New ways to image and target tumour hypoxia and its molecular responses

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ABSTRACT

Tumour hypoxia and its molecular responses have been shown to be associated with poor prognosis. Detection of hypoxia, preferably in a non-invasive manner, could therefore predict treatment outcome and serve as a tool to individualize treatment. This review gives an overview of recent literature on hypoxia imaging markers currently used in clinical trials. Furthermore, recent progress made in targeting hypoxia (hypoxia-activated prodrugs) or hypoxia response (carbonic anhydrase IX inhibitors) is summarized. Last, window-of-opportunity trials implementing non-invasive imaging are proposed as an important tool to prove anti-tumour efficacy of experimental drugs early during drug development.
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It is well established that tumours are not a collection of relatively homogeneous cancer cells, but act as organs with a complexity that might even exceed that of healthy tissues. Therefore to understand the biology of a tumour both the different individual cell types within a tumour as well as its microenvironment need to be studied [1]. Within this review, we will focus on hypoxia, a common characteristic of solid tumours, which has been associated with poor prognosis [2]. Detection of hypoxia, preferably in a non-invasive manner, could predict treatment outcome and serve as a tool to support treatment decisions. Such non-invasive imaging approaches that are routinely available in clinical practice including positron emission tomography (PET), magnetic resonance imaging (MRI) and perfusion computed tomography (CT) are able to accurately and reliably image hypoxia in tumours. Over the last decade, these diagnostic techniques are developing into versatile tools integrated in treatment monitoring, outcome prediction and treatment targeting. A meta-analysis evaluating the relationship between hypoxia imaging and outcome after radiation treatment demonstrated a uniform tendency for poor response when tumours were hypoxic. This was not only observed for widely used hypoxic PET tracers, but also when hypoxia was indirectly evaluated using perfusion-CT or DCE-MRI [3].

While the prognostic significance of tumoural hypoxia on outcome has been established more than two decades ago only recently compounds are being tested in clinical trials that enable monitoring and selective elimination of hypoxic tumours cells. Here we will provide an update on the current status of hypoxia imaging agents and strategies to combat tumour hypoxia.

Hypoxia PET imaging tracers

Multiple PET tracers suitable for the detection of hypoxia have been developed, validated and shown to exhibit different characteristics. The ideal hypoxia tracer has complete clearance of unbound tracer at time of imaging, thus only bound in oxygen deprived tissues resulting in high signal to noise ratios [3]. We recently reviewed the PET hypoxia tracers that were validated in preclinical and clinical studies and reported accurate quantification methods and clinical applications [4]. The most investigated PET hypoxia tracer is fluoromisonidazole (FMISO). However, due to concerns regarding FMISO stability, metabolite formation and slow clearance properties [5,6], alternative hypoxia PET tracers with different clearance and hydrophilicity characteristics have been developed trying to overcome these limitations:

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fluoroazomycin arabinoside (FAZA), fluoroerythronitroimidazole (FETNIM) and fluorinated etanidazole derivatives (EF1, EF3, EF5), which all have been extensively reviewed previously [3,4,7].

More recently, the hydrophilic flortanidazole (HX4), with preferred pharmacokinetics and clearance properties, has been synthesized through click-chemistry [8] showing 82% intact and 84% unmetabolized tracer at 2 h post injection (h p.i.) in plasma and urine respectively [9]. HX4 has been evaluated in a preclinical rhabdomyosarcoma rat tumour model, where binding was causally dependent on tumoural oxygenation status. Furthermore, a significant spatial relationship at tumour-microregional level between HX4 distribution and the exogenous hypoxia marker pimonidazole staining was observed [10,11]. Studies in primates and healthy volunteers [9] and in patients with histologically proven solid cancer [12] provided evidence for a good safety profile. Recently, in non-small cell lung cancer patients, image contrast was found to be superior 4 h p.i. compared with earlier time points and uptake patterns were strongly correlated between two scans [13]. Overlap studies between HX4 and the metabolism tracer FDG indicated that on average 24% of the hypoxic volume is outside the FDG volume [14]. Similar results have been obtained for head and neck cancer patients [15], suggesting that hypoxia PET imaging provides complementary information to FDG imaging.

Due to the large heterogeneity in uptake, differences in tumour and animal models, different time points of imaging and anaesthesia observed in the literature, it is difficult to compare different hypoxia markers. Although characterization of new hypoxia markers should be preferably performed in multiple cancer models, highly additive data can be expected from comparisons of different tracers within the same tumour models [3,11]. Recently, we performed a comparative study characterizing the clinically approved hypoxia markers FMISO, FAZA and HX4 on tumour to blood ratio (TBR), reproducibility and reversibility within a rat rhabdomyosarcoma model [16]. Blood clearance for FAZA and HX4 became similar 3 h p.i., while for FMISO as expected clearance from normal tissues was significantly lower. Differences in tumour uptake resulted in significantly higher TBR for HX4 compared to the other tracers. Reproducibility and spatial overlap between two PET acquisitions over a 48 h time period was high for both FMISO and HX4. Furthermore, decreasing the hypoxic fraction using carbogen resulted in loss of FMISO uptake, while increased hypoxia achieved by breathing 7% oxygen, further enhanced FAZA and HX4 uptake. Another study performed a similar comparison in a SQ20b head and neck xenograft mouse model and found similar tumour to muscle ratios for FMISO, FAZA and HX4 [10]. However, these results were obtained at 80–90 min p.i., a time point which is probably too early for evaluation since normal tissue clearance is still ongoing. A comparative study in head and neck cancer patients found similar tumour to muscle ratios for HX4 imaging at 1.5 h p.i. and FMISO imaging at 2 h p.i. [17]. For HX4 higher uptake and increasing ratios would be expected at later time points based on our clinical results [13]. Recently a simulation study, comparing FMISO, FAZA and HX4 based on their respective physical and chemical properties, revealed that tracer clearance and diffusion are the major parameters influencing image contrast. Highest clearance and image contrast was observed for HX4, but also the largest patient-to-patient variation, which might be a concern for clinical imaging to define tumour hypoxia based on a reliable threshold value [18].

Current available tracers have proven to be reliable for evaluation of tumour hypoxia, although with inherent problems resulting in clinical limitations. Alternative tracers, such as HX4, are promising with respect to deliver higher contrast images, whereas FMISO remains a robust reproducible hypoxia marker. It is not inconceivable that more tracers will be developed; but currently existing PET tracers should rather be used in clinic with standardized protocols enabling comparisons between different institutes. Furthermore, applicability and clinical validation should be proven in multiple cancer types and tracers need be tested with respect to their prognostic and predictive value.

Hypoxia targeting

The compelling evidence for hypoxia in tumour tissue and its therapeutic importance makes hypoxia a high priority target for cancer therapy. Bioreductive prodrugs selectively activated under hypoxia and drugs that inhibit molecular targets in hypoxic cells (vide infra) are currently extensively investigated. A recent overview described the challenges and opportunities of these strategies [19]. The clinically most advanced hypoxia-activated prodrug is tirapazamine (TPZ). Although promising results have been reported in a number of Phase 2 trials, TPZ failed in several Phase 3 clinical trials since no survival benefit was observed when incorporated into standard therapy regimens. Possible explanations are its poor tumour penetration, low *in vivo* potency at tolerable doses and unacceptable toxicity levels and lack of patient selection with high levels of tumour hypoxia [20]. A more potent hypoxia-activated prodrug currently undergoing early clinical testing is TH-302. It is a 2-nitroimidazole conjugated to bromo-isophosphoramide mustard, which is released and activated upon very low levels of oxygen [21] and diffuses to surrounding cells creating a cytotoxic bystander effect [19]. TH-302 displayed clinical activity when used as single agent, which makes it unique compared to earlier generation hypoxia-activated cytotoxins which demonstrate anti-tumour activity only when used in combination with radiation or chemotherapy [22]. Furthermore, TH-302 efficacy was correlated with the hypoxic fraction across different tumour models [23–26]. Phase 1 trials have proved TH-302 safety with nausea, vomiting and fatigue as the most frequently occurring toxicities. Other trials successfully combined TH-302 with doxorubicin in patients with advanced soft tissue sarcoma [27] or with gemcitabine in patients with advanced pancreatic cancer [28]. A phase 3 double-blind, placebo-controlled trial has been initiated in which patients with advanced pancreatic cancer were randomized to gemcitabine combined with TH-302 or placebo [29]. Recently our group has evaluated the efficacy of TH-302 in a rat rhabdomyosarcoma and a human H460 xenograft model, using growth delay as endpoint. TH-302 in both models significantly inhibited tumour growth and markedly sensitized tumours to radiation. Furthermore, the therapeutic effect of TH-302 was dependent on the tumour oxygenation status prior to local radiotherapy that was modified by either carbogen (to improve oxygenation) or low oxygen containing gas (to increase hypoxia) breathing [30].

Increasing tumour oxygenation has shown potential for improving radiotherapy efficacy in several randomized clinical trials [31,32]. In spite of positive results, these strategies using hyperbaric oxygen or carbogen combined with vasodilating agents have not gained clinical traction due to practical limitations, toxicity and relatively modest clinical benefit [33]. An alternative strategy to achieve improved tumour oxygenation is to decrease cellular oxygen consumption using for example metformin, an inhibitor of the mitochondrial NADH dehydrogenase, also known as complex 1, activity in the mitochondrial electron transport respiration chain [34]. Recently, it has been demonstrated that metformin increases tumour response to radiotherapy, through a reduction in oxygen consumption and improved tumour oxygenation [35]. For future personalized cancer medicine, evaluation of hypoxia biomarkers and patient stratification will be essential to apply hypoxia targeting treatments to change radiotherapy response.

Hypoxia molecular response

As tumours progress from an early to later stage disease, supply of oxygen becomes limited. Cancer cells must therefore alter their metabolism to an anaerobic glycolytic phenotype, resulting in a less efficient energy production and intracellular acidosis. In order to survive cancer cells must adapt to this acidic microenvironment, which helps promote metastases [36]. One of the important molecular responses to hypoxia is the stabilization of the hypoxia inducible factor (HIF)-1α, enabling interaction with HIF-1β. The complex translocates to the nucleus where it binds to hypoxia responsive elements (HRE) in the promoter region of target genes, such as vascular endothelial growth factor (VEGF), glucose transporters 1 (GLUT-1) and carbonic anhydrase IX (CAIX) [37]. CAIX as well as other membrane transporters, like the sodium-proton exchanger 1 (NHE-1) and the monocarboxylate transporters (MCT), are upregulated to counteract the hypoxia-induced intracellular acidosis. CAIX is a tumour specific dimeric membrane bound zinc metallo-enzyme, which catalyses the reversible hydration of carbon dioxide to bicarbonate and a proton to help maintain the cells pH homeostasis [38]. High tumoural CAIX expression has been associated with poor prognosis, tumour progression and aggressiveness [39]. Inhibition of its function would therefore be a promising anticancer approach to target the hypoxic compartment of tumours.

CAIX imaging tracers

A molecular imaging approach based on selective ligands to accessible proteins overexpressed at sites of hypoxia is desired. Such an agent could help physicians to decide which patients would benefit from adjuvant hypoxia-targeted therapy, e.g. anti-CAIX therapy. One strategy is using antibodies or antibody fragments targeted against transmembrane CAIX expression. The highly specific antibody M75 recognizes the extracellular proteoglycan-like domain of CAIX and is used for Western blotting and immunohistochemistry [40]. Specific accumulation of iodine-125 radiolabelled derivative has been observed in HT29 tumour-bearing mice [41]. Independently, the monoclonal G250 antibody was developed as a biomarker for renal cell carcinoma [42]. A chimeric version of G250 (cG250) has been radiolabelled with iodine-124, zirconium-89 or indium-111 for diagnostic purposes [43]. However, no apparent correlation has been observed between cG250 uptake and pimonidazole labelling or CAIX staining in head and neck tumour models attributed to the large interval between cG250 injection and immunohistochemical evaluation [44]. Pepsin degradation of intact cG250 antibodies resulted in $F(ab')_2$ fragments with a smaller molecular weight resulting in faster clearance from blood and healthy tissues. Zirconium-89 labelled cG250-$F(ab')_2$ fragments were found to spatially correlate with CAIX expression [45]. Furthermore, fully human CAIX single-chain variable fragment (scFv) minibodies have been generated using phage-display technology. They recognize the extracellular carbonic anhydrase domain, but do not inhibit CAIX activity and do not bind to the other transmembrane enzyme CAXII [46]. Recently, near-infrared (NIR) fluorescent monoclonal antibodies against CAIX and CAXII have been successfully tested for the non-invasive detection of breast cancer metastasis [47]. A dual labelled antibody combining indium-11 or iodine-125 nuclear with NIR imaging has proven feasible for preoperative and intraoperative detection of CAIX expressing renal cell carcinomas [48,49].

A second approach is the use of small molecules specifically targeting the active site of CAIX. Several classes of small molecules with low (nM) affinity have been extensively described, but due to the high degree of homology among CA isotypes, small molecules are generally not specific for one isoform [50]. To prevent interaction with the intracellular CA isoforms charged species or bulky groups such as FITC, albumin or sugar moieties are added to the small molecule. These strategies prevent transportation across membranes, but do not guarantee selectivity between CAIX and CAXII, both transmembrane enzymes with their catalytic domains oriented extracellularly. Attempts to design specific compounds targeting each isoform separately have been recently reviewed [51].

We and others have demonstrated *in vitro* that small molecule binding requires not only CAIX expression but also its hypoxic activation [52,53]. This offers a big advantage compared with antibodies against CAIX, since these small molecules can distinguish cells that are currently hypoxic from those that were previously hypoxic, while antibodies do not since their long half-life after reoxygenation [54]. *In vivo*, we have reported significant accumulation of fluorescent sulfonamides in HT29 xenografts, which was causally related with tumour oxygenation. Furthermore, bound sulfonamide decreased rapidly upon tumour reoxygenation [55]. Similar results have been obtained using fluorescent acetazolamide derivatives showing preferential targeting of CAIX overexpressing SK-RC-52 renal cell xenografts [56]. Recently, a series of sulfonamide derivatives conjugated with NIR fluorescent dyes having up to 50-fold higher selectivity for CAIX compared to the intracellular and other transmembrane isoforms has been designed. High tumoural uptake with little accumulation in other organs, except for the kidneys, has been observed using fluorescence molecular tomography [57]. Several synthesis strategies have been proposed to enable nuclear imaging, however most attempts were not successful in showing specific enhanced tumour uptake. We have synthesized and evaluated a technecium-99 m labelled sulfonamide for visualization of CAIX expression by SPECT imaging. Despite favourable affinity values maximum tumour uptake was low (<0.5% ID/g) even after varying physicochemical properties of the molecules [58,59]. A range of sulfonamides conjugated metal complexes have recently been designed for metallic radionuclide imaging. Although high *in vitro* uptake was observed, cellular binding between CAIX positive and negative cell lines was not obviously different [60]. Besides metal chelation approaches, direct fluorine-18 radiolabelling of CAIX targeted molecules has been assessed. Several compounds have been synthesized, such as 7-(2-fluoroethoxy)coumarin (FEC) and U-104 [61], the tertiary sulfonamides 4a-c [62] and VM4-037A [63]. Although all derivatives showed good affinity for CAIX with excellent plasma stability, uptake in HT-29 xenografts was minimal which precludes their application as CAIX imaging agents.

CAIX targeting

Inhibiting CAIX can be done either by the use of monoclonal antibodies or with small molecule inhibitors. Antibody approaches are mostly based on the concept of antibody dependent cell cytotoxicity (ADCC). A leading example is cG250, marketed as RENCAREX®, which is extensively investigated as an anticancer immunotherapy [64]. Phase 1 and 2 trials have demonstrated safety and efficacy as monotherapy or in combination with interferon (IFN)-α for the treatment of renal cell carcinoma (RCC) [65]. This antibody was also tested in the double-blind, placebo-controlled phase 3 aRISER trial for adjuvant therapy of clear cell RCC, but as announced by WILEX AG the antibody failed to meet the primary endpoint, since no improvement in median disease-free survival was observed compared to placebo. Several new antibodies currently tested in preclinical settings show promising results regarding anti-tumour effects [66,67].

Specific inhibition of different carbonic anhydrase isoforms using small molecules is an active field of research and has been extensively reviewed [50,68,69]. Membrane-impermeable acetazolamide derivatives [56] and aromatic sulfonamides [70] were able to reduce tumour growth and proliferation. Treatment of mammary-tumour bearing mice with CAIX-specific sulfonamide and glycosylcoumarin inhibitors resulted in a significant reduction in tumour growth and lung metastasis formation [71]. One of the several potent CAIX inhibitors identified from a DNA-encoded chemical library screen has shown high and specific accumulation in tumour models [72]. A new class of sulfamate inhibitors proved to be excellent candidates for low dosage anti-metastatic drugs [73], but were ineffective in reducing primary tumour growth [73,74]. Combining small molecules targeting CAIX with conventional therapies might yield even better efficacy. Recently, a CAIX dependent sensitizing effect of indanesulfonamides [75] and acetazolamide [76] on respectively radio- and chemotherapy has been demonstrated. Similarly combination of paclitaxel with orally administered U-104 significantly affected primary tumour growth and metastasis formation by reducing the breast cancer stem cell population [77]. Nitroimidazole and sulfamide based dual targeting drugs reduced hypoxic extracellular acidification *in vitro*, inhibited tumour growth at low dosage and sensitized tumours to both radiation [78] and doxorubicin [79]. This dual-targeting strategy appeared to be more effective than single targeting molecules. Recently, a family of novel small-molecule drug conjugates comprising of a linker cleavable in the extracellular space and a potent cytotoxic payload targeting CAIX has been designed and characterized. The disulfide-linked conjugate with maytansinoid DM1 as cytotoxic payload and an acetazolamide derivative as the targeting ligand has shown potent anti-tumour effects in renal cell carcinoma models with only minimal toxicity [80]. These results indicate that targeted delivery of potent cytotoxic agents using CAIX directed ligands may provide therapeutic benefits over current standard of care. The first clinical trial (NCT02215850) testing a small molecule CAIX inhibitor, named SLC-0111, is currently ongoing and is focused on testing the safety in subjects with advanced solid tumours.

Window-of-opportunity trial

Although there is a high number of new promising anti-cancer agents under preclinical and clinical investigation, the success rate of approved drugs for clinical practice has not been significantly increased. Improved clinical trial designs, such as 'window-of-opportunity' trials will help to select effective drugs at an earlier stage and to identify patients which potentially will benefit of the drug. In this trial, the patient agrees to delay combined conventional anti-cancer therapy to first receive the experimental drug, with the aim to obtain knowledge about anti-tumour activity in a disease state that is not disturbed by previous or simultaneous treatments [81]. The question has been raised whether these trials should be more widely applied in early phases of drug development knowing the progress in imaging and monitoring tumour progression [82] to prevent expensive long-lasting classical clinical testing of inefficacious drugs. Using this trial approach, hypoxia imaging can be used as a biomarker of response, especially suitable in the context of testing hypoxia (response) targeting drugs. Upon patient inclusion, baseline hypoxia should be acquired followed by the experimental targeting drug. A post-treatment hypoxia PET scan will assess the effect of the single treatment by comparison of the hypoxic fractions between the two scans (Fig. 1). This window-of-opportunity trial can precede a phase 1 trial testing safety of the experimental drug in combination with conventional treatment, e.g. radiotherapy, or a randomized phase 2 clinical trial.

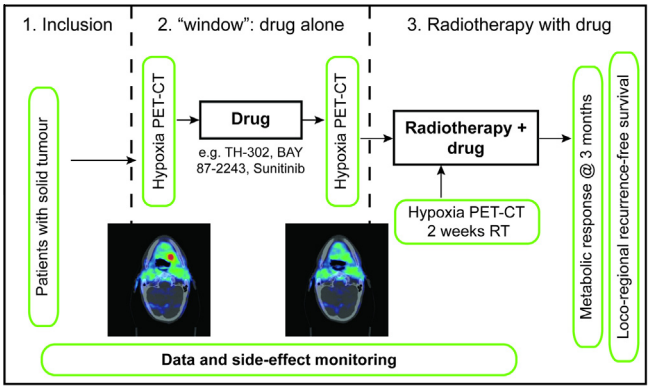

Fig. 1. Window-of-opportunity trial concept implementing non-invasive hypoxia imaging before and after drug administration followed by phase 1 or 2 trial combining radiotherapy and experimental drug. Additional hypoxia imaging can be included in step 3 for early response monitoring. HX4 hypoxia PET-CT images from a patient with head and neck cancer are shown as proof of concept.

We have used this concept preclinically to proof efficacy of the hypoxia-activated cytotoxic prodrug TH-302. The hypoxic fraction assessed with HX4 PET imaging in the rhabdomyosarcoma model was significantly reduced at day 4 upon TH-302 treatment, while vehicle treatment was ineffective. Additionally, TH-302 was not only effective as monotherapy, but also sensitized tumours to a single dose of radiation [30]. Similarly, BAY 87-2243, an inhibitor of mitochondrial complex 1, resulted in reduced HIF-1α activity and pimonidazole binding prior to radiotherapy improving local tumour control [83,84]. Its efficacy has also been shown by a dramatic reduction in FAZA PET signal before significant changes in tumour volume were observed [85]. Finally sunitinib treatment resulted in improved tumour oxygenation as FAZA uptake in Caki-1 renal cell xenografts [86] and in patients with soft-tissue sarcomas [87] was significantly reduced during therapy. Upon withdrawal of sunitinib therapy, FAZA uptake increased again, indicating a rebound in tumour hypoxia. These examples clearly highlight the importance of imaging the hypoxic fraction of tumours to monitor treatment response.

Conclusions

Current clinically available hypoxia PET tracers, although showing different characteristics, have proven to be reliable for evaluation of tumour hypoxia. Much progress has been made in the synthesis and evaluation of high affinity small molecules targeting CAIX. Nevertheless, proper clinically-suited diagnostic tools are still lacking. The window-of-opportunity trial concept implementing non-invasive imaging to monitor treatment response is an important tool to provide evidence of anti-tumour efficacy in earlier stages of drug development.

Conflict of interest statement

None of the authors have any conflict of interest to declare.

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References

[1] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
[2] Nordsmark M, Bentzen SM, Rudat V, et al. Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 2005;77:18–24.
[3] Horsman MR, Mortensen LS, Petersen JB, Busk M, Overgaard J. Imaging hypoxia to improve radiotherapy outcome. Nat Rev Clin Oncol 2012;9:674–87.
[4] Peeters SG, Zegers CM, Yaromina A, Van Elmpt W, Dubois L, Lambin P. Current preclinical and clinical applications of hypoxia PET imaging using 2-nitroimidazoles. Q J Nucl Med Mol Imaging 2015;59:39–57.
[5] Rasey JS, Koh WJ, Evans ML, et al. Quantifying regional hypoxia in human tumors with positron emission tomography of [18F]fluoromisonidazole: a pretherapy study of 37 patients. Int J Radiat Oncol Biol Phys 1996;36:417–28.
[6] Krohn KA, Link JM, Mason RP. Molecular imaging of hypoxia. J Nucl Med 2008;49:129S–48S.
[7] Lopci E, Grassi I, Chiti A, et al. PET radiopharmaceuticals for imaging of tumor hypoxia: a review of the evidence. Am J Nucl Med Mol Imaging 2014;4:365–84.
[8] Kolb HC, Finn MG, Sharpless KB. Click chemistry: diverse chemical function from a few good reactions. Angew Chem Int Ed Engl 2001;40:2004–21.
[9] Doss M, Zhang JJ, Belanger MJ, et al. Biodistribution and radiation dosimetry of the hypoxia marker 18F-HX4 in monkeys and humans determined by using whole-body PET/CT. Nucl Med Commun 2010;31:1016–24.
[10] Carlin S, Zhang H, Reese M, Ramos NN, Chen Q, Ricketts SA. A comparison of the imaging characteristics and microregional distribution of 4 hypoxia PET tracers. J Nucl Med 2014;55:515–21.
[11] Dubois LJ, Lieuwes NG, Janssen MH, et al. Preclinical evaluation and validation of [18F]HX4, a promising hypoxia marker for PET imaging. Proc Natl Acad Sci U S A 2011;108:14620–5.
[12] van Loon J, Janssen MH, Ollers M, et al. PET imaging of hypoxia using [18F] HX4: a phase I trial. Eur J Nucl Med Mol Imaging 2010;37:1663–8.
[13] Zegers CM, van Elmpt W, Wierts R, et al. Hypoxia imaging with [(1)(8)F]HX4 PET in NSCLC patients: defining optimal imaging parameters. Radiother Oncol 2013;109:58–64.
[14] Zegers CM, van Elmpt W, Reymen B, et al. In vivo quantification of hypoxic and metabolic status of NSCLC tumors using [18F]HX4 and [18F]FDG-PET/CT imaging. Clin Cancer Res 2014;20:6389–97.
[15] Zegers CM, van Elmpt W, Hoebers FJ, et al. Imaging of tumour hypoxia and metabolism in patients with head and neck squamous cell carcinoma. Acta Oncol 2015:1–7.
[16] Peeters SG, Zegers CM, Lieuwes NG, et al. A comparative study of the hypoxia PET tracers [(1)(8)F]HX4, [(1)(8)F]FAZA, and [(1)(8)F]FMISO in a preclinical tumor model. Int J Radiat Oncol Biol Phys 2015;91:351–9.
[17] Chen L, Zhang Z, Kolb HC, Walsh JC, Zhang J, Guan Y. (1)(8)F-HX4 hypoxia imaging with PET/CT in head and neck cancer: a comparison with (1)(8)F-FMISO. Nucl Med Commun 2012;33:1096–102.
[18] Wack LJ, Monnich D, van Elmpt W, et al. Comparison of [18F]-FMISO, [18F]-FAZA and [18F]-HX4 for PET imaging of hypoxia – a simulation study. Acta Oncol 2015:1–8.
[19] Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer 2011;11:393–410.
[20] Reddy SB, Williamson SK. Tirapazamine: a novel agent targeting hypoxic tumor cells. Expert Opin Investig Drugs 2009;18:77–87.
[21] Meng F, Evans JW, Bhupathi D, et al. Molecular and cellular pharmacology of the hypoxia-activated prodrug TH-302. Mol Cancer Ther 2012;11:740–51.
[22] Bennewith KL, Dedhar S. Targeting hypoxic tumour cells to overcome metastasis. BMC Cancer 2011;11:504.
[23] Cardenas-Rodriguez J, Li Y, Galons JP, et al. Imaging biomarkers to monitor response to the hypoxia-activated prodrug TH-302 in the MiaPaCa2 flank xenograft model. Magn Reson Imaging 2012;30:1002–9.
[24] Hu J, Van Valckenborgh E, Dehui X. Synergistic induction of apoptosis in multiple myeloma cells by bortezomib and hypoxia-activated prodrug TH-302, in vivo and in vitro. Mol Cancer Ther 2013.
[25] Liu Q, Sun JD, Wang J, et al. TH-302, a hypoxia-activated prodrug with broad in vivo preclinical combination therapy efficacy: optimization of dosing regimens and schedules. Cancer Chemother Pharmacol 2012;69:1487–98.
[26] Sun JD, Liu Q, Wang J, et al. Selective tumor hypoxia targeting by hypoxia-activated prodrug TH-302 inhibits tumor growth in preclinical models of cancer. Clin Cancer Res 2012;18:758–70.
[27] Chawla SP, Cranmer LD, Van Tine BA, et al. Phase II study of the safety and antitumor activity of the hypoxia-activated prodrug TH-302 in combination with doxorubicin in patients with advanced soft tissue sarcoma. J Clin Oncol 2014;32:3299–306.
[28] Borad MJ, Reddy SG, Bahary N, et al. Randomized phase II trial of gemcitabine plus TH-302 versus gemcitabine in patients with advanced pancreatic cancer. J Clin Oncol 2015;33:1475–81.
[29] Van Cutsem E, Fram R, Schlichting M, Ryan D. P-0173 Phase 3 trial of gemcitabine and TH-302 compared with gemcitabine and placebo in patients with pancreatic adenocarcinoma: the MAESTRO trial.. Ann Oncol 2013;24:iv38–iv121.
[30] Peeters SG, Zegers CM, Biemans R. TH-302 in combination with radiotherapy enhances the therapeutic outcome and is associated with pretreatment [18F] HX4 hypoxia PET imaging. Clin Cancer Res 2015.
[31] Janssens GO, Rademakers SE, Terhaard CH, et al. Accelerated radiotherapy with carbogen and nicotinamide for laryngeal cancer: results of a phase III randomized trial. J Clin Oncol 2012;30:1777–83.
[32] Overgaard J. Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck–a systematic review and meta-analysis. Radiother Oncol 2011;100:22–32.
[33] Overgaard J. Hypoxic radiosensitization: adored and ignored. J Clin Oncol 2007;25:4066–74.
[34] van Gisbergen MW, Voets AM, Starmans MH, et al. How do changes in the mtDNA and mitochondrial dysfunction influence cancer and cancer therapy? Challenges, opportunities and models. Mutat Res, Rev Mutat Res 2015;764:16–30.
[35] Zannella VE, Dal Pra A, Muaddi H, et al. Reprogramming metabolism with metformin improves tumor oxygenation and radiotherapy response. Clin Cancer Res 2013;19:6741–50.
[36] Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004;4:891–9.
[37] Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer 2008;8:851–64.
[38] Alterio V, Hilvo M, Di Fiore A, et al. Crystal structure of the catalytic domain of the tumor-associated human carbonic anhydrase IX. Proc Natl Acad Sci U S A 2009;106:16233–8.
[39] Potter CP, Harris AL. Diagnostic, prognostic and therapeutic implications of carbonic anhydrases in cancer. Br J Cancer 2003;89:2–7.
[40] Pastorekova S, Zavadova Z, Kostal M, Babusikova O, Zavada J. A novel quasi-viral agent, MaTu, is a two-component system. Virology 1992;187:620–6.
[41] Chrastina A, Zavada J, Parkkila S, et al. Biodistribution and pharmacokinetics of 125I-labeled monoclonal antibody M75 specific for carbonic anhydrase IX, an intrinsic marker of hypoxia, in nude mice xenografted with human colorectal carcinoma. Int J Cancer 2003;105:873–81.
[42] Oosterwijk E, Ruiter DJ, Hoedemaeker PJ, et al. Monoclonal antibody G 250 recognizes a determinant present in renal-cell carcinoma and absent from normal kidney. Int J Cancer 1986;38:489–94.
[43] Cheal SM, Punzalan B, Doran MG, et al. Pairwise comparison of 89Zr- and 124I-labeled cG250 based on positron emission tomography imaging and nonlinear immunokinetic modeling: in vivo carbonic anhydrase IX receptor binding and internalization in mouse xenografts of clear-cell renal cell carcinoma. Eur J Nucl Med Mol Imaging 2014;41:985–94.
[44] Troost EG, Bussink J, Kaanders JH, et al. Comparison of different methods of CAIX quantification in relation to hypoxia in three human head and neck tumor lines. Radiother Oncol 2005;76:194–9.
[45] Hoeben BA, Kaanders JH, Franssen GM, et al. PET of hypoxia with 89Zr-labeled cG250-F(ab')2 in head and neck tumors. J Nucl Med 2010;51:1076–83.
[46] Ahlskog JK, Schliemann C, Marlind J, et al. Human monoclonal antibodies targeting carbonic anhydrase IX for the molecular imaging of hypoxic regions in solid tumours. Br J Cancer 2009;101:645–57.
[47] Tafreshi NK, Bui MM, Bishop K, et al. Noninvasive detection of breast cancer lymph node metastasis using carbonic anhydrases IX and XII targeted imaging probes. Clin Cancer Res 2012;18:207–19.
[48] Muselaers CH, Rijpkema M, Bos DL. Radionuclide and fluorescence imaging of clear cell renal cell carcinoma using dual labeled anti-carbonic anhydrase IX antibody G250. J Urol 2015.
[49] Muselaers CH, Stillebroer AB, Rijpkema M, et al. Optical imaging of renal cell carcinoma with anti-carbonic anhydrase IX monoclonal antibody girentuximab. J Nucl Med 2014;55:1035–40.
[50] Supuran CT. Carbonic anhydrases: novel therapeutic applications for inhibitors and activators. Nat Rev Drug Discov 2008;7:168–81.
[51] Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, De Simone G. Multiple binding modes of inhibitors to carbonic anhydrases: how to design specific drugs targeting 15 different isoforms? Chem Rev 2012;112:4421–68.
[52] Dubois L, Douma K, Supuran CT, et al. Imaging the hypoxia surrogate marker CA IX requires expression and catalytic activity for binding fluorescent sulfonamide inhibitors. Radiother Oncol 2007;83:367–73.
[53] Svastova E, Hulikova A, Rafajova M, et al. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. FEBS Lett 2004;577:439–45.
[54] Rafajova M, Zatovicova M, Kettmann R, Pastorek J, Pastorekova S. Induction by hypoxia combined with low glucose or low bicarbonate and high posttranslational stability upon reoxygenation contribute to carbonic anhydrase IX expression in cancer cells. Int J Oncol 2004;24:995–1004.
[55] Dubois L, Lieuwes NG, Maresca A, et al. Imaging of CA IX with fluorescent labelled sulfonamides distinguishes hypoxic and (re)-oxygenated cells in a xenograft tumour model. Radiother Oncol 2009;92:423–8.
[56] Ahlskog JK, Dumelin CE, Trussel S, Marlind J, Neri D. In vivo targeting of tumor-associated carbonic anhydrases using acetazolamide derivatives. Bioorg Med Chem Lett 2009;19:4851–6.
[57] Groves K, Bao B, Zhang J, et al. Synthesis and evaluation of near-infrared fluorescent sulfonamide derivatives for imaging of hypoxia-induced carbonic anhydrase IX expression in tumors. Bioorg Med Chem Lett 2012;22:653–7.

[58] Akurathi V, Dubois L, Celen S, et al. Development and biological evaluation of (9)(9)mTc-sulfonamide derivatives for in vivo visualization of CA IX as surrogate tumor hypoxia markers. Eur J Med Chem 2014;71:374–84.
[59] Akurathi V, Dubois L, Lieuwes NG, et al. Synthesis and biological evaluation of a 99mTc-labelled sulfonamide conjugate for in vivo visualization of carbonic anhydrase IX expression in tumor hypoxia. Nucl Med Biol 2010;37:557–64.
[60] Dilworth JR, Pascu SI, Waghorn PA, et al. Synthesis of sulfonamide conjugates of Cu(II), Ga(III), In(III), Re(V) and Zn(II) complexes: carbonic anhydrase inhibition studies and cellular imaging investigations. Dalton Trans 2015;44:4859–73.
[61] Pan J, Lau J, Mesak F, et al. Synthesis and evaluation of 18F-labeled carbonic anhydrase IX inhibitors for imaging with positron emission tomography. J Enzyme Inhib Med Chem 2014;29:249–55.
[62] Lau J, Pan J, Zhang Z, et al. Synthesis and evaluation of (18)F-labeled tertiary benzenesulfonamides for imaging carbonic anhydrase IX expression in tumours with positron emission tomography. Bioorg Med Chem Lett 2014;24:3064–8.
[63] Peeters SG, Dubois L, Lieuwes NG. [F]VM4-037 microPET imaging and biodistribution of two in vivo CAIX-expressing tumor models. Mol Imaging Biol 2015.
[64] Surfus JE, Hank JA, Oosterwijk E, et al. Anti-renal-cell carcinoma chimeric antibody G250 facilitates antibody-dependent cellular cytotoxicity with in vitro and in vivo interleukin-2-activated effectors. J Immunother Emphasis Tumor Immunol 1996;19:184–91.
[65] Siebels M, Rohrmann K, Oberneder R, et al. A clinical phase I/II trial with the monoclonal antibody cG250 (RENCAREX(R)) and interferon-alpha-2a in metastatic renal cell carcinoma patients. World J Urol 2011;29:121–6.
[66] Birkhauser FD, Koya RC, Neufeld C, et al. Dendritic cell-based immunotherapy in prevention and treatment of renal cell carcinoma: efficacy, safety, and activity of Ad-GM.CAIX in immunocompetent mouse models. J Immunother 2013;36:102–11.
[67] Chang DK, Moniz RJ, Xu Z, et al. Human anti-CAIX antibodies mediate immune cell inhibition of renal cell carcinoma in vitro and in a humanized mouse model in vivo. Mol Cancer 2015;14:119.
[68] McDonald PC, Winum JY, Supuran CT, Dedhar S. Recent developments in targeting carbonic anhydrase IX for cancer therapeutics. Oncotarget 2012;3:84–97.
[69] Neri D, Supuran CT. Interfering with pH regulation in tumours as a therapeutic strategy. Nat Rev Drug Discov 2011;10:767–77.
[70] Cianchi F, Vinci MC, Supuran CT, et al. Selective inhibition of carbonic anhydrase IX decreases cell proliferation and induces ceramide-mediated apoptosis in human cancer cells. J Pharmacol Exp Ther 2010;334:710–9.
[71] Lou Y, McDonald PC, Oloumi A, et al. Targeting tumor hypoxia: suppression of breast tumor growth and metastasis by novel carbonic anhydrase IX inhibitors. Cancer Res 2011;71:3364–76.
[72] Buller F, Mannocci L, Zhang Y, Dumelin CE, Scheuermann J, Neri D. Design and synthesis of a novel DNA-encoded chemical library using Diels-Alder cycloadditions. Bioorg Med Chem Lett 2008;18:5926–31.
[73] Gieling RG, Babur M, Mamnani L, et al. Antimetastatic effect of sulfamate carbonic anhydrase IX inhibitors in breast carcinoma xenografts. J Med Chem 2012;55:5591–600.
[74] Meijer TW, Bussink J, Zatovicova M, et al. Tumor microenvironmental changes induced by the sulfamate carbonic anhydrase IX inhibitor S4 in a laryngeal tumor model. PLoS ONE 2014;9:e108068.
[75] Dubois L, Peeters S, Lieuwes NG, et al. Specific inhibition of carbonic anhydrase IX activity enhances the in vivo therapeutic effect of tumor irradiation. Radiother Oncol 2011;99:424–31.
[76] Gieling RG, Parker CA, De Costa LA, et al. Inhibition of carbonic anhydrase activity modifies the toxicity of doxorubicin and melphalan in tumour cells in vitro. J Enzyme Inhib Med Chem 2013;28:360–9.
[77] Lock FE, McDonald PC, Lou Y, et al. Targeting carbonic anhydrase IX depletes breast cancer stem cells within the hypoxic niche. Oncogene 2013;32:5210–9.
[78] Dubois L, Peeters SG, van Kuijk SJ, et al. Targeting carbonic anhydrase IX by nitroimidazole based sulfamides enhances the therapeutic effect of tumor irradiation: a new concept of dual targeting drugs. Radiother Oncol 2013;108:523–8.
[79] Rami M, Dubois L, Parvathaneni NK, et al. Hypoxia-targeting carbonic anhydrase IX inhibitors by a new series of nitroimidazole-sulfonamides/sulfamides/sulfamates. J Med Chem 2013;56:8512–20.
[80] Krall N, Pretto F, Decurtins W, Bernardes GJ, Supuran CT, Neri D. A small-molecule drug conjugate for the treatment of carbonic anhydrase IX expressing tumors. Angew Chem Int Ed Engl 2014;53:4231–5.
[81] Orloff J, Douglas F, Pinheiro J, et al. The future of drug development: advancing clinical trial design. Nat Rev Drug Discov 2009;8:949–57.
[82] Glimelius B, Lahn M. Window-of-opportunity trials to evaluate clinical activity of new molecular entities in oncology. Ann Oncol 2011;22:1717–25.
[83] Helbig L, Koi L, Bruchner K, et al. BAY 87–2243, a novel inhibitor of hypoxia-induced gene activation, improves local tumor control after fractionated irradiation in a schedule-dependent manner in head and neck human xenografts. Radiat Oncol 2014;9:207.
[84] Helbig L, Koi L, Bruchner K, et al. Hypoxia-inducible factor pathway inhibition resolves tumor hypoxia and improves local tumor control after single-dose irradiation. Int J Radiat Oncol Biol Phys 2014;88:159–66.
[85] Chang E, Liu H, Unterschemmann K, et al. 18F-FAZA PET imaging response tracks the reoxygenation of tumors in mice upon treatment with the mitochondrial complex I inhibitor BAY 87–2243. Clin Cancer Res 2015;21:335–46.
[86] Chapman DW, Jans HS, Ma I, et al. Detecting functional changes with [(18)F]FAZA in a renal cell carcinoma mouse model following sunitinib therapy. Eur J Nucl Med Mol Imaging 2014;4:27.
[87] Lewin J, Khamly KK, Young RJ, et al. A phase Ib/II translational study of sunitinib with neoadjuvant radiotherapy in soft-tissue sarcoma. Br J Cancer 2014;111:2254–61.