies [81].

1.4.3 Acid-activated agents

Development of agents that are only active under relatively acidic conditions is an area of active investigation. These include agents that are acid-labile and deliver therapy selectively to acidic microenvironment, as well as agents that are activated under acidic microenvironments.

Acid-labile agents include nanoparticles and labile linkers on antibody drug conjugates, ADC. Acid-labile nanoparticles employ multiple different platforms and chemistries intended to dissolve in mildly acidic conditions found in tumors, and this rapidly evolving field has been the subject of many reviews [93, 113, 114]. Notably, many of these contain either PET or activatable MR imaging moieties that allow monitoring of targeting and activation [94, 115]. Antibody drug conjugates are designed to target cell surface receptors and release their chemotherapeutic drugs following cleavage of an acid-labile linker on the cell surface or in an endosome. There are currently four approved ADCs for cancers and there are currently about 60 ADCs in clinical trials [116]. The chemistries of acid-labile linkers have a long history and continue to evolve in order to fine-tune the kinetics and pH profile of these linkers [117, 118].

For acid-activated agents, Engelman and colleagues have developed a series of peptides, called “pH low inserting peptides” or pHLIPs, whose configuration changes under mildly acidic conditions and promotes the insertion of the peptide stably across the plasma membrane [119]. Notably, there is tremendous flexibility to tune the pH at which these are activated and the payloads that they contain, which can be imaging moieties, therapeutic agents, or both [120] .

The most well-developed acid-activated agents, and the ones with the most promise for clinical translation, however, are the so-called proton pump inhibitors, PPIs. PPIs (i.e., omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole) are used worldwide as very potent antacids. These are over-the-counter medications and are very well tolerated, even with chronic treatments [121, 122]. PPIs are tetracyclic sulfenamides that are activated by protonation to become sulfhydryl (e.g. cysteine) reagents. They were developed to be activated by stomach acid and bind irreversibly to inhibit the gastric H^+/K^+-ATPase. In cancer cells, PPIs have been shown to increase the pH of lysosomes due to the inhibition of V-ATPase, and targeting in an acidic milieu may be due to the increased lysosomal-endosomal turnover, described above [123, 124]. PPIs may be used a monotherapy or in combination with chemo- or immunotherapies with improved responses [52, 90, 125].

Similar observations were made in clinical studies in household pets with advanced or chemo-refractory tumors, where PPIs achieved long-term responses with improved performance status when combined with either standard treatment [126] or metronomic regimens [91]. These studies carry a significant translational value considering that cancers in companion animals are spontaneous and can share many similarities with human tumors [127]. Notably, cancer is the principal cause of death in pet dogs, with an incidence among certain breeds such as Golden Retrievers and Bernese Mountain Dogs approaching an incidence of 50% [128]. In dogs and cats, the progression of cancer is extremely rapid, with an aggressive behavior that frequently results in poor responses to therapy. This could be partially ascribed to their compressed lifespan compared with humans; however, if we consider this from a metabolic point of view, it could be induced and influenced by a baseline metabolism that is more acid than that of herbivores and primates. In particular, it has been underlined by a recent work that the gastric pH of dogs and cats is much more acid than omnivorous and herbivorous species, potentially laying the base for an acid milieu favoring the occurrence of neoplasia. Likewise, in terms of incidence, cancer is infrequently reported in horses, accounting for less than 5% of the surgeries performed at referral institutions [129], compared with dogs whose incidence, accordingly to the cancer registries, is around 45%, despite being their lifespan much shorter than that of horses’ [130].This could also explain the extreme effectiveness of alkaline therapy in such species when combined to the conventional chemotherapy [131].

Because of their widespread use, there are a number of population-based studies showing beneficial effects of PPIs in the management of cancer. A total of 64,234 women

diagnosed with breast cancer between 2004 and 2013, accrued by a recent observational case-control study, were selected as cases, and an equal number of healthy women as controls. Logistic regression modeling analysis revealed breast cancer patients were 25% less likely to have had prior PPI exposure [132]. A dose-response effect was also detected, with the highest effect, 35% lower PPI odds (95%CI 0.61–0.70) among patients in the highest exposure category suggesting that women at a higher-than-average risk of breast cancer may benefit from PPI prescriptions if they have medical conditions that could benefit from PPIs. The high safety profile, low cost, and widespread long-term usage of PPIs make them ideal candidates for further exploration into their anti-cancer effects. A retrospective meta-analysis of 596 previously untreated head and neck squamous cell carcinoma (HNSCC) patients revealed a strong univariate association between PPI use and improved overall survival ($P < 0.001$) [133]. A retrospective analysis of patients with refractory GI cancer showed that the addition of PPI to chemotherapy significantly increased the time to progression [134].

1.4.4 Targeting acidogenic metabolism

The "reverse pH gradient (acid outside, alkaline inside)" in tumors is maintained by increased expression and/or activity of various plasma membrane transporters and acid efflux proteins that control pH homeostasis, including vacuolar-type H^+-ATPase, monocarboxylate transporters (MCTs), Na^+–H^+ exchangers (NHEs), and carbonic anhydrases (CAs) [1, 135–139] (Fig. 1). Disrupting pH homeostasis by inhibiting these transporters and exchangers has been suggested as a therapeutic strategy, and some of these inhibitors are in clinical trials [140, 141]. While NHE1 is ubiquitously expressed in cancer and normal cells, it appears to play more of an essential role in cancers. For example, knocking out NHE1 has a greater impact on tumor growth if combined with mutations that increase the lactic acid load [142]. Further, silencing of NHE1 or MCT4 expression reduced the pH gradient and limited tumor growth in similar xenograft models [143, 144]. Amiloride, the first NHE inhibitor developed was shown to affect the metastatic process by decreasing vasoendothelial growth factor (VEGF) production and the activity of urokinase-type plasminogen activator (μPA), metalloproteinases (MMP), and other proteases [145]. Since then, more potent and specific NHE1 inhibitors have been developed (e.g. ethyl isopropyl-, hexamethyl-, or dimethyl-amilorides) [146, 147]. These potassium-sparing diuretics have antineoplastic and anti-metastatic properties and are well tolerated and safe [148–151]. In clinical trials, cariporide, a non-amiloride-based NHE1 inhibitor provided protection to the myocardium during ischemic-reperfusion injury, yet had a small therapeutic window [70]. However, there is potential for repurposing cariporide as an anti-cancer agent as it is very effective in initiating internal acidification of cancer cells by inhibiting NHE1-mediated H^+ efflux leading to cancer cell death [70, 152, 153]. The challenge with using NHE1 inhibitors is to identify rational combinations that will increase the therapeutic efficacy.

Monocarboxylate transporters (MCTs) are crucial players in regulating pH homeostasis by facilitating the export of lactic acid from glycolytic cancer cells or to facilitate uptake of lactate or pyruvate as energy sources. Among the 14 isoforms identified, MCT1 and 4 are lactate/H^+ symporters broadly expressed in cancers and are associated with cancer aggressiveness and prognosis in many cancer types [154–156]. Although it is thought that MCT1 is mainly involved in lactate uptake and MCT4 in lactate export, it is becoming increasingly appreciated that these permeases primarily respond to the lactate, pyruvate, and H^+ concentrations on each side of the membrane [150, 157, 158]. Specific siRNA silencing of MCT1 in malignant human glioma cells demonstrated a decrease of pHi by 0.6 units and rapid cell death, confirming the key role of MCTs in pHi regulation [159]. Although the first generation small molecule MCT inhibitors, the cinnamates, were effective in lowering intracellular pH and cell viability, they were not specific enough for further clinical development [24, 160, 161]. Blocking MCT1/2 in Ras-transformed fibroblast CCL39 cells expressing MCT1 and 2 with AR-C155858, a specific MCT1/2 inhibitor developed by Astra Zeneca, suppressed lactate export, glycolysis, and strongly reduced pHi and cell growth when cells were forced to use glycolysis in response to oligomycin treatment. In addition, ectopic expression of MCT4 in these cells conferred resistance to MCT1/2 inhibition and reestablished tumorigenicity, stressing the role of MCT4 in pHi regulation [162]. This was further confirmed using a 31P magnetic resonance spectroscopy by overexpressing MCT4 in Ras-transformed cells, showing that intracellular pH was elevated to alkaline levels while extracellular pH was acidic [143]. Moreover, MCT4-depleted breast cancer cells showed increased cell death and reduced tumor growth due to elevated levels of reactive oxygen species and decreased intracellular pH [163]. AZD3965, a more specific and potent inhibitor of MCT1 derived from AR-C155858, has shown promise in preclinical studies of small cell lung cancer, colon cancer, and lymphoma [71–73, 164]. As expected, the major metabolic consequences of MCT1 inhibition were lactate accumulation, a decrease in intracellular pH, and dependence on mitochondrial metabolism which sensitized the cells to mitochondrial complex 1 inhibitors, metformin, or phenformin. AZD3965 is currently undergoing phase I/II clinical trials in the UK for patients with solid tumors, prostate cancer, and diffuse large-cell B lymphoma [76, 165]. In addition, syrosingopine, an antihypertensive drug, is reported to be a dual inhibitor of MCT1 and MCT4 and elicited synthetic lethality with metformin due to NAD^+ depletion in cancer cells [77]. Interestingly, in a recent study, MCT1/2

inhibitor (SR13800) was successfully employed to lower intracellular pH and was efficient in inhibiting proliferation in breast cancer cells when combined with depletion of GAPDH. This synthetic lethal approach was particularly effective in breast cancer cells adapted to hypoxia or low extracellular pH and that display aggressive phenotypes [107]. Taken together, these studies strongly suggest the potential of therapeutically targeting metabolic vulnerabilities under acidosis.

Carbonic anhydrase IX is an attractive target for the purpose of reducing tumor acidity. As described above, CA9 is a major contributor to extracellular acidosis, it is a negative prognostic marker in many cancers and is strongly associated with increased invasion and metastasis [166, 167]. Further, its normal tissue expression is restricted to the upper GI tract and gall bladder (www.proteinatlas.org). Because of its attractiveness as a target, it has been a drug target since its discovery by Pastorek and Pastorekova in 1994 [168]. In general, CA inhibitors contain sulfonamide groups that target the active site [169]. Exofacial CAs, such as CA9 and CA12, can be selectively targeted by appending a large hydrophilic or halogenated group to reduce internalization [170]. The status of CA9 and CA12 inhibitors and their role as anti-cancer agents have recently been reviewed [171, 172]. Importantly, cancer cells adapted to acidosis are particularly susceptible to CA9 inhibition [173]. *In vivo*, CA9 knockdown or specific CA9 inhibitors (CAI17) similarly suppressed tumor growth and metastasis in aggressive breast tumor models (4T1, MDAmb231) [174]. A CA9/CA12-specific inhibitor SLC0111 was additive to temozolomide in delaying the growth of GBM *in vivo* [69]. A phase I trial of SLC0111 (NCT02215850) was completed last year, and a new trial is set to open in combination with gemcitabine in pancreatic cancer (NCT03450018).

2 Conclusions

It has almost been a century since the Nobelist, Otto Warburg, first described the phenomenon of *aerobic glycolysis* in cancers, and he, at that time, postulated that this would result in acid-base imbalances. It has only been in the last quarter century that tools have been developed which interrogate the acid-base balance of tumors. It has only been in the last decade that it has become more widely appreciated that solid tumors (and even those residing in bone marrow) can be profoundly acidic and that, on one hand, adaptation to this acidosis provides the cancer cells with an evolutionary fitness advantage, but on the other hand, this also exposes them to therapeutic vulnerabilities. Herein, we have described some of these adaptations and therapeutic approaches either to reduce the acidosis itself to eliminate the fitness advantage or to exploit the acid-induced vulnerabilities.

Funding This work was supported by the Anticancer Fund (RJG), US PHS NIH grants R01 CA077575 (RJG), U54 CA193489 (RJG), and the Florida Health grant 8BC04 (SRP/RJG); the Italian Ministry of Health (SF); and Grants-in-Aid from Japan Society of the Promotion of Science, JSPS KAKENHI grant numbers JP15K15034 and 18H03182 (YM).

Compliance with ethical standards

Conflict of interest Dr. Gillies reports a COI with Helix Biopharma, with whom he is a consultant and investor.

References

1. Hinton, A., Sennoune, S. R., Bond, S., Fang, M., Reuveni, M., Sahagian, G. G., Jay, D., Martinez-Zaguilan, R., & Forgac, M. (2009). Function of a subunit isoforms of the V-ATPase in pH homeostasis and in vitro invasion of MDA-MB231 human breast cancer cells. *The Journal of Biological Chemistry, 284*, 16400–16408. https://doi.org/10.1074/jbc.M901201200.
2. Toyomura, T., Oka, T., Yamaguchi, C., Wada, Y., & Futai, M. (2000). Three subunit a isoforms of mouse vacuolar H(+)-ATPase. Preferential expression of the a3 isoform during osteoclast differentiation. *The Journal of Biological Chemistry, 275*, 8760–8765.
3. Boedtkjer, E., Moreira, J. M. A., Mele, M., Vahl, P., Wielenga, V. T., Christiansen, P. M., Jensen, V. E. D., Pedersen, S. F., & Aalkjaer, C. (2013). Contribution of Na^+,HCO_3^--cotransport to cellular pH control in human breast cancer: a role for the breast cancer susceptibility locus NBCn1 (SLC4A7). *International Journal of Cancer, 132*, 1288–1299. https://doi.org/10.1002/ijc.27782.
4. Lee, S., Mele, M., Vahl, P., Christiansen, P. M., Jensen, V. E. D., & Boedtkjer, E. (2015). Na^+,HCO_3^--cotransport is functionally upregulated during human breast carcinogenesis and required for the inverted pH gradient across the plasma membrane. *Pflügers Archiv, 467*, 367–377. https://doi.org/10.1007/s00424-014-1524-0.
5. Lee, S., Axelsen, T. V., Andersen, A. P., Vahl, P., Pedersen, S. F., & Boedtkjer, E. (2016). Disrupting Na^+,HCO_3^--cotransporter NBCn1 (Slc4a7) delays murine breast cancer development. *Oncogene, 35*, 2112–2122. https://doi.org/10.1038/onc.2015.273.
6. Ames, S., Pastorekova, S., & Becker, H. M. (2018). The proteoglycan-like domain of carbonic anhydrase IX mediates non-catalytic facilitation of lactate transport in cancer cells. *Oncotarget, 9*, 27940–27957. https://doi.org/10.18632/oncotarget.25371.
7. Mahon, B. P., Bhatt, A., Socorro, L., Driscoll, J. M., Okoh, C., Lomelino, C. L., Mboge, M. Y., Kurian, J. J., Tu, C., Agbandje-McKenna, M., Frost, S. C., & McKenna, R. (2016). The structure of carbonic anhydrase IX is adapted for low-pH catalysis. *Biochemistry, 55*, 4642–4653. https://doi.org/10.1021/acs.biochem.6b00243.
8. Lee, S. H., McIntyre, D., Honess, D., Hulikova, A., Pacheco-Torres, J., Cerdán, S., Swietach, P., Harris, A. L., & Griffiths, J. R. (2018). Carbonic anhydrase IX is a pH-stat that sets an acidic tumour extracellular pH in vivo. *British Journal of Cancer, 119*, 622–630. https://doi.org/10.1038/s41416-018-0216-5.
9. Bartosova, M., et al. (2002). Expression of carbonic anhydrase IX in breast is associated with malignant tissues and is related to overexpression of c-erbB2. *The Journal of Pathology, 197*, 314–321. https://doi.org/10.1002/path.1120.
10. Shiozaki, A., Hikami, S., Ichikawa, D., Kosuga, T., Shimizu, H., Kudou, M., Yamazato, Y., Kobayashi, T., Shoda, K., Arita, T.,

Konishi, H., Komatsu, S., Kubota, T., Fujiwara, H., Okamoto, K., Kishimoto, M., Konishi, E., Marunaka, Y., & Otsuji, E. (2018). Anion exchanger 2 suppresses cellular movement and has prognostic significance in esophageal squamous cell carcinoma. *Oncotarget, 9*, 25993–26006. https://doi.org/10.18632/oncotarget.25417.

11. Anemone, A., Consolino, L., Arena, F., Capozza, M., & Longo, D. V. (2019). Imaging tumor acidosis: a survey of the available techniques for mapping in vivo tumor pH. *Cancer Metastasis Rev.* https://doi.org/10.1007/s10555-019-09782-9.
12. Gillies, R. J., Brown, J. S., Anderson, A. R. A., & Gatenby, R. A. (2018). Eco-evolutionary causes and consequences of temporal changes in intratumoural blood flow. *Nature Reviews. Cancer, 18*, 576–585. https://doi.org/10.1038/s41568-018-0030-7.
13. Wang, L. X., Liu, K., Lin, G. W., & Zhai, R. Y. (2012). Solitary necrotic nodules of the liver: histology and diagnosis with CT and MRI. *Hepatitis Monthly, 12*, e6212. https://doi.org/10.5812/hepatmon.6212.
14. Heidenreich, A., Ravery, V., & European Society of Oncological, U. (2004). Preoperative imaging in renal cell cancer. *World Journal of Urology, 22*, 307–315. https://doi.org/10.1007/s00345-004-0411-2.
15. Mohlin, S., Wigerup, C., Jogi, A., & Pahlman, S. (2017). Hypoxia, pseudohypoxia and cellular differentiation. *Experimental Cell Research, 356*, 192–196. https://doi.org/10.1016/j.yexcr.2017.03.007.
16. Barathova, M., Takacova, M., Holotnakova, T., Gibadulinova, A., Ohradanova, A., Zatovicova, M., Hulikova, A., Kopacek, J., Parkkila, S., Supuran, C. T., Pastorekova, S., & Pastorek, J. (2008). Alternative splicing variant of the hypoxia marker carbonic anhydrase IX expressed independently of hypoxia and tumour phenotype. *British Journal of Cancer, 98*, 129–136. https://doi.org/10.1038/sj.bjc.6604111.
17. Gatenby, R. A., & Gillies, R. J. (2004). Why do cancers have high aerobic glycolysis? *Nature Reviews. Cancer, 4*, 891–899. https://doi.org/10.1038/nrc1478.
18. Longo, D. L., Bartoli, A., Consolino, L., Bardini, P., Arena, F., Schwaiger, M., & Aime, S. (2016). In vivo imaging of tumor metabolism and acidosis by combining PET and MRI-CEST pH imaging. *Cancer Research, 76*, 6463–6470. https://doi.org/10.1158/0008-5472.CAN-16-0825.
19. Helmlinger, G., Yuan, F., Dellian, M., & Jain, R. K. (1997). Interstitial pH and pO2 gradients in solid tumors in vivo: high-resolution measurements reveal a lack of correlation. *Nature Medicine, 3*, 177–182.
20. Hashim, A. I., Zhang, X., Wojtkowiak, J. W., Martinez, G. V., & Gillies, R. J. (2011). Imaging pH and metastasis. *NMR in Biomedicine, 24*, 582–591. https://doi.org/10.1002/nbm.1644.
21. Swietach, P., Vaughan-Jones, R. D., Harris, A. L., & Hulikova, A. (2014). The chemistry, physiology and pathology of pH in cancer. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 369*, 20130099. https://doi.org/10.1098/rstb.2013.0099.
22. Gatenby, R. A., & Gawlinski, E. T. (1996). A reaction-diffusion model of cancer invasion. *Cancer Research, 56*, 5745–5753.
23. Stubbs, M., McSheehy, P. M., Griffiths, J. R., & Bashford, C. L. (2000). Causes and consequences of tumour acidity and implications for treatment. *Molecular Medicine Today, 6*, 15–19.
24. Halestrap, A. P., & Wilson, M. C. (2012). The monocarboxylate transporter family–role and regulation. *IUBMB Life, 64*, 109–119. https://doi.org/10.1002/iub.572.
25. Faubert, B., Li, K. Y., Cai, L., Hensley, C. T., Kim, J., Zacharias, L. G., Yang, C., Do, Q. N., Doucette, S., Burguete, D., Li, H., Huet, G., Yuan, Q., Wigal, T., Butt, Y., Ni, M., Torrealba, J., Oliver, D., Lenkinski, R. E., Malloy, C. R., Wachsmann, J. W., Young, J. D., Kernstine, K., & DeBerardinis, R. J. (2017). Lactate metabolism in human lung tumors. *Cell, 171*, 358–371 e359. https://doi.org/10.1016/j.cell.2017.09.019.
26. Nelson, S. J., Kurhanewicz, J., Vigneron, D. B., Larson, P. E. Z., Harzstark, A. L., Ferrone, M., van Criekinge, M., Chang, J. W., Bok, R., Park, I., Reed, G., Carvajal, L., Small, E. J., Munster, P., Weinberg, V. K., Ardenkjaer-Larsen, J. H., Chen, A. P., Hurd, R. E., Odegardstuen, L. I., Robb, F. J., Tropp, J., & Murray, J. A. (2013). Metabolic imaging of patients with prostate cancer using hyperpolarized [1-(1)(3)C]pyruvate. *Science Translational Medicine, 5*, 198ra108. https://doi.org/10.1126/scitranslmed.3006070.
27. Puppulin, L., Hosogi, S., Sun, H., Matsuo, K., Inui, T., Kumamoto, Y., Suzaki, T., Tanaka, H., & Marunaka, Y. (2018). Bioconjugation strategy for cell surface labelling with gold nanostructures designed for highly localized pH measurement. *Nature Communications, 9*, 5278. https://doi.org/10.1038/s41467-018-07726-5.
28. Morita, T., Nagaki, T., Fukuda, I., & Okumura, K. (1992). Clastogenicity of low pH to various cultured mammalian cells. *Mutation Research, 268*, 297–305.
29. Gillies, R. J., Verduzco, D., & Gatenby, R. A. (2012). Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. *Nature Reviews. Cancer, 12*, 487–493. https://doi.org/10.1038/nrc3298.
30. Corbet, C., Draoui, N., Polet, F., Pinto, A., Drozak, X., Riant, O., & Feron, O. (2014). The SIRT1/HIF2alpha axis drives reductive glutamine metabolism under chronic acidosis and alters tumor response to therapy. *Cancer Research, 74*, 5507–5519. https://doi.org/10.1158/0008-5472.CAN-14-0705.
31. Zhang, H. Y., Hormi-Carver, K., Zhang, X., Spechler, S. J., & Souza, R. F. (2009). In benign Barrett's epithelial cells, acid exposure generates reactive oxygen species that cause DNA double-strand breaks. *Cancer Research, 69*, 9083–9089. https://doi.org/10.1158/0008-5472.CAN-09-2518.
32. Delikatny, E. J., Chawla, S., Leung, D. J., & Poptani, H. (2011). MR-visible lipids and the tumor microenvironment. *NMR in Biomedicine, 24*, 592–611. https://doi.org/10.1002/nbm.1661.
33. Glunde, K., Guggino, S. E., Solaiyappan, M., Pathak, A. P., Ichikawa, Y., & Bhujwalla, Z. M. (2003). Extracellular acidification alters lysosomal trafficking in human breast cancer cells. *Neoplasia, 5*, 533–545.
34. Damaghi, M., Tafreshi, N. K., Lloyd, M. C., Sprung, R., Estrella, V., Wojtkowiak, J. W., Morse, D. L., Koomen, J. M., Bui, M. M., Gatenby, R. A., & Gillies, R. J. (2015). Chronic acidosis in the tumour microenvironment selects for overexpression of LAMP2 in the plasma membrane. *Nature Communications, 6*, 8752. https://doi.org/10.1038/ncomms9752.
35. Rothberg, J. M., Bailey, K. M., Wojtkowiak, J. W., Ben-Nun, Y., Bogyo, M., Weber, E., Moin, K., Blum, G., Mattingly, R. R., Gillies, R. J., & Sloane, B. F. (2013). Acid-mediated tumor proteolysis: contribution of cysteine cathepsins. *Neoplasia, 15*, 1125–1137.
36. Rozhin, J., Sameni, M., Ziegler, G., & Sloane, B. F. (1994). Pericellular pH affects distribution and secretion of cathepsin B in malignant cells. *Cancer Research, 54*, 6517–6525.
37. Wojtkowiak, J. W., Rothberg, J. M., Kumar, V., Schramm, K. J., Haller, E., Proemsey, J. B., Lloyd, M. C., Sloane, B. F., & Gillies, R. J. (2012). Chronic autophagy is a cellular adaptation to tumor acidic pH microenvironments. *Cancer Research, 72*, 3938–3947.
38. Walton, Z. E., Patel, C. H., Brooks, R. C., Yu, Y., Ibrahim-Hashim, A., Riddle, M., Porcu, A., Jiang, T., Ecker, B. L., Tameire, F., Koumenis, C., Weeraratna, A. T., Welsh, D. K., Gillies, R., Alwine, J. C., Zhang, L., Powell, J. D., & Dang, C. V. (2018). Acid suspends the circadian clock in hypoxia through inhibition of mTOR. *Cell, 174*, 72–87 e32. https://doi.org/10.1016/j.cell.2018.05.009.

39. Logozzi, M., Angelini, D. F., Iessi, E., Mizzoni, D., di Raimo, R., Federici, C., Lugini, L., Borsellino, G., Gentilucci, A., Pierella, F., Marzio, V., Sciarra, A., Battistini, L., & Fais, S. (2017). Increased PSA expression on prostate cancer exosomes in in vitro condition and in cancer patients. *Cancer Letters, 403*, 318–329. https://doi.org/10.1016/j.canlet.2017.06.036.
40. Parolini, I., Federici, C., Raggi, C., Lugini, L., Palleschi, S., de Milito, A., Coscia, C., Iessi, E., Logozzi, M., Molinari, A., Colone, M., Tatti, M., Sargiacomo, M., & Fais, S. (2009). Microenvironmental pH is a key factor for exosome traffic in tumor cells. *The Journal of Biological Chemistry, 284*, 34211–34222. https://doi.org/10.1074/jbc.M109.041152.
41. Ratajczak, J., Wysoczynski, M., Hayek, F., Janowska-Wieczorek, A., & Ratajczak, M. Z. (2006). Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. *Leukemia, 20*, 1487–1495. https://doi.org/10.1038/sj.leu.2404296.
42. Ban, J. J., Lee, M., Im, W., & Kim, M. (2015). Low pH increases the yield of exosome isolation. *Biochemical and Biophysical Research Communications, 461*, 76–79. https://doi.org/10.1016/j.bbrc.2015.03.172.
43. Logozzi, M., Mizzoni, D., Angelini, D., di Raimo, R., Falchi, M., Battistini, L., & Fais, S. (2018). Microenvironmental pH and exosome levels interplay in human cancer cell lines of different histotypes. *Cancers (Basel), 10*. https://doi.org/10.3390/cancers10100370.
44. Busco, G., Cardone, R. A., Greco, M. R., Bellizzi, A., Colella, M., Antelmi, E., Mancini, M. T., Dell'Aquila, M. E., Casavola, V., Paradiso, A., & Reshkin, S. J. (2010). NHE1 promotes invadopodial ECM proteolysis through acidification of the peri-invadopodial space. *The FASEB Journal, 24*, 3903–3915. https://doi.org/10.1096/fj.09-149518.
45. Boedtkjer, E., Bentzon, J. F., Dam, V. S., & Aalkjaer, C. N.+. (2016). HCO_3^--cotransporter NBCn1 increases pH_i gradients, filopodia and migration of smooth muscle cells and promotes arterial remodeling. *Cardiovascular Research, 111*, 227–239. https://doi.org/10.1093/cvr/cvw079.
46. Lloyd, M. C., Alfarouk, K. O., Verduzco, D., Bui, M. M., Gillies, R. J., Ibrahim, M. E., Brown, J. S., & Gatenby, R. A. (2014). Vascular measurements correlate with estrogen receptor status. *BMC Cancer, 14*, 279. https://doi.org/10.1186/1471-2407-14-279.
47. Lloyd, M. C., Cunningham, J. J., Bui, M. M., Gillies, R. J., Brown, J. S., & Gatenby, R. A. (2016). Darwinian dynamics of intratumoral heterogeneity: not solely random mutations but also variable environmental selection forces. *Cancer Research, 76*, 3136–3144. https://doi.org/10.1158/0008-5472.CAN-15-2962.
48. Estrella, V., Chen, T., Lloyd, M., Wojtkowiak, J., Cornnell, H. H., Ibrahim-Hashim, A., Bailey, K., Balagurunathan, Y., Rothberg, J. M., Sloane, B. F., Johnson, J., Gatenby, R. A., & Gillies, R. J. (2013). Acidity generated by the tumor microenvironment drives local invasion. *Cancer Research, 73*, 1524–1535. https://doi.org/10.1158/0008-5472.CAN-12-2796.
49. Gatenby, R. A., Gawlinski, E. T., Gmitro, A. F., Kaylor, B., & Gillies, R. J. (2006). Acid-mediated tumor invasion: a multidisciplinary study. *Cancer Research, 66*, 5216–5223. https://doi.org/10.1158/0008-5472.CAN-05-4193.
50. Pilon-Thomas, S., Kodumudi, K. N., el-Kenawi, A. E., Russell, S., Weber, A. M., Luddy, K., Damaghi, M., Wojtkowiak, J. W., Mulé, J. J., Ibrahim-Hashim, A., & Gillies, R. J. (2016). Neutralization of tumor acidity improves antitumor responses to immunotherapy. *Cancer Research, 76*, 1381–1390. https://doi.org/10.1158/0008-5472.CAN-15-1743.
51. Lardner, A. (2001). The effects of extracellular pH on immune function. *Journal of Leukocyte Biology, 69*, 522–530.
52. Calcinotto, A., Filipazzi, P., Grioni, M., Iero, M., de Milito, A., Ricupito, A., Cova, A., Canese, R., Jachetti, E., Rossetti, M., Huber, V., Parmiani, G., Generoso, L., Santinami, M., Borghi, M., Fais, S., Bellone, M., & Rivoltini, L. (2012). Modulation of microenvironment acidity reverses anergy in human and murine tumor-infiltrating T lymphocytes. *Cancer Research, 72*, 2746–2756. https://doi.org/10.1158/0008-5472.CAN-11-1272.
53. Grundstrom, S., Dohlsten, M., & Sundstedt, A. (2000). IL-2 unresponsiveness in anergic CD4+ T cells is due to defective signaling through the common gamma-chain of the IL-2 receptor. *Journal of Immunology, 164*, 1175–1184.
54. Wells, A. D., Walsh, M. C., Sankaran, D., & Turka, L. A. (2000). T cell effector function and anergy avoidance are quantitatively linked to cell division. *Journal of Immunology, 165*, 2432–2443.
55. Demotte, N., Stroobant, V., Courtoy, P. J., van der Smissen, P., Colau, D., Luescher, I. F., Hivroz, C., Nicaise, J., Squifflet, J. L., Mourad, M., Godelaine, D., Boon, T., & van der Bruggen, P. (2008). Restoring the association of the T cell receptor with CD8 reverses anergy in human tumor-infiltrating lymphocytes. *Immunity, 28*, 414–424. https://doi.org/10.1016/j.immuni.2008.01.011.
56. Dvorak, H. F. (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *The New England Journal of Medicine, 315*, 1650–1659. https://doi.org/10.1056/NEJM198612253152606.
57. Ashby, B. S. (1966). pH studies in human malignant tumours. *Lancet, 2*, 312–315.
58. Dong, L., Li, Z., Leffler, N. R., Asch, A. S., Chi, J. T., & Yang, L. V. (2013). Acidosis activation of the proton-sensing GPR4 receptor stimulates vascular endothelial cell inflammatory responses revealed by transcriptome analysis. *PLoS One, 8*, e61991. https://doi.org/10.1371/journal.pone.0061991.
59. Silva, A. S., Yunes, J. A., Gillies, R. J., & Gatenby, R. A. (2009). The potential role of systemic buffers in reducing intratumoral extracellular pH and acid-mediated invasion. *Cancer Research, 69*, 2677–2684. https://doi.org/10.1158/0008-5472.CAN-08-2394.
60. Ibrahim Hashim, A., Cornnell, H. H., Coelho Ribeiro, M. L., Abrahams, D., Cunningham, J., Lloyd, M., Martinez, G. V., Gatenby, R. A., & Gillies, R. J. (2011). Reduction of metastasis using a non-volatile buffer. *Clinical & Experimental Metastasis, 28*, 841–849. https://doi.org/10.1007/s10585-011-9415-7.
61. Robey, I. F., Baggett, B. K., Kirkpatrick, N. D., Roe, D. J., Dosescu, J., Sloane, B. F., Hashim, A. I., Morse, D. L., Raghunand, N., Gatenby, R. A., & Gillies, R. J. (2009). Bicarbonate increases tumor pH and inhibits spontaneous metastases. *Cancer Research, 69*, 2260–2268. https://doi.org/10.1158/0008-5472.CAN-07-5575.
62. Ibrahim-Hashim, A., Cornnell, H. H., Abrahams, D., Lloyd, M., Bui, M., Gillies, R. J., & Gatenby, R. A. (2012). Systemic buffers inhibit carcinogenesis in TRAMP mice. *The Journal of Urology, 188*, 624–631. https://doi.org/10.1016/j.juro.2012.03.113.
63. Ibrahim-Hashim, A., Robertson-Tessi, M., Enriquez-Navas, P. M., Damaghi, M., Balagurunathan, Y., Wojtkowiak, J. W., Russell, S., Yoonseok, K., Lloyd, M. C., Bui, M. M., Brown, J. S., Anderson, A. R. A., Gillies, R. J., & Gatenby, R. A. (2017). Defining cancer subpopulations by adaptive strategies rather than molecular properties provides novel insights into intratumoral evolution. *Cancer Research, 77*, 2242–2254. https://doi.org/10.1158/0008-5472.CAN-16-2844.
64. Pilot, C., Mahipal, A., & Gillies, R. J. (2018). Buffer therapy → buffer diet. *Journal of Nutrition & Food Sciences, 8*, 684–688.
65. Tian, B., Wong, W. Y., Hegmann, E., Gaspar, K., Kumar, P., & Chao, H. (2015). Production and characterization of a camelid single domain antibody-urease enzyme conjugate for the treatment of cancer. *Bioconjugate Chemistry, 26*, 1144–1155. https://doi.org/10.1021/acs.bioconjchem.5b00237.

66. Ramlau, R., et al. (2017). Phase I/II dose escalation study of L-DOS47 as a monotherapy in non-squamous non-small cell lung cancer patients. *Journal of Thoracic Oncology, 12*, S1017–S1072.
67. Bushinsky, D. A., Hostetter, T., Klaerner, G., Stasiv, Y., Lockey, C., McNulty, S., Lee, A., Parsell, D., Mathur, V., Li, E., Buysse, J., & Alpern, R. (2018). Randomized, controlled trial of TRC101 to increase serum bicarbonate in patients with CKD. *Clinical Journal of the American Society of Nephrology, 13*, 26–35. https://doi.org/10.2215/CJN.07300717.
68. Kazokaite, J., Aspatwar, A., Parkkila, S., & Matulis, D. (2017). An update on anticancer drug development and delivery targeting carbonic anhydrase IX. *PeerJ, 5*, e4068. https://doi.org/10.7717/peerj.4068.
69. Boyd, N. H., Walker K., Fried J., Hackney J. R., McDonald P. C., Benavides G. A., Spina R., Audia A., Scott S. E., Libby C. J., Tran A. N., Bevensee M. O., Griguer C., Nozell S., Gillespie G. Y., Nabors B., Bhat K. P., Bar E. E., Darley-Usmar V., Xu B., Gordon E., Cooper S. J., Dedhar S., Hjelmeland A. B.. (2017) Addition of carbonic anhydrase 9 inhibitor SLC-0111 to temozolomide treatment delays glioblastoma growth in vivo. *JCI Insight* 2, doi: https://doi.org/10.1172/jci.insight.92928.
70. Harguindey, S., et al. (2013). Cariporide and other new and powerful NHE1 inhibitors as potentially selective anticancer drugs - an integral molecular/biochemical/metabolic/clinical approach after one hundred years of cancer research. *Journal of Translational Medicine, 11*, 282. https://doi.org/10.1186/1479-5876-11-282.
71. Polanski, R., Hodgkinson, C. L., Fusi, A., Nonaka, D., Priest, L., Kelly, P., Trapani, F., Bishop, P. W., White, A., Critchlow, S. E., Smith, P. D., Blackhall, F., Dive, C., & Morrow, C. J. (2014). Activity of the monocarboxylate transporter 1 inhibitor AZD3965 in small cell lung cancer. *Clinical Cancer Research, 20*, 926–937. https://doi.org/10.1158/1078-0432.CCR-13-2270.
72. Beloueche-Babari, M., Wantuch, S., Casals Galobart, T., Koniordou, M., Parkes, H. G., Arunan, V., Chung, Y. L., Eykyn, T. R., Smith, P. D., & Leach, M. O. (2017). MCT1 inhibitor AZD3965 increases mitochondrial metabolism, facilitating combination therapy and noninvasive magnetic resonance spectroscopy. *Cancer Research, 77*, 5913–5924. https://doi.org/10.1158/0008-5472.CAN-16-2686.
73. Noble, R. A., Bell, N., Blair, H., Sikka, A., Thomas, H., Phillips, N., Nakjang, S., Miwa, S., Crossland, R., Rand, V., Televantou, D., Long, A., Keun, H. C., Bacon, C. M., Bomken, S., Critchlow, S. E., & Wedge, S. R. (2017). Inhibition of monocarboxyate transporter 1 by AZD3965 as a novel therapeutic approach for diffuse large B-cell lymphoma and Burkitt lymphoma. *Haematologica, 102*, 1247–1257. https://doi.org/10.3324/haematol.2016.163030.
74. Draoui, N., Schicke, O., Seront, E., Bouzin, C., Sonveaux, P., Riant, O., & Feron, O. (2014). Antitumor activity of 7-aminocarboxycoumarin derivatives, a new class of potent inhibitors of lactate influx but not efflux. *Molecular Cancer Therapeutics, 13*, 1410–1418. https://doi.org/10.1158/1535-7163.MCT-13-0653.
75. Doherty, J. R., Yang, C., Scott, K. E. N., Cameron, M. D., Fallahi, M., Li, W., Hall, M. A., Amelio, A. L., Mishra, J. K., Li, F., Tortosa, M., Genau, H. M., Rounbehler, R. J., Lu, Y., Dang, C. V., Kumar, K. G., Butler, A. A., Bannister, T. D., Hooper, A. T., Unsal-Kacmaz, K., Roush, W. R., & Cleveland, J. L. (2014). Blocking lactate export by inhibiting the Myc target MCT1 disables glycolysis and glutathione synthesis. *Cancer Research, 74*, 908–920. https://doi.org/10.1158/0008-5472.CAN-13-2034.
76. Marchiq, I., & Pouyssegur, J. (2016). Hypoxia, cancer metabolism and the therapeutic benefit of targeting lactate/H(+) symporters. *J Mol Med (Berl), 94*, 155–171. https://doi.org/10.1007/s00109-015-1307-x.
77. Benjamin, D., Robay, D., Hindupur, S. K., Pohlmann, J., Colombi, M., el-Shemerly, M. Y., Maira, S. M., Moroni, C., Lane, H. A., & Hall, M. N. (2018). Dual inhibition of the lactate transporters MCT1 and MCT4 is synthetic lethal with metformin due to NAD+ depletion in cancer cells. *Cell Reports, 25*, 3047–3058 e3044. https://doi.org/10.1016/j.celrep.2018.11.043.
78. Mele, L., Paino, F., Papaccio, F., Regad, T., Boocock, D., Stiuso, P., Lombardi, A., Liccardo, D., Aquino, G., Barbieri, A., Arra, C., Coveney, C., la Noce, M., Papaccio, G., Caraglia, M., Tirino, V., & Desiderio, V. (2018). A new inhibitor of glucose-6-phosphate dehydrogenase blocks pentose phosphate pathway and suppresses malignant proliferation and metastasis in vivo. *Cell Death & Disease, 9*, 572. https://doi.org/10.1038/s41419-018-0635-5.
79. Cremon, C., Stanghellini, V., Barbaro, M. R., Cogliandro, R. F., Bellacosa, L., Santos, J., Vicario, M., Pigrau, M., Alonso Cotoner, C., Lobo, B., Azpiroz, F., Bruley des Varannes, S., Neunlist, M., DeFilippis, D., Iuvone, T., Petrosino, S., di Marzo, V., & Barbara, G. (2017). Randomised clinical trial: the analgesic properties of dietary supplementation with palmitoylethanolamide and polydatin in irritable bowel syndrome. *Alimentary Pharmacology & Therapeutics, 45*, 909–922. https://doi.org/10.1111/apt.13958.
80. Indraccolo, U., Indraccolo, S. R., & Mignini, F. (2017). Micronized palmitoylethanolamide/trans-polydatin treatment of endometriosis-related pain: a meta-analysis. *Annali dell'Istituto Superiore di Sanità, 53*, 125–134. https://doi.org/10.4415/ANN_17_02_08.
81. Hitosugi, T., Zhou, L., Elf, S., Fan, J., Kang, H. B., Seo, J. H., Shan, C., Dai, Q., Zhang, L., Xie, J., Gu, T. L., Jin, P., Alečković, M., LeRoy, G., Kang, Y., Sudderth, J. A., DeBerardinis, R. J., Luan, C. H., Chen, G. Z., Muller, S., Shin, D. M., Owonikoko, T. K., Lonial, S., Arellano, M. L., Khoury, H. J., Khuri, F. R., Lee, B. H., Ye, K., Boggon, T. J., Kang, S., He, C., & Chen, J. (2012). Phosphoglycerate mutase 1 coordinates glycolysis and biosynthesis to promote tumor growth. *Cancer Cell, 22*, 585–600. https://doi.org/10.1016/j.ccr.2012.09.020.
82. Liberti, M. V., Dai, Z., Wardell, S. E., Baccile, J. A., Liu, X., Gao, X., Baldi, R., Mehrmohamadi, M., Johnson, M. O., Madhukar, N. S., Shestov, A. A., Chio, I. I. C., Elemento, O., Rathmell, J. C., Schroeder, F. C., McDonnell, D. P., & Locasale, J. W. (2017). A predictive model for selective targeting of the Warburg effect through GAPDH inhibition with a natural product. *Cell Metabolism, 26*, 648–659 e648. https://doi.org/10.1016/j.cmet.2017.08.017.
83. Harriman, G., Greenwood, J., Bhat, S., Huang, X., Wang, R., Paul, D., Tong, L., Saha, A. K., Westlin, W. F., Kapeller, R., & Harwood, H. J., Jr. (2016). Acetyl-CoA carboxylase inhibition by ND-630 reduces hepatic steatosis, improves insulin sensitivity, and modulates dyslipidemia in rats. *Proceedings of the National Academy of Sciences of the United States of America, 113*, E1796–E1805. https://doi.org/10.1073/pnas.1520686113.
84. Kim, C. W., Addy, C., Kusunoki, J., Anderson, N. N., Deja, S., Fu, X., Burgess, S. C., Li, C., Ruddy, M., Chakravarthy, M., Previs, S., Milstein, S., Fitzgerald, K., Kelley, D. E., & Horton, J. D. (2017). Acetyl CoA carboxylase inhibition reduces hepatic steatosis but elevates plasma triglycerides in mice and humans: a bedside to bench investigation. *Cell Metabolism, 26*, 576. https://doi.org/10.1016/j.cmet.2017.08.011.
85. Kall, S. L., Delikatny, E. J., & Lavie, A. (2018). Identification of a unique inhibitor-binding site on choline kinase alpha. *Biochemistry, 57*, 1316–1325. https://doi.org/10.1021/acs.biochem.7b01257.
86. Lacal, J. C., & Campos, J. M. (2015). Preclinical characterization of RSM-932A, a novel anticancer drug targeting the human choline kinase alpha, an enzyme involved in increased lipid metabolism of cancer cells. *Molecular Cancer Therapeutics, 14*, 31–39. https://doi.org/10.1158/1535-7163.MCT-14-0531.

87. Kumar, M., Arlauckas, S. P., Saksena, S., Verma, G., Ittyerah, R., Pickup, S., Popov, A. V., Delikatny, E. J., & Poptani, H. (2015). Magnetic resonance spectroscopy for detection of choline kinase inhibition in the treatment of brain tumors. *Molecular Cancer Therapeutics, 14*, 899–908. https://doi.org/10.1158/1535-7163.MCT-14-0775.
88. Mazarico, J. M., Sanchez-Arevalo Lobo, V. J., Favicchio, R., Greenhalf, W., Costello, E., Carrillo-de Santa Pau, E., Marques, M., Lacal, J. C., Aboagye, E., & Real, F. X. (2016). Choline kinase alpha (CHKalpha) as a therapeutic target in pancreatic ductal adenocarcinoma: expression, predictive value, and sensitivity to inhibitors. *Molecular Cancer Therapeutics, 15*, 323–333. https://doi.org/10.1158/1535-7163.MCT-15-0214.
89. Zaytseva, Y. Y., Rychahou, P. G., le, A. T., Scott, T. L., Flight, R. M., Kim, J. T., Harris, J., Liu, J., Wang, C., Morris, A. J., Sivakumaran, T. A., Fan, T., Moseley, H., Gao, T., Lee, E. Y., Weiss, H. L., Heuer, T. S., Kemble, G., & Evers, M. (2018). Preclinical evaluation of novel fatty acid synthase inhibitors in primary colorectal cancer cells and a patient-derived xenograft model of colorectal cancer. *Oncotarget, 9*, 24787–24800. https://doi.org/10.18632/oncotarget.25361.
90. Wang, B. Y., Zhang, J., Wang, J. L., Sun, S., Wang, Z. H., Wang, L. P., Zhang, Q. L., Lv, F. F., Cao, E. Y., Shao, Z. M., Fais, S., & Hu, X. C. (2015). Intermittent high dose proton pump inhibitor enhances the antitumor effects of chemotherapy in metastatic breast cancer. *Journal of Experimental & Clinical Cancer Research, 34*, 85. https://doi.org/10.1186/s13046-015-0194-x.
91. Spugnini, E. P., Buglioni, S., Carocci, F., Francesco, M., Vincenzi, B., Fanciulli, M., & Fais, S. (2014). High dose lansoprazole combined with metronomic chemotherapy: a phase I/II study in companion animals with spontaneously occurring tumors. *Journal of Translational Medicine, 12*, 225. https://doi.org/10.1186/s12967-014-0225-y.
92. Walsh, M., Fais, S., Spugnini, E. P., Harguindey, S., Abu Izneid, T., Scacco, L., Williams, P., Allegrucci, C., Rauch, C., & Omran, Z. (2015). Proton pump inhibitors for the treatment of cancer in companion animals. *Journal of Experimental & Clinical Cancer Research, 34*, 93. https://doi.org/10.1186/s13046-015-0204-z.
93. Upreti, M., Jyoti, A., & Sethi, P. (2013). Tumor microenvironment and nanotherapeutics. *Transl Cancer Res, 2*, 309–319. https://doi.org/10.3978/j.issn.2218-676X.2013.08.11.
94. Barkey, N. M., Preihs, C., Cornnell, H. H., Martinez, G., Carie, A., Vagner, J., Xu, L., Lloyd, M. C., Lynch, V. M., Hruby, V. J., Sessler, J. L., Sill, K. N., Gillies, R. J., & Morse, D. L. (2013). Development and in vivo quantitative magnetic resonance imaging of polymer micelles targeted to the melanocortin 1 receptor. *Journal of Medicinal Chemistry, 56*, 6330–6338. https://doi.org/10.1021/jm4005576.
95. Cheng, R., Meng, F., Deng, C., Klok, H. A., & Zhong, Z. (2013). Dual and multi-stimuli responsive polymeric nanoparticles for programmed site-specific drug delivery. *Biomaterials, 34*, 3647–3657. https://doi.org/10.1016/j.biomaterials.2013.01.084.
96. Zhu, L., & Torchilin, V. P. (2013). Stimulus-responsive nanopreparations for tumor targeting. *Integr Biol (Camb), 5*, 96–107. https://doi.org/10.1039/c2ib20135f.
97. Castaneda, L., et al. (2013). Acid-cleavable thiomaleamic acid linker for homogeneous antibody-drug conjugation. *Chem Commun (Camb), 49*, 8187–8189. https://doi.org/10.1039/c3cc45220d.
98. Lambert, J. M., & Morris, C. Q. (2017). Antibody-drug conjugates (ADCs) for personalized treatment of solid tumors: a review. *Advances in Therapy, 34*, 1015–1035. https://doi.org/10.1007/s12325-017-0519-6.
99. Rinaldi, F., Hanieh, P. N., del Favero, E., Rondelli, V., Brocca, P., Pereira, M. C., Andreev, O. A., Reshetnyak, Y. K., Marianecci, C., & Carafa, M. (2018). Decoration of nanovesicles with pH (low) insertion peptide (pHLIP) for targeted delivery. *Nanoscale Research Letters, 13*, 391. https://doi.org/10.1186/s11671-018-2807-8.
100. Tang, H., Zhao, W., Yu, J., Li, Y., & Zhao, C. (2018). Recent development of pH-responsive polymers for cancer nanomedicine. *Molecules, 24*. https://doi.org/10.3390/molecules24010004.
101. Wyatt, L. C., Lewis, J. S., Andreev, O. A., Reshetnyak, Y. K., & Engelman, D. M. (2018). Applications of pHLIP technology for cancer imaging and therapy: (trends in biotechnology 35, 653-664, 2017). *Trends in Biotechnology, 36*, 1300. https://doi.org/10.1016/j.tibtech.2017.11.005.
102. Wyatt, L. C., Moshnikova, A., Crawford, T., Engelman, D. M., Andreev, O. A., & Reshetnyak, Y. K. (2018). Peptides of pHLIP family for targeted intracellular and extracellular delivery of cargo molecules to tumors. *Proceedings of the National Academy of Sciences of the United States of America, 115*, E2811–E2818. https://doi.org/10.1073/pnas.1715350115.
103. Burns, K. E., Robinson, M. K., & Thevenin, D. (2015). Inhibition of cancer cell proliferation and breast tumor targeting of pHLIP-monomethyl auristatin E conjugates. *Molecular Pharmaceutics, 12*, 1250–1258. https://doi.org/10.1021/mp500779k.
104. Antosh, M. P., Wijesinghe, D. D., Shrestha, S., Lanou, R., Huang, Y. H., Hasselbacher, T., Fox, D., Neretti, N., Sun, S., Katenka, N., Cooper, L. N., Andreev, O. A., & Reshetnyak, Y. K. (2015). Enhancement of radiation effect on cancer cells by gold-pHLIP. *Proceedings of the National Academy of Sciences of the United States of America, 112*, 5372–5376. https://doi.org/10.1073/pnas.1501628112.
105. Yu, M., Guo, F., Wang, J., Tan, F., & Li, N. (2015). Photosensitizer-loaded pH-responsive hollow gold nanospheres for single light-induced photothermal/photodynamic therapy. *ACS Applied Materials & Interfaces, 7*, 17592–17597. https://doi.org/10.1021/acsami.5b05763.
106. Yu, M., Guo, F., Wang, J., Tan, F., & Li, N. (2016). A pH-driven and photoresponsive nanocarrier: remotely-controlled by near-infrared light for stepwise antitumor treatment. *Biomaterials, 79*, 25–35. https://doi.org/10.1016/j.biomaterials.2015.11.049.
107. Persi, E., Duran-Frigola, M., Damaghi, M., Roush, W. R., Aloy, P., Cleveland, J. L., Gillies, R. J., & Ruppin, E. (2018). Systems analysis of intracellular pH vulnerabilities for cancer therapy. *Nature Communications, 9*, 2997. https://doi.org/10.1038/s41467-018-05261-x.
108. Rahier, N. J., Molinier, N., Long, C., Deshmukh, S. K., Kate, A. S., Ranadive, P., Verekar, S. A., Jiotode, M., Lavhale, R. R., Tokdar, P., Balakrishnan, A., Meignan, S., Robichon, C., Gomes, B., Aussagues, Y., Samson, A., Sautel, F., & Bailly, C. (2015). Anticancer activity of koningic acid and semisynthetic derivatives. *Bioorganic & Medicinal Chemistry, 23*, 3712–3721. https://doi.org/10.1016/j.bmc.2015.04.004.
109. Kumagai, S., Narasaki, R., & Hasumi, K. (2008). Glucose-dependent active ATP depletion by koningic acid kills high-glycolytic cells. *Biochemical and Biophysical Research Communications, 365*, 362–368. https://doi.org/10.1016/j.bbrc.2007.10.199.
110. Peng, X., Gong, F., Chen, Y., Jiang, Y., Liu, J., Yu, M., Zhang, S., Wang, M., Xiao, G., & Liao, H. (2014). Autophagy promotes paclitaxel resistance of cervical cancer cells: involvement of Warburg effect activated hypoxia-induced factor 1-alpha-mediated signaling. *Cell Death & Disease, 5*, e1367. https://doi.org/10.1038/cddis.2014.297.
111. Du, Q. H., Peng, C., & Zhang, H. (2013). Polydatin: a review of pharmacology and pharmacokinetics. *Pharmaceutical Biology, 51*, 1347–1354. https://doi.org/10.3109/13880209.2013.792849.

112. Jones, N. P., & Schulze, A. (2012). Targeting cancer metabolism–aiming at a tumour's sweet-spot. *Drug Discovery Today, 17*, 232–241. https://doi.org/10.1016/j.drudis.2011.12.017.
113. Shi, J., Kantoff, P. W., Wooster, R., & Farokhzad, O. C. (2017). Cancer nanomedicine: progress, challenges and opportunities. *Nature Reviews. Cancer, 17*, 20–37. https://doi.org/10.1038/nrc.2016.108.
114. Uthaman, S., Huh, K. M., & Park, I. K. (2018). Tumor microenvironment-responsive nanoparticles for cancer theragnostic applications. *Biomater Res, 22*, 22. https://doi.org/10.1186/s40824-018-0132-z.
115. Sun, X., du, R., Zhang, L., Zhang, G., Zheng, X., Qian, J., Tian, X., Zhou, J., He, J., Wang, Y., Wu, Y., Zhong, K., Cai, D., Zou, D., & Wu, Z. (2017). A pH-responsive yolk-like nanoplatform for tumor targeted dual-mode magnetic resonance imaging and chemotherapy. *ACS Nano, 11*, 7049–7059. https://doi.org/10.1021/acsnano.7b02675.
116. Tsuchikama, K., & An, Z. (2018). Antibody-drug conjugates: recent advances in conjugation and linker chemistries. *Protein & Cell, 9*, 33–46. https://doi.org/10.1007/s13238-016-0323-0.
117. Jain, N., Smith, S. W., Ghone, S., & Tomczuk, B. (2015). Current ADC linker chemistry. *Pharmaceutical Research, 32*, 3526–3540. https://doi.org/10.1007/s11095-015-1657-7.
118. Lambert, J. M., & Berkenblit, A. (2018). Antibody-drug conjugates for cancer treatment. *Annual Review of Medicine, 69*, 191–207. https://doi.org/10.1146/annurev-med-061516-121357.
119. Reshetnyak, Y. K., Andreev, O. A., Segala, M., Markin, V. S., & Engelman, D. M. (2008). Energetics of peptide (pHLIP) binding to and folding across a lipid bilayer membrane. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 15340–15345. https://doi.org/10.1073/pnas.0804746105.
120. Wyatt, L. C., Lewis, J. S., Andreev, O. A., Reshetnyak, Y. K., & Engelman, D. M. (2017). Applications of pHLIP technology for cancer imaging and therapy. *Trends in Biotechnology, 35*, 653–664. https://doi.org/10.1016/j.tibtech.2017.03.014.
121. Izumi, H., Torigoe, T., Ishiguchi, H., Uramoto, H., Yoshida, Y., Tanabe, M., Ise, T., Murakami, T., Yoshida, T., Nomoto, M., & Kohno, K. (2003). Cellular pH regulators: potentially promising molecular targets for cancer chemotherapy. *Cancer Treatment Reviews, 29*, 541–549.
122. Taylor, S., Spugnini, E. P., Assaraf, Y. G., Azzarito, T., Rauch, C., & Fais, S. (2015). Microenvironment acidity as a major determinant of tumor chemoresistance: proton pump inhibitors (PPIs) as a novel therapeutic approach. *Drug Resistance Updates, 23*, 69–78. https://doi.org/10.1016/j.drup.2015.08.004.
123. De Milito, A., et al. (2010). pH-dependent antitumor activity of proton pump inhibitors against human melanoma is mediated by inhibition of tumor acidity. *International Journal of Cancer, 127*, 207–219. https://doi.org/10.1002/ijc.25009.
124. De Milito, A., Marino, M. L., & Fais, S. (2012). A rationale for the use of proton pump inhibitors as antineoplastic agents. *Current Pharmaceutical Design, 18*, 1395–1406.
125. Ferrari, S., Perut, F., Fagioli, F., Brach del Prever, A., Meazza, C., Parafioriti, A., Picci, P., Gambarotti, M., Avnet, S., Baldini, N., & Fais, S. (2013). Proton pump inhibitor chemosensitization in human osteosarcoma: from the bench to the patients' bed. *Journal of Translational Medicine, 11*, 268. https://doi.org/10.1186/1479-5876-11-268.
126. Spugnini, E. P., Baldi, A., Buglioni, S., Carocci, F., Milesi de Bazzichini, G., Betti, G., Pantaleo, I., Menicagli, F., Citro, G., & Fais, S. (2011). Lansoprazole as a rescue agent in chemoresistant tumors: a phase I/II study in companion animals with spontaneously occurring tumors. *Journal of Translational Medicine, 9*, 221. https://doi.org/10.1186/1479-5876-9-221.
127. Ranieri, G., Gadaleta, C. D., Patruno, R., Zizzo, N., Daidone, M. G., Hansson, M. G., Paradiso, A., & Ribatti, D. (2013). A model of study for human cancer: spontaneous occurring tumors in dogs. Biological features and translation for new anticancer therapies. *Critical Reviews in Oncology/Hematology, 88*, 187–197. https://doi.org/10.1016/j.critrevonc.2013.03.005.
128. Dobson, J. M. (2013). Breed-predispositions to cancer in pedigree dogs. *ISRN Vet Sci, 2013*, 941275. https://doi.org/10.1155/2013/941275.
129. Johnson, P. J. (1998). Dermatologic tumors (excluding sarcoids). *The Veterinary Clinics of North America. Equine Practice, 14*, 625–658, viii.
130. Merlo, D. F., Rossi, L., Pellegrino, C., Ceppi, M., Cardellino, U., Capurro, C., Ratto, A., Sambucco, P. L., Sestito, V., Tanara, G., & Bocchini, V. (2008). Cancer incidence in pet dogs: findings of the Animal Tumor Registry of Genoa, Italy. *Journal of Veterinary Internal Medicine, 22*, 976–984. https://doi.org/10.1111/j.1939-1676.2008.0133.x.
131. Kosugi, Y., Yamamoto, S., Sano, N., Furuta, A., Igari, T., Fujioka, Y., & Amano, N. (2015). Evaluation of acid tolerance of drugs using rats and dogs controlled for gastric acid secretion. *Journal of Pharmaceutical Sciences, 104*, 2887–2893. https://doi.org/10.1002/jps.24401.
132. Chen, C. H., Lee, C. Z., Lin, Y. C., Kao, L. T., & Lin, H. C. (2018). Negative association of proton pump inhibitors with subsequent development of breast cancer: a nationwide population-based study. *Journal of Clinical Pharmacology, 59*, 350–355. https://doi.org/10.1002/jcph.1329.
133. Papagerakis, S., Bellile, E., Peterson, L. A., Pliakas, M., Balaskas, K., Selman, S., Hanauer, D., Taylor, J. M. G., Duffy, S., & Wolf, G. (2014). Proton pump inhibitors and histamine 2 blockers are associated with improved overall survival in patients with head and neck squamous carcinoma. *Cancer Prevention Research (Philadelphia, Pa.), 7*, 1258–1269. https://doi.org/10.1158/1940-6207.CAPR-14-0002.
134. Falcone, R., Roberto, M., D'Antonio, C., Romiti, A., Milano, A., Onesti, C. E., Marchetti, P., & Fais, S. (2016). High-doses of proton pump inhibitors in refractory gastro-intestinal cancer: a case series and the state of art. *Digestive and Liver Disease, 48*, 1503–1505. https://doi.org/10.1016/j.dld.2016.08.126.
135. Casey, J. R., Grinstein, S., & Orlowski, J. (2010). Sensors and regulators of intracellular pH. *Nature Reviews. Molecular Cell Biology, 11*, 50–61. https://doi.org/10.1038/nrm2820.
136. Martinez-Zaguilan, R., Lynch, R. M., Martinez, G. M., & Gillies, R. J. (1993). Vacuolar-type H(+)-ATPases are functionally expressed in plasma membranes of human tumor cells. *The American Journal of Physiology, 265*, C1015–C1029. https://doi.org/10.1152/ajpcell.1993.265.4.C1015.
137. Sennoune, S. R., Bakunts, K., Martínez, G. M., Chua-Tuan, J. L., Kebir, Y., Attaya, M. N., & Martínez-Zaguilán, R. (2004). Vacuolar H+-ATPase in human breast cancer cells with distinct metastatic potential: distribution and functional activity. *American Journal of Physiology. Cell Physiology, 286*, C1443–C1452. https://doi.org/10.1152/ajpcell.00407.2003.
138. Cardone, R. A., Casavola, V., & Reshkin, S. J. (2005). The role of disturbed pH dynamics and the Na+/H+ exchanger in metastasis. *Nature Reviews. Cancer, 5*, 786–795. https://doi.org/10.1038/nrc1713.
139. Stock, C., & Pedersen, S. F. (2017). Roles of pH and the Na(+)/H(+) exchanger NHE1 in cancer: from cell biology and animal models to an emerging translational perspective? *Seminars in Cancer Biology, 43*, 5–16. https://doi.org/10.1016/j.semcancer.2016.12.001.
140. Mullard, A. (2016). Cancer metabolism pipeline breaks new ground. *Nature Reviews. Drug Discovery, 15*, 735–737. https://doi.org/10.1038/nrd.2016.223.

141. Neri, D., & Supuran, C. T. (2011). Interfering with pH regulation in tumours as a therapeutic strategy. *Nature Reviews. Drug Discovery, 10*, 767–777. https://doi.org/10.1038/nrd3554.
142. Pouyssegur, J., Franchi, A., & Pages, G. (2001). pHi, aerobic glycolysis and vascular endothelial growth factor in tumour growth. *Novartis Found Symp, 240*, 186–196; discussion 196–188.
143. Chiche, J., Fur, Y. L., Vilmen, C., Frassineti, F., Daniel, L., Halestrap, A. P., Cozzone, P. J., Pouysségur, J., & Lutz, N. W. (2012). In vivo pH in metabolic-defective Ras-transformed fibroblast tumors: key role of the monocarboxylate transporter, MCT4, for inducing an alkaline intracellular pH. *International Journal of Cancer, 130*, 1511–1520. https://doi.org/10.1002/ijc.26125.
144. Reshkin, S. J., et al. (2000). Na+/H+ exchanger-dependent intracellular alkalinization is an early event in malignant transformation and plays an essential role in the development of subsequent transformation-associated phenotypes. *The FASEB Journal, 14*, 2185–2197. https://doi.org/10.1096/fj.00-0029com.
145. Provost, J. J., & Wallert, M. A. (2013). Inside out: targeting NHE1 as an intracellular and extracellular regulator of cancer progression. *Chemical Biology & Drug Design, 81*, 85–101. https://doi.org/10.1111/cbdd.12035.
146. Commisso, C., Davidson, S. M., Soydaner-Azeloglu, R. G., Parker, S. J., Kamphorst, J. J., Hackett, S., Grabocka, E., Nofal, M., Drebin, J. A., Thompson, C. B., Rabinowitz, J. D., Metallo, C. M., Vander Heiden, M. G., & Bar-Sagi, D. (2013). Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells. *Nature, 497*, 633–637. https://doi.org/10.1038/nature12138.
147. Hosogi, S., Miyazaki, H., Nakajima, K. I., Ashihara, E., Niisato, N., Kusuzaki, K., & Marunaka, Y. (2012). An inhibitor of Na(+)/H(+) exchanger (NHE), ethyl-isopropyl amiloride (EIPA), diminishes proliferation of MKN28 human gastric cancer cells by decreasing the cytosolic Cl(−) concentration via DIDS-sensitive pathways. *Cellular Physiology and Biochemistry, 30*, 1241–1253. https://doi.org/10.1159/000343315.
148. Kellen, J. A., Mirakian, A., & Kolin, A. (1988). Antimetastatic effect of amiloride in an animal tumour model. *Anticancer Research, 8*, 1373–1376.
149. Matthews, H., Ranson, M., & Kelso, M. J. (2011). Anti-tumour/metastasis effects of the potassium-sparing diuretic amiloride: an orally active anti-cancer drug waiting for its call-of-duty? *International Journal of Cancer, 129*, 2051–2061. https://doi.org/10.1002/ijc.26156.
150. Dimmer, K. S., Friedrich, B., Lang, F., Deitmer, J. W., & Broer, S. (2000). The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. *The Biochemical Journal, 350*(Pt 1), 219–227.
151. Amith, S. R., Wilkinson, J. M., & Fliegel, L. (2016). KR-33028, a potent inhibitor of the Na(+)/H(+) exchanger NHE1, suppresses metastatic potential of triple-negative breast cancer cells. *Biochemical Pharmacology, 118*, 31–39. https://doi.org/10.1016/j.bcp.2016.08.010.
152. Di Sario, A., et al. (2007). Selective inhibition of ion transport mechanisms regulating intracellular pH reduces proliferation and induces apoptosis in cholangiocarcinoma cells. *Digestive and Liver Disease, 39*, 60–69. https://doi.org/10.1016/j.dld.2006.07.013.
153. Lv, C., Yang, X., Yu, B., Ma, Q., Liu, B., & Liu, Y. (2012). Blocking the Na+/H+ exchanger 1 with cariporide (HOE642) reduces the hypoxia-induced invasion of human tongue squamous cell carcinoma. *International Journal of Oral and Maxillofacial Surgery, 41*, 1206–1210. https://doi.org/10.1016/j.ijom.2012.03.001.
154. Counillon, L., Bouret, Y., Marchiq, I., & Pouyssegur, J. (2016). Na(+)/H(+) antiporter (NHE1) and lactate/H(+) symporters (MCTs) in pH homeostasis and cancer metabolism. *Biochimica et Biophysica Acta, 1863*, 2465–2480. https://doi.org/10.1016/j.bbamcr.2016.02.018.
155. Miranda-Goncalves, V., et al. (2013). Monocarboxylate transporters (MCTs) in gliomas: expression and exploitation as therapeutic targets. *Neuro-Oncology, 15*, 172–188. https://doi.org/10.1093/neuonc/nos298.
156. Pinheiro, C., Longatto-Filho, A., Azevedo-Silva, J., Casal, M., Schmitt, F. C., & Baltazar, F. (2012). Role of monocarboxylate transporters in human cancers: state of the art. *Journal of Bioenergetics and Biomembranes, 44*, 127–139. https://doi.org/10.1007/s10863-012-9428-1.
157. Manning Fox, J. E., Meredith, D., & Halestrap, A. P. (2000). Characterisation of human monocarboxylate transporter 4 substantiates its role in lactic acid efflux from skeletal muscle. *The Journal of Physiology, 529*(Pt 2), 285–293.
158. Ullah, M. S., Davies, A. J., & Halestrap, A. P. (2006). The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. *The Journal of Biological Chemistry, 281*, 9030–9037. https://doi.org/10.1074/jbc.M511397200.
159. Mathupala, S. P., Parajuli, P., & Sloan, A. E. (2004). Silencing of monocarboxylate transporters via small interfering ribonucleic acid inhibits glycolysis and induces cell death in malignant glioma: an in vitro study. *Neurosurgery, 55*, 1410–1419; discussion 1419.
160. Fang, J., Quinones, Q. J., Holman, T. L., Morowitz, M. J., Wang, Q., Zhao, H., Sivo, F., Maris, J. M., & Wahl, M. L. (2006). The H+-linked monocarboxylate transporter (MCT1/SLC16A1): a potential therapeutic target for high-risk neuroblastoma. *Molecular Pharmacology, 70*, 2108–2115. https://doi.org/10.1124/mol.106.026245.
161. Halestrap, A. P. (2012). The monocarboxylate transporter family–structure and functional characterization. *IUBMB Life, 64*, 1–9. https://doi.org/10.1002/iub.573.
162. Le Floch, R., et al. (2011). CD147 subunit of lactate/H+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 16663–16668. https://doi.org/10.1073/pnas.1106123108.
163. Baenke, F., Dubuis, S., Brault, C., Weigelt, B., Dankworth, B., Griffiths, B., Jiang, M., Mackay, A., Saunders, B., Spencer-Dene, B., Ros, S., Stamp, G., Reis-Filho, J. S., Howell, M., Zamboni, N., & Schulze, A. (2015). Functional screening identifies MCT4 as a key regulator of breast cancer cell metabolism and survival. *The Journal of Pathology, 237*, 152–165. https://doi.org/10.1002/path.4562.
164. Marchiq, I., Le Floch, R., Roux, D., Simon, M. P., & Pouyssegur, J. (2015). Genetic disruption of lactate/H+ symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin. *Cancer Research, 75*, 171–180. https://doi.org/10.1158/0008-5472.CAN-14-2260.
165. Martinez-Outschoorn, U. E., Peiris-Pages, M., Pestell, R. G., Sotgia, F., & Lisanti, M. P. (2017). Cancer metabolism: a therapeutic perspective. *Nature Reviews. Clinical Oncology, 14*, 11–31. https://doi.org/10.1038/nrclinonc.2016.60.
166. Supuran, C. T., & Winum, J. Y. (2015). Carbonic anhydrase IX inhibitors in cancer therapy: an update. *Future Medicinal Chemistry, 7*, 1407–1414. https://doi.org/10.4155/fmc.15.71.
167. McDonald, P. C., Winum, J. Y., Supuran, C. T., & Dedhar, S. (2012). Recent developments in targeting carbonic anhydrase IX for cancer therapeutics. *Oncotarget, 3*, 84–97. https://doi.org/10.18632/oncotarget.422.
168. Pastorek, J., et al. (1994). Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase and a putative helix-loop-helix DNA binding segment. *Oncogene, 9*, 2877–2888.

169. Supuran, C. T., Scozzafava, A., & Casini, A. (2003). Carbonic anhydrase inhibitors. *Medicinal Research Reviews, 23*, 146–189. https://doi.org/10.1002/med.10025.

170. Ilies, M. A., Vullo, D., Pastorek, J., Scozzafava, A., Ilies, M., Caproiu, M. T., Pastorekova, S., & Supuran, C. T. (2003). Carbonic anhydrase inhibitors. Inhibition of tumor-associated isozyme IX by halogenosulfanilamide and halogenophenylaminobenzolamide derivatives. *Journal of Medicinal Chemistry, 46*, 2187–2196. https://doi.org/10.1021/jm021123s.

171. Supuran, C. T. (2018). Carbonic anhydrase inhibitors as emerging agents for the treatment and imaging of hypoxic tumors. *Expert Opinion on Investigational Drugs, 27*, 963–970. https://doi.org/10.1080/13543784.2018.1548608.

172. Supuran, C. T. (2017). Carbonic anhydrase inhibition and the management of hypoxic tumors. *Metabolites, 7*. https://doi.org/10.3390/metabo7030048.

173. Andreucci, E., Peppicelli, S., Carta, F., Brisotto, G., Biscontin, E., Ruzzolini, J., Bianchini, F., Biagioni, A., Supuran, C. T., & Calorini, L. (2017). Carbonic anhydrase IX inhibition affects viability of cancer cells adapted to extracellular acidosis. *J Mol Med (Berl), 95*, 1341–1353. https://doi.org/10.1007/s00109-017-1590-9.

174. Lou, Y., McDonald, P. C., Oloumi, A., Chia, S., Ostlund, C., Ahmadi, A., Kyle, A., auf dem Keller, U., Leung, S., Huntsman, D., Clarke, B., Sutherland, B. W., Waterhouse, D., Bally, M., Roskelley, C., Overall, C. M., Minchinton, A., Pacchiano, F., Carta, F., Scozzafava, A., Touisni, N., Winum, J. Y., Supuran, C. T., & Dedhar, S. (2011). Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors. *Cancer Research, 71*, 3364–3376. https://doi.org/10.1158/0008-5472.CAN-10-4261.