



Universiteit  
Leiden  
The Netherlands

## Targeting tumor-associated acidity in cancer immunotherapy

Lacroix, R.; Rozeman, E.A.; Kreutz, M.; Renner, K.; Blank, C.U.

### Citation

Lacroix, R., Rozeman, E. A., Kreutz, M., Renner, K., & Blank, C. U. (2018). Targeting tumor-associated acidity in cancer immunotherapy. *Cancer Immunology, Immunotherapy*, 67(9), 1331-1348. doi:10.1007/s00262-018-2195-z

Version: Not Applicable (or Unknown)

License: [Licensed under Article 25fa Copyright Act/Law \(Amendment Taverne\)](#)

Downloaded from: <https://hdl.handle.net/1887/3627642>

**Note:** To cite this publication please use the final published version (if applicable).



# Targeting tumor-associated acidity in cancer immunotherapy

Ruben Lacroix<sup>1</sup> · Elisa A. Rozeman<sup>2</sup> · Marina Kreutz<sup>3</sup> · Kathrin Renner<sup>3</sup> · Christian U. Blank<sup>1,2</sup>

Received: 16 January 2018 / Accepted: 29 June 2018 / Published online: 5 July 2018  
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

## Abstract

Checkpoint inhibitors, such as cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed cell death-1 (PD-1) monoclonal antibodies have changed profoundly the treatment of melanoma, renal cell carcinoma, non-small cell lung cancer, Hodgkin lymphoma, and bladder cancer. Currently, they are tested in various tumor entities as monotherapy or in combination with chemotherapies or targeted therapies. However, only a subgroup of patients benefit from checkpoint blockade (combinations). This raises the question, which all mechanisms inhibit T cell function in the tumor environment, restricting the efficacy of these immunotherapeutic approaches. Serum activity of lactate dehydrogenase, likely reflecting the glycolytic activity of the tumor cells and thus acidity within the tumor microenvironment, turned out to be one of the strongest markers predicting response to checkpoint inhibition. In this review, we discuss the impact of tumor-associated acidity on the efficacy of T cell-mediated cancer immunotherapy and possible approaches to break this barrier.

**Keywords** Cancer · Immune therapy · Checkpoint blockade · Acidity · Lactic acid · Metabolism

## Abbreviations

CA	Carbonic anhydrase
CEST-MRI	Chemical exchange saturation transfer-MRI
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
DCA	Dichloroacetate
DNP-MRSI	Dynamic nuclear polarization-MRSI
MCT	Monocarboxylate transporter
NHE	Sodium proton exchanger
NKT cell	Natural killer T cell
PET	Positron emission tomography
PPI	Proton pump inhibitor
TME	Tumor microenvironment
T <sub>reg</sub>	Regulatory T cells
V-ATPase	Vacuolar-type H <sup>+</sup> -ATPase

## Introduction

Cancer is a relentless disease capable of adapting to a multitude of therapies; therefore, we are urgently in need of novel treatment strategies. One angle is to exploit the power of the immune system. Its role in tumor control was already proposed over a century ago [1]. Yet, findings from the last decades conclusively show its involvement in tumor control, with the discovery of neo-antigen specific immune cells in patients cementing its importance [2]. However, tumors use a myriad of strategies to circumvent immune pressure; accordingly, “avoiding immune destruction” is acknowledged as a hallmark of cancer [3]. Currently, the most successful immunotherapeutic strategy against cancer is to target immune checkpoints. These are “switches” that can either promote or inhibit the activity of immune cells. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), an inhibitory surface receptor, was first therapeutically exploited. A monoclonal antibody against this protein (Ipilimumab) changed profoundly systemic treatment of late stage melanoma, leading to long-term survival in a portion of patients [4]. Targeting the Programmed Cell Death Protein (PD-1)/PD-1 ligand 1 (PD-L1) pathway with monoclonal antibodies (nivolumab, pembrolizumab) further enhanced prognosis for melanoma patients [5–7]. First trials combining PD-1 and CTLA-4 blockade revealed additional clinical benefit [8–10]. The success of immune checkpoint targeting

✉ Christian U. Blank  
c.blank@nki.nl

<sup>1</sup> Department of Molecular Oncology and Immunology, Netherlands Cancer Institute, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands

<sup>2</sup> Department of Medical Oncology, Netherlands Cancer Institute, Amsterdam, The Netherlands

<sup>3</sup> Department of Internal Medicine III, University Hospital Regensburg, Regensburg, Germany

in melanoma is being translated to other tumor types such as non-small cell lung cancer, renal cell carcinoma, bladder cancer and breast cancer with varying success [11–15].

Still, immunotherapy is successful only in a subset of patients. This is due to the many mechanisms that tumors adopt to blunt anti-tumor immunity. These can be tumor cell intrinsic such as loss of antigen presentation or impaired responsiveness to interferons (IFN) [16–18]. While others are imposed by the tumor microenvironment (TME) such as stromal barriers [19], insufficient vascularization [20], hypoxia [21], nutrient shortage [22, 23], and lactate accumulation and concomitant acidification [24].

Acidification of the TME has been shown to promote angiogenesis [25–28], invasion [29–33] and metastasis [34–38]. Not surprisingly, tumor acidity is strongly linked to a poor prognosis [39–44]. Furthermore, acidity is associated with resistance to chemotherapy [45–48], radiation therapy [49, 50], and reduced survival after surgery [51, 52]. More recently, it has been recognized that the acidic nature of the tumor limits the efficacy of checkpoint therapies [53–55]. Thus, it is likely that the low intra-tumoral pH also downregulates anti-tumor immune responses. Median extracellular pH inside patients' tumors is between 6.9 and 7.0 (compared to 7.3–7.4 in normal tissue) [56]. At the same time, intracellular tumor pH remains unaffected [57]. In murine cancer models, the extracellular pH is reported to be even lower (6.2–6.9) [58]. Acidity is a consequence of tumor metabolism. It predominately originates from the fermentation of glucose to lactate, which is secreted in co-transport with a proton, below referred to as lactic acid [59–61]. Hypoxia as a consequence of inadequate vascularization can force tumor cells to anaerobic glycolysis. However, hypoxia and acidity have been established as independent parameters [62, 63]. Aerobic glycolysis appears to be favored also by oxygenated tumor cells. This metabolic preference, the so-called “Warburg effect”, is named after Otto Warburg, the first scientist who observed aberrant glucose metabolism in tumors [64]. Glycolysis is hypothesized to rapidly supply both the energy and the carbon source obligate for proliferation and adaptation to an ever-changing tumor microenvironment [65–67]. Tumor cells were shown to import exogenous lactate. Moreover, inhibiting lactate uptake debilitated tumor growth, testifying to the importance of this metabolic reprogramming [68–71]. Lactate uptake by tumor cells was recently demonstrated in vivo in patients with lung cancer [72]. Oncogenic mutations are at the root of this metabolic inclination [73–79]. As aerobic glycolysis is considerably less energy efficient compared to oxidative phosphorylation, an accelerated glycolytic flux is required. This necessitates a high rate of glucose uptake and lactic acid secretion that can be up to 30-fold the rate found in healthy tissues [80]. As a consequence, lactic acid accumulates inside the TME (mean 6.0 mM for non-metastatic lesions; 12.5 mM for metastatic

lesions [44]) resulting in a low pH. Recently, the combination of 2-deoxy-2-[fluorine-18]fluoro-D-glucose Positron emission tomography ( $^{18}\text{F}$ -FDG PET) and acid-sensitive MRI was used to elegantly demonstrate the overlap between areas of high glucose uptake and areas of acidity [81]. Still, glycolysis deficient tumors were, despite lacking lactic acid production, able to acidify their surroundings [82–84]. An alternative source of acidity is  $\text{CO}_2$  production by oxidative phosphorylation. The importance of the export of this molecule is indicated by reduced tumor growth when membrane carbonic anhydrases or bicarbonate transporters were inhibited [85, 86]. Considering both, the physical and the financial toxicities of checkpoint modulating therapies, one might want to exclude patients harboring acidic tumors or pre-treat them with anti-acidic therapies to ensure efficacy. Therefore, it is imperative to be able to diagnose tumor acidity. Here, we will discuss methods of establishing tumor acidity, followed by reviewing negative effects of acidity on the anti-tumor immune response and outcome of immune-based therapies. Finally, we outline therapeutic options that can counteract acidity and could promote the efficacy of immune therapies.

## Techniques to diagnose tumor acidity

Tumor acidity can be characterized in situ by technical adaptations of magnetic resonance imaging (MRI) and positron emission tomography (PET). Chemical exchange saturation transfer MRI (CEST-MRI) is a highly sensitive technique based on saturation of exchangeable protons on a molecule of interest at a frequency different from water protons. A detectable signal is generated when a proton on the molecule is transferred to the surrounding water. The molecule of interest can be one of the clinically approved pH-sensitive agents to allow for pH measurement. Murine studies revealed the potency of CEST-MRI. For example, tumor pH was mapped with ultra-high resolution ( $<0.6\text{ mm}^2$ ) in a broad spectrum of murine models [81, 87–92] and confirmed by pH electrode measurements [90]. CEST-MRI makes use of clinical grade equipment, facilitating its introduction into the clinic. A substantial step was recently taken when CEST-MRI was used in a clinical setting to accurately determine the pH of urine inside the bladder of a human volunteer [93]. In addition, PET can be modified to approximate tumor acidity. Advantages over MR-based methods are the high signal-to-noise ratio and the wide availability. One modification that allows PET to be used for pH estimation utilizes  $^{18}\text{F}$ -FDG derivatives that can only be transported inside the cell under acidic conditions [94]. Another adaptation makes use of radiolabeled peptides that preferentially insert in cell membranes in acidic environments. The latter method was used to map acidity in a variety of murine tumors [95, 96].

Alternative methods for determining tumor acidity focus on the detection of lactate as it is a reliable surrogate for acidity [59–61]. Lactate can be detected in cryopreserved tumor material with enzymatic assays [97]. Additionally, bioluminescence imaging can be employed to visualize lactate distribution [98]. However, the latter technique necessitates skill and specialized equipment. Implementation of enzymatic assays into clinical routine is further complicated by the requirement to freeze samples instantly after the biopsy is taken. In addition, tumors are known to be heterogeneous, and as a result the probed area may not be representative of the whole tumor. To overcome these problems, tumor-wide lactate can be detected in situ with MRI. Quantification of lactate with  $^1\text{H}$ -MRI has been tested in patients with brain malignancies [99, 100], revealing that lactate is a marker of progression, inversely correlated with response to a combination of radiotherapy, temozolomide and enzastaurin [101, 102]. A limitation of  $^1\text{H}$ -MRI is the low specificity for lactate, preventing its use in tumors originating from tissues other than the brain [103, 104]. CEST-MRI grants detection of lactate with improved sensitivity [105]. As a proof of concept, CEST-MRI was recently used to detect lactate in muscle tissue of human volunteers [106]. Dynamic nuclear polarization-MRSI (DNP-MRSI) is a different MRI technique that can be used to quantify lactate. It derives its sensitivity from the injection of substrates with hyperpolarized nuclear spins. An advantage of using DNP-MRSI is that the fate of the labeled substrates can be followed.

Lactate measurements obtained with this method could be used to monitor development, progression, and response to therapy in murine models of cancer [107–114]. Currently, the technique is being prepared for entry into clinical routine [115]. A major hurdle was taken with the development of hyperpolarization equipment compatible with clinically available MRI scanners [116, 117]. Additionally, a recent study with the aim of detecting lactate levels in prostate cancer patients demonstrated safety, feasibility and sensitivity of DNP-MRSI [118]. Alternatively, lactate dehydrogenase (LDH), the enzyme responsible for the conversion of pyruvate to lactate, can be taken as a surrogate for the presence of lactate. LDH can be detected with immunohistochemistry if there is a biopsy available [119]. Due to accessibility it is more common in the clinic to measure the activity of LDH in patient blood. Studies indicate a correlation between serum and tumor LDH [120–123]. It is generally assumed that tumor LDH corresponds to intra-tumor lactate levels, yet, only anecdotal evidence exists for this [36, 124, 125]. On the other hand, serum LDH is an indicator of cell death [126, 127]. Therefore, it is conceivable that LDH release reflects tumor necrosis, which is expected to be higher in larger tumors [128]. However, recent evidence showed that there is no correlation between tumor burden and serum LDH [129,

130]. Notwithstanding, both tumor lactate and blood LDH activity share prognostic value in cancer [37, 43, 44, 131].

Which of these techniques should be used? Lactate detection in biopsies or LDH activity in the serum is both good starting points for a basic understanding of tumor acidity. Yet, more insight can be gained using advanced techniques. DNP-MRSI can be employed to measure lactate and other metabolites to monitor tumor progression or response to anti-glycolytic drugs. However, this method requires introduction of new machinery into the clinic. On the other hand, pH measurements with CEST-MRI, a technique which relies on clinically available resources and contrast agents, could be used to select patients for anti-acidic drugs in the near future.

### Tumor acidity as a predictive and prognostic marker for IT

Analysis of tumor acidity in patients treated with checkpoint inhibitors has not yet been established in clinical research. All studies, so far, are restricted to analyzing LDH activity in peripheral blood. In melanoma, LDH activity is an established prognostic marker for survival and is embedded in the AJCC-staging criteria [132]. Our group was one of the first showing that high LDH activity in peripheral blood correlated strongly with a negative outcome (in terms of objective response rate, progression free survival and overall survival) upon immunotherapy with ipilimumab in advanced melanoma patients [133]. The same was found later for melanoma patients treated with pembrolizumab [55], and for the combination of CTLA-4 and PD-1 blockade [134]. Furthermore, LDH correlates with outcome upon CTLA-4 and PD-1 blockade in uveal melanoma [135] and PD-1 blockade in non-small cell lung cancer [136]. Interestingly, high LDH activity in melanoma patients also impairs progression free survival and overall survival upon chemotherapy, and single BRAF or combined BRAF + MEK inhibition [129, 137, 138]. In contrast to checkpoint inhibitors, patients with elevated LDH activity initially do respond to BRAF + MEK inhibition [138]. Moreover, targeted therapy rapidly lowers LDH levels [139] and increased.

T-cell infiltration into the tumor [140–142]. Based on these data LDH has been incorporated into the Cancer Immunogram, a concept summarizing the requirements for an efficient anti-tumor immune response [143]. Thus, targeted therapy might be a promising combination partner for checkpoint inhibition by lowering tumor acidity, which is currently tested in several clinical trials (NCT02968303, NCT02631447, NCT02902029).

## Effects of acidity on the immune system

Tumor acidity and high LDH activity have been established as negative prognostic factors long ago [39–44]. Yet, only recently, studies have been uncovering the effects of acidity on blunting the anti-tumor immune response. T cells are thought to be crucial for effecting the anti-tumor immune response. Their importance is reflected by the prognostic power of CD8+ T cell infiltration in patients [144–146]. Furthermore, increased frequency of tumor infiltrating T cells (TIL) is associated with improved response to immune-based therapies [147]. One of the earliest studies showing the effect of low pH on T cells in vitro was performed by Bosticardo et al. in 2001. Their pioneering work showed that at pH 6.6, which is close to the physiological range of tumors [56], CD3+ T cell proliferation, cytokine production and cytotoxicity were impaired [148]. Subsequent studies which explored both CD4+ and CD8+ T cell function in medium acidified with either lactic acid or hydrochloric acid confirmed these original findings [24, 149–152].

These studies suggest that the effects of lactic acid are a consequence of the concomitant acidification rather than of the lactate molecule itself. This claim is further supported by the fact that buffering lactic acid to neutral pH abrogates its negative effects [24, 150]. Moreover, equal concentrations of sodium lactate had no negative impact on CD8+ T cells [24, 149]. Interestingly, subsequent culturing in fresh medium for 24 h reversed functional impairment [24, 149–152]. Whether reduced CD3+ T cell function is a consequence of increased cell death is under discussion. Several works argue that survival is initially unaffected [149, 151, 152, Lacroix and Blank, unpublished], but reduced after long-time culture in acidic medium [24, 153]. Comparable to T cells, natural killer (NK) and natural killer T (NKT) cells displayed reduced function when cultured at low pH in vitro [153–156]. Impaired viability of NK cells cultured in lactic acid was also reported [153]. The effects of lactic acid on lymphocytes are increasingly studied in physiological settings. This line of research started with Dröge et al. who showed in the late eighties that systemic injection of lactate did not impair priming of lymphocytes [157]. Our group (Kreutz lab) was one of the first focusing on the consequences on distinct immune subsets. We demonstrated, by applying an inhibitor of LDH-A in a tumor-T cell co culture, that tumor-derived lactic acid can inhibit CD8+ T cell function [24]. Subsequently, we demonstrated the negative impact of tumor-derived lactic acid on anti-tumor immunity in vivo [153]. LDH-A deficient tumors grew markedly slower than control tumors in immunocompetent mice, but not in mice lacking T and NK cells. Reduced interferon

gamma (IFN $\gamma$ ) and Granzyme B production by NK cells and both CD4+ and CD8+ T cells were identified as mechanisms of the adverse effect of lactic acid. In line with these data, increased NK cell activity and extended survival were observed in a murine B cell lymphoma model when tumor acidity was counteracted with sodium bicarbonate [158]. Expression of LDH-A in human melanomas negatively correlates with survival and expression of T cell activation markers, suggesting the relevance of these findings for immunotherapy in humans [153]. In contrast to conventional T cells, regulatory T cells (T<sub>reg</sub>) thrive in high lactate environments. The master T<sub>reg</sub> transcription factor forkhead box P3 (FoxP3) skews cellular metabolism away from glycolysis towards oxidative phosphorylation, negating the need to export lactate for their function [159]. Vice versa, inhibition of T cell glycolysis, as is proposed to be a consequence of lactic acid, leads to FoxP3 expression and induction of regulatory T cells [160–162]. Recent findings propose that lactate uptake might even be essential for immune suppression by T<sub>reg</sub> [163]. Adaptation of T<sub>reg</sub> to function in high concentrations of lactic acid could be understood from the fact that tumors and sites of trauma share the abundance of this molecule.

Monocytes and macrophages produced less pro-inflammatory cytokines when cultured in the presence of lactic acid [164–166], instead factors that promote tumor progression were being produced such as interleukin-17 (IL-17), interleukin 23 (IL-23), arginase (ARG1) and vascular endothelial growth factor (VEGF) [167–175]. Furthermore, priming of T cells was impaired in vitro [170]. No cell death was reported in vitro. The consequences of lactic acid on myeloid cells in vivo are subjected to increased study. Lactate prevented inflammation in a murine colitis model [176], presumably partly due to its anti-inflammatory effect on Macrophages [177]. Lewis lung carcinomas developed more rapidly in immunocompetent mice when cells were co-injected with macrophages cultured in medium with lactic acid [169]. This experiment showed for the first time that lactic acid can skew macrophages towards a *bona fide* pro-tumor phenotype. Lower frequencies of myeloid cells were found in glycolysis deficient tumors [153, 154]. While LDH-A deletion in myeloid cells themselves decreased tumor angiogenesis and boosted levels of intra tumor PD1<sup>+</sup> T cells. An explanation for the latter findings might be deduced from the in vitro observation that LDH-A deficient macrophages were more readily skewed towards an anti-tumor phenotype [178]. It was recently found in biopsies from patients with head and neck cancers that lactic acid was correlated with the levels of pro-tumor (CD163<sup>+</sup>) macrophages [97]. Altogether, these data firmly establish the importance of lactic acid in skewing macrophages towards a pro-tumor role.

In dendritic cells (DC), lactic acid impaired induction of monocyte-derived DCs [179], cytokine production and priming of T cells [177, 180, 181]. DCs extracted from murine gliomas were unable to produce interleukin 12 (IL-12) upon Toll-like receptor stimulation *ex vivo*. Functionality could be restored by treating mice with the glycolytic inhibitor Diclofenac [182]. Interestingly, and in contrast to its many inhibitory effects, DC antigen uptake, processing and expression of costimulatory molecules are reported to be higher in acidic conditions [183, 184].

Altered activity of immune cells in acidic conditions is partially a consequence of impaired metabolism. T cells [185, 186], NK cells [187, 188], monocytes [165], macrophages [166, 189] and DCs [189, 190] increase glycolytic rates after activation to support their function. Conversely, inhibiting glycolysis leads to decreased or alternative functionality of these immune cells [165, 166, 187, 188, 190–192]. Efficient disposal of lactic acid is a prerequisite for sustaining high glycolytic rates [193]. Immune cells unable to export lactic acid displayed decreased functionality [194, 195]. Reduced export of endogenous lactic acid is a consequence of high concentrations of extracellular lactic acid [24, 153, 165]. In line with these findings, reduced (glycolytic) metabolism is observed in immune cells cultured with lactic acid [152, 153, 165, 166]. These sequences of events may act as a common mechanism of metabolic inhibition by lactic acid preceding impaired function. The molecular consequences of lactic acidosis remain to be studied in detail. Lactate was shown to activate extracellular signal-regulated kinases (ERK)/Signal transducer and activator of transcription 3 (STAT3) signaling in bone marrow-derived macrophages [167]. Yet, in T cells no activation of ERK signaling was observed. Neither was the

phosphorylation of mitogen-activated protein kinase kinase (MEK), Protein kinase B (AKT/PKB) and proteins downstream of the T Cell receptor (TCR) affected. Rather, lactic acid led to a rapid reduction in phosphorylation of mitogen-activated protein kinase 8 (JNK), c-Jun, and p38 MAP kinase [149], proteins that are linked to cytokine production [196]. In contrast to these data, we showed that of the latter proteins only phosphorylation of p38 was reduced. Instead, we showed reduced upregulation of the transcription factor nuclear factor of activated T-cells (NFAT) upon culturing with lactic acid. NFAT is upregulated upon T cell activation and involved in IFN $\gamma$  signaling [197]. It is well known that acidity downregulates protein synthesis via the mechanistic target of rapamycin (mTOR) pathway [198, 199]. This pathway links metabolism to immune cell function and is a pivotal regulator of the immune response [200, 201], suggesting that acidity could impair immune cell function via mTOR signaling. Indeed, impaired NKT cell functionality as a consequence of acidity was mediated via the mTOR pathway [156]. An overview of the effects of lactic acid on immune cells is given in Table 1.

## Anti-acidic interventions combined with immune therapy

Acidity (indirectly measured by LDH levels) is associated with poor response to immune-based therapies in cancer [55, 133–136]. This might be in part due to the inhibitory effect on immune effectors as described afore. Thus, there is a clear rationale for combining immune therapies with compounds that prevent or counteract acidity. One option is to prevent (lactic) acid from being deposited in the tumor

**Table 1** Functional and metabolic consequences of acidity on immune cell populations (OxPhos: oxidative phosphorylation)

Immune population	Functional effects	Metabolic effects	Pathways affected
Tumor cell	↑ PD-L1 [178]		HIF-1a [202]
Effector T cell	↓ Proliferation [153] ↓ Effector cytokines [153] ↓ Cytotoxicity [153] ↑ FoxP3 (induction of T <sub>reg</sub> ) [160–162]	↓ Glycolysis [153]	↓ NFAT [153]
Regulatory T cell	↑ Immune suppression [163]	↓ Glycolysis [159] ↑ OxPhos [159]	↓ Myc [159]
NKT cell	↓ Effector cytokines [156]		↓ mTOR [156]
NK cell	↓ Effector cytokines [153–155] ↑ Apoptosis [153]	↓ metabolism [153]	↓ NFAT [153]
Monocyte	↓ Inflammatory cytokines (CCL2, TNF $\alpha$ ) [164, 165] ↑ Pro-tumoral factors (IL-23) [168]	↓ Glycolysis [165]	↓ AKT/NF $\kappa$ B [164]
Macrophage	↓ Inflammatory cytokines (IL-1b, IL-6, IL-12) [166] ↓ T cell priming [170] ↑ Pro-tumoral factors (IL-17, IL-23, ARG1, VEGF) [169]	↓ Glycolysis [166]	↓ ERK/STAT3
Dendritic cell	↓ T cell priming [180, 182] ↑ Pro-tumoral factors (IL-10) [181]		

microenvironment; production and export could be blocked by inhibiting glycolysis or monocarboxylate transporters (MCT's), respectively. A second opportunity is to inhibit transporters used by tumors to transfer excess protons out of the cell such as proton pumps and carbonic anhydrases. Alternatively, systemic alkaline supplementation can buffer tumor acidity. These strategies demonstrated efficacy in combination with conventional anti-cancer treatments in clinical trials. For instance, high-dose omeprazole improved efficacy of chemotherapy in breast cancer [203]. Whereas buffering the tumor with sodium carbonate increased the impact of chemoembolization in hepatocellular carcinoma [204]. A myriad of other combination therapies containing anti-acidic interventions is currently being evaluated in clinical trials (among others: NCT01791595, NCT01748500, NCT01069081, NCT01163903). A concise review covering the current state of de-acidifying drugs in preclinical and clinical studies was recently published by Tomas Koltai [205]. The impact of anti-acidic drugs on immune therapies is currently being subjected to intense study.

### Glycolysis inhibitors

Tumor acidity can be prevented by blocking glycolysis. Compounds that inhibit key players in this metabolic pathway are being investigated as anti-tumor drugs. For example, dichloroacetate (DCA) shifts metabolism away from glycolysis, thereby reducing the production of lactic acid and increasing tumor pH [110, 206, 207]. DCA showed potent anti-tumor effects in preclinical studies [208–210]. Opposing to earlier claims made that glycolysis inhibition affects macrophage function; it was shown that DCA rescued lactic acid-induced impairment of priming by murine macrophages *in vitro*. Furthermore, lower arginase expression was detected inside tumors from mice treated with DCA. Treatment with DCA synergized with poly I:C TLR stimulation to control the growth of subcutaneous tumors. Contrary to expectations, intra-tumor levels of lactic acid did not change upon DCA treatment, suggesting alternative immune potentiating mechanisms of the compound [170]. Altogether these data led to DCA being tested in cancer patients [211]. Exposing T cells to DCA *in vitro* did not reduce survival and proliferation. However, within human CD4+ T cells, DCA led to induction of regulatory T cells and interleukin 10 (IL-10) production at the expense of IFN $\gamma$  production [160–162]. Thus, in our view, this molecule might be counterproductive in cancer therapy. The widely used drug diclofenac was recently shown to reduce lactate production by tumor cells *in vitro* and *in vivo* [182, 212]. Furthermore, diclofenac generated a more immune permissive environment in murine gliomas characterized by increased DC function and lower levels and activation of T<sub>reg</sub>. Still, combining diclofenac with an immune potentiating TLR stimulus did

not increase survival of glioma-bearing mice [182]. In contrast, diclofenac exerted a positive impact on the response to anti-CTLA-4 and anti-PD1 therapy *in vivo* in a 4T1 model (Renner et al., data submitted). Yet, direct incubation of murine T cells with diclofenac dose-dependently reduced proliferation and cytokine secretion [182]. A variety of studies show a dependence of murine T cells on glycolysis. For instance, LDH-A knock-out murine CD4<sup>+</sup> T cells exhibited reduced cytokine production [213]. However, the importance of glycolysis for effector functions is questioned in human T cells, as effector functions are preserved in low glucose conditions [214, 215]. In our opinion, diclofenac or derivatives deserve further studying in combination with immune based therapies.

### Lactate transport inhibitors

Molecules that block the export of lactic acid by tumor cells can prevent tumors from becoming acidic. Export of lactic acid occurs mainly via the monocarboxylate transporters (MCTs). The importance of these proteins for tumor cells is illustrated by accumulation of intracellular lactate and growth reduction *in vivo* following impairment of MCT or its essential subunit CD147/BASIGIN [193, 216–220]. A MCT-1/MCT-2 inhibitor (AZD3965) is currently tested in phase I clinical trials for advanced solid tumors and diffuse large B cell lymphomas (NCT01791595). One concern might be that MCT inhibitors impair the functionality of T cells. Indeed, intracellular acidification and reduced proliferation was observed in T cells after inhibition of MCT1 and 2 with AR-C155858. Yet interleukin 2 (IL-2) production was conserved [195]. One study showed that anti-PD1 combined with an inhibitor specific for lactate uptake (7-ACC) generated superior tumor control compared to the single agents in the murine B16 melanoma model. Of note, this intervention did not reduce tumor acidity. Instead its efficacy is based on reducing the suppressive capability of T<sub>reg</sub>'s. Conventional T cells will not be affected by this intervention [163].

There might be a window where MCT blockade reduces T cell proliferation without affecting cytokine production. In that way, lactate transport inhibitors might become effective combination partners of checkpoint inhibition.

### Proton transport inhibitors

Besides MCT's, tumor cells use Vacuolar-type H<sup>+</sup>-ATPase's (V-ATPase), sodium proton exchangers (NHE's) and carbonic anhydrases (CA's) to dispose of excess protons. Compounds that inhibit V-ATPase's (PPI's) such as esomeprazole and pantoprazole are currently widely used in the clinic for gastric protection. Inhibiting V-ATPase's demonstrated anti-tumor effects *in vitro* [221–223]. <sup>31</sup>P-MRSI in combination with the cell impermeable pH reporter 3-aminopropyl

phosphonate (3-APP) was used to show that a single dose of esomeprazole rapidly increased the pH of engrafted murine (12.5 mg/kg;  $\text{pH}_c$  6.5–7) and human (2.5 mg/kg;  $\text{pH}_c$  6.55–6.85) melanomas. The increase in pH was maintained for several hours [151, 223]. Esomeprazole was combined with adoptive cell transfer (ACT), a therapy based on transfer of tumor reactive immune cells, in mice carrying B16 melanomas. Addition of the PPI resulted in increased frequency of IFN $\gamma$  producing TIL's. Of note, both transferred and endogenous T cells profited from the PPI treatment. The combination of ACT and PPI improved survival of tumor-bearing mice [151].

A different series of studies focused on the application of PPI's in T cell lymphoma. The tumor used in these studies strongly suppresses myelopoiesis. Dosing the mice with Pantoprazole rescued myelopoiesis and led to increased infiltration of anti-tumor macrophages in the tumor. Notably, a general shift away from immunosuppressive cytokines was observed in the TME, suggesting also other immune populations could benefit from PPI therapy [224, 225]. Although some studies dating back to the late 1990s suggest that direct inhibition of V-ATPase's in T cells might inhibit function and viability [226, 227]. Carbonic anhydrases (CA's) allow tumor cells to dispose of protons. Genetic knock down of these enzymes markedly reduced outgrowth of murine tumors [85, 86]. Acetazolamide is a clinically approved pan-CA inhibitor that showed anti-tumor activity in vitro, but remains to be tested in vivo [228–230]. A different class of proton exporters used by tumors is the sodium proton exchangers (NHE's). The relevance of these transporters for tumor cells is demonstrated by impaired tumor progression after genetic ablation or small molecule inhibition [86, 231–234]. Cariporide is a NHE inhibitor with reported anti-tumor activity in vitro [235, 236]. This inhibitor never made it to the clinic after failed clinical trials for ischemic cardiac events [237]. The current knowledge on inhibitors of proton pumps, carbonic anhydrases and sodium proton exchangers certainly supports further research on these molecules as partners for immune therapies.

## Buffer therapies

A straight forward approach to counter tumor acidity is systemic buffering. Sodium bicarbonate is a non-toxic compound widely used to neutralize stomach acid. It was demonstrated in mouse models that bicarbonate increased intra-tumoral pH and suppressed tumor progression [35]. A concise summary of the impact of systemic buffers on tumors was published recently by Faes and Dormond [238]. Currently, the consequences of systemic buffering on immunity are being scrutinized in preclinical studies.

It was shown that raising tissue pH with sodium bicarbonate led to increased function of NK cells and a significant

survival benefit in an endogenous model of murine B cell lymphoma. This advantage was abrogated when either NK cells or T cells were depleted, showing that the effects of systemic buffering were immune mediated, and may not be confined to a single immune population [158]. Bicarbonate improved both CD8<sup>+</sup> T cell infiltration and tumor control in the BRAF<sup>V600E</sup>/PTEN<sup>ko</sup>/ $\beta$  catenin-driven Yumm 1.1 melanoma model but not in BRAF<sup>wt</sup> B16 melanomas. Therefore, the authors combined sodium bicarbonate with immune therapies in B16 melanoma. Neither addition of bicarbonate to anti-CTLA-4 nor to the combination of CTLA-4 and PD-1 blockade led to a significant reduction in tumor size. However, buffering improved tumor control by PD-1 blockade to the level of the dual checkpoint blockade. The anti-PD-1/anti-CTLA-4 combination is approved for the treatment of melanoma, but is accompanied by severe adverse effects. Testing of anti-PD1 in combination with bicarbonate might therefore be clinically relevant. In a subsequent experiment, buffering substances were given together with adoptive T cell transfer. Systemic buffering increased persistence of transferred cells and resulted in an increased percentage of long-term surviving mice in comparison to mice that received adoptive cell transfer alone [152]. Furthermore, buffer therapy increased infiltration of transferred immune cells into the tumor in a xenograft model of hepatocellular carcinoma. Moreover, these TILs stained more positive for the cytotoxic marker perforin. In addition here, the combination of cell transfer and buffer therapy showed superior tumor control [239].

The question remains if systemic buffering is a clinically applicable strategy. In all studies, mice received drinking water with sodium bicarbonate at a concentration of 200 mM (16.8 g/l), leading to an intake of ~3.5 g/kg [35]. Only a single study reported toxicity at this concentration [240]. Murine data was used to simulate the sodium carbonate intake required for a human to achieve similar results. They concluded that a dosing of 1.1–1.7 g/kg was needed, far beyond the 0.5 g/kg that their model deemed safe [241]. High bicarbonate intake has been evaluated with athletes to counteract the negative effects of acidosis on performance. The tested doses were between 0.2 and 0.5 g/kg and were maintained for 5–6 days without side effects [242, 243]. A phase I clinical trial was recently completed where the participants received 0.5 g/kg bicarbonate daily for 90 days [NCT02531919], unfortunately no results were published. Side effects of systemic alkalization could be reduced by selecting a buffer substance with a more suitable acid dissociation constant ( $\text{pK}_a$ ) [241, 244]. While the undesirable effects of large sodium intake could be circumvented by choosing a buffer without a counter ion, such as 2-imidazole-1-yl-3-ethoxycarbonylpropionic acid (IEPA) or tris(hydroxymethyl)aminomethane (THAM, Tris). Both molecules prevented cancer metastasis in murine models



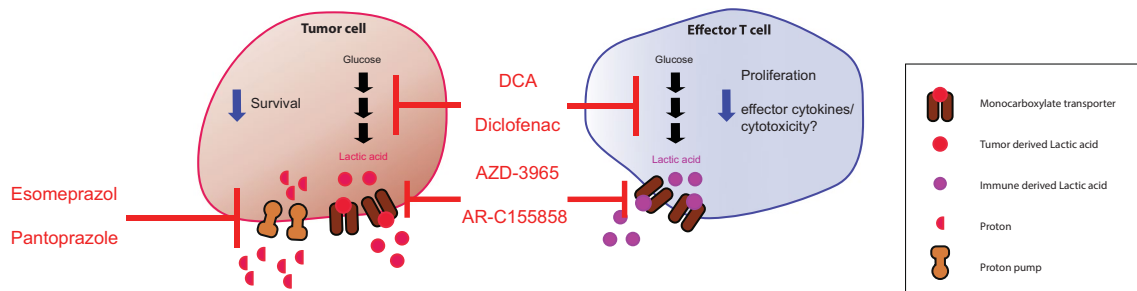
[245, 246]. Alternatively, novel means of administration could reduce systemic consequences. For instance, calcium carbonate nanoparticles were synthesized that, when dosed intravenously, release carbonate proportionate to the surrounding acidity, increasing the pH to a maximum of 7.4 [247]. Buffering therapies show promising results in pre-clinical cancer studies, while reports from other fields demonstrated clinical feasibility. Additionally, novel compounds and ways of administration could make buffering safer and easier. Hence, the combination of buffering agents and immune therapies especially deserves further investigation, having in our view a realistic chance to translate to the clinic. An overview of the discussed anti-acidic strategies is given in Table 1 and graphically represented in Fig. 1.

## Conclusion

Characteristic for tumors is their high rate of aerobic glycolysis. As a consequence, high amounts of lactic acid are produced, resulting in an acidic tumor environment. Acidity, correlates with both impaired prognosis and lower response

rates to immune-based therapies such as checkpoint inhibitors in cancer.

This might be in part due to the detrimental effects acidity has on immune effector cells. On the other hand, subpopulations of immune cells that do well in acidic conditions have acquired immune suppressive or tissue regenerating properties (Table 1). Targeting tumor acidity might thus be a promising approach to improve efficacy of immunotherapies. Several strategies are discussed in this review (Fig. 1; Table 2). As T cells rely on aerobic glycolysis and subsequent export of lactic acid to exert their function, caution is warranted when glycolysis inhibitors are to be combined with immune therapies. Immune cells appear less dependent on proton transporters to maintain their intracellular pH. PPI's for example might therefore be a promising partner for immune therapies. A different strategy to reduce tumor acidity without apparent negative effects on immune cells is systemic buffering. This intervention has been shown to improve endogenous anti-tumor immune responses, the effect of checkpoint inhibition and the efficacy of adoptive T cell therapies. As knowledge on tumor acidity is coming of age, its relevance in tumor immune evasion becomes clearer. We propose, therefore, that tumor acidity needs to



**Fig. 1** The effects of anti-acidic strategies on tumor and T cells. While targeting mediators of tumor acidity could lead to increased effect of immune based therapies, direct detrimental effects on T cell function are reported as well. Effects of anti-acidic interventions are

denoted inside the cell. Downwards facing arrows indicate a decrease. Systemic buffer therapies are omitted since there are no negative consequences for T cell function reported

**Table 2** Summary of tested combinations of anti-acidic partners with immune therapies

Compound	Effect on tumor acidity	Used in combination with which type of Immune therapy	Effect on immune cells	Effect on tumor control
DCA [170]	No difference in lactate	Poly I:C	↓ Arginase activity	Reduction in outgrowth of murine B16 and EG7
Esomeprazole (PPI) [151]	↑ pH	Adoptive cell transfer	↑ Persistence of transferred cells ↑ Immune cell function	Extended survival in murine B16.OVA
Sodium bicarbonate [152]	↑ pH	Anti-PD1 Adoptive T Cell transfer	↑ CD8 <sup>+</sup> infiltration	Extended survival in murine B16 and Panc02
Sodium bicarbonate [239]	↑ pH	Adoptive T and NK cell transfer	↑ Persistence of transferred cells ↑ Immune cell function	Reduction in outgrowth of murine HepG2

be considered as biomarker and that it should be targeted in combination with checkpoint inhibition or cellular therapies. We envision that tumors from patients with increased LDH activity will be examined with cutting-edge magnetic resonance-based imaging techniques to confirm tumor acidity. Subsequently, these patients will receive treatment regimens incorporating anti-acidic compounds. We hope that the diagnostic and therapeutic options proposed here will pave the way towards personalized immunotherapy and improve so the patients' outcome.

**Author contributions** Ruben Lacroix wrote the majority of the review and processed writing and suggestions by the co-authors. Elisa A Roze-man wrote the chapter "Tumor acidity as a predictive and prognostic marker for IT" and provided comments for the rest of the review. Kathrin Renner contributed considerable to the chapters "Anti-acidic interventions combined with immune therapy" and "Effects of acidity on the immune system" and provided input for other parts. The whole was done under the supervision of and final inspection by Marina Kreutz and Christian Blank.

**Funding** No relevant funding.

## Compliance with ethical standards

**Conflict of interest** Christian U. Blank receives grants and/or research support from Novartis and BMS, and has received honoraria or consultation fees for MSD, BMS, Roche, Novartis, GSK, Pfizer and Lilly. The other authors declare that they have no conflict of interest.

## References

- Ribatti D (2017) The concept of immune surveillance against tumors. The first theories. *Oncotarget* 8(4):7175–7180. <https://doi.org/10.18632/oncotarget.12739>
- Linnemann C, van Buuren MM, Bies L, Verdegaal EM, Schotte R, Calis JJ, Behjati S, Velds A, Hilkmann H, Atmioui DE, Visser M, Stratton MR, Haanen JB, Spits H, van der Burg SH, Schumacher TN (2015) High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. *Nat Med* 21(1):81–85. <https://doi.org/10.1038/nm.3773>
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
- Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, Patt D, Chen TT, Berman DM, Wolchok JD (2015) Pooled analysis of long-term survival data from phase II and Phase III trials of ipilimumab in unresectable or metastatic melanoma. *J Clin Oncol* 33(17):1889–1894. <https://doi.org/10.1200/JCO.2014.56.2736>
- Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, Weber JS, Joshua AM, Hwu WJ, Gangadhar TC, Patnaik A, Dronca R, Zarour H, Joseph RW, Boasberg P, Chmielowski B, Mateus C, Postow MA, Gergich K, Ellassaiss-Schaap J, Li XN, Iannone R, Ebbinghaus SW, Kang SP, Daud A (2014) Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet* 384(9948):1109–1117. [https://doi.org/10.1016/S0140-6736\(14\)60958-2](https://doi.org/10.1016/S0140-6736(14)60958-2)
- Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Mateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A, investigators K- (2015) Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med* 372(26):2521–2532. <https://doi.org/10.1056/NEJMoa1503093>
- Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, Hoeller C, Khushalani NI, Miller WH Jr, Lao CD, Linette GP, Thomas L, Lorigan P, Grossmann KF, Hassel JC, Maio M, Sznol M, Ascierto PA, Mohr P, Chmielowski B, Bryce A, Svane IM, Grob JJ, Krackhardt AM, Horak C, Lambert A, Yang AS, Larkin J (2015) Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol* 16(4):375–384. [https://doi.org/10.1016/S1470-2045\(15\)70076-8](https://doi.org/10.1016/S1470-2045(15)70076-8)
- Hodi FS, Chesney J, Pavlick AC, Robert C, Grossmann KF, McDermott DF, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor DR, Salama AK, Taylor MH, Ott PA, Horak C, Gagnier P, Jiang J, Wolchok JD, Postow MA (2016) Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. *Lancet Oncol* 17(11):1558–1568. [https://doi.org/10.1016/S1470-2045\(16\)30366-7](https://doi.org/10.1016/S1470-2045(16)30366-7)
- Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS, Wolchok JD (2015) Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med* 373(1):23–34. <https://doi.org/10.1056/NEJMoa1504030>
- Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, Lao CD, Wagstaff J, Schadendorf D, Ferrucci PF, Smylie M, Dummer R, Hill A, Hogg D, Haanen J, Carlino MS, Bechter O, Maio M, Marquez-Rodas I, Guidoboni M, McArthur G, Lebbe C, Ascierto PA, Long GV, Cebon J, Sosman J, Postow MA, Callahan MK, Walker D, Rollin L, Bhone R, Hodi FS, Larkin J (2017) Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 377(14):1345–1356. <https://doi.org/10.1056/NEJMoa1709684>
- Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, Barlesi F, Kohlhaufl M, Arrieta O, Burgio MA, Fayette J, Lena H, Poddubskaya E, Gerber DE, Gettinger SN, Rudin CM, Rizvi N, Crino L, Blumenschein GR Jr, Antonia SJ, Dorange C, Harbison CT, Graf Finckenstein F, Brahmer JR (2015) Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 373(17):1627–1639. <https://doi.org/10.1056/NEJMoa1507643>
- Gettinger SN, Horn L, Gandhi L, Spigel DR, Antonia SJ, Rizvi NA, Powderly JD, Heist RS, Carvajal RD, Jackman DM, Sequist LV, Smith DC, Leming P, Carbone DP, Pinder-Schenck MC, Topalian SL, Hodi FS, Sosman JA, Sznol M, McDermott DF, Pardoll DM, Sankar V, Ahlers CM, Salvati M, Wigginton JM, Hellmann MD, Kollia GD, Gupta AK, Brahmer JR (2015) Overall survival and long-term safety of nivolumab (anti-programmed death 1 antibody, BMS-936558, ONO-4538) in patients with previously treated advanced non-small-cell lung cancer. *J Clin Oncol* 33(18):2004–2012. <https://doi.org/10.1200/JCO.2014.58.3708>
- Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, Tsykodi SS, Sosman JA, Procopio G, Plimack

- ER, Castellano D, Choueiri TK, Gurney H, Donskov F, Bono P, Wagstaff J, Gauler TC, Ueda T, Tomita Y, Schutz FA, Kollmannsberger C, Larkin J, Ravaud A, Simon JS, Xu LA, Waxman IM, Sharma P, CheckMate I (2015) Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med* 373(19):1803–1813. <https://doi.org/10.1056/NEJMoa1510665>
14. Bellmunt J, Powles T, Vogelzang NJ (2017) A review on the evolution of PD-1/PD-L1 immunotherapy for bladder cancer: the future is now. *Cancer Treat Rev* 54:58–67. <https://doi.org/10.1016/j.ctrv.2017.01.007>
  15. Hartkopf AD, Taran FA, Wallwiener M, Walter CB, Kramer B, Grischke EM, Brucker SY (2016) PD-1 and PD-L1 immune checkpoint blockade to treat breast cancer. *Breast Care* 11(6):385–390. <https://doi.org/10.1159/000453569>
  16. Khong HT, Wang QJ, Rosenberg SA (2004) Identification of multiple antigens recognized by tumor-infiltrating lymphocytes from a single patient: tumor escape by antigen loss and loss of MHC expression. *J Immunother* 27(3):184–190
  17. Jager E, Ringhoffer M, Karbach J, Arand M, Oesch F, Knuth A (1996) Inverse relationship of melanocyte differentiation antigen expression in melanoma tissues and CD8+ cytotoxic-T-cell responses: evidence for immunoselection of antigen-loss variants in vivo. *Int J Cancer* 66 (4):470–476. [https://doi.org/10.1002/\(SICI\)1097-0215\(19960516\)66:4%3C470::AID-IJC10%3E3.0.CO;2-C](https://doi.org/10.1002/(SICI)1097-0215(19960516)66:4%3C470::AID-IJC10%3E3.0.CO;2-C)
  18. Shin DS, Zaretsky JM, Escuin-Ordinas H, Garcia-Diaz A, Huelieskovian S, Kalbasi A, Grasso CS, Hugo W, Sandoval S, Torrejon DY, Palaskas N, Rodriguez GA, Parisi G, Azhdam A, Chmielowski B, Cherry G, Seja E, Berent-Maoz B, Shintaku IP, Le DT, Pardoll DM, Diaz LA Jr, Tumei PC, Graeber TG, Lo RS, Comin-Anduix B, Ribas A (2017) Primary resistance to PD-1 blockade mediated by JAK1/2 mutations. *Cancer Discov* 7(2):188–201. <https://doi.org/10.1158/2159-8290.CD-16-1223>
  19. Salmon H, Franciszkiewicz K, Damotte D, Dieu-Nosjean MC, Validire P, Trautmann A, Mami-Chouaib F, Donnadieu E (2012) Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J Clin Invest* 122(3):899–910. <https://doi.org/10.1172/JCI45817>
  20. Siemann DW (2011) The unique characteristics of tumor vasculature and preclinical evidence for its selective disruption by tumor-vascular disrupting agents. *Cancer Treat Rev* 37(1):63–74. <https://doi.org/10.1016/j.ctrv.2010.05.001>
  21. Westendorf AM, Skibbe K, Adamczyk A, Buer J, Geffers R, Hansen W, Pastille E, Jendrossek V (2017) Hypoxia enhances immunosuppression by inhibiting CD4+ effector T cell function and promoting T<sub>reg</sub> activity. *Cell Physiol Biochem* 41(4):1271–1284. <https://doi.org/10.1159/000464429>
  22. Sukumar M, Roychoudhuri R, Restifo NP (2015) Nutrient competition: a new axis of tumor immunosuppression. *Cell* 162(6):1206–1208. <https://doi.org/10.1016/j.cell.2015.08.064>
  23. Timosenko E, Hadjinicolaou AV, Cerundolo V (2017) Modulation of cancer-specific immune responses by amino acid degrading enzymes. *Immunotherapy* 9(1):83–97. <https://doi.org/10.2217/imt-2016-0118>
  24. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, Gottfried E, Schwarz S, Rothe G, Hoves S, Renner K, Timischl B, Mackensen A, Kunz-Schughart L, Andreesen R, Krause SW, Kreutz M (2007) Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood* 109(9):3812–3819. <https://doi.org/10.1182/blood-2006-07-035972>
  25. Hunt TK, Aslam RS, Beckert S, Wagner S, Ghani QP, Hussain MZ, Roy S, Sen CK (2007) Aerobically derived lactate stimulates revascularization and tissue repair via redox mechanisms. *Antioxid Redox Signal* 9(8):1115–1124. <https://doi.org/10.1089/ars.2007.1674>
  26. Vegran F, Boidot R, Michiels C, Sonveaux P, Feron O (2011) Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-kappaB/IL-8 pathway that drives tumor angiogenesis. *Cancer Res* 71(7):2550–2560. <https://doi.org/10.1158/0008-5472.CAN-10-2828>
  27. Sonveaux P, Copetti T, De Saedeleer CJ, Vegran F, Verrax J, Kennedy KM, Moon EJ, Dhup S, Danhier P, Frerart F, Gallez B, Ribeiro A, Michiels C, Dewhirst MW, Feron O (2012) Targeting the lactate transporter MCT1 in endothelial cells inhibits lactate-induced HIF-1 activation and tumor angiogenesis. *PLoS one* 7(3):e33418. <https://doi.org/10.1371/journal.pone.0033418>
  28. Shi Q, Le X, Wang B, Abbruzzese JL, Xiong Q, He Y, Xie K (2001) Regulation of vascular endothelial growth factor expression by acidosis in human cancer cells. *Oncogene* 20(28):3751–3756. <https://doi.org/10.1038/sj.onc.1204500>
  29. Goetze K, Walenta S, Ksiazkiewicz M, Kunz-Schughart LA, Mueller-Klieser W (2011) Lactate enhances motility of tumor cells and inhibits monocyte migration and cytokine release. *Int J Oncol* 39(2):453–463. <https://doi.org/10.3892/ijo.2011.1055>
  30. Martinez-Zaguilan R, Seftor EA, Seftor RE, Chu YW, Gillies RJ, Hendrix MJ (1996) Acidic pH enhances the invasive behavior of human melanoma cells. *Clin Exp Metastasis* 14(2):176–186
  31. Kato Y, Nakayama Y, Umeda M, Miyazaki K (1992) Induction of 103-kDa gelatinase/type IV collagenase by acidic culture conditions in mouse metastatic melanoma cell lines. *J Biol Chem* 267(16):11424–11430
  32. Kato Y, Ozawa S, Tsukuda M, Kubota E, Miyazaki K, St-Pierre Y, Hata R (2007) Acidic extracellular pH increases calcium influx-triggered phospholipase D activity along with acidic sphingomyelinase activation to induce matrix metalloproteinase-9 expression in mouse metastatic melanoma. *FEBS J* 274(12):3171–3183. <https://doi.org/10.1111/j.1742-4658.2007.05848.x>
  33. Coman D, Huang Y, Rao JU, De Feyter HM, Rothman DL, Juchem C, Hyder F (2016) Imaging the intratumoral-peritumoral extracellular pH gradient of gliomas. *NMR Biomed* 29(3):309–319. <https://doi.org/10.1002/nbm.3466>
  34. Chen Y, Kung HN, Chen CH, Huang SH, Chen KH, Wang SM (2011) Acidic extracellular pH induces p120-catenin-mediated disruption of adherens junctions via the Src kinase-PKCdelta pathway. *FEBS Lett* 585(4):705–710. <https://doi.org/10.1016/j.febslet.2011.01.022>
  35. Robey IF, Baggett BK, Kirkpatrick ND, Roe DJ, Dosescu J, Sloane BF, Hashim AI, Morse DL, Raghunand N, Gatenby RA, Gillies RJ (2009) Bicarbonate increases tumor pH and inhibits spontaneous metastases. *Cancer Res* 69(6):2260–2268. <https://doi.org/10.1158/0008-5472.CAN-07-5575>
  36. Rizwan A, Serganova I, Khanin R, Karabeber H, Ni X, Thakur S, Zakian KL, Blasberg R, Koutcher JA (2013) Relationships between LDH-A, lactate, and metastases in 4T1 breast tumors. *Clin Cancer Res* 19(18):5158–5169. <https://doi.org/10.1158/1078-0432.CCR-12-3300>
  37. Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW, Mueller-Klieser W (2001) Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. *Int J Radiat Oncol Biol Phys* 51(2):349–353
  38. Walenta S, Salameh A, Lyng H, Evensen JF, Mitze M, Rofstad EK, Mueller-Klieser W (1997) Correlation of high lactate levels in head and neck tumors with incidence of metastasis. *Am J Pathol* 150(2):409–415
  39. Liu R, Cao J, Gao X, Zhang J, Wang L, Wang B, Guo L, Hu X, Wang Z (2016) Overall survival of cancer patients with serum lactate dehydrogenase greater than 1000 IU/L. *Tumor Biol* 37(10):14083–14088
  40. Li G, Wang Z, Xu J, Wu H, Cai S, He Y (2016) The prognostic value of lactate dehydrogenase levels in colorectal cancer:

- a meta-analysis. *BMC Cancer* 16:249. <https://doi.org/10.1186/s12885-016-2276-3>
41. Walenta S, Schroeder T, Mueller-Klieser W (2004) Lactate in solid malignant tumors: potential basis of a metabolic classification in clinical oncology. *Curr Med Chem* 11(16):2195–2204
  42. Walenta S, Wetterling M, Lehrke M, Schwickert G, Sundfor K, Rofstad EK, Mueller-Klieser W (2000) High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. *Cancer Res* 60(4):916–921
  43. Zhang J, Yao YH, Li BG, Yang Q, Zhang PY, Wang HT (2015) Prognostic value of pretreatment serum lactate dehydrogenase level in patients with solid tumors: a systematic review and meta-analysis. *Sci Rep* 5:9800. <https://doi.org/10.1038/srep09800>
  44. Walenta S, Mueller-Klieser WF (2004) Lactate: mirror and motor of tumor malignancy. *Semin Radiat Oncol* 14(3):267–274. <https://doi.org/10.1016/j.semradonc.2004.04.004>
  45. Wen Q, Meng X, Xie P, Wang S, Sun X, Yu J (2017) Evaluation of factors associated with platinum-sensitivity status and survival in limited-stage small cell lung cancer patients treated with chemoradiotherapy. *Oncotarget* 8(46):81405–81418. <https://doi.org/10.18632/oncotarget.19073>
  46. Sauvant C, Nowak M, Wirth C, Schneider B, Riemann A, Gekle M, Thews O (2008) Acidosis induces multi-drug resistance in rat prostate cancer cells (AT1) in vitro and in vivo by increasing the activity of the p-glycoprotein via activation of p38. *Int J Cancer* 123(11):2532–2542. <https://doi.org/10.1002/ijc.23818>
  47. Wachsberger PR, Landry J, Storck C, Davis K, O'Hara MD, Owen CS, Leeper DB, Coss RA (1997) Mammalian cells adapted to growth at pH 6.7 have elevated HSP27 levels and are resistant to cisplatin. *Int J Hyperth* 13(3):251–255; (discussion 257–259)
  48. Phua LC, Goh S, Tai DWM, Leow WQ, Alkaff SMF, Chan CY, Kam JH, Lim TKH, Chan ECY (2018) Metabolomic prediction of treatment outcome in pancreatic ductal adenocarcinoma patients receiving gemcitabine. *Cancer Chemother Pharmacol* 81(2):277–289. <https://doi.org/10.1007/s00280-017-3475-6>
  49. Park HJ, Lee SH, Chung H, Rhee YH, Lim BU, Ha SW, Griffin RJ, Lee HS, Song CW, Choi EK (2003) Influence of environmental pH on G2-phase arrest caused by ionizing radiation. *Radiat Res* 159(1):86–93
  50. Lee HS, Park HJ, Lyons JC, Griffin RJ, Auger EA, Song CW (1997) Radiation-induced apoptosis in different pH environments in vitro. *Int J Radiat Oncol Biol Phys* 38(5):1079–1087
  51. Shih CC, Lee TS, Tsuang FY, Lin PL, Cheng YJ, Cheng HL, Wu CY (2017) Pretreatment serum lactate level as a prognostic biomarker in patients undergoing supratentorial primary brain tumor resection. *Oncotarget* 8(38):63715–63723. <https://doi.org/10.18632/oncotarget.18891>
  52. Yuan ZY, Gao SG, Mu JW, Xue Q, Mao YS, Wang DL, Zhao J, Gao YS, Huang JF, He J (2016) Prognostic value of preoperative serum lactate dehydrogenase in thymic carcinoma. *J Thorac Dis* 8(9):2464–2472. <https://doi.org/10.21037/jtd.2016.08.56>
  53. Martens A, Wistuba-Hamprecht K, Geukes Foppen M, Yuan J, Postow MA, Wong P, Romano E, Khammari A, Dreno B, Capone M, Ascierto PA, Di Giacomo AM, Maio M, Schilling B, Sucker A, Schadendorf D, Hassel JC, Eigentler TK, Martus P, Wolchok JD, Blank C, Pawelec G, Garbe C, Weide B (2016) Baseline peripheral blood biomarkers associated with clinical outcome of advanced melanoma patients treated with ipilimumab. *Clin Cancer Res* 22(12):2908–2918. <https://doi.org/10.1158/1078-0432.CCR-15-2412>
  54. Damuzzo V, Solito S, Pinton L, Carrozzo E, Valpione S, Pigozzo J, Arboretti Giancristofaro R, Chiarion-Sileni V, Mandruzzato S (2016) Clinical implication of tumor-associated and immunological parameters in melanoma patients treated with ipilimumab. *Oncoimmunology* 5(12):e1249559. <https://doi.org/10.1080/2162402X.2016.1249559>
  55. Weide B, Martens A, Hassel JC, Berking C, Postow MA, Bisschop K, Simeone E, Mangana J, Schilling B, Di Giacomo AM, Brenner N, Kahler K, Heinzerling L, Gutzmer R, Bender A, Gebhardt C, Romano E, Meier F, Martus P, Maio M, Blank C, Schadendorf D, Dummer R, Ascierto PA, Hospers G, Garbe C, Wolchok JD (2016) Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab. *Clin Cancer Res* 22(22):5487–5496. <https://doi.org/10.1158/1078-0432.CCR-16-0127>
  56. Wike-Hooley JL, Haveman J, Reinhold HS (1984) The relevance of tumour pH to the treatment of malignant disease. *Radiother Oncol* 2(4):343–366
  57. Gerweck LE, Seetharaman K (1996) Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. *Cancer Res* 56(6):1194–1198
  58. Gillies RJ, Raghunand N, Karczmar GS, Bhujwala ZM (2002) MRI of the tumor microenvironment. *J Magn Reson Imaging* 16(4):430–450. <https://doi.org/10.1002/jmri.10181>
  59. Volk T, Jahde E, Fortmeyer HP, Glusenkamp KH, Rajewsky MF (1993) pH in human tumour xenografts: effect of intravenous administration of glucose. *Br J Cancer* 68(3):492–500
  60. Jain RK, Shah SA, Finney PL (1984) Continuous noninvasive monitoring of pH and temperature in rat Walker 256 carcinoma during normoglycemia and hyperglycemia. *J Natl Cancer Inst* 73(2):429–436
  61. Kallinowski F, Tyler G, Mueller-Klieser W, Vaupel P (1989) Growth-related changes of oxygen consumption rates of tumor cells grown in vitro and in vivo. *J Cell Physiol* 138(1):183–191. <https://doi.org/10.1002/jcp.1041380124>
  62. Yaromina A, Quennet V, Zips D, Meyer S, Shakirin G, Walenta S, Mueller-Klieser W, Baumann M (2009) Co-localisation of hypoxia and perfusion markers with parameters of glucose metabolism in human squamous cell carcinoma (hSCC) xenografts. *Int J Radiat Biol* 85(11):972–980. <https://doi.org/10.3109/09553000903232868>
  63. Hunt TK, Aslam R, Hussain Z, Beckert S (2008) Lactate, with oxygen, incites angiogenesis. *Adv Exp Med Biol* 614:73–80. [https://doi.org/10.1007/978-0-387-74911-2\\_9](https://doi.org/10.1007/978-0-387-74911-2_9)
  64. Warburg O, Wind F, Negelein E (1927) The metabolism of tumors in the Body. *J Gen Physiol* 8(6):519–530
  65. Epstein T, Gatenby RA, Brown JS (2017) The Warburg effect as an adaptation of cancer cells to rapid fluctuations in energy demand. *PLoS one* 12(9):e0185085. <https://doi.org/10.1371/journal.pone.0185085>
  66. Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324(5930):1029–1033. <https://doi.org/10.1126/science.1160809>
  67. San-Millan I, Brooks GA (2017) Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. *Carcinogenesis* 38(2):119–133. <https://doi.org/10.1093/carcin/bgw127>
  68. Kennedy KM, Scarbrough PM, Ribeiro A, Richardson R, Yuan H, Sonveaux P, Landon CD, Chi JT, Pizzo S, Schroeder T, Dewhirst MW (2013) Catabolism of exogenous lactate reveals it as a legitimate metabolic substrate in breast cancer. *PLoS one* 8(9):e75154. <https://doi.org/10.1371/journal.pone.0075154>
  69. Park S, Chang CY, Safi R, Liu X, Baldi R, Jasper JS, Anderson GR, Liu T, Rathmell JC, Dewhirst MW, Wood KC, Locasale JW, McDonnell DP (2016) ERR $\alpha$ -regulated lactate metabolism contributes to resistance to targeted therapies in breast cancer. *Cell Rep* 15(2):323–335. <https://doi.org/10.1016/j.celrep.2016.03.026>
  70. Corbet C, Bastien E, Draoui N, Doix B, Mignon L, Jordan BF, Marchand A, Vanherck JC, Chaltin P, Schakman O, Becker HM, Riand O, Feron O (2018) Interruption of lactate uptake

- by inhibiting mitochondrial pyruvate transport unravels direct antitumor and radiosensitizing effects. *Nat Commun* 9(1):1208. <https://doi.org/10.1038/s41467-018-03525-0>
71. 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. *Mol Cancer Ther* 13(6):1410–1418. <https://doi.org/10.1158/1535-7163.MCT-13-0653>
  72. Faubert B, Li KY, Cai L, Hensley CT, Kim J, Zacharias LG, Yang C, Do QN, Doucette S, Burguete D, Li H, Huet G, Yuan Q, Wigal T, Butt Y, Ni M, Torrealba J, Oliver D, Lenkinski RE, Malloy CR, Wachsmann JW, Young JD, Kernstine K, DeBerardinis RJ (2017) Lactate metabolism in human lung tumors. *Cell* 171(2):358–371 e359. <https://doi.org/10.1016/j.cell.2017.09.019>
  73. Kerr EM, Gaude E, Turrell FK, Frezza C, Martins CP (2016) Mutant Kras copy number defines metabolic reprogramming and therapeutic susceptibilities. *Nature* 531(7592):110–113. <https://doi.org/10.1038/nature16967>
  74. Brunelli L, Caiola E, Marabese M, Brogginini M, Pastorelli R (2016) Comparative metabolomics profiling of isogenic KRAS wild type and mutant NSCLC cells in vitro and in vivo. *Sci Rep* 6:28398. <https://doi.org/10.1038/srep28398>
  75. Gaglio D, Metallo CM, Gameiro PA, Hiller K, Danna LS, Balestrieri C, Alberghina L, Stephanopoulos G, Chiaradonna F (2011) Oncogenic K-Ras decouples glucose and glutamine metabolism to support cancer cell growth. *Mol Syst Biol* 7:523. <https://doi.org/10.1038/msb.2011.56>
  76. Ward PS, Thompson CB (2012) Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer cell* 21(3):297–308. <https://doi.org/10.1016/j.ccr.2012.02.014>
  77. Shin PJ, Zhu Z, Camarda R, Bok RA, Zhou AY, Kurhanewicz J, Goga A, Vigneron DB (2017) Cancer recurrence monitoring using hyperpolarized [1-(13)C]pyruvate metabolic imaging in murine breast cancer model. *Magn Reson Imaging* 43:105–109. <https://doi.org/10.1016/j.mri.2017.07.014>
  78. Chiche J, Le Fur Y, Vilmen C, Frassinetti F, Daniel L, Halestrap AP, Cozzzone PJ, Pouyssegur J, Lutz NW (2012) In vivo pH in metabolic-defective Ras-transformed fibroblast tumors: key role of the monocarboxylate transporter, MCT4, for inducing an alkaline intracellular pH. *Int J Cancer* 130(7):1511–1520. <https://doi.org/10.1002/ijc.26125>
  79. Singh KB, Hahm ER, Rigatti LH, Normolle DP, Yuan JM, Singh SV (2018) Inhibition of Glycolysis in Prostate Cancer Chemoprevention by Phenethyl Isothiocyanate. *Cancer Prev Res (Phila)*. <https://doi.org/10.1158/1940-6207.CAPR-17-0389>
  80. Holm E, Hagmuller E, Staedt U, Schlickeiser G, Gunther HJ, Leweling H, Tokus M, Kollmar HB (1995) Substrate balances across colonic carcinomas in humans. *Cancer Res* 55(6):1373–1378
  81. Longo DL, 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 Res* 76(22):6463–6470. <https://doi.org/10.1158/0008-5472.CAN-16-0825>
  82. Helmlinger G, Sckell A, Dellian M, Forbes NS, Jain RK (2002) Acid production in glycolysis-impaired tumors provides new insights into tumor metabolism. *Clin Cancer Res* 8(4):1284–1291
  83. Yamagata M, Hasuda K, Stamato T, Tannock IF (1998) The contribution of lactic acid to acidification of tumours: studies of variant cells lacking lactate dehydrogenase. *Br J Cancer* 77(11):1726–1731
  84. Newell K, Franchi A, Pouyssegur J, Tannock I (1993) Studies with glycolysis-deficient cells suggest that production of lactic acid is not the only cause of tumor acidity. *Proc Natl Acad Sci USA* 90(3):1127–1131
  85. Chiche J, Ilc K, Laferriere J, Trottier E, Dayan F, Mazure NM, Brahimi-Horn MC, Pouyssegur J (2009) Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. *Cancer Res* 69(1):358–368. <https://doi.org/10.1158/0008-5472.CAN-08-2470>
  86. Parks SK, Pouyssegur J (2015) The Na(+)/HCO3(−) co-transporter SLC4A4 plays a role in growth and migration of colon and breast cancer cells. *J Cell Physiol* 230(8):1954–1963. <https://doi.org/10.1002/jcp.24930>
  87. Longo DL, Sun PZ, Consolino L, Michelotti FC, Uggeri F, Aime S (2014) A general MRI-CEST ratiometric approach for pH imaging: demonstration of in vivo pH mapping with iobitridol. *J Am Chem Soc* 136(41):14333–14336. <https://doi.org/10.1021/ja5059313>
  88. Chen LQ, Howison CM, Jeffery JJ, Robey IF, Kuo PH, Pagel MD (2014) Evaluations of extracellular pH within in vivo tumors using acidoCEST MRI. *Magn Reson Med* 72(5):1408–1417. <https://doi.org/10.1002/mrm.25053>
  89. Moon BF, Jones KM, Chen LQ, Liu P, Randtke EA, Howison CM, Pagel MD (2015) A comparison of iopromide and iopamidol, two acidoCEST MRI contrast media that measure tumor extracellular pH. *Contrast Media Mol Imaging* 10(6):446–455. <https://doi.org/10.1002/cmml.1647>
  90. Chen LQ, Randtke EA, Jones KM, Moon BF, Howison CM, Pagel MD (2015) Evaluations of tumor acidosis within in vivo tumor models using parametric maps generated with acidoCEST MRI. *Mol Imaging Biol* 17(4):488–496. <https://doi.org/10.1007/s11307-014-0816-2>
  91. Chen M, Chen C, Shen Z, Zhang X, Chen Y, Lin F, Ma X, Zhuang C, Mao Y, Gan H, Chen P, Zong X, Wu R (2017) Extracellular pH is a biomarker enabling detection of breast cancer and liver cancer using CEST MRI. *Oncotarget* 8(28):45759–45767. <https://doi.org/10.18632/oncotarget.17404>
  92. Akhenblit PJ, Hanke NT, Gill A, Persky DO, Howison CM, Pagel MD, Baker AF (2016) Assessing metabolic changes in response to mTOR inhibition in a mantle cell lymphoma xenograft model using AcidoCEST MRI. *Mol Imaging*. <https://doi.org/10.1177/1536012116645439>
  93. Muller-Lutz A, Khalil N, Schmitt B, Jellus V, Pentang G, Oeltzschner G, Antoch G, Lanzman RS, Wittsack HJ (2014) Pilot study of Iopamidol-based quantitative pH imaging on a clinical 3T MR scanner. *Magma* 27(6):477–485. <https://doi.org/10.1007/s10334-014-0433-8>
  94. Flavell RR, Truillet C, Regan MK, Ganguly T, Blecha JE, Kurhanewicz J, VanBrocklin HF, Keshari KR, Chang CJ, Evans MJ, Wilson DM (2016) Caged [(18)F]FDG glycosylamines for imaging acidic tumor microenvironments using positron emission tomography. *Bioconjugate Chem* 27(1):170–178. <https://doi.org/10.1021/acs.bioconjchem.5b00584>
  95. Demoin DW, Wyatt LC, Edwards KJ, Abdel-Atti D, Sarparanta M, Pourat J, Longo VA, Carlin SD, Engelman DM, Andreev OA, Reshetnyak YK, Viola-Villegas N, Lewis JS (2016) PET imaging of extracellular pH in tumors with (64)Cu- and (18)F-labeled pHLIP peptides: a structure-activity optimization study. *Bioconjugate Chem* 27(9):2014–2023. <https://doi.org/10.1021/acs.bioconjchem.6b00306>
  96. Viola-Villegas NT, Carlin SD, Ackerstaff E, Sevak KK, Divilov V, Serganova I, Kruchevsky N, Anderson M, Blasberg RG, Andreev OA, Engelman DM, Koutcher JA, Reshetnyak YK, Lewis JS (2014) Understanding the pharmacological properties of a metabolic PET tracer in prostate cancer. *Proc Natl Acad Sci USA* 111(20):7254–7259. <https://doi.org/10.1073/pnas.1405240111>
  97. Ohashi T, Aoki M, Tomita H, Akazawa T, Sato K, Kuze B, Mizuta K, Hara A, Nagaoka H, Inoue N, Ito Y (2017) M2-like

- macrophage polarization in high lactic acid-producing head and neck cancer. *Cancer Sci* 108(6):1128–1134. <https://doi.org/10.1111/cas.13244>
98. Walenta S, Schroeder T, Mueller-Klieser W (2002) Metabolic mapping with bioluminescence: basic and clinical relevance. *Biomol Eng* 18(6):249–262
  99. Madan A, Ganji SK, An Z, Choe KS, Pinho MC, Bachoo RM, Maher EM, Choi C (2015) Proton T2 measurement and quantification of lactate in brain tumors by MRS at 3 T in vivo. *Magn Reson Med* 73(6):2094–2099. <https://doi.org/10.1002/mrm.25352>
  100. Payne GS, Harris LM, Cairns GS, Messiou C, deSouza NM, Macdonald A, Saran F, Leach MO (2016) Validating a robust double-quantum-filtered (1) H MRS lactate measurement method in high-grade brain tumours. *NMR Biomed* 29(10):1420–1426. <https://doi.org/10.1002/nbm.3587>
  101. Harris LM, Tunariu N, Messiou C, Hughes J, Wallace T, DeSouza NM, Leach MO, Payne GS (2015) Evaluation of lactate detection using selective multiple quantum coherence in phantoms and brain tumours. *NMR Biomed* 28(3):338–343. <https://doi.org/10.1002/nbm.3255>
  102. Nelson SJ, Kadambi AK, Park I, Li Y, Crane J, Olson M, Molinaro A, Roy R, Butowski N, Cha S, Chang S (2017) Association of early changes in 1H MRSI parameters with survival for patients with newly diagnosed glioblastoma receiving a multimodality treatment regimen. *Neuro-Oncol* 19(3):430–439. <https://doi.org/10.1093/neuonc/now159>
  103. Payne GS, deSouza NM, Messiou C, Leach MO (2015) Single-shot single-voxel lactate measurements using FOCI-LASER and a multiple-quantum filter. *NMR Biomed* 28(4):496–504. <https://doi.org/10.1002/nbm.3276>
  104. Kobus T, Wright AJ, Van Asten JJ, Heerschap A, Scheenen TW (2014) In vivo (1) H MR spectroscopic imaging of aggressive prostate cancer: can we detect lactate? *Magn Reson Med* 71(1):26–34. <https://doi.org/10.1002/mrm.24635>
  105. Zhang L, Martins AF, Mai Y, Zhao P, Funk AM, Clavijo Jordan MV, Zhang S, Chen W, Wu Y, Sherry AD (2017) Imaging extracellular lactate in vitro and in vivo using CEST MRI and a paramagnetic shift reagent. *Chemistry* 23(8):1752–1756. <https://doi.org/10.1002/chem.201604558>
  106. DeBrosse C, Nanga RP, Bagga P, Nath K, Haris M, Marincola F, Schnall MD, Hariharan H, Reddy R (2016) Erratum: lactate chemical exchange saturation transfer (LATEST) imaging in vivo: a biomarker for LDH activity. *Sci Rep* 6:21813. <https://doi.org/10.1038/srep21813>
  107. Venkatesh HS, Chaumeil MM, Ward CS, Haas-Kogan DA, James CD, Ronen SM (2012) Reduced phosphocholine and hyperpolarized lactate provide magnetic resonance biomarkers of PI3K/Akt/mTOR inhibition in glioblastoma. *Neuro-Oncol* 14(3):315–325. <https://doi.org/10.1093/neuonc/nor209>
  108. Radoul M, Chaumeil MM, Eriksson P, Wang AS, Phillips JJ, Ronen SM (2016) MR studies of glioblastoma models treated with dual PI3K/mTOR inhibitor and temozolomide: metabolic changes are associated with enhanced survival. *Mol Cancer Ther* 15(5):1113–1122. <https://doi.org/10.1158/1535-7163.MCT-15-0769>
  109. Di Galleonardo V, Aldeborgh HN, Miloushev V, Folkers KM, Granlund K, Tap WD, Lewis JS, Weber WA, Keshari KR (2017) Multinuclear NMR and MRI reveal an early metabolic response to mTOR inhibition in sarcoma. *Cancer Res* 77(11):3113–3120. <https://doi.org/10.1158/0008-5472.CAN-16-3310>
  110. Park JM, Recht LD, Josan S, Merchant M, Jang T, Yen YF, Hurd RE, Spielman DM, Mayer D (2013) Metabolic response of glioma to dichloroacetate measured in vivo by hyperpolarized (13)C magnetic resonance spectroscopic imaging. *Neuro-Oncol* 15(4):433–441. <https://doi.org/10.1093/neuonc/nos319>
  111. Park JM, Spielman DM, Josan S, Jang T, Merchant M, Hurd RE, Mayer D, Recht LD (2016) Hyperpolarized (13)C-lactate to (13)C-bicarbonate ratio as a biomarker for monitoring the acute response of anti-vascular endothelial growth factor (anti-VEGF) treatment. *NMR Biomed* 29(5):650–659. <https://doi.org/10.1002/nbm.3509>
  112. Day SE, Kettunen MI, Cherukuri MK, Mitchell JB, Lizak MJ, Morris HD, Matsumoto S, Koretsky AP, Brindle KM (2011) Detecting response of rat C6 glioma tumors to radiotherapy using hyperpolarized [1- 13C]pyruvate and 13C magnetic resonance spectroscopic imaging. *Magn Reson Med* 65(2):557–563. <https://doi.org/10.1002/mrm.22698>
  113. Saito K, Matsumoto S, Takakusagi Y, Matsuo M, Morris HD, Lizak MJ, Munasinghe JP, Devasahayam N, Subramanian S, Mitchell JB, Krishna MC (2015) 13C-MR spectroscopic imaging with hyperpolarized [1-13C]pyruvate detects early response to radiotherapy in SCC tumors and HT-29 tumors. *Clin Cancer Res* 21(22):5073–5081. <https://doi.org/10.1158/1078-0432.CCR-14-1717>
  114. Ravoori MK, Singh SP, Lee J, Bankson JA, Kundra V (2017) In vivo assessment of ovarian tumor response to tyrosine kinase inhibitor pazopanib by using hyperpolarized (13)C-pyruvate MR spectroscopy and (18)F-FDG PET/CT imaging in a mouse model. *Radiology* 285(3):830–838. <https://doi.org/10.1148/radio.12017161772>
  115. Zaccagna F, Grist JT, Deen SS, Woitek R, Lechermann LM, McLean MA, Basu B, Gallagher FA (2018) Hyperpolarized carbon-13 magnetic resonance spectroscopic imaging: a clinical tool for studying tumour metabolism. *Br J Radiol* 91(1085):20170688. <https://doi.org/10.1259/bjr.20170688>
  116. Ardenkjaer-Larsen JH, Leach AM, Clarke N, Urbahn J, Anderson D, Skloss TW (2011) Dynamic nuclear polarization polarizer for sterile use intent. *NMR Biomed* 24(8):927–932. <https://doi.org/10.1002/nbm.1682>
  117. von Morze C, Reed GD, Larson PE, Mammoli D, Chen AP, Tropp J, Van Criekinge M, Ohliger MA, Kurhanewicz J, Vigneron DB, Merritt ME (2018) In vivo hyperpolarization transfer in a clinical MRI scanner. *Magn Reson Med* 80(2):480–487. <https://doi.org/10.1002/mrm.27154>
  118. Nelson SJ, Kurhanewicz J, Vigneron DB, Larson PE, Harzstark AL, Ferrone M, van Criekinge M, Chang JW, Bok R, Park I, Reed G, Carvajal L, Small EJ, Munster P, Weinberg VK, Ardenkjaer-Larsen JH, Chen AP, Hurd RE, Odegardstuen LI, Robb FJ, Tropp J, Murray JA (2013) Metabolic imaging of patients with prostate cancer using hyperpolarized [1-(1)(3)C]pyruvate. *Sci Transl Med* 5(198):198ra108. <https://doi.org/10.1126/scitranslmed.3006070>
  119. Penheiter AR, Deelchand DK, Kittelson E, Damgard SE, Murphy SJ, O'Brien DR, Bamlet WR, Passow MR, Smyrk TC, Couch FJ, Vasmatzis G, Port JD, Marjanska M, Carlson SK (2018) Identification of a pyruvate-to-lactate signature in pancreatic intraductal papillary mucinous neoplasms. *Pancreatol* 18(1):46–53. <https://doi.org/10.1016/j.pan.2017.11.006>
  120. Goldman RD, Kaplan NO, Hall TC (1964) Lactic dehydrogenase in human neoplastic tissues. *Cancer Res* 24:389–399
  121. Wood DC, Varela V, Palmquist M, Weber F (1973) Serum lactic dehydrogenase and isoenzyme changes in clinical cancer. *J Surg Oncol* 5(3):251–257
  122. Balinsky D, Greengard O, Cayanis E, Head JF (1984) Enzyme activities and isozyme patterns in human lung tumors. *Cancer Res* 44(3):1058–1062
  123. Balinsky D, Platz CE, Lewis JW (1983) Isozyme patterns of normal, benign, and malignant human breast tissues. *Cancer Res* 43(12 Pt 1):5895–5901
  124. Serrao EM, Kettunen MI, Rodrigues TB, Dzien P, Wright AJ, Gopinathan A, Gallagher FA, Lewis DY, Frese KK, Almeida J,

- Howat WJ, Tuveson DA, Brindle KM (2016) MRI with hyperpolarised [1-13C]pyruvate detects advanced pancreatic preneoplasia prior to invasive disease in a mouse model. *Gut* 65(3):465–475. <https://doi.org/10.1136/gutjnl-2015-310114>
125. Serganova I, Rizwan A, Ni X, Thakur SB, Vider J, Russell J, Blasberg R, Koutcher JA (2011) Metabolic imaging: a link between lactate dehydrogenase A, lactate, and tumor phenotype. *Clin Cancer Res* 17(19):6250–6261. <https://doi.org/10.1158/1078-0432.CCR-11-0397>
  126. Cui J, Xiong J, Zhang Y, Peng T, Huang M, Lin Y, Guo Y, Wu H, Wang C (2017) Serum lactate dehydrogenase is predictive of persistent organ failure in acute pancreatitis. *J Crit Care* 41:161–165. <https://doi.org/10.1016/j.jcrc.2017.05.001>
  127. Green H, Tobar A, Gafter-Gvili A, Leibovici L, Klein T, Rahamimov R, Mor E, Grossman A (2017) Serum lactate dehydrogenase is elevated in ischemic acute tubular necrosis but not in acute rejection in kidney transplant patients. *Progr Transplant* 27(1):53–57. <https://doi.org/10.1177/1526924816664089>
  128. Milross CG, Tucker SL, Mason KA, Hunter NR, Peters LJ, Milas L (1997) The effect of tumor size on necrosis and polarographically measured pO<sub>2</sub>. *Acta Oncol* 36(2):183–189
  129. Agarwala SS, Keilholz U, Gilles E, Bedikian AY, Wu J, Kay R, Stein CA, Itri LM, Suci S, Eggermont AM (2009) LDH correlation with survival in advanced melanoma from two large, randomised trials (Oblimersen GM301 and EORTC 18951). *Eur J Cancer* 45(10):1807–1814. <https://doi.org/10.1016/j.ejca.2009.04.016>
  130. Dercle L, Ammari S, Champiat S, Massard C, Ferte C, Taihi L, Seban RD, Aspeslagh S, Mahjoubi L, Kamsu-Kom N, Robert C, Marabelle A, Schlumberger M, Soria JC, Postel-Vinay S (2016) Rapid and objective CT scan prognostic scoring identifies metastatic patients with long-term clinical benefit on anti-PD-1/L1 therapy. *Eur J Cancer* 65:33–42. <https://doi.org/10.1016/j.ejca.2016.05.031>
  131. Dong T, Liu Z, Xuan Q, Wang Z, Ma W, Zhang Q (2017) Tumor LDH-A expression and serum LDH status are two metabolic predictors for triple negative breast cancer brain metastasis. *Sci Rep* 7(1):6069. <https://doi.org/10.1038/s41598-017-06378-7>
  132. Gershenwald JE, Scolyer RA, Hess KR, Sondak VK, Long GV, Ross MI, Lazar AJ, Faries MB, Kirkwood JM, McArthur GA, Haydu LE, Eggermont AM, Flaherty KT, Balch CM, Thompson JF, for members of the American Joint Committee on Cancer Melanoma Expert P, the International Melanoma D, Discovery P (2017) Melanoma staging: evidence-based changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA Cancer J Clin* 67(6):472–492. <https://doi.org/10.3322/caac.21409>
  133. Kelderman S, Heemskerk B, van Tinteren H, van den Brom RR, Hospers GA, van den Eertwegh AJ, Kapiteijn EW, de Groot JW, Soetekouw P, Jansen RL, Fiets E, Furness AJ, Renn A, Krzystanek M, Szallasi Z, Lorigan P, Gore ME, Schumacher TN, Haanen JB, Larkin JM, Blank CU (2014) Lactate dehydrogenase as a selection criterion for ipilimumab treatment in metastatic melanoma. *Cancer Immunol Immunother* 63(5):449–458. <https://doi.org/10.1007/s00262-014-1528-9>
  134. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Wagstaff J, Hogg D, Hill A, Carlino MS, Wolter P, Lebbé C, Schachter J, Thomas L, Hassel JC, Lorigan P, Walker D, Jiang J, Hodi FS, Wolchok JD (abstract 3003) (2015) Efficacy and safety in key patient subgroups of nivolumab (NIVO) alone or combined with ipilimumab (IPI) versus IPI alone in treatment-naïve patients with advanced melanoma (MEL) (CheckMate 067). The European Cancer Congress 2015
  135. Heppt MV, Heinzerling L, Kahler KC, Forschner A, Kirchnerberger MC, Loquai C, Meissner M, Meier F, Terheyden P, Schell B, Herbst R, Goppner D, Kiecker F, Rafei-Shamsabadi D, Haferkamp S, Huber MA, Utikal J, Ziemer M, Bumer I, Pfeiffer C, Schad SG, Schmid-Tannwald C, Tietze JK, Eigentler TK, Berking C (2017) Prognostic factors and outcomes in metastatic uveal melanoma treated with programmed cell death-1 or combined PD-1/cytotoxic T-lymphocyte antigen-4 inhibition. *Eur J Cancer* 82:56–65. <https://doi.org/10.1016/j.ejca.2017.05.038>
  136. Taniguchi Y, Tamiya A, Isa SI, Nakahama K, Okishio K, Shirogama T, Suzuki H, Inoue T, Tamiya M, Hirashima T, Imamura F, Atagi S (2017) Predictive factors for poor progression-free survival in patients with non-small cell lung cancer treated with nivolumab. *Anticancer Res* 37(10):5857–5862. <https://doi.org/10.21873/anticancerres.12030>
  137. Larkin J, Ascierto PA, Dreno B, Atkinson V, Liszkay G, Maio M, Mandalà M, Demidov L, Stroyakovskiy D, Thomas L, de la Cruz-Merino L, Dutriaux C, Garbe C, Sovak MA, Chang I, Choong N, Hack SP, McArthur GA, Ribas A (2014) Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med* 371(20):1867–1876. <https://doi.org/10.1056/NEJMoA1408868>
  138. Long GV, Grob JJ, Nathan P, Ribas A, Robert C, Schadendorf D, Lane SR, Mak C, Legenne P, Flaherty KT, Davies MA (2016) Factors predictive of response, disease progression, and overall survival after dabrafenib and trametinib combination treatment: a pooled analysis of individual patient data from randomised trials. *Lancet Oncol* 17(12):1743–1754. [https://doi.org/10.1016/S1470-2045\(16\)30578-2](https://doi.org/10.1016/S1470-2045(16)30578-2)
  139. Tsao SC, Weiss J, Hudson C, Christophi C, Cebon J, Behren A, Dobrovic A (2015) Monitoring response to therapy in melanoma by quantifying circulating tumour DNA with droplet digital PCR for BRAF and NRAS mutations. *Sci Rep* 5:11198. <https://doi.org/10.1038/srep11198>
  140. Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, Mitra D, Boni A, Newton LP, Liu C, Peng W, Sullivan RJ, Lawrence DP, Hodi FS, Overwijk WW, Lizee G, Murphy GF, Hwu P, Flaherty KT, Fisher DE, Wargo JA (2013) BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res* 19(5):1225–1231. <https://doi.org/10.1158/1078-0432.CCR-12-1630>
  141. Deken MA, Gadiot J, Jordanova ES, Lacroix R, van Gool M, Kroon P, Pineda C, Geukes Foppen MH, Scolyer R, Song JY, Verbrugge I, Hoeller C, Dummer R, Haanen JB, Long GV, Blank CU (2016) Targeting the MAPK and PI3K pathways in combination with PD1 blockade in melanoma. *Oncoimmunology* 5(12):e1238557. <https://doi.org/10.1080/2162402X.2016.1238557>
  142. Kakavand H, Wilmott JS, Menzies AM, Vilain R, Haydu LE, Yearley JH, Thompson JF, Kefford RF, Hersey P, Long GV, Scolyer RA (2015) PD-L1 expression and tumor-infiltrating lymphocytes define different subsets of MAPK inhibitor-treated melanoma patients. *Clin Cancer Res* 21(14):3140–3148. <https://doi.org/10.1158/1078-0432.CCR-14-2023>
  143. Blank CU, Haanen JB, Ribas A, Schumacher TN (2016) CANCER IMMUNOLOGY. The “cancer immunogram”. *Science* 352(6286):658–660. <https://doi.org/10.1126/science.aaf2834>
  144. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, Lugli A, Zlobec I, Hartmann A, Bifulco C, Nagtegaal ID, Palmqvist R, Masucci GV, Botti G, Tatangelo F, Delrio P, Maio M, Laghi L, Grizzi F, Asslaber M, D’Arrigo C, Vidal-Vanaclocha F, Zavadova E, Chouchane L, Ohashi PS, Hafezi-Bakhtiari S, Wouters BG, Roehrl M, Nguyen L, Kawakami Y, Hazama S, Okuno K, Ogino S, Gibbs P, Waring P, Sato N, Torigoe T, Itoh K, Patel PS, Shukla SN, Wang Y, Kopetz S, Sinicrope FA, Scripcariu V, Ascierto PA, Marincola FM, Fox BA, Pages F (2014) Towards the introduction of the ‘Immunoscore’ in the

- classification of malignant tumours. *J Pathol* 232(2):199–209. <https://doi.org/10.1002/path.4287>
145. Mlecnik B, Bindea G, Angell HK, Maby P, Angelova M, Tougeron D, Church SE, Lafontaine L, Fischer M, Fredriksen T, Sasso M, Bilocq AM, Kirilovsky A, Obenauf AC, Hamieh M, Berger A, Bruneval P, Tuech JJ, Sabourin JC, Le Pessot F, Mauillon J, Rafii A, Laurent-Puig P, Speicher MR, Trajanoski Z, Michel P, Sesboue R, Frebourg T, Pages F, Valge-Archer V, Latouche JB, Galon J (2016) Integrative analyses of colorectal cancer show immunoscore is a stronger predictor of patient survival than microsatellite instability. *Immunity* 44(3):698–711. <https://doi.org/10.1016/j.immuni.2016.02.025>
  146. Jochems C, Schlom J (2011) Tumor-infiltrating immune cells and prognosis: the potential link between conventional cancer therapy and immunity. *Exp Biol Med* (Maywood) 236(5):567–579. <https://doi.org/10.1258/ebm.2011.011007>
  147. Tumei PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 515(7528):568–571. <https://doi.org/10.1038/nature13954>
  148. Bosticardo M, Ariotti S, Losana G, Bernabei P, Forni G, Novelli F (2001) Biased activation of human T lymphocytes due to low extracellular pH is antagonized by B7/CD28 costimulation. *Eur J Immunol* 31(9):2829–2838. [https://doi.org/10.1002/1521-4141\(200109\)31:9%3C2829::AID-IMMU2829%3E3.0.CO;2-U](https://doi.org/10.1002/1521-4141(200109)31:9%3C2829::AID-IMMU2829%3E3.0.CO;2-U)
  149. Mendler AN, Hu B, Prinz PU, Kreutz M, Gottfried E, Noessner E (2012) Tumor lactic acidosis suppresses CTL function by inhibition of p38 and JNK/c-Jun activation. *Int J Cancer* 131(3):633–640. <https://doi.org/10.1002/ijc.26410>
  150. Nakagawa Y, Negishi Y, Shimizu M, Takahashi M, Ichikawa M, Takahashi H (2015) Effects of extracellular pH and hypoxia on the function and development of antigen-specific cytotoxic T lymphocytes. *Immunol Lett* 167(2):72–86. <https://doi.org/10.1016/j.imlet.2015.07.003>
  151. 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 Res* 72(11):2746–2756. <https://doi.org/10.1158/0008-5472.CAN-11-1272>
  152. Pilon-Thomas S, Kodumudi KN, El-Kenawi AE, Russell S, Weber AM, Luddy K, Damaghi M, Wojtkowiak JW, Mule JJ, Ibrahim-Hashim A, Gillies RJ (2016) Neutralization of tumor acidity improves antitumor responses to immunotherapy. *Cancer Res* 76(6):1381–1390. <https://doi.org/10.1158/0008-5472.CAN-15-1743>
  153. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, Matos C, Bruss C, Klobuch S, Peter K, Kastenberger M, Bogdan C, Schleicher U, Mackensen A, Ullrich E, Fichtner-Feigl S, Kesselring R, Mack M, Ritter U, Schmid M, Blank C, Dettmer K, Oefner PJ, Hoffmann P, Walenta S, Geissler EK, Pouyssegur J, Villunger A, Steven A, Seliger B, Schreml S, Haferkamp S, Kohl E, Karrer S, Berneburg M, Herr W, Mueller-Klieser W, Renner K, Kreutz M (2016) LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metabol* 24(5):657–671. <https://doi.org/10.1016/j.cmet.2016.08.011>
  154. Husain Z, Huang Y, Seth P, Sukhatme VP (2013) Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *J Immunol* 191(3):1486–1495. <https://doi.org/10.4049/jimmunol.1202702>
  155. Loeffler DA, Juneau PL, Heppner GH (1991) Natural killer-cell activity under conditions reflective of tumor micro-environment. *Int J Cancer* 48(6):895–899
  156. Xie D, Zhu S, Bai L (2016) Lactic acid in tumor microenvironments causes dysfunction of NKT cells by interfering with mTOR signaling. *Sci China Life Sci* 59(12):1290–1296. <https://doi.org/10.1007/s11427-016-0348-7>
  157. Droge W, Roth S, Altmann A, Mihm S (1987) Regulation of T-cell functions by L-lactate. *Cell Immunol* 108(2):405–416
  158. Potzl J, Roser D, Bankel L, Homberg N, Geishauser A, Brenner CD, Weigand M, Rocken M, Mocikat R (2017) Reversal of tumor acidosis by systemic buffering reactivates NK cells to express IFN-gamma and induces NK cell-dependent lymphoma control without other immunotherapies. *Int J Cancer* 140(9):2125–2133. <https://doi.org/10.1002/ijc.30646>
  159. Angelin A, Gil-de-Gomez L, Dahiya S, Jiao J, Guo L, Levine MH, Wang Z, Quinn WJ 3rd, Kopinski PK, Wang L, Akimova T, Liu Y, Bhatti TR, Han R, Laskin BL, Baur JA, Blair IA, Wallace DC, Hancock WW, Beier UH (2017) Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metabol* 25(6):1282–1293e1287. <https://doi.org/10.1016/j.cmet.2016.12.018>
  160. Eleftheriadis T, Pissas G, Karioti A, Antoniadis G, Antoniadis N, Liakopoulos V, Stefanidis I (2013) Dichloroacetate at therapeutic concentration alters glucose metabolism and induces regulatory T-cell differentiation in alloreactive human lymphocytes. *J Basic Clin Physiol Pharmacol* 24(4):271–276. <https://doi.org/10.1515/jbcpp-2013-0001>
  161. Eleftheriadis T, Sounidaki M, Pissas G, Antoniadis G, Liakopoulos V, Stefanidis I (2016) In human alloreactive CD4(+) T-cells, dichloroacetate inhibits aerobic glycolysis, induces apoptosis and favors differentiation towards the regulatory T-cell subset instead of effector T-cell subsets. *Mol Med Rep* 13(4):3370–3376. <https://doi.org/10.3892/mmr.2016.4912>
  162. Ostroukhova M, Goplen N, Karim MZ, Michalec L, Guo L, Liang Q, Alam R (2012) The role of low-level lactate production in airway inflammation in asthma. *Am J Physiol Lung Cell Mol Physiol* 302(3):L300–L307. <https://doi.org/10.1152/ajplu.00221.2011>
  163. McLane W, Whetstone R, Deshpande R, Menk A, Scharping N, Morrison B, Gellhaus Wendell S, Delgoffe G (2017) Lactic acid as a mediator of metabolic symbiosis between regulatory T Cells and the tumor microenvironment. In: 32nd annual meeting and pre-conference programs of the society for immunotherapy of cancer (SITC 2017, Abstract). National harbor, Maryland
  164. Peter K, Rehli M, Singer K, Renner-Sattler K, Kreutz M (2015) Lactic acid delays the inflammatory response of human monocytes. *Biochem Biophys Res Commun* 457(3):412–418. <https://doi.org/10.1016/j.bbrc.2015.01.005>
  165. Dietl K, Renner K, Dettmer K, Timischl B, Eberhart K, Dorn C, Hellerbrand C, Kastenberger M, Kunz-Schughart LA, Oefner PJ, Andreesen R, Gottfried E, Kreutz MP (2010) Lactic acid and acidification inhibit TNF secretion and glycolysis of human monocytes. *J Immunol* 184(3):1200–1209. <https://doi.org/10.4049/jimmunol.0902584>
  166. Errea A, Cayet D, Marchetti P, Tang C, Kluzza J, Offermanns S, Sirard JC, Rumbo M (2016) Lactate inhibits the pro-inflammatory response and metabolic reprogramming in murine macrophages in a GPR81-independent manner. *PLoS one* 11(11):e0163694. <https://doi.org/10.1371/journal.pone.0163694>
  167. Mu X, Shi W, Xu Y, Xu C, Zhao T, Geng B, Yang J, Pan J, Hu S, Zhang C, Zhang J, Wang C, Shen J, Che Y, Liu Z, Lv Y, Wen H, You Q (2018) Tumor-derived lactate induces M2 macrophage polarization via the activation of the ERK/STAT3 signaling pathway in breast cancer. *Cell Cycle* 17(4):428–438. <https://doi.org/10.1080/15384101.2018.1444305>



168. Shime H, Yabu M, Akazawa T, Kodama K, Matsumoto M, Seya T, Inoue N (2008) Tumor-secreted lactic acid promotes IL-23/IL-17 proinflammatory pathway. *J Immunol* 180(11):7175–7183
169. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, Cline GW, Phillips AJ, Medzhitov R (2014) Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 513(7519):559–563. <https://doi.org/10.1038/nature13490>
170. Ohashi T, Akazawa T, Aoki M, Kuze B, Mizuta K, Ito Y, Inoue N (2013) Dichloroacetate improves immune dysfunction caused by tumor-secreted lactic acid and increases antitumor immunoreactivity. *Int J Cancer* 133(5):1107–1118. <https://doi.org/10.1002/ijc.28114>
171. Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, Basham B, McClanahan T, Kastelein RA, Oft M (2006) IL-23 promotes tumour incidence and growth. *Nature* 442(7101):461–465. <https://doi.org/10.1038/nature04808>
172. Takahashi H, Numasaki M, Lotze MT, Sasaki H (2005) Interleukin-17 enhances bFGF-, HGF- and VEGF-induced growth of vascular endothelial cells. *Immunol Lett* 98(2):189–193. <https://doi.org/10.1016/j.imlet.2004.11.012>
173. Goel HL, Mercurio AM (2013) VEGF targets the tumour cell. *Nat Rev Cancer* 13(12):871–882. <https://doi.org/10.1038/nrc3627>
174. Kerbel RS (2008) Tumor angiogenesis. *N Engl J Med* 358(19):2039–2049. <https://doi.org/10.1056/NEJMra0706596>
175. Polat MF, Taysi S, Polat S, Boyuk A, Bakan E (2003) Elevated serum arginase activity levels in patients with breast cancer. *Surg Today* 33(9):655–661. <https://doi.org/10.1007/s00595-002-2563-2>
176. Iraporda C, Romanin DE, Bengoa AA, Errea AJ, Cayet D, Foli-gne B, Sirard JC, Garrote GL, Abraham AG, Rumbo M (2016) Local treatment with lactate prevents intestinal inflammation in the TNBS-induced colitis model. *Front Immunol* 7:651. <https://doi.org/10.3389/fimmu.2016.00651>
177. Iraporda C, Errea A, Romanin DE, Cayet D, Pereyra E, Pignatario O, Sirard JC, Garrote GL, Abraham AG, Rumbo M (2015) Lactate and short chain fatty acids produced by microbial fermentation downregulate proinflammatory responses in intestinal epithelial cells and myeloid cells. *Immunobiology* 220(10):1161–1169. <https://doi.org/10.1016/j.imbio.2015.06.004>
178. Seth P, Csizmadia E, Hedblom A, Vuerich M, Xie H, Li M, Longhi MS, Wegiel B (2017) Deletion of lactate dehydrogenase-A in myeloid cells triggers antitumor immunity. *Cancer Res* 77(13):3632–3643. <https://doi.org/10.1158/0008-5472.CAN-16-2938>
179. Selleri S, Bifsha P, Civini S, Pacelli C, Dieng MM, Lemieux W, Jin P, Bazin R, Patey N, Marincola FM, Moldovan F, Zaouter C, Trudeau LE, Benabdhalha B, Louis I, Beausejour C, Stroncek D, Le Deist F, Haddad E (2016) Human mesenchymal stromal cell-secreted lactate induces M2-macrophage differentiation by metabolic reprogramming. *Oncotarget* 7(21):30193–30210. <https://doi.org/10.18632/oncotarget.8623>
180. Gottfried E, Kunz-Schughart LA, Ebner S, Mueller-Klieser W, Hoves S, Andreesen R, Mackensen A, Kreutz M (2006) Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. *Blood* 107(5):2013–2021. <https://doi.org/10.1182/blood-2005-05-1795>
181. Nasi A, Fekete T, Krishnamurthy A, Snowden S, Rajnavolgyi E, Catrina AI, Wheelock CE, Vivar N, Rethi B (2013) Dendritic cell reprogramming by endogenously produced lactic acid. *J Immunol* 191(6):3090–3099. <https://doi.org/10.4049/jimmunol.1300772>
182. Chirasani SR, Leukel P, Gottfried E, Hochrein J, Stadler K, Neumann B, Oefner PJ, Gronwald W, Bogdahn U, Hau P, Kreutz M, Grauer OM (2013) Diclofenac inhibits lactate formation and efficiently counteracts local immune suppression in a murine glioma model. *Int J Cancer* 132(4):843–853. <https://doi.org/10.1002/ijc.27712>
183. Vermeulen M, Giordano M, Trevani AS, Sedlik C, Gamberale R, Fernandez-Calotti P, Salamone G, Raiden S, Sanjurjo J, Gef-fner JR (2004) Acidosis improves uptake of antigens and MHC class I-restricted presentation by dendritic cells. *J Immunol* 172(5):3196–3204
184. Tong J, Wu WN, Kong X, Wu PF, Tian L, Du W, Fang M, Zheng F, Chen JG, Tan Z, Gong F (2011) Acid-sensing ion channels contribute to the effect of acidosis on the function of dendritic cells. *J Immunol* 186(6):3686–3692. <https://doi.org/10.4049/jimmunol.1001346>
185. Wang R, Green DR (2012) Metabolic reprogramming and metabolic dependency in T cells. *Immunol Rev* 249(1):14–26. <https://doi.org/10.1111/j.1600-065X.2012.01155.x>
186. Gerriets VA, Rathmell JC (2012) Metabolic pathways in T cell fate and function. *Trends Immunol* 33(4):168–173. <https://doi.org/10.1016/j.it.2012.01.010>
187. Donnelly RP, Loftus RM, Keating SE, Liou KT, Biron CA, Gardiner CM, Finlay DK (2014) mTORC1-dependent metabolic reprogramming is a prerequisite for NK cell effector function. *J Immunol* 193(9):4477–4484. <https://doi.org/10.4049/jimmunol.1401558>
188. Keating SE, Zaiatz-Bittencourt V, Loftus RM, Keane C, Brennan K, Finlay DK, Gardiner CM (2016) Metabolic reprogramming supports IFN-gamma production by CD56bright NK cells. *J Immunol* 196(6):2552–2560. <https://doi.org/10.4049/jimmunol.1501783>
189. Kelly B, O'Neill LA (2015) Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res* 25(7):771–784. <https://doi.org/10.1038/cr.2015.68>
190. Krawczyk CM, Holowka T, Sun J, Blagih J, Amiel E, DeBerardinis RJ, Cross JR, Jung E, Thompson CB, Jones RG, Pearce EJ (2010) Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 115(23):4742–4749. <https://doi.org/10.1182/blood-2009-10-249540>
191. Liu RT, Zhang M, Yang CL, Zhang P, Zhang N, Du T, Ge MR, Yue LT, Li XL, Li H, Duan RS (2018) Enhanced glycolysis contributes to the pathogenesis of experimental autoimmune neuritis. *J Neuroinflamm* 15(1):51. <https://doi.org/10.1186/s12974-018-1095-7>
192. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, Chi H (2011) HIF1 $\alpha$ -dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and T<sub>reg</sub> cells. *J Exp Med* 208(7):1367–1376. <https://doi.org/10.1084/jem.20110278>
193. Doherty JR, Yang C, Scott KE, Cameron MD, Fallahi M, Li W, Hall MA, Amelio AL, Mishra JK, Li F, Tortosa M, Genau HM, Rounbehler RJ, Lu Y, Dang CV, Kumar KG, Butler AA, Bannister TD, Hooper AT, Unsal-Kacmaz K, Roush WR, Cleveland JL (2014) Blocking lactate export by inhibiting the Myc target MCT1 disables glycolysis and glutathione synthesis. *Cancer Res* 74(3):908–920. <https://doi.org/10.1158/0008-5472.CAN-13-2034>
194. Tan Z, Xie N, Banerjee S, Cui H, Fu M, Thanickal VJ, Liu G (2015) The monocarboxylate transporter 4 is required for glycolytic reprogramming and inflammatory response in macrophages. *J Biol Chem* 290(1):46–55. <https://doi.org/10.1074/jbc.M114.603589>
195. Murray CM, Hutchinson R, Bantick JR, Belfield GP, Benjamin AD, Brazma D, Bundick RV, Cook ID, Craggs RI, Edwards S, Evans LR, Harrison R, Holness E, Jackson AP, Jackson CG, Kingston LP, Perry MW, Ross AR, Rugman PA, Sidhu SS, Sullivan M, Taylor-Fishwick DA, Walker PC, Whitehead YM,

- Wilkinson DJ, Wright A, Donald DK (2005) Monocarboxylate transporter MCT1 is a target for immunosuppression. *Nat Chem Biol* 1(7):371–376
196. Rincon M, Davis RJ (2009) Regulation of the immune response by stress-activated protein kinases. *Immunol Rev* 228(1):212–224. <https://doi.org/10.1111/j.1600-065X.2008.00744.x>
197. Teixeira LK, Fonseca BP, Vieira-de-Abreu A, Barboza BA, Robbs BK, Bozza PT, Viola JP (2005) IFN-gamma production by CD8+ T cells depends on NFAT1 transcription factor and regulates Th differentiation. *J Immunol* 175(9):5931–5939
198. Pouyssegur J, Chambard JC, Franchi A, Paris S, Van Obberghen-Schilling E (1982) Growth factor activation of an amiloride-sensitive Na<sup>+</sup>/H<sup>+</sup> exchange system in quiescent fibroblasts: coupling to ribosomal protein S6 phosphorylation. *Proc Natl Acad Sci USA* 79(13):3935–3939
199. Balgi AD, Diering GH, Donohue E, Lam KK, Fonseca BD, Zimmerman C, Numata M, Roberge M (2011) Regulation of mTORC1 signaling by pH. *PLoS one* 6(6):e21549. <https://doi.org/10.1371/journal.pone.0021549>
200. Linke M, Fritsch SD, Sukhbaatar N, Hengstschlager M, Weichhart T (2017) mTORC1 and mTORC2 as regulators of cell metabolism in immunity. *FEBS Lett* 591(19):3089–3103. <https://doi.org/10.1002/1873-3468.12711>
201. Powell JD, Pollizzi KN, Heikamp EB, Horton MR (2012) Regulation of immune responses by mTOR. *Annu Rev Immunol* 30:39–68. <https://doi.org/10.1146/annurev-immunol-020711-075024>
202. De Saedeleer CJ, Copetti T, Porporato PE, Verrax J, Feron O, Sonveaux P (2012) Lactate activates HIF-1 in oxidative but not in Warburg-phenotype human tumor cells. *PLoS one* 7(10):e46571. <https://doi.org/10.1371/journal.pone.0046571>
203. Wang BY, Zhang J, Wang JL, Sun S, Wang ZH, Wang LP, Zhang QL, Lv FF, Cao EY, Shao ZM, Fais S, Hu XC (2015) Erratum to: Intermittent high dose proton pump inhibitor enhances the antitumor effects of chemotherapy in metastatic breast cancer. *J Exp Clin Cancer Res* 34:109. <https://doi.org/10.1186/s13046-015-0220-z>
204. Chao M, Wu H, Jin K, Li B, Wu J, Zhang G, Yang G, Hu X (2016) A nonrandomized cohort and a randomized study of local control of large hepatocarcinoma by targeting intratumoral lactic acidosis. *Elife*. <https://doi.org/10.7554/eLife.15691>
205. Koltai T (2016) Cancer: fundamentals behind pH targeting and the double-edged approach. *Onco Targets Ther* 9:6343–6360. <https://doi.org/10.2147/OTT.S115438>
206. Anemone A, Consolino L, Conti L, Reineri F, Cavallo F, Aime S, Longo DL (2017) In vivo evaluation of tumour acidosis for assessing the early metabolic response and onset of resistance to dichloroacetate by using magnetic resonance pH imaging. *Int J Oncol* 51(2):498–506. <https://doi.org/10.3892/ijo.2017.4029>
207. Lin G, Hill DK, Andrejeva G, Boulton JK, Troy H, Fong AC, Orton MR, Panek R, Parkes HG, Jafar M, Koh DM, Robinson SP, Judson IR, Griffiths JR, Leach MO, Eykyn TR, Chung YL (2014) Dichloroacetate induces autophagy in colorectal cancer cells and tumours. *Br J Cancer* 111(2):375–385. <https://doi.org/10.1038/bjc.2014.281>
208. Sun RC, Fadia M, Dahlstrom JE, Parish CR, Board PG, Blackburn AC (2010) Reversal of the glycolytic phenotype by dichloroacetate inhibits metastatic breast cancer cell growth in vitro and in vivo. *Breast Cancer Res Treat* 120(1):253–260. <https://doi.org/10.1007/s10549-009-0435-9>
209. Madhok BM, Yeluri S, Perry SL, Hughes TA, Jayne DG (2010) Dichloroacetate induces apoptosis and cell-cycle arrest in colorectal cancer cells. *Br J Cancer* 102(12):1746–1752. <https://doi.org/10.1038/sj.bjc.6605701>
210. Kumar A, Kant S, Singh SM (2012) Novel molecular mechanisms of antitumor action of dichloroacetate against T cell lymphoma: Implication of altered glucose metabolism, pH homeostasis and cell survival regulation. *Chem Biol Interact* 199(1):29–37. <https://doi.org/10.1016/j.cbi.2012.06.005>
211. Dunbar EM, Coats BS, Shroods AL, Langae T, Lew A, Forder JR, Shuster JJ, Wagner DA, Stacpoole PW (2014) Phase I trial of dichloroacetate (DCA) in adults with recurrent malignant brain tumors. *Invest New Drugs* 32(3):452–464. <https://doi.org/10.1007/s10637-013-0047-4>
212. Gottfried E, Lang SA, Renner K, Bosserhoff A, Gronwald W, Rehli M, Einhell S, Gedig I, Singer K, Seilbeck A, Mackensen A, Grauer O, Hau P, Dettmer K, Andreesen R, Oefner PJ, Kreutz M (2013) New aspects of an old drug—diclofenac targets MYC and glucose metabolism in tumor cells. *PLoS one* 8(7):e66987. <https://doi.org/10.1371/journal.pone.0066987>
213. Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO (2016) Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science* 354(6311):481–484. <https://doi.org/10.1126/science.aaf6284>
214. Renner K, Geiselhoringer AL, Fante M, Bruss C, Farber S, Schonhammer G, Peter K, Singer K, Andreesen R, Hoffmann P, Oefner P, Herr W, Kreutz M (2015) Metabolic plasticity of human T cells: Preserved cytokine production under glucose deprivation or mitochondrial restriction, but 2-deoxy-glucose affects effector functions. *Eur J Immunol* 45(9):2504–2516. <https://doi.org/10.1002/eji.201545473>
215. Tripmacher R, Gaber T, Dziurla R, Haupt T, Erekul K, Grutzkau A, Tschirschmann M, Scheffold A, Radbruch A, Burmester GR, Buttgerit F (2008) Human CD4(+) T cells maintain specific functions even under conditions of extremely restricted ATP production. *Eur J Immunol* 38(6):1631–1642. <https://doi.org/10.1002/eji.200738047>
216. Morais-Santos F, Granja S, Miranda-Goncalves V, Moreira AH, Queiros S, Vilaca JL, Schmitt FC, Longatto-Filho A, Paredes J, Baltazar F, Pinheiro C (2015) Targeting lactate transport suppresses in vivo breast tumour growth. *Oncotarget* 6(22):19177–19189. <https://doi.org/10.18632/oncotarget.3910>
217. Schneiderhan W, Scheler M, Holzmann KH, Marx M, Gschwend JE, Bucholz M, Gress TM, Seufferlein T, Adler G, Oswald F (2009) CD147 silencing inhibits lactate transport and reduces malignant potential of pancreatic cancer cells in vivo and in vitro models. *Gut* 58(10):1391–1398. <https://doi.org/10.1136/gut.2009.181412>
218. Belouche-Babari M, Wantuch S, Casals Galobart T, Konioridou M, Parkes HG, Arunan V, Chung YL, Eykyn TR, Smith PD, Leach MO (2017) MCT1 inhibitor AZD3965 increases mitochondrial metabolism, facilitating combination therapy and noninvasive magnetic resonance spectroscopy. *Cancer Res* 77(21):5913–5924. <https://doi.org/10.1158/0008-5472.CAN-16-2686>
219. Le Floch R, Chiche J, Marchiq I, Naiken T, Ilc K, Murray CM, Critchlow SE, Roux D, Simon MP, Pouyssegur J (2011) CD147 subunit of lactate/H<sup>+</sup> symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. *Proc Natl Acad Sci USA* 108(40):16663–16668. <https://doi.org/10.1073/pnas.1106123108>
220. Marchiq I, Le Floch R, Roux D, Simon MP, Pouyssegur J (2015) Genetic disruption of lactate/H<sup>+</sup> symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin. *Cancer Res* 75(1):171–180. <https://doi.org/10.1158/0008-5472.CAN-14-2260>
221. Cotter K, Capecci J, Sennoune S, Huss M, Maier M, Martinez-Zaguilan R, Forgacs M (2015) Activity of plasma membrane V-ATPases is critical for the invasion of MDA-MB231 breast cancer cells. *J Biol Chem* 290(6):3680–3692. <https://doi.org/10.1074/jbc.M114.611210>

222. Chueca E, Apostolova N, Esplugues JV, Garcia-Gonzalez MA, Lanás A, Piazuelo E (2016) Proton pump inhibitors display anti-tumor effects in Barrett's adenocarcinoma cells. *Front Pharmacol* 7:452. <https://doi.org/10.3389/fphar.2016.00452>
223. De Milito A, Canese R, Marino ML, Borghi M, Iero M, Villa A, Venturi G, Lozupone F, Iessi E, Logozzi M, Della Mina P, Santinami M, Rodolfo M, Podo F, Rivoltini L, Fais S (2010) pH-dependent antitumor activity of proton pump inhibitors against human melanoma is mediated by inhibition of tumor acidity. *Int J Cancer* 127(1):207–219. <https://doi.org/10.1002/ijc.25009>
224. Vishvakarma NK, Singh SM (2010) Immunopotentiating effect of proton pump inhibitor pantoprazole in a lymphoma-bearing murine host: Implication in antitumor activation of tumor-associated macrophages. *Immunol Lett* 134(1):83–92. <https://doi.org/10.1016/j.imlet.2010.09.002>
225. Vishvakarma NK, Singh SM (2011) Augmentation of myelopoiesis in a murine host bearing a T cell lymphoma following in vivo administration of proton pump inhibitor pantoprazole. *Biochimie* 93(10):1786–1796. <https://doi.org/10.1016/j.biochi.2011.06.022>
226. Togashi K, Kataoka T, Nagai K (1997) Characterization of a series of vacuolar type H(+)-ATPase inhibitors on CTL-mediated cytotoxicity. *Immunol Lett* 55(3):139–144
227. Togashi K, Kataoka T, Nagai K (1997) Concanamycin A, a vacuolar type H(+)-ATPase inhibitor, induces cell death in activated CD8(+). *CTL Cytotechnol* 25(1–3):127–135. <https://doi.org/10.1023/A:1007995212658>
228. Noma N, Fujii G, Miyamoto S, Komiya M, Nakanishi R, Shimura M, Tanuma SI, Mutoh M (2017) Impact of acetazolamide, a carbonic anhydrase inhibitor, on the development of intestinal polyps in min mice. *Int J Mol Sci* 18 (4). <https://doi.org/10.3390/ijms18040851>
229. Parkkila S, Rajaniemi H, Parkkila AK, Kivela J, Waheed A, Pastorekova S, Pastorek J, Sly WS (2000) Carbonic anhydrase inhibitor suppresses invasion of renal cancer cells in vitro. *Proc Natl Acad Sci USA* 97(5):2220–2224. <https://doi.org/10.1073/pnas.040554897>
230. Mohammadpour R, Safarian S, Ejeian F, Sheikholya-Lavasani Z, Abdolmohammadi MH, Sheinabi N (2014) Acetazolamide triggers death inducing autophagy in T-47D breast cancer cells. *Cell Biol Int* 38(2):228–238. <https://doi.org/10.1002/cbin.10197>
231. Amith SR, Wilkinson JM, Baksh S, Fliegel L (2015) The Na(+)/H(+) exchanger (NHE1) as a novel co-adjuvant target in paclitaxel therapy of triple-negative breast cancer cells. *Oncotarget* 6(2):1262–1275. <https://doi.org/10.18632/oncotarget.2860>
232. Amith SR, Wilkinson JM, Fliegel L (2016) Na+/H+ exchanger NHE1 regulation modulates metastatic potential and epithelial-mesenchymal transition of triple-negative breast cancer cells. *Oncotarget* 7(16):21091–21113. <https://doi.org/10.18632/oncotarget.8520>
233. Amith SR, Fliegel L (2016) The Na+/H+ exchanger in metastasis. *Aging (Albany NY)* 8(7):1291. <https://doi.org/10.18632/aging.101002>
234. Reshkin SJ, Bellizzi A, Caldeira S, Albarani V, Malanchi I, Poignee M, Alunni-Fabbroni M, Casavola V, Tommasino M (2000) Na+/H+ exchanger-dependent intracellular alkalization is an early event in malignant transformation and plays an essential role in the development of subsequent transformation-associated phenotypes. *FASEB J* 14(14):2185–2197. <https://doi.org/10.1096/fj.00-0029com>
235. 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. *Int J Oral Maxillofac Surg* 41(10):1206–1210. <https://doi.org/10.1016/j.ijom.2012.03.001>
236. Di Sario A, Bendia E, Omenetti A, De Minicis S, Marzioni M, Kleemann HW, Candelaresi C, Saccomanno S, Alpini G, Benedetti A (2007) Selective inhibition of ion transport mechanisms regulating intracellular pH reduces proliferation and induces apoptosis in cholangiocarcinoma cells. *Dig Liver Dis* 39(1):60–69. <https://doi.org/10.1016/j.dld.2006.07.013>
237. Mentzer RM Jr, Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B, Haverich A, Knight J, Menasche P, Myers ML, Nicolau J, Simoons M, Thulin L, Weisel RD, Investigators ES (2008) Sodium–hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: results of the EXPEDITION study. *Ann Thorac Surg* 85(4):1261–1270. <https://doi.org/10.1016/j.athoracsur.2007.10.054>
238. Dormond SFaO (2015) Systemic buffers in cancer therapy: the example of sodium bicarbonate; stupid idea or wise remedy? *Med Chem* 5:540–544. <https://doi.org/10.4172/2161-0444.1000314>
239. Yuan YH, Zhou CF, Yuan J, Liu L, Guo XR, Wang XL, Ding Y, Wang XN, Li DS, Tu HJ (2016) NaHCO<sub>3</sub> enhances the antitumor activities of cytokine-induced killer cells against hepatocellular carcinoma HepG2 cells. *Oncol Lett* 12(5):3167–3174. <https://doi.org/10.3892/ol.2016.5112>
240. Azzarito T, Lugini L, Spugnini EP, Canese R, Gugliotta A, Fidanza S, Fais S (2016) Effect of modified alkaline supplementation on syngenic melanoma growth in CB57/BL mice. *PloS one* 11(7):e0159763. <https://doi.org/10.1371/journal.pone.0159763>
241. Martin NK, Robey IF, Gaffney EA, Gillies RJ, Gatenby RA, Maini PK (2012) Predicting the safety and efficacy of buffer therapy to raise tumour pH: an integrative modelling study. *Br J Cancer* 106(7):1280–1287. <https://doi.org/10.1038/bjc.2012.58>
242. Mc Naughton L, Thompson D (2001) Acute versus chronic sodium bicarbonate ingestion and anaerobic work and power output. *J Sports Med Phys Fitness* 41(4):456–462
243. Mueller SM, Gehrig SM, Frese S, Wagner CA, Boutellier U, Toigo M (2013) Multiday acute sodium bicarbonate intake improves endurance capacity and reduces acidosis in men. *J Int Soc Sports Nutr* 10(1):16. <https://doi.org/10.1186/1550-2783-10-16>
244. Silva AS, Yunes JA, Gillies RJ, Gatenby RA (2009) The potential role of systemic buffers in reducing intratumoral extracellular pH and acid-mediated invasion. *Cancer Res* 69(6):2677–2684. <https://doi.org/10.1158/0008-5472.CAN-08-2394>
245. Ibrahim Hashim A, Cornnell HH, Coelho Ribeiro Mde L, Abrahams D, Cunningham J, Lloyd M, Martinez GV, Gatenby RA, Gillies RJ (2011) Reduction of metastasis using a non-volatile buffer. *Clin Exp Metastasis* 28(8):841–849. <https://doi.org/10.1007/s10585-011-9415-7>
246. Ibrahim-Hashim A, Abrahams D, Enriquez-Navas PM, Luddy K, Gatenby RA, Gillies RJ (2017) Tris-base buffer: a promising new inhibitor for cancer progression and metastasis. *Cancer Med* 6(7):1720–1729. <https://doi.org/10.1002/cam4.1032>
247. Som A, Raliya R, Tian L, Akers W, Ippolito JE, Singamaneni S, Biswas P, Achilefu S (2016) Monodispersed calcium carbonate nanoparticles modulate local pH and inhibit tumor growth in vivo. *Nanoscale* 8(25):12639–12647. <https://doi.org/10.1039/c5nr06162h>