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Causes, Consequences, and Therapy of Tumors Acidosis

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Abstract

While cancer is commonly described as “a disease of the genes”, it is also associated with massive metabolic re-programming 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) development of acid-activatable drugs and nanomedicines, and 4) inhibiting metabolic processes responsible for generating acids in the first place.

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INTRODUCTION

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 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 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 net generation of one H^+ equivalent and is thus also acidifying.

Metabolic acid loads occur intracellularly, and cells have evolved robust and redundant mechanisms to export H^+ and maintain intracellular pH within strict bounds (Figure 1). Active (ATP-requiring) H^+ equivalent transporters include: 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 re-hydrated extracellularly (producing a H^+) via membrane bound, exofacial carbonic anhydrases (CA4, CA9, or CA12)⁶. Of these, CA9 is active at very low pH values⁷ and is considered a “pH-stat” responsible for acidifying the extracellular microenvironment⁸. CA9 has long been known to be a negative prognostic indicator in breast and other cancers⁹.

Anion exchanger 2 (AE2) participates in the exchange of Cl^- with HCO_3^- , regulating acid-base balance in the intracellular space and micro-environments 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 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 could be used as diagnostic, predictive, and/or response biomarkers, have been comprehensively reviewed elsewhere¹¹.

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 characteristics¹². 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. Necrotic core are 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 MRI¹⁸. Acidosis is also exacerbated by poor perfusion, as pH decreases with increasing distance from blood vessels¹⁹. This results in the accumulation of acids in the extracellular environment and an acidic tumor pHe, 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 (Figure 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 membrane²⁴. A recent study using ¹³C 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 MCT1²⁵. Similarly, the conversion of ¹³C pyruvate to ¹³C lactate (and vice versa) can be monitored in human patients using dynamic nuclear hyperpolarized magnetic resonance imaging²⁶. This release of acid by fermentative cells, results in profound acidity in areas that are proximal to the cell membrane. A recent study using Raman spectroscopy with gold nanoparticles reported an NHE dependent extremely low pH (~6.0) within 20 nM from the plasma membrane of tumor cells²⁷. This information would be helpful for design of pH-dependent drug development.

Consequences of tumor acidosis

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 (Figure 3). At the genomic level, acidosis can induce genome instability through chromosome breakages and translocations driving somatic evolution²⁸. Acidosis in the microenvironment provides a strong evolutionary selection pressure that contributes to the emergence of aggressive, therapy resistant clones²⁹. 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 acids³⁰. Increased oxidative flux increases reactive oxygen species³¹, 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 reversal of FAS inhibition and driving fatty acid synthesis that can paradoxically occur simultaneously with β -ox³⁰. This may be related to the significant increase in the abundance of adiposomes, which is a rapid and reversible response to acidosis³².

One of the most profound adaptations to acidosis is an increase in lysosomogenesis and redistribution of lysosomes from a peri-nuclear 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 redistribution of the lysosomal protein LAMP2 to the plasma membrane where it protects from acid hydrolysis³⁴. LAMP2 is also a key participant in inducing autophagy and acid-adapted cells are known to be chronically autophagic³⁷. The redistribution of lysosomes has also been associated with separation of mTORC from its regulatory complex, including RHEB³⁸, 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 communication⁴¹ and indeed, acidosis has been shown to stabilize and increase exosomal RNA and protein content⁴². 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 osteosarcoma⁴³. The results obtained using 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.

Invasion and metastasis.—Acidosis is a potent stimulator of local invasion (Figure 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 trailing 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.

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- γ and IL-2, up-regulation 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 a transient ischemia-driven tissue acidification, which resolves as the wound heals⁵⁷. The role of acidification in physiological wound healing is not known with certainty, but recent data suggest that acidification stimulates production of inflammatory cytokines by the stroma or endothelium⁵⁸. As these induce neo-angiogenesis, the acidosis is reduced and inflammation resolves. In tumors, this acidification never resolves.

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.

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) direct 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 (Figure 4).

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 regulated⁵⁹. 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 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 SAEs⁶⁴.

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 cancers⁶⁵. Once at the target, the urease converts endogenous urea to 2NH_4^+ and 1HCO_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 target⁶⁷. It has just completed a phase III (NCT03317444), but has not yet been investigated in cancer.

Targeting Metabolic Vulnerabilities—As described above, adaptation to acidic microenvironment involves significant metabolic re-programming. 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. 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 survival at low, but not neutral, pH⁶⁸. 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 GAPDH⁶⁹. Using machine learning, pharmacogenomics and metabolomics, a recent study demonstrated that cytotoxic effect of KA treatment is heterogeneous and is determined by the quantitative extent of Warburg Effect; glucose uptake and lactate secretion⁷⁰. This study extended the

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^{71,72}.

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 properties⁷³. Polydatin has been used as an anti-cancer agent for years, without knowledge of its biochemical target⁷⁴. Recently, it has been shown to be a potent inhibitor of G6PD, the rate limiting step to enter the Pentose Phosphate Pathway, PPP⁷⁵. 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 overexpression of G6PD. Further, it is well-tolerated in vivo and a phase II trial (albeit not in cancer) has been completed⁷⁶. 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 studies⁷⁷.

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^{78–80}. Notably, many of these contain either PET or activatable MR imaging moieties that allow monitoring of targeting and activation^{81, 82}. 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 trials⁸³. The chemistries of acid labile linkers has a long history and continues to evolve in order to fine tune the kinetics and pH profile of these linkers^{84,85}

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 membrane⁸⁶. 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 both⁸⁷.

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^{88, 89}. PPIs are Tetracyclic Sulfenamides that activated by protonation to become sulhdryl (e.g. cysteine) reagents. They were developed to be activated by stomach acid and bind irreversibly to and inhibit the gastric H^+/K^+ ATPase. In cancer cells, PPIs have been shown to increase the pH of lysosomes due to inhibition of V-ATPase, and targeting in an acidic milieu may be due to the increased lysosomal-endosomal turnover, described above^{90, 91}. PPIs may be used a monotherapy or in combination with chemo- or immune-therapies with improved responses^{52, 92, 93}.

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 treatment⁹⁴ or metronomic regimens⁹⁵. These studies carry a significant translational value considering that cancers in companion animals are spontaneous and can share many similarities with human tumors⁹⁶. 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%⁹⁷. 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 to 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 institutions⁹⁸, compared to dogs whose incidence, accordingly to the cancer registries, is around 45%, despite being their lifespan much shorter than horses⁹⁹. This could also explain the extreme effectiveness of alkaline therapy in such species when combined to conventional chemotherapy¹⁰⁰.

Because of their widespread use, there are a number of population-based studies showing beneficial effects of PPIs in the management of cancer. A recent observational case-control study accrued 64,234 women diagnosed with breast cancer between 2004 and in 2013 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 exposure¹⁰¹. 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 makes them ideal candidates for further exploration into their anticancer effects. A retrospective meta-analysis of 596 previously untreated head and neck squamous cell carcinoma (HNSCC) patients revealed a strong univariate association between PPIs use and improved overall survival ($P<0.001$)¹⁰². A retrospective analysis of patients with refractory GI cancer showed that the addition of PPI to chemotherapy significantly increased the time to progression¹⁰³.

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, 104–108} (Figure 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^{109, 110}. 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 load¹¹¹. Further, silencing of NHE1 or MCT4 expression reduced the pH gradient and limited tumor growth in similar xenograft models^{112, 113}. Amiloride, the first NHE inhibitor developed was shown to affect metastatic process by decreasing vasoendothelial growth factor (VEGF) production and the activity of urokinase-type plasminogen activator (uPA), metalloproteinases (MMP) and other proteases¹¹⁴. Since then, more potent and specific NHE1 inhibitors have been developed (e.g. ethylisopropyl- Hexamethyl- or dimethyl- amilorides)^{115, 116}. These potassium sparing diuretic have antineoplastic and anti-metastatic properties and are well tolerated and safe^{117–120}. In clinical trials, cariporide, a non-amiloride based NHE1 inhibitor provided protection to the myocardium during ischemic-reperfusion injury, yet had a small therapeutic window¹²¹. 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^{121–123}. 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^{124–126}. 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^{119, 127, 128}. 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 regulation¹²⁹. 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, 130, 131}. 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 regulation¹³². This was further confirmed using 31P magnetic resonance spectroscopy by overexpressing MCT4 in Ras transformed cells, showing that intracellular pH was elevated to alkaline levels while extracellular pH was acidic¹¹². 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 pH¹³³. 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^{134–137}. As expected, the major metabolic consequences of MCT1 inhibition were lactate accumulation, decrease in intracellular pH as well as 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^{138, 139}. 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 cells¹⁴⁰. 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 phenotypes⁶⁸. 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^{141, 142}. 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 1994¹⁴³. In general, CA inhibitors contain sulfonamide groups that target the active site¹⁴⁴. Exofacial CAs, such as CA9 and CA12, can be selectively targeted by appending a large hydrophilic or halogenated group to reduce internalization¹⁴⁵. The status of CA9 and CA12 inhibitors and their role as anti-cancer agents has recently been reviewed^{146, 147}. Importantly, cancer cells adapted to acidosis are particularly susceptible to CA9 inhibition¹⁴⁸. In vivo, CA9 knockdown or specific CA9 inhibitors (CAI17) similarly suppressed tumor growth and metastasis in aggressive breast tumor models (4T1, MDAMB231)¹⁴⁹. A CA9/CA12 specific inhibitor SLC0111 was additive to temozolomide in delaying growth of GBM in vivo¹⁵⁰. 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).

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 with which to 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 to either reduce the acidosis itself to eliminate the fitness advantage, or to exploit the acid-induced vulnerabilities.

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Figure 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 CO_2 , and re-hydrated with exofacial carbonic anhydrases

Figure 2: Acid phenotypic heterogeneity and metabolic cooperativity in tumors (counterclockwise from lower left). The diffusion distance of O₂ 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 CO₂. 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.

Figure 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.

Figure 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 as well as bone, where the tumor generated acidity can be a potent effector of bone pain via ASICs (figure1). Local invasion is associated also with 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 leads to poorer perfusion. Finally, acid is a potent inhibitor of effector T cell function, inhibiting immune surveillance.

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 & 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&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 NCT00165594 NCT00165880 151
CA9/CA12	SLC0111	Clinical	Phase 1	NCT02215850 NCT03450018 150
NHE1	Cariporide	Preclinical		121
Lactate transport				
MCT1	AZD3965	Preclinical& Clinical	Ongoing; will be concluded in August 2019.	NCT01791595 134, 135, 137
MCT1&2	AR-C155858	Preclinical		152
MCT1&2	SR13800	Preclinical		153
MCT4	AZ93	Preclinical		138
	Syrosingopine	Preclinical	Inhibits MCT1 also	140
3. Targeting metabolic vulnerabilities under acidosis				
A) Pentose Phosphate Pathway				
Polydatin		Preclinical & clinical	Phase II; well tolerated as analgesic in irritable bowel syndrome and endometriosis	75 75, 76, 154
PGAM1	PGMI-004A	Preclinical		77
B) Glycolysis				
GAPDH	Koningic acid	Preclinical		70
C) Lipid synthesis				
ACC(AcetylCoA Carboxylase)	NDI-010976(ND-630, GS-0976 or firsocostat	Preclinical& Clinical (PhaseII)for NASH	Allosteric inhibitor; prevents dimerization of ACC, reduce FA synthesis, induce β ox	155
	MK4074	Clinical	Liver specific ACC1&2 inhibitor, for liver steatosis	156 NCT01431521 Phase 1 completed, no data available

Metabolic Pathway targeted	Agent	Stage of development	Comments	References
Choline kinase	TCD-717	TCD-717 in clinical development, breast, lung and colon	Promising phase I data	157
	RSM932A	Preclinical		158
	MN58b	Preclinical		159, 160
Fatty Acid	TVB-2640	Clinical Phase II		NCT03032484
Synthase(FASN)	TVB-3664	Preclinical		161
<u>Mevalonate Pathway</u>				
HMG-CoA reductase	Simvastatin	Phase II clinical Trial	Breast cancer stage I-II, preventing liver cancer in patients with liver cirrhosis	NCT00334542
<u>D) Lipid oxidation</u>				
CPT1	Etomoxir, Perhexiline	Clinical	Perhexilin is approved for antiangina therapy	NCT02431221
<u>4. Acid activated agents</u>				
Proton Pump Inhibitors H ⁺ /K ⁺ ATPase	Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole & Rabeprazole	Clinical, Phase II completed	Intermittent high dose PPI enhance the antitumor effects of chemotherapy in metastatic breast cancer patients	NCT01069081 93, 95, 162
Nanoparticles	Liposomes, micelles, polymeric nanoparticles	In vitro, preclinical	Drug release under acidic pH.	79, 81, 163, 164
ADCs	Acid labile linkers (Eg. Hydrazon e)	Clinical	Many ADCs have been approved by FDA. ADCs with drug release at acidic pH are under development	165, 166
pHLIPS	pHLIP MMAE conjugates,	Preclinical	Intracellular delivery of therapeutic agents	167–171
	HAuNS-pHLIP-Ce6 particles			172–174