POSTER

poster #1

EFFECT OF CCL2 ON MONOCYTES AND METASTATIC BREAST CANCER

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Introduction: In the tumor microenvironment, cancer cells coexist with host stroma and immune cells, including myeloid cells. While some host cells inhibit cancer growth, others produce several soluble factors that promote tumor growth and progression. In turn, cancer cells provide growth factors, cytokines, and chemokines that attract host cells and promote their proliferation and survival. This crosstalk contributes to tumorigenesis and progression via enhanced angiogenesis, proliferation and invasion. The factors that contribute to this vicious cycle of cooperation are poorly understood, yet their identification is of primary importance to understanding malignant progression and to developing new mechanism-based therapies for cancer

Methods: Here we focus on the cellular and molecular mechanisms of metastasis and on the roles of CCL2 in this multi-step process. To address our questions, we used multiphoton intravital imaging; FACS; IHC; confocal microscopy.

Results: The CC chemokine ligand 2 (CCL2) and its receptor CCR2 have recently been shown to play key roles in promoting tumorigenesis and metastasis. Notably, CCL2 is upregulated in invasive breast carcinoma. Overexpression of CCL2 recruits inflammatory monocytes (CD11b+, Gr-1+ and CCR2+) to the primary tumor and to metastatic sites. By neutralizing CCL2 with an antibody, tumor growth was delayed and metastases were reduced. By using intravital imaging on the primary tumor we could detect morphological changes and reduced motility of tumor cells, upon anti-CCL2 treatment. These features correlated with decrease of macrophages within the tumor microenvironment upon antibody treatment.

Conclusions: These findings suggest that CCR2+ monocytes, might play different roles in tumor growth and metastasis.

poster #2

CHARACTERISATION AND DETECTION OF LIVER METASTASIS WITH CEA-SPECIFIC CU-64-DOTA LABELLED M5A-ANTIBODY *IN VIVO*

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Introduction: Metastasis is one of the hallmarks of cancer, but with the currently available imaging methods, liver metastases are still challenging to detect. Hence, novel tools for detection of micro metastases in the liver need to be established. The GPI-anchored surface protein Carcinoembryonic Antigen (CEA) is a differentiation antigen of colon mucosa expressed in a tissue-specific manner. CEA is an attractive target for novel PET-tracers, since its expression stays stable during local progress and metastasis, i.e. about 95% of metastases of CEA-positive primary colon tumours have been shown to be CEA-positive. In addition, neoexpression of CEA has been described in several carcinomas of other tissues [1]. To closely mirror the clinical setting, we have used the fully humanised CEA-specific antibody M5A (T84.66 specificity) to detect CEA-expressing liver metastases in a syngeneic orthotopic tumour model.

Methods: The metastasizing tumour cell lines MC38 (CEA-negative) or its CEA transfected derivative, C15A3 (CEApositive) were injected intrasplenically into CEA-transgenic C57BL/6-mice expressing the antigen in a human-like tissue-specific fashion [2]. Development of liver metastases was monitored early by whole-body micro-CT. Subsequently, mice were injected with 20µg of Cu-64-DOTA-labelled M5A-antibody or a Cu-64-DOTA-labelled control antibody. 3h, 24h and 48h post injection PET- and MRT-images were acquired. For blocking experiments, 500µg of unlabelled M5A-antibody was injected 3h prior to injection of Cu-DOTA labelled M5A-antibody. Livers were dissected and H&E and immunohistochemistry stainings as well as autoradiographies were prepared.

Results: The %ID/cc in CEA positive tumours was highest 24h after tracer injection (16.5% ± 6.3%) and significantly increased compared to healthy liver tissue in the same animals (8.6% ± 0.9%), as well as to CEA negative tumours in control animals (5.5% ± 0.6%). The ratio of the %ID/cc between C15A3 tumours and healthy liver tissue was determined as 1.9 ± 0.7, while the ratio of MC38 tumours and liver was significantly lower (0.8 ± 0.1). Liver metastases could be clearly distinguished from liver tissue in PET. Immunohistochemistry stainings with M5A-antibody showed homogeneous CEA expression within metastases, but intratumoural antibody distribution showed to be inhomogeneous in PET. This finding was verified in the autoradiographies and is likely due to the binding barrier effect. Blocking of the epitope with unlabelled M5A-antibody reduced the %ID/cc from 16.5% to 4.3% in CEA positive tumours 24h after injection of labelled M5A-antibody, showing its specific binding. An unspecific control antibody showed significantly lower uptake in CEA positive tumours in terms of %ID/cc (5.3% ± 0.5%).

Conclusions: Although the unspecific background in healthy liver tissue was as high as expected, we were able to detect liver metastasis with a Cu-64-DOTA-labelled antibody in PET for the first time. These results confirm the high specificity of the M5A-antibody in a novel tumour model superior to the artifical subcutaneous tumour models. This work gives evidence that the detection and characterisation of liver metastases with Cu-64-DOTA labelled antibodies is possible.

Special thanks to John E. Shively, Paul Yazaki, David Colcher and Andrew Raubitschek from the Graduate School of the City of Hope Land Beckman Research Institute, City of Hope National Medical Center, Duarte, CA 91010, USA, for supply of M5A.

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poster #3

VISUALIZING METASTASIS WITH NANOPARTICLES – AN EARLY INSIGHT

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Introduction: Today imaging cancer, herewith metastases are commonly based on positron emission tomography (PET) with radionucleotids. A negative side effect of numerous examinations during treatment is the exposition of patients to high doses of radioactivity. To reduce exposure other approaches for visualization and characterization of tumors and metastasis are needed. Magnetic resonance imaging (MRI) combined with specific nontoxic probes is a promising procedure. Super paramagnetic iron oxide particles (SPIO) reduce T2* effects in MRI and thus image properties. Specific SPIO against a cancer marker accumulate in tumor and metastases expressing the antigen and therefore can be detected with MRI. For a proof of principle study we combined a monoclonal antibody against the human epithelial adhesion molecule (EpCAM) which is highly expressed in several tumor entities with SPIO for *in vitro* and *in vivo* testing of imaging possibilities. Besides SPIO we used a parallel approach with Quantum Dots (QD) to fully investigate the behavior of nanoparticles *in vitro* and *in vivo* via fluorescence.

Methods: Nanoparticles were encapsulated with an amphiphilic polymer and covalently bound to specific (MOC31) and unspecific (MOPC) antibodies. Specific binding of probes was tested *in vitro* with colon carcinoma cell line HT29 (high expression of EpCAM) and an EpCAM knock down HT29 cell line with different concentrations of SPIO and QD and analyzed with MRI or FACS and confocal microscopy respectively. For *in vivo* analysis probes were tested with HT 29 tumor xenografts in immunodeficient mice. MRI (SPIO), *in vivo* fluorescence and intravital microscopy (QD) were used to examine specific binding.

Results: Specificity of encapsulated nanoparticles bound to MOC31 could be visualized by reduced T2* values in MRI in MOC31-SPIO incubated HT 29 cells but not with MOPC-SPIO. Furthermore FACS analysis showed a difference between HT 29 cells blocked with MOC31 before incubation with MOC31-QD to MOPC blocked cells. Nanoparticles accumulated in liver, lung and to a lower extend in tumors.

Conclusions: The use of nanoparticles in imaging is promising but enhancement of probes is necessary to gain specific accumulation *in vivo* in tumors. Reducing the size of specific nanoparticles via the use of single-chain variable fragment (scFv) or designed ankyrin repeat proteins (DARPin) is a favorable approach.

poster #4

LONGITUDINAL NON-INVASIVE PROBING OF ORTHOTOPIC HUMAN ACUTE MYELOID LEUKEMIA DEVELOPMENT AND DISSEMINATION THANKS TO NEAR-INFRARED-2-DEOXYGLUCOSE (NIR-2DG): SPECIFICITY, EFFICIENCY AND EXPLOITABILITY

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Introduction: In order to analyze the invasion and "metastatic" properties of human leukemia cells toward different bones, spleen as well as non-hematopoietic organs it is critically needed to develop non-invasive imaging technologies for pre-clinical longitudinal studies. Human acute myeloid leukemia (hAML) originates from a rare population of leukemia stem cells (LSCs) (Bonnet & Dick 1996, Nature). Orthotopic xenotransplantation of LSCs in highly immunocompromised mice allows to faithfully recapitulate many features of the human disease, therefore allowing to analyze its physiopathology and assess new therapeutic strategies in a clinically relevant setting. Unfortunately, longitudinal monitoring of AML development, spreading and response to treatment remains a challenging task involving invasive bone marrow (BM) punctures. Furthermore such sampling only provide a snapshot in a given bone. Because gene transfer into LSCs impedes on their capability to engraft, bioluminescence (BLI) tracking cannot be implemented routinely for primary AML. We previously showed that near-infrared (NIR) staining of leukemic cells with lipophilic dyes was not a satisfying option for this application neither (Lassailly F et al, Blood 2010). Since glucose transporter expression and / or metabolism rate are frequently increased in various cancers, including leukemia, we wondered whether NIR-2-Deoxyglucose (NIR-2DG) could be a reliable tool for longitudinal quantification of human leukemia development and response to treatment.

Methods: As a starting point to assess NIR-2DG, we generated 2 mouse models based on orthotopic transplantation of hAML cell lines lentivirally transduced with luciferase. After administration of NIR-2DG, mice were imaged using NIR imaging (reflectance imaging,spectral unmixing and fluorescence tomography). Bioluminescence imaging (planar and diffuse luminescent tomography), was used as a positive control to locate leukemia cells and analyze the co-localization with NIR signals. Healthy animals injected with the same amounts of probe were used as stringent negative controls.

Results: Starting with mice at an advanced stage of leukemia development, we observed a clear AML related increase of NIR signal as compared to healthy controls, associated with a good colocalization of BLI and NIR signals. After detailed flow cytometry analysis of different bones, we demonstrate that the frontoparietal bones of the skull, called calvaria, are a good BM compartment to analyze leukemia development. These, and other superficial sites of the skeleton therefore offer a convenient window to analyze the bone marrow. We exploited these anatomical sites to determine if evolution of NIR signal intensity over time would be useful for monitoring AML progression / response to treatment. Only the terminal stages of the disease gave rise to significant NIR signal increase.

Conclusions: We conclude that NIR-2DG provides good enough data to make a convincing proof of principle. However, considering the dynamic range of quantification, it is unlikely that this strategy can be exploited to monitor the development of AML and / or its response to treatment. More importantly, these results underline the importance to include exploitability studies in order to demonstrate, beside extreme cases, how useful is the technology to assess biological questions.

Acknowledgement: Authors a very grateful to PerkinElmer and LiCor Biosciences for kindly providing the NIR probes and to BRU for technical support.

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poster #5

KINOME WIDE SCREENING FOR REGULATORS OF FOCAL ADHESIONS DYNAMICS IDENTIFIES PFKFB2 AS A NOVEL DETERMINANT OF BREAST CANCER PROGRESSION

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Introduction: An essential step in metastasis formation includes tumor cell migration and invasion. This requires the plasticity of matrix adhesions structures. The migration of tumor cells is indeed highly controlled by the assembly/disassembly of those adhesions. These consist of cytoskeletal structural components, adaptor proteins, tyrosine kinases and phosphatases that together form the so-called integrin adhesome. Although some adhesome components are known to be essential in metastasis formation, it remains unclear how exactly the adhesion-mediated signaling controls the diversity of migratory and invasive behaviours of tumor cells.

Methods: To provide a systematic analysis of genes that regulate matrix adhesion dynamics, we performed a high content screen with MCF7 breast epithelial cells, using siRNAs targeting human genes encoding phosphatases and kinases. We did setup an image-based fixed assay that allows the quantification of the assembly and disassembly of the matrix adhesions in MCF7 cells using confocal microscopy. We applied the nocodazole assay described earlier by Ezratty and coworkers (Ezratty et al.,2005). Addition of nocodazole resulted in adhesion assembly while its washout provoked adhesion disassembly (Le Devedec et al., 2012). Under these conditions we fixed and stained the knockdown cells for vinculin a marker of matrix adhesions.

Results: The primary screen involved the identification of hits that impair focal adhesion assembly and/or disassembly. A validation of the hits yielded high confidence genes; some were further studied using time lapse microscopy of adhesion dynamics and tumor cell migration in different cell-lines. Importantly, one of the validated candidate genes PFKFB2, which is involved in the regulation of cell metabolism, correlated with breast cancer patient metastasis free survival.

Conclusions: Our results indicate the feasibility of automated high content imaging-based screening to identify novel clinically relevant cancer metastasis associated genes.

Acknowledgement: This work was financially supported by grants from the Dutch Cancer Society (UL2007-3860), the EU FP7 Health Program MetaFight project (Grant agreement no.201862) and EU FP7 Health Program Systems Microscopy NoE project (Grant agreement no.258068).

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poster #6

DEVELOPMENT OF A NEW MOLECULAR IMAGING APPROACH FOR EARLY DETECTION OF LUNG METASTASIS

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Introduction: Previously, we have demonstrated that antibody-conjugated microparticles of iron oxide (MPIO) targeting vascular cell adhesion molecule-1 (VCAM-1) enable early detection of brain metastases in mice *in vivo* using MRI, as a consequence of VCAM-1 upregulation on tumour-associated vessels[1-3]. We have also demonstrated early upregulation of VCAM-1 in lung tissue containing metastases. The objective of this work, therefore, was to determine whether VCAMMPIO bind to sites of metastasis in the lungs and, thus, could enable early detection of these tumours.

Methods: 8-10 weeks old, female BALB/c mice were used throughout. Lung metastasis: Mice were injected i.v. with 500,000 4T1-GFP cells. At day 5, 7, 9, 10 and 14 after metastasis induction, mice were injected i.v. with VCAM-MPIO or IgG-MPIO (4000µg Fe/kg) and killed 1, 3, 5, 7 or 24 hours later. Tissue from metastatic model has been acquired at early time points after administration of 4T1 cells; day 1, day 2, day 3 and day 4 post i.v. of 4T1 cells. At the desired time point mice were killed, the lungs transcardially perfused with heparinase saline, followed by fixation with 5ml of 4% PFA, inflated with 4% PFA and post fixed in 4% PFA overnight. Positive control: Mice were injected i.p. with LPS (0.5mg/ml in 200ml) to induce systemic inflammation 4h prior to i.v. injection of VCAM-MPIO or IgG-MPIO (4000µg and 75µg of Fe/kg). 1h post-VCAM-MPIO injection the animals were killed and lungs processed as above. Dose response: mice were injected i.v. with decreasing doses of VCAM-MPIO (4000, 1000, 400, 200, 100, 75 and 50µg Fe/kg body weight). One hour post-injection mice were killed, the lungs transcardially perfused heparinase saline and inflated with 4% PFA. Samples were embedded in 1% agarose and T2*-weighted MR images acquired. Subsequently, lungs were assessed immunohistochemically.

Results: We have demonstrated expression of VCAM-1 on the lung vasculature, including microvasculature, by immunohistochemical assessment, both in the positive control model and in the metastatic model. Specifically for the metastatic model we observe that VCAM-1 expression is upregulated on the endothelium of vessels and capillaries, within and around micrometastases. VCAM-MPIO binding is consistently observed after i.v. administration of 4T1-GFP cells, from as early as 5 days (<50um diameter of colony) up to 14 days after tumour cell injection (>800um diameter of colony). IgG-MPIO showed no specific binding in either the positive control or metastatic models.

Conclusions: These data suggest that VCAM-MPIO bind specifically to VCAM-1-positive vessels in lungs associated with metastases. Bright air-space lung imaging using hyperpolarised xenon is in development to enable *in vivo* imaging of VCAM-MPIO binding[4]. Early detection of lung metastases could greatly enhance therapeutic efficacy.

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poster #7

MR IMAGING OF OVARIAN CARCINOMA BEARING MICE AFTER INTRAVENOUS ADMINISTRATION OF DUAL-MODE FOLATE TARGETED SLNS

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Introduction: One of the major uptake systems utilized by eukaryotic cells is the high affinity folate receptor called folate-binding protein (FBP) (i). In particular the α-isoform tends to be over expressed in malignant tissues as ovarian carcinoma cells. Nanoparticles have emerged as a promising new drug delivery and diagnostic imaging technologies with potential for very selective delivery of therapeutic agents and targeting for exquisite diagnostic imaging of tumour (ii). Such agents provide several advantages over traditional compounds. Mainly, a single nanoparticle can contain a high payload of the contrast-generating material, which greatly improves the MRI detection. Aim of the study was the *in vivo* evaluation of folate targeted Solid Lipid Nanoparticles, (p)SLNs, with MRI at 7T on IGROV-1 ovarian carcinoma bearing mice.

Methods: Ten million IGROV-1 cells were resuspended in 1:1 Matrigel™ : RPMI-1640 medium and injected subcutaneously in the right flank of each 6 weeks old female mouse. Targeted (p) SLNs were loaded with a gadolinium complex, flurophore rhodamine and folate in the lipidic membrane. Untargeted particles without folate were used as control. Both (p)SLNs were i.v. administered at a sub-clinical gadolinium dose of 0.05 mmol/kg. MR images were acquired at 7T at different time points after administration (30 min, 4 and 24 h). MRI analysis was based on recognition, on the entire tumour, of the pixels exceeding the 40 % of enhancement. Fluorescence evaluation on tumour sections was scored by visual inspection (0<score<3).

Results: Statistical analysis highlighted that the number of pixels exceeding the 40% of enhancement in the group of animals receiving Rhodamine-folate-Gd-loaded-(p)SLNs was statistically different at 4 and 24 h after treatment ($p < 0.001$) from the group injected with Rhodamine-Gd-loaded-(p)SLNs. The mean values of the fluorescence scoring for (p)SLNs and Folate-(p) SLN groups also showed differences, although with borderline statistical significance (pvalue = 0.05, *t-test*).

Conclusions: These results suggested the hypothesis that the folate moiety recognizes and binds the folate receptor overexpressed in the IGROV-1 tumours, supporting the use of nanoparticles platform for molecular imaging applications. Moreover, the use of a high gadolinium payload in nanoparticles allows the possibility of reducing the injected dose without a substantial reduction of the contrast agent efficacy.

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poster #8

EX VIVO ASSAYS FOR THE DNA DAMAGE RESPONSE IN HUMAN TUMOR TISSUE SAMPLES

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Introduction: The DNA damage response is a highly conserved and important cluster of pathways in eukaryotic cells. Extensive knowledge of these pathways has been acquired in laboratory settings using cell lines and mouse models, particularly in DNA double strand break repair. However, much less is known for tissues and tumors in humans. Several human tumors arise from DNA damage response defects, e.g. BRCA deficient breast and ovarian tumors.

Methods: We are developing methods to determine DNA damage repair in viable slices from breast tumor specimens that have been cultured ex vivo. We study aspects of DNA repair by monitoring the accumulation (foci) and/or disappearance of proteins at sites of induced DNA double stranded breaks by immunofluorescent imaging.

Results: We have optimized culture methods in order to extend ex vivo life span of the tumor slices up to 144 hours with minimal decrease in replicating cells (measured by EdU incorporation) and minimal increase in apoptosis. We show accumulation (foci) and disappearance of 53bp1 protein at sites of induced double stranded breaks indicating DNA repair. To specifically measure activity of homologous recombination in these tumor slices we showed that Rad51 foci only appear in S/G2 cells after ionizing radiation induced DNA damage. We showed that this functional assay could distinguish BRCA1 deficient tumors from non BRCA1 deficient tumors in breast cancer xenograft mouse models.

Conclusions: The evaluation of homologous recombination in tumor samples might help to identify patients that could benefit from treatment with specific therapeutic drugs e.g. Platinum drugs (DNA interstrand crosslinkers) and PARPinhibitors. Similar assays may also be developed to determine sensitivity for known chemotherapeutic drugs to improve the choice for specific treatment in individual patients.

poster #9

MULTIMODALITY IMAGING OF A DNA REPAIR DEFICIENT PREMATURE AGING MOUSE MODEL

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Introduction: The prevalence of cardiovascular diseases (CVD), a leading cause of death, increases with the average age of the population. Furthermore, aging remains the most significant risk factor for CVD, even after correction for classic cardiovascular risk factors. DNA is constantly damaged by both extrinsic as well as intrinsic sources (e.g. ROS), which when left unrepaired, has devastating consequences for organismal health, leading in the end to cancer and age-related disease, including CVD. The ERCC1$^{-/d7}$ mouse has a deficiency in DNA repair and as a result shows a premature aging phenotype, including growth retardation, neurological degeneration, cardiovascular aging and a shortened lifespan. A better understanding of age-related changes in cardiovascular structures and function is required to successively treat or prevent cardiovascular disease in the elderly population. In order to find biomarkers related to vascular alterations and age related diseases, we will test the use of near infrared fluorescent (NIRF) probes to facilitate non-invasive imaging of processes concerned with aging and vascular instability. The development of new non-invasive diagnostic methods could lead to better treatment options in the future.

Methods: ERCC1$^{-/d7}$ mice, which display premature features of aging, and ERCC1$^{+/+}$ mice will be imaged *in vivo* and *ex vivo* with NIRF probes that target markers of aging and vascular alterations. *In vivo* molecular imaging will be combined with micro-CT imaging for 3D computation. NIRF probes used in this study include markers for proteolytic activity, oxidative stress, apoptosis and kidney dysfunction. Cardiovascular and renal tissue will be harvested and examined for potential markers of cellular senescence, DNA damage, and aging.

Results: With the use of multimodality imaging, we expect to find processes and biomarkers related to vascular instability and aging. Additionally, new treatment options can be explored which target these processes.

Conclusions: Non-invasive imaging, using NIRF probes, may enable longitudinal imaging of processes concerned with aging and vascular instability. Consequently, disease progression can be monitored over time and the effect of (new) pharmacotherapy can be studied

poster #10

HIGHLY SENSITIVE ENDOGENOUS MRI CONTRAST TO MAP VARIATIONS IN TUMOR OXYGENATION: MONITORING OF RESPONSE TO CARBOGEN BREATHING AND TO THE ANTI-VASCULAR AGENT CA4

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Introduction: Oxygen level is a key factor for tumor response to therapy. Improving tumor oxygenation at the time of treatment could lead to an improved response to therapy. In order to individualize the treatments and select patients who could benefit from tumor reoxygenation, there is a critical need for methods able to monitor dynamically and noninvasively tumor oxygenation. However, non-invasive tissue oxygenation mapping remains challenging, especially in the clinical setting. We recently developed a sensitive non-invasive magnetic resonance (MR) method to map variations in tissue oxygenation, a technique based on the measurement of the R_1 of the lipid protons that we named 'MOBILE' for Mapping of Oxygen By Imaging Lipids relaxation Enhancement[1]. The purpose of the current work was to assess the sensitivity and the quantitative properties of MOBILE by direct comparison with quantitative techniques (i.e. EPR oximetry, and OxyLite™ probes) in two mammary tumor models, in response to: (1) a hyperoxic breathing challenge and (2) the administration of an anti-vascular agent (CA4).

Methods: Mammary tumor models (NT-2 and Human MDA-MB-231 cells) were implanted orthotopically in FBV and FVB/N mice, respectively. Mice were anesthetized using isoflurane and the MR compatible OxyLite probe was inserted into the tumor. MRI experiments were performed with a 11.7T (Bruker, Biospec), and with a quadrature ^{1}H volume coil (for MOBILE acquisitions). EPR experiments were subsequently performed on the same tumors using a 1.1GHz Magnettech in vivo system 24h after injection of a paramagnetic oxygen reporter probe. Measurements were acquired before and during carbogen breathing or before and 4h after CA4 administration.

Results: Our results demonstrate that MOBILE is able to monitor positive changes in tumor oxygenation during carbogen breathing, and to monitor negative changes in tumor oxygenation following administration of CA4. The best correlation was observed between MOBILE and EPR oximetry. Indeed, the *intrinsic* values for "R_1 lipids" could be positively correlated with the values assessed using EPR oximetry with a slope of 0.01744±0.00656 (r^2=0.5407, p=0.0376). Using OxyLite probes, some trends were found between DR_1 Lipids and DpO_2, presenting a positive linear fit with a slope of 0.0027±0.0023 (r^2=0.22, p=0.11). Difference in sampling volumes, invasiveness, and tumor heterogeneity might be responsible for the lack of significant correlation between OxyLite and MOBILE measurements.

Conclusions: Our data demonstrate intrinsic quantitative properties for MOBILE, as well as its ability to dynamically follow positive and negative changes in tumor oxygenation. MOBILE is currently being compared to ^{19}F-MRI relaxometry after administration of the oxygen reporter probe 15C5. Indeed, ^{19}F oximetry presents the advantage of being able to map tumor oxygenation and therefore to probe tumor heterogeneity and to be simultaneously comparable to MOBILE. Knowing that MOBILE has been translated into the clinical setting on healthy volunteers at 3T[1], the technique holds promises for future human oncologic applications.

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poster #11

RADIATION SENSITIVITY IN HNSCC AND THE ROLE OF CELLULAR OXYGEN SENSORS

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Introduction: Head and neck squamous cell carcinomas (HNSCC) are known to be hypoxic causing resistance to radiotherapy and therefore poor prognostic to patients. Previously, it has been shown how the accumulation of hypoxia PET tracer fluorine 18 labeled 2-nitroimidazol EF5 (^{18}F-EF5) varies in HNSCC patients (1). In our laboratory, we have previously found that ^{18}F-EF5 uptake was phenotype specific in UT-SCC cell lines (University Turku HNSCC) correlating better to aggressive phenotype than hypoxia. It has been hypothesized that the regulators of hypoxia inducible factors (HIFs), prolyl hydroxylases (PHDs), affect the aggressiveness of HNSCC. PHDs serve as cellular oxygen sensors as they use oxygen as a cofactor in an enzymatic hydroxylation of HIF. Also the expression of PHD2 has been shown to predict radiation sensitivity among HNSCC patients (2). In this study, our aim was to investigate the role of PHDs in UTSCC cell lines as well as their impact on radiation sensitivity.

Methods: The three isoforms of PHDs and Hif-1α was silenced from UT-SCC cells lines using siRNA. The growth rate of silenced UT-SCC cells was observed for 12 days. To study the role of PHD3 in radiation sensitivity, clonogenic survival assay was used in PHD3 silenced UT-SCC cells. The expression of PHD isoforms and Hif-1α was detected by immunoblots from a gene silenced UT-SCC cells after culturing them under hypoxia.

Results: We have previously detected upregulation of PHD enzymes in HNSCC tumors. In UT-SCC cells, the depletion of PHD1 reduces the proliferation rate and reduced the expression of PHD3 in hypoxia. In our preliminary studies, PHD depleted UT-SCC cells were more sensitive to radiation therapy. According to our observation, PHD1 and PHD3 also seem to have an effect on cell metabolism.

Conclusions: Our preliminary results indicate that PHD expression in HNSCC is critical for cell survival and the PHD1 seems to affect the expression of PHD3. This study also suggests that loss of PHD3 affects the radiation sensitivity. The impact of PHDs on the uptake of ^{18}F-EF5 will be evaluated.

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poster #12

MRI BIOMARKERS FOR THE IDENTIFICATION OF HIGH INTENSITY FOCUSED ULTRASOUND-TREATED TUMOR TISSUE

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Introduction: High Intensity Focused Ultrasound (HIFU) is an emerging technique for the treatment of solid tumors. The aim of this project is to identify suitable Magnetic Resonance Imaging (MRI) biomarkers that can be used to distinguish HIFU-treated tumor tissue from residual/recurrent non-treated tumor tissue. As a first approach, multiparametric MR analysis was performed based on T1, T2, Apparent Diffusion Coefficient (ADC) and Magnetization Transfer Ratio (MTR) maps that were acquired longitudinally after HIFU treatment. Secondly, more advanced MRI parameters are studied for their sensitivity to HIFU-induced tissue necrosis. It has been shown that Amide Proton Transfer (APT) imaging is sensitive to tumor tissue necrosis after radiation therapy [1]. Therefore, APT imaging was applied to assess whether this technique could give complementary yet distinct information about tumor tissue status after HIFU treatment.

Methods:Tumormodel: CT26 colon carcinoma-bearing Balb/c mice. The HIFU treatment consisted of partial tumor ablation. Multiparametric MRI analysis: MRI was performed before (n=14), 1h after (n=14) and 72h after HIFU (n=7). The MRI protocol consisted of multi-slice assessment of T1, T2, ADC and MTR. Image clustering using the Iterative Self Organizing Data Analysis (ISODATA) technique [2] was performed on feature vectors consisting of subsets of MRI parameters. ISODATA-derived HIFUtreated tumor fractions were quantitatively compared to histology-derived non-viable tumor fractions. APT imaging: APT-MRI was performed before (n=15), 1h after (n=15) and 72h after HIFU (n=8). The effects of HIFU treatment on tumor APT intensity were studied by analysis of the APT intensity distribution in the tumor pixels before and after HIFU.

Results: Multiparametric MRI analysis: The highest one-to-one correspondence (R^2=0.77) between the ISODATA-derived HIFU-treated and histology-derived non-viable tumor fractions was observed for feature vector {T1, T2, ADC}. R1 (1/T1), R2 (1/T2), ADC and MTR each were significantly increased in the ISODATA-defined HIFU-treated tumor tissue at 1h after HIFU treatment as compared to non-treated tumor tissue. R1, ADC and MTR were also significantly increased at 72h after HIFU APT imaging: A pronounced decrease in APT intensity was observed in distinct regions of tumor tissue after HIFU treatment. A pronounced shift towards lower APT intensity values (-10 to -2%) was observed after HIFU compared to before HIFU. This range of APT intensity values was assumed to be related to HIFU-induced changes. A significant increase in the fraction of pixels within this HIFU-related APT intensity range was observed both at 1h (37.0±16.3%) and 72 h (49.0±16.4%) after HIFU compared to before HIFU (21.9±16.3%).

Conclusions: These results show that HIFU-treated tumor tissue can be distinguished from non-treated tumor tissue using multiparametric MRI analysis based on T1, T2 and ADC. Furthermore, significant changes in tumor APT intensity were observed after HIFU treatment, which indicates that APT imaging could potentially be a novel biomarker for identification of HIFU-treated tumor tissue. In the future, the proposed MR biomarkers will be combined into a single protocol to determine a robust set of biomarkers for HIFU treatment evaluation.

Acknowledgement: This research was supported by the Center for Translational Molecular Medicine (VOLTA).

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poster #13

MULTICOLOR BIOLUMINESCENCE IMAGING OF NFKB ACTIVATION IN A BREAST CANCER CELL LINE FOR SCREENING ANALOGUES OF CURCUMIN

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Introduction: Amongst the 10 biological hallmarks of cancer, sustained proliferative signalling and inflammation can be linked to NFkB pathway that is commonly activated in breast cancer cells (1). Curcumin and curcumin analogues have been discovered that directly or indirectly suppress NF-κB at key points along the activation pathway and they have been examined for chemoprevention, chemosensitization and for use as adjuvants (2). Here we generated a multicolor bioluminescent breast cancer cell line enabling simultaneous investigation of transcription factor NF-κB signalling and apoptosis (3).

Methods: The human breast cancer cell line (MDA-MD-231) was genetically modified to express a green, a red and a blue emitting luciferase to monitor cell number and viability, NF-κB promoter activity and to perform specific cell sorting and detection, respectively. The stable triple color cell line was used to test efficacy of curcumin analogues. Cells were cultured in a black 96 well plate at a density of 5000/well, stimulated with 10ng/ml of TNFa and treated with curcumin or curcumin analogues (e.g. demethoxycurcumin, bis-demethoxycurcumin, EF24 and EF31) .Signals were collected from live cells using a luminograph (Ivis Spectrum) and a series of band pass filters (20nm). Information about cell viability and NF-κB signalling can be evaluated at the same time by spectrally resolving the light emitted by green CBG99 and red PpyRE9 luciferase reporters, after addition of the single shared substrate D-luciferin in live cells. MTS assay was performed to test cell proliferation and Apoptosis was monitored by determination of caspase 3/7 activity. For this, DEVD-luciferin, which can only be used as substrate after removal of the DEVD moiety by the caspase enzyme, was added to the cells.

Results: The cell line showed a 70 fold induction of NF-κB promoter activity 24 hours after TNFa stimulation. By correcting the signals for cell viability the assay allowed to calculate the effect on NF-κB signaling for all the compounds tested over a wide range of concentrations. EF31 showed the lowest IC50 (0.8mM) in proliferation assay and the lowest IC50 (3.2 mM) on TNFa induced NF-κB activation. EF31 was also inducing the highest Caspase3/7 activity demonstrating to be the most potent curcumin analogue among the tested ones.

Conclusions: Our new method provides an imaging platform for the identification and optimization of compounds active on NF-κB signaling and apoptosis. The possibility of evaluating these processes at the same time, using a stable cell line, represents a great advantage in terms of predictability, time and cost.

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poster #14

A MAJORITY OF THE DIFFERENTIALLY EXPRESSED GENES IS DOWN-REGULATED DURING MALIGNANT TRANSFORMATION IN A FOUR STAGE HUMAN CELL LINE MODEL

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Introduction: We have combined a quantitative transcriptomics strategy using deep RNA sequencing with an antibodybased protein profiling approach to study a human fibroblast cell line model representing four different stages on the route to malignancy. The cell line model was created in order to mimic the process of malignant transformation and goes from primary contact-inhibited cells, through immortalized and transformed cells to finally metastatic cells.

Methods: The methods used in this study include paired end RNA sequencing with following bioinformatics analyses, network analysis of protein-protein interactions and immunofluorescence stainings of cells and confocal imaging.

Results: The RNA sequencing revealed that approximately 6% (n=1357) of the human protein coding genes was differentially expressed in at least one of the steps from normal to metastatic cells, with a majority of the genes being down-regulated. The group of up-regulated genes was highly enriched for genes involved in proliferative activity while the down-regulated genes were overrepresented by secreted proteins or proteins exposed on the cell surface, supporting the principle of dedifferentiation during malignant transformation. Morphological changes and differential expression patterns were successfully validated on the protein level with immunofluorescence stainings of the different cells in the model.

Conclusions: We here demonstrate how a transcriptomics approach with following protein analysis can be used to define the changes that accompany the mechanisms related to immortalization, transformation and invasion/metastasis separately. The majority of the molecular changes accompanying malignant transformation was shown to occur on the outside of the cell and many genes were identified as candidates for follow-up studies to further investigate their role in tumor formation.

Acknowledgement: We are grateful to Dr William C. Hahn (Harvard Medical School, Boston, MA, USA) for the BJ cell line model. The authors wish to acknowledge SciLifeLab (Science for Life Laboratory Stockholm) and SNISS for help with massively parallel sequencing and bioinformatics analysis and the entire staff of the Human Protein Atlas project. This work was supported by grants from the Knut and Alice Wallenberg Foundation and the strategic grant Science for Life Laboratory.

poster #15

DELINEATION OF MICROSCOPIC ORAL SQUAMOUS CELL CARCINOMA USING A NEAR-INFRARED FLUORESCENT ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR NANOBODY

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Introduction: Despite of the primary objective of achieving macroscopic clearance of 1 cm in the surgical management of oropharyngeal or oral squamous cell carcinoma (OSCC), the presence of tumor at the margin has been reported in 16%.[1] Numerous reports have indicated that involved margins implies a worse prognosis but incorporation of wider surgical margins will bring about functional impairment.[2,3] Therefore, delineation of the tumor during operation may promise an improvement of radical resections, thus increasing patients' survival rates. Being widely overexpressed in oral squamous cell carcinomas, the epidermal growth factor receptor (EGFR) serves as a very interesting target in intra-operative fluorescence imaging. In this study, nanobodies, the smallest functional antigen binding fragments derived from a naturally occurring single-chain antibody, were used to delineate the primary tumor and cervical lymph node metastases. Nanobodies have proved to be stable, soluble, are rapidly eliminated and have demonstrated efficient and specific tumor targeting.[4]

Methods: The EGFR specific nanobody 7D12 and the nonspecific control R2 were conjugated to the IRDye 800CW (LI-COR, Lincoln, NE, USA). OSC-19-luc2-cGFP (OSC) and SW620 cells were assessed for their EGFR expression using an anti-EGFR antibody (Ab528, Santa Cruz, CA, USA). Binding studies with both nanobodies were done using the Odyssey (LI-COR). To correct for the amount of cells the nucleic acid stain TO-PRO-3 (Invitrogen, Carlsbad, CA, USA) was used. *In vivo*, OSC cells were orthotopically injected in the tip of the tongue. The size of the primary tumor and microscopic cervical lymph node metastases were followed by bioluminescence. 7D12-CW800, R2-CW800 and the IRDye 800CW carboxylate were intravenously injected and *in vivo* and *ex vivo* fluorescence was acquired using the IVIS spectrum (Caliper Life Science, Hopkinton, MA, USA) and FLARE intraoperative near-infrared fluorescence imaging system.

Results: The *in vitro* binding assay clearly demonstrated specific binding of 7D12-CW800 to the EGFR overexpressing cell line OSC. In the EGFR negative cell line SW620, no difference in fluorescence intensity was seen between 7D12CW800 and R2-CW800. *In vivo,* in contrast to R2-CW800 and CW800 carboxylate, 7D12-CW800 could clearly delineate the primary tumor and microscopic metastases using the FLARE intraoperative near-infrared fluorescence imaging system. Furthermore, tumor sections showed tumor specific binding of 7D12-CW800 that was homogenously distributed throughout the tumors.

Conclusions: The anti-EGFR nanobody 7D12-CW800 offers a clear visualization of primary orthotopic oral squamous cell carcinomas and microscopic lymph node metastases using the FLARE intraoperative near-infared fluorescence imaging system.

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poster #16

RNA-APTAMERS AGAINST HUMAN RTKS AS TOOLS FOR IN VIVO CANCER CELL MOLECULAR IMAGING

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Introduction: Expression of specific molecular markers and metabolic activities of a given tumor may be studied by non-invasive imaging. The assessment of these parameters may allow to evaluate the response of a given tumor to therapeutics. Thus, the development of imaging agents that couple high specificity for the biomarker with low toxicity and high sensitivity is necessary to improve diagnosis and therapy for clinical applications. In this respect, aptamers represent an emerging attractive class of targeting molecules for the specific recognition of tumor-specific biomarkers. Aptamers are short single-stranded oligonucleotides ligands, with high affinity and selectivity to their target. Because of their appropriate *in vivo* chemical stability, lack of immunogenicity and toxic side-effects these molecules are promising imaging agents.

Methods: We have internally labeled the anti-EGFR, anti-Axl and anti-PDGFR aptamers with Alexa-fluorescent probes and incubated each aptamer on human cancer cells to investigate aptamers binding and internalization by confocal microscopy. For in vivo imaging the labeled aptamers were intravenous injected in nude mice, previously inoculated with RTK-positive and RTK-negative cells. The fluorescent signal has been detected and analyzed under *IVIS Spectrum* in vivo pre-clinical imaging system for assessing aptamer biodistribution and anti-tumor effect. Further, we are investigating the functionality of nanoparticle-aptamer bioconjugates with the anti-RTKs aptamers for cancer imaging. QDs were encapsulated inside the liposome and the anti-EGFR aptamer conjugated with PEG molecules on the surface of liposome. The nanoparticle-aptamer bioconjugates were incubated with EGFR-positive A549 and EGFR-negative MCF-7 cells and signals were observed under fluorescent microscope.

Results: Here we report aptamers capability to spread into the body and to specifically accumulate in the tumor and to inhibit *in vivo* tumor growth. In addition, a specific distribution of the nanoparticles was observed on the A549 cells while the green fluorescent signals on MCF-7 cells were negligible.

Conclusions: Our results indicate the above aptamer-based molecules as potential tools for in vivo imaging applications.

Acknowledgement: This work was supported by: CNR, AICR No 11-0075 (LC), MIUR grant, MERIT RBNE08YFN3_001 (VdF), AIRC No 11781 (LC), Compagnia di San Paolo and from the Italian Ministry of Economy and Finance to the CNR for the Project FaReBio di Qualità. AR is recipient of a AIRC-Marie Curie fellowship.

poster #17

DIFFERENCE OF ^{18}F-FLT UPTAKE AND DW-MRI IN VARIOUS LUNG CARCINOMA XENOGRAFTS

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Introduction: Lung cancer is the most common cause of cancer-related death worldwide. For defining treatment strategies non-invasive longitudinal assessment of tumour progression is important. This may be performed by employing molecular imaging techniques, such as positron emission tomography (PET) and magnetic resonance imaging (MRI). The aim of this study was to evaluate glucose metabolism and tumour proliferation by 2deoxy2[18]F]fluoro-D-glucose (^{18}F-FDG) and 3´-deoxy-3´-[^{18}F] fluoro-L-thymidine (^{18}F-FLT) µPET in conjunction with µMRI in various lung cancer cell lines grown *in vivo* with respect to tumour type-dependent differences.

Methods: Five lung cancer cell lines (A549, HTB56, EBC1, H1975, NCI H82) were implanted subcutaneously in NMRI nude mice (n=3 tumours per mouse, n=6 mice per cell line). Tumour growth was followed by caliper measurements. Two and four weeks after implantation ^{18}F-FDG- and ^{18}F-FLT-PET were performed employing a high-resolution quadHIDAC µPET camera (spatial resolution 0.7 mm FWHM). In some animals (n=19) T2 weighted morphological MRI was performed at 9.4 T (Bruker Biospec, in plane resolution 137 µm, slice thickness 1 mm) and the apparent diffusion coefficient (ADC) was determined by diffusion weighted (DW) MRI. Histology was accomplished to correlate findings with tumour proliferation (Ki67), human equilibrative nucleoside transporter 1 (hENT1) and thymidine kinase 1 (TK1) expression, and cell death (caspase 3 and TUNEL).

Results: Most tumours showed similar growth *in vivo*. The investigated xenografts varied with respect to intratumoural homogeneity, clearly visible on µMR as well as on µPET images. This finding could be confirmed by immunohistochemistry. Furthermore, ADC values within proliferative regions of H1975 and A549 tumours were higher when compared to HTB56, EBC1, and NCI H82, indicating necrosis. Cell death in these regions (scored by visual observation of caspase 3 staining and TUNEL) did not seem to correlate with this finding. The uptake of ^{18}F-FLT differed significantly between cell lines, with H1975 and A549 demonstrating the highest radiotracer accumulation. Differences in ^{18}F-FDG uptake were only marginal. The Ki67 index positively correlated with ^{18}F-FDG retention. In contrast, there seemed to be a negative correlation of Ki67 with ^{18}F-FLT uptake. High ^{18}F-FLT levels in A549 and H1975 tumours could not be explained by immunohistochemical stainings of TK1 and hENT1. Quantification of these proteins by means of western blot is still ongoing. Furthermore, we are currently analysing BrdU incorporation and thymidine levels in tumours to explain variances in ^{18}F-FLT uptake.

Conclusions: Tumour tissue heterogeneity varies considerably between different xenografts arising from established lung cancer cell lines. Furthermore, these xenografts differ in their *in vivo* uptake of ^{18}F-FLT for a yet unknown reason. There is no positive correlation of tracer uptake with proliferation as determined by Ki67 index or growth rate. These data suggest that differences between tumours, e.g. variances in ^{18}F-FLT uptake, are hard to predict and have to be taken into account when employing imaging modalities for treatment planning in clinical situations.

Acknowledgement: Supported in part by EU FP7 IMI project QuIC-ConCePT; grant agreement N° 115151.

poster #18

HER-2/*NEU* ONCOGENE EFFECTS ON MICRORNA LEVELS AND ITS ROLE IN ANCHORAGE INDEPENDENT GROWTH BY LOSS OF CELL ADHESION

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Introduction: Receptor tyrosine kinases are essential for mediating signals from the extracellular environment into a cell to induce changes in cell physiology, metabolism, proliferation and differentiation. The HER-2/*neu* oncogene is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinase genes and has been shown to be significantly associated with shorter time to relapse and overall survival in breast cancer. The HER-2/*neu* oncogene has been implicated in transformation primarily for its role in mediation of growth factor signal transduction in mitogenic pathways. However, studies have also shown that overexpression of HER-2/*neu* confers cell lines with more invasive and metastatic phenotypes in addition to accelerated growth. Furthermore, metastases are found more frequently in patients with HER-2/*neu* overexpression. HER-2/*neu* overexpression, therefore, may play a role in dynamic interactions between the transformed cell and its extracellular environment. My laboratory is exploring the ways that HER-2/*neu* signal transduction cascades mediate anchorage independent survival by the downstream effects on microRNA expression. Recent evidence has been reported that certain microRNA levels are altered by tyrosine kinase activity.

Methods: Two mouse anti-human monoclonal antibodies (mAbs) to the HER-2/neu extracellular domain have been described previously (1). Standard cell culture methods were used to grow and seed SKBR-3 human breast cancer cells overexpressing HER-2/neu receptor as well as NIH3T3 cells overexpressing human HER-2/neu for the following assays. We used the MTS Cell Proliferation Assay (Promega, Madison, WI) to assess downstream effects of these mAbs. microRNA expression levels were assessed using a TaqMan microRNA PCR assay (Applied Biosystems, Foster City, CA). Quantitative PCR was used as described in the manufacturer's protocol. Cell adhesion assays were used using adhesion proteins plated in 48 well plates. 0.5% Crystal violet was used to assess remaining cells for quantification of cell adhesion.

Results: One of the antibodies, 8H11, has an agonistic effect on SKBR-3 cell lines. Cells increased their proliferation rate by 16%. The other, 10H8, inhibits cell proliferation of SKBR-3 cells, but to a lesser degree than trastuzumab (16% and 30%, respectively). We tested the effects of these two antibodies to assess the alteration of miRNA levels in cells overexpressing HER-2/*neu* receptor levels. The 8H11 antibody, as an agonist, increases the expression of miRNA21, a miRNA known to have an oncogenic phenotype (2). The other antibody, 10H8, significantly downregulates miRNA21 expression, similar to trastuzumab. We investigated the effect of HER-2/*neu* overexpression on cell adhesion in parental NIH 3T3 cells and in NIH 3T3 cells stably transfected with a HER-2/*neu* expression vector. HER-2/*neu* overexpression was associated with a relative loss of cell adhesion to each of several extracellular matrix (ECM) proteins. Cell-matrix adhesion on vitronectin and denatured collagen, type I was selectively impaired by HER-2/*neu* overexpression.

Conclusions: These findings suggest that an oncogene product enables a cell to evade growth regulation by disruption of cell-matrix interactions, a key initial component of the metastatic cascade. The mechanism of interaction may be related to altered expression of microRNA levels. In our human PET/CT scans of a 64-Cu-trastuzumab positron emission tracer, we detect locally invasive cells preferentially in the spreading tumor (that lacks FDG tracer uptake) and this supports our cell culture findings *in vivo*.

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poster #19

IMAGING THE CONSEQUENCES OF THE HYPOXIC TUMOUR ENVIRONMENT AT THE CELLULAR LEVEL

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Introduction: Hypoxia is a well-characterized parameter of the tumour microenvironment that influences cancer progression and response to treatment. Cells adapt to hypoxia via the control of a broad range of genes encoding proteins involved in energy metabolism, cell survival, autophagy and apoptosis. This transcriptional response is mediated by the intracellular hypoxia-inducible transcription factor (HIF). Most studies on HIF regulation have been performed using bulk cell analysis techniques and fixed end-point assays. However, observation of protein localisation and levels in single living cells have uncovered complex dynamics which have not yet been explored for hypoxia-dependent signalling.

Methods: Here we have used time lapse confocal microscopy in hypoxic and normoxic environment to image, with a high temporal resolution over a long period of time, fluorescent tagged HIF-1α & HIF-2α levels and localization in singe living cells. HIF transcriptional activity was also measured in single cells by using time-lapse wide field microscopy.

Results: We observed for the first time discrete single or repetitive pulses of HIF-1α and -2α accumulation in hypoxic environment (1% O_2). We carried real-time luminescence experiments at a single cell levels using the HRE-luciferase reporter and we showed that the transiency observed for HIFα accumulation was also present at the transcriptional level. We have developed new mathematical models based on the data generated by the single cell imaging of HIF. We have demonstrated computationally and experimentally a key role for the prolyl hydroxylase PHD2 as a negative feedback regulating HIF timing and controlling the associated cell fate. Indeed, PHD2 has an essential role in maintaining HIF-1a accumulation transient, which has important consequences on preventing cell death caused by an excessive and long lasting HIF-1α accumulation. We further used the mathematical model to predict how hypoxia/re-oxygenation intensity and kinetics as well as repetitive hypoxic pulses found in tumours can affect HIF dynamics.

Conclusions: We have demonstrated computationally and experimentally a key role for PHD2 as a negative feedback regulating HIF timing and controlling the associated cell fate. This understanding of the role of HIF dynamics on cell fate is likely to have an important impact on the design of drug targeting HIF for cancer progression.

Acknowledgement: We thank BBSRC for funding.

poster #20

ASSESSMENT OF ANTI-ANGIOGENIC DRUG ACTIVITY USING DYNAMIC CONTRAST-ENHANCED MICRO-CT CORRELATES WITH 3-DIMENSIONAL FLUORESCENCE ULTRAMICROSCOPY IN BREAST CANCER XENOGRAFTS

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Introduction: Imaging of tumor microvasculature plays a crucial role in understanding angiogenesis and anti-angiogenic drug mechanisms[1]. While many methods exist to quantify angiogenesis *ex vivo*, it is desirable to use non-invasive methods for the assessment of anti-angiogenic drug activity *in vivo*[2,3]. Dynamic contrast-enhanced micro-CT (DCE micro-CT) offers a promising method to obtain *in vivo* functional information of tumor perfusion in cancer xenograft models[4]. In this study, we apply DCE micro-CT to quantify tumor vascular changes after short-term anti-angiogenic therapy in orthotopic breast cancer xenografts. For purpose of validation, the results are compared to the findings obtained by 3D high-resolution fluorescence ultramicroscopy (UM) of excised tumor tissue.

Methods: SCID/beige mice were orthotopically injected with human breast cancer cells (KPL-4) and split into a treatment and a control group. For anti-angiogenic treatment a monoclonal antibody targeting the human vascular endothelial growth factor (VEGF) was used. A non-specific IgG1 was used as control. Antibodies were administered weekly for a treatment period of two weeks and imaging was performed on the study endpoint. First, mice underwent DCE micro-CT imaging measuring the functional parameters relative blood volume (rBV) and vascular permeability (K). Then, after tumor explantation, the tumor vessel architecture as well as tumor volume was characterized performing quantitative UM. In order to address intra-tumoral heterogeneity, the quantitative results of both imaging methods were analyzed and compared in three distinct volumes of interest – tumor center, tumor periphery, and total tumor.

Results: Based on DCE micro-CT, we found that contrast enhancement was most strongly reduced in the periphery of tumors of the treatment group. As compared to control a significant reduction of mean rBV and mean K of 28% and 14% was observed, respectively. In contrast, an increase of tumor contrast enhancement was observable in the tumor center, which corresponded to a gain of rBV and K of 35% and 28%, respectively. Global assessment of the total tumor did not yield significant differences between both groups. Subsequent analysis of the tumor vascular architecture with UM confirmed a dense and highly irregular vessel structure in the tumor periphery of the control group, whereas tumor centers widely lack vascular perfusion. On the contrary, tumors of the treatment group showed a clearly reduced vessel density with a homogeneously distributed vessel structure throughout the entire tumor volume. Analysis of the tumor volumes did not show significant differences between treated and control group. These findings are in good agreement with the observations made by DCE micro-CT and confirm early vascular changes after anti-angiogenic drug treatment before drug efficacy becomes evident by tumor growth inhibition.

Conclusions: In conclusion, these data demonstrate that DCE micro-CT is an excellent method to non-invasively assess early anti-angiogenic drug effects in preclinical orthotopic tumor models. Hence, DCE micro CT may serve as good biomarker for prediction of therapeutic efficacy in clinical trials.

This work was funded by the Roche Postdoc Fellowship (RPF) program.

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poster #21

CHARACTERIZATION OF DENDRITIC CONTRAST AGENTS FOR DCE-MRI OF THE TUMOR VASCULATURE

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Dynamic Contrast-Enhanced Magnetic Resonance Imaging (DCE-MRI) is a promising tool for tumor treatment evaluation to assess treatment-induced vascular changes. DCE-MRI with macromolecular rather than low-molecular-weight contrast agents may reflect specific vascular parameters more accurately[1]. Dendritic MRI contrast agents are especially suitable for evaluation of the dependence of pharmacokinetic parameters on contrast agent size, because of their tunable size. In this study dendritic contrast agents of various molecular weight, including albumin-binding contrast agents, were characterized *in vitro* and *in vivo*. Based on this study an optimal set of contrast agents for characterization of tumor vasculature can be selected.

Poly(propylene imine) (PPI) dendrimers of generation G0-G5 were synthesized by SyMO-Chem BV: G2PPIRhoB$_1$-GdDOTA$_4$-PEG$_3$, G3-PPI-RhoB$_1$-GdDOTA$_8$-PEG$_7$, G4-PPI-RhoB$_1$-GdDOTA$_4$-PEG$_{15}$, G5-PPI-RhoB$_3$-GdDOTA$_{31}$-PEG$_{30}$, G0-PPI-GdDOTA$_1$-Palm$_1$, G1-PPI-GdDOTA$_3$-Palm$_1$, G2-PPI-GdDOTA$_7$-Palm$_1$, G2-PPI-RhoB$_1$-GdDOTA$_6$-Palm$_1$. The palmitoyl moiety facilitates murine serum albumin (MSA) binding Relaxivity measurements were performed to investigate aggregation behavior and contrast agent efficacy. Albumin binding was investigated using proton relaxation enhancement (PRE) measurements. With this method the enhancement in relaxation rate R_1 (s^{-1}) as a function of [MSA] at fixed [Gd^{3+}] was measured to provide e.g. the binding strength nK_A (n: number of binding sites) CT26 colon carcinoma bearing Balb/c mice (n=3/agent) were injected with one of the dendrimers or Gd-DOTA (0.03 mmol Gd/kg) and blood samples were taken until 24h after injection. R_1 (s^{-1}) values were determined on a 6.3T Bruker Biospec and ΔR_1 (s^{-1}) values were calculated as R_1 post – R_1 pre-injection. Distribution half-lives ($t_{½,\alpha}$) and elimination half-lives ($t_{½,\beta}$) were determined by fitting the data to a bi-exponential decay model. Biodistribution was assessed by Inductively Coupled Plasma (ICP) analysis of tumor/organs/tissue excised 24h after injection (%ID/g tissue).

Dendrimer relaxivity generally increased with molecular weight. PRE measurements below the critical aggregation concentration (CAC) showed the highest binding strength for G0-PPI-GdDOTA$_1$-Palm$_1$ (nK_A=17.4 10^4 M^{-1}) compared to G1-PPI-GdDOTA$_3$-Palm$_1$ (nK_A=0.15 10^4 M^{-1}), G2-PPI-GdDOTA$_7$-Palm$_1$ (nK_A=0.50 10^4 M^{-1}), G2PPIRhoB$_1$GdDOTA$_6$-Palm$_1$ (nK_A=0.82 10^4 M^{-1}) and the clinically used Ablavar® (nK_A=0.09 10^4 M^{-1}).Relatively long elimination half-lives of the albumin-binding dendrimers (G1-PPI-GdDOTA$_3$-Palm$_1$: 9.53±2.00h, G2PPIGdDOTA$_7$-Palm$_1$: 5.73±1.11h and G2-PPI-RhoB$_1$-GdDOTA$_8$-Palm$_1$: 1.61±0.41h) were observed. In general elimination half-lives were dependent on molecular weight for the non-palmitoyl containing dendrimers (G2: 0.77±0.18h, G3-PPI-RhoB$_1$-GdDOTA$_8$-PEG$_7$: 0.82±0.93h, G4-PPI-RhoB$_1$-GdDOTA$_4$-PEG$_{15}$: 2.87±2.75h, G5PPIRhoB$_3$GdDOTA$_{31}$PEG$_{30}$: 1.60±0.35h) and longer compared to Gd-DOTA (0.31±0.11h).Tumor/muscle uptake ratios determined by ICP were generally higher for the dendrimers (G1-PPI-GdDOTA$_3$-Palm$_1$: 4.50±1.07, G2-PPI-GdDOTA$_7$-Palm$_1$: 4.29±1.01, G2-PPI-RhoB$_1$-GdDOTA$_6$-Palm$_1$: 2.82±1.48, G2PPIRhoB$_1$GdDOTA$_4$PEG$_3$: 2.35±0.74, G3-PPI-RhoB$_1$-GdDOTA$_8$-PEG$_7$: 4.61±0.63, G4PPIRhoB$_1$GdDOTA$_4$PEG$_{15}$: 3.46±0.97, G5-PPI-RhoB$_3$-GdDOTA$_{31}$-PEG$_{30}$: 3.46±1.37) than Gd-DOTA (2.51±2.04), with the highest ratio for G3-PPI-RhoB$_1$-GdDOTA$_8$-PEG$_7$. Mice injected with G0-PPI-GdDOTA$_1$-Palm$_1$ were sacrificed prematurely due to toxicity issues.

The dendritic contrast agents provide a large range in blood circulation times. In addition, a higher tumor uptake of the dendrimers compared to Gd-DOTA was observed. These properties imply that these agents may possess a range of tumor wash-in and wash-out rates, making them suitable for investigation of the dependence of pharmacokinetic parameters on contrast agent size. In a next step, DCE-MRI with a selection of agents will be performed to characterize tumor vasculature and its response to treatment.

This research was supported by the Center for Translational Molecular Medicine (VOLTA).

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poster #22

IMAGING ERPC3-TREATMENT RESPONSE SHOWS REDUCED TSPO SIGNAL AND DEMONSTRATES GLIOMA INVASION OF MICROGLIA/MACROPHAGES AND ASTROCYTES IN THE TUMOR CORE

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Introduction: In 2012, the mean survival rate from glioblastoma at time of diagnosis is 15 months. New treatments are urgently required. The alkylphosphocholine Erufosine (ErPC3) has been shown to induce apoptosis in otherwise highly apoptosis-resistant glioma cell lines. In addition, the mitochondrial 18kDa Translocator Protein (TSPO) seems to be required for apoptosis induction by ErPC3 (*1*). Recently, we have shown that [^{18}F]DPA-714, a TSPO radioligand, is capable to image glioma and quantify its growth using positron emission tomography (PET) (*2*). Gliomas are not exclusively composed of cancer cells but also of cells of the tumor microenvironment (TME), i.e. endothelial cells, immune cells and fibroblasts. Recently, it has become clear that the TME plays an important role in both disease progression and response to therapy. In the present study, we investigated the activity of ErPC3 on rat 9L glioma, *in vitro* and *in vivo* using [^{18}F]DPA-714 PET-imaging. In addition, immunohistochemistry was employed to evaluate changes on cells of the TME, in particular microglia/macrophages and astrocytes.

Methods: 9L glioma cells were incubated with ErPC3 and treatment efficacy was characterized by cell viability, cell proliferation and apoptosis assays. *In vivo*, 9L rat glioma cells were stereotactically implanted into the striatum of Fischer rats. Glioma growth was monitored by [^{18}F]DPA-714 PET before and after one and two weeks of ErPC3-treatment. Rats received either ErPC3 (40mg/kg body weight) or sham-treatment at 48h intervals and for a maximum of 2 weeks, eleven days after tumor implantation. In order to investigate ErPC3 effect on tumor and cells of the TME we performed Hematoxylin/Eosin-staining, TUNEL-assay and immunohistochemistry for TSPO, CD11b and GFAP as marker for TSPO positive cells, microglia/macrophages and astrocytes, respectively.

Results: ErPC3 treatment demonstrates a significant decrease in 9L cell proliferation and viability ($p<0.001$), as well as a significant increase in apoptosis ($p<0.001$) and caspase-3 activation. *In vivo*, PET imaging shows a significant reduction in [^{18}F]DPA-714 uptake after ErPC3 treatment ($p<0.05$). Immunohistochemistry data reveal reduced tumor volume and increased cell death in ErPC3 treated animals. The treatment effect was further accompanied by infiltration of inflammatory cells and astrocytes, demonstrating an increased number of microglia/macrophages and astrocyte density in the tumor core of ErPC3-treated tumors.

Conclusions: Results demonstrate a significant antitumor effect of ErPC3 *in vitro* and *in vivo* and a change in the TME with a strong infiltration of TSPO-positive microglia/macrophages and reactive astrocytes into the tumor core. TSPO imaging using [^{18}F]DPA-714 allows effective monitoring and quantification of disease progression and response to ErPC3 therapy in intracranial 9L gliomas.

Acknowledgement: This work has been supported by the Joint INCa/DAAD Translational Research Programme on Cancer and the EU 7th Framework Programme under grant agreement n° 278850 (INMiND). The authors are grateful to Dr. M. Higuchi for providing the anti-TSPO antibody NP155 and Genzyme for providing Erufosine.

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poster #23

TARGETING OF ACTIVATED LEUKOCYTES BY AN IMAGING PEPTIDE SPECIFICALLY BINDING TO A B2-INTEGRIN ENABELING OPTICAL- AND RADIO-IMAGING OF INFLAMMATORY LOCI IN VIVO

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Introduction: We have reported the bicyclic peptide CPCFLLGCC (LLG) as a ligand for activated leukocyte integrins. Here, we present the use of radionuclide- or fluorophore-labeled LLG peptide in visualizing accumulated active leukocytes in inflammation and infection. Results from three different animal species with three different leukocyte accumulating models show the ability of LLG to target imaging molecules to inflamed tissues with infiltration of activated leukocytes. The peptide CPCFLLGCC (LLG), known to bind activated b2-integrins in vitro, was fused with green fluorescent protein (GFP) to test the ability of our construct to target and bind activated leukocytes in a skin lesion mouse model.

Methods: Proof of concept: Ex vivo targetingWe demonstrated the LLG peptide as a targeting molecule screening leukemia patient samples by the BRASIL bacteriophage biopanning method. In vivo targeting in infiltration model. The in vivo targeting of activated leukocytes with LLG was demonstrated with a leukocyte infiltration model. Injecting sterile-filtered thioglycolate broth (TG) into mouse peritoneal cavity induces sterile peritonitis Targeting to bacterial infection and inflammation LLG targeting to sites of inflammation was examined in New Zealand White rabbits by inducing an abscess with Escherichia coli (1 x 1011 CFU, colony-forming units) injected into the left thigh muscle *In vivo* targeting in infiltration model The *in vivo* targeting of activated leukocytes with LLG was demonstrated with a leukocyte infiltration model. Injecting sterile-filtered thioglycolate broth (TG) into mouse peritoneal cavity induces sterile peritonitis. Biodistribution of the iodinated LLG peptide and its conjugates. Balb/c mice received 40 µg iodinated LLG peptide or its conjugates (Peg-LLG, M-LLG, L-LLG) via tail vein, sacrified and the harvested organs were gamma-counted.Iodine-125-YADGACPCFLLGCC and iodine-125-GST-LLG were labeled using the iodogen method. Competition assays with the constructs showed the peptide had retained its function. In-111-DTPA-conjugates of the tested peptides were produced using a standard DTPA-conjugation protocol and subsequent labeling with In-111 SPECT imaging of the three animal models were performed.

Results: .LLG phage showed clear selectivity for activated leukocytes in AML patient samples. Synthetic LLG-C4 peptide blocked the phage indicating binding specificity. White blood cells from healthy donors did not bind LLG-C4 phage. We confirmed the ability of LLG phage to recognize leukemic cells by studying several cell lines showing variable levels of binding of LLG phage to granulocytic OCI-AML3 cells..From three different animal species with three different leukocyte accumulating models show the ability of LLG to target imaging molecules to inflamed tissues with infiltration of activated leukocytes. The LLG peptide effectively targets both bacteria induced and sterile inflammation. Our data suggests utility of our fluorescently fused peptide as well as the radiolabeled variants of LLG to be efficient and robust tools for both the detection of and targeting to sites of robust leukocyte activation in response to inflammation. These results are promising in view of finding novel therapeutic approaches by which anti-inflammatory and neuroprotective compounds can be targeted to brain areas at risk for further tissue damage and delayed cell injury.

Conclusions: We demonstrated the targeting ability of the CPCFLLGCC peptide in different animal species and different disease models using radionuclide- and fluorophore-labeled peptide constructs. The peptide proved very efficient in blocking neutrophils in the peritoneal infiltration model. LLG showed avid binding to neutrophils, the major players in the first line of defense, assumingly due to their rapid activation. In the ear-inflammation model, where the inflamed tissue is poorly vascularised, the targeting of the peptide was less efficient. In the rabbit *E. coli* infection model the LLG-peptide showed efficient targeting of infiltrating leukocytes, proving to be one of the best targeting molecules of dozens tested thus far with this model.

poster #24

INFLUENCE OF THE ADMINISTRATION ROUTE ON THE TREATMENT EFFICIENCY OF TUMOR-ASSOCIATED ANTIGEN (TAA) SPECIFIC TH1 CELLS IN AN ANIMAL MODEL FOR PANCREATIC CANCER

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Introduction: TAA-specific Th1 cells could serve as a promising new approach for immunotherapy of cancer patients. For the clinical application adequate treatment schemes have to be established defining the right administration route, dosage, injection frequency or adjunction with other immunotherapeutic agents or chemotherapeutics like the leucocyte depleting agent cyclophosphamide. Furthermore, the mode of action of TAA-specific Th1 cells during immunotherapy is not well understood and has to be further characterized in preclinical settings. In this approach we compared the influence of the administration route on TAA-specific Th1 cell-treatment efficiency in an endogenous animal model for pancreatic cancer (RIP1-Tag2).

Methods: Irradiated (2Gy) RIP1-Tag2-mice were treated weekly *i.v.* or *i.p.* with 10^7 Tag2-Th1 cells until the age of 14 weeks. The tumor size progression and treatment efficiency were determined by 7 T small-animal Magnetic Resonance Imaging (MRI). In addition blood glucose levels (BGL) serve in this animal model as a marker for tumor burden and progression. Untreated animals die at 14 weeks of age due to hypoglycemia. We further analyzed homing properties of *i.v./i.p.* transferred Cy5-fluorescence labeled Tag2-Th1 cells in 14 weeks old Tag2-Th1 cell-treated RIP1-Tag2-mice with Optical Imaging (OI) and Fluorescence Microscopy (FM). To characterize the Tag2-Th1 cell-mediated antitumoral effect, we further performed H&E-histology of the T-cell homing sites and immunohistochemistry of the pancreatic tissue.

Results: BGL in *i.p.* treated mice were 82±5 mg/dl and slightly increased in *i.v.* treated mice to 93±4 mg/dl, compared to 41±3 mg/dl in SHAM-treated mice at 14 weeks of age. In SHAM-treated 14 weeks old RIP1-Tag2-mice the mean tumor volume was 30.0±8.6 mm³, in *i.p.* treated mice 15.2±4.4 mm³ while tumor volume was lowest in *i.v.* treated mice with 5.8±1.7 mm³ (MRI). OI revealed strong homing to the tumor site only of *i.p.* transferred Cy5-labeled Tag2-Th1 cells, whereas *i.v.* administration leads to very low cell accumulations at the tumor site. FM analysis confirmed the OI results. As further homing sites could be the lung, liver, and spleen identified after *i.v.* administration. *I.p.* transferred Tag2-Th1 cells homed mainly to the lymph nodes, peripancreatic-lymphatic-tissue, and spleen. CD3- and B220-immunohistochemistry of the pancreatic tissue showed an infiltration of host B- and T-cells in *i.v.* Tag2-Th1 cell-treated RIP1-Tag2-mice, whereas after *i.p.* Tag2-Th1 cell-treatment host immune cells accumulated circularly around the tumors. No infiltration in the complete pancreatic tissue of host immune cells could be observed after SHAM-treatment. H&E-staining of the thymus revealed, that weekly *i.v.* treatment with Tag2-Th1 cells induced an atrophy and *i.p.* treatment resulted in a depletion. No histological altertions were found after SHAM-treatment. The treatment with Tag2-Th1 cells had no influences on other T cell homing sites (lung, liver, spleen).

Conclusions: Thus, *i.v.* administration of Tag2-Th1 cells is more efficient in RIP1-Tag2-mice than *i.p.* treatment despite a lack of migration to the tumor site. The differences in the antitumoral effects and mobilization of host immune cells to the tumor tissue are associated with the administration route. Future experiments will characterize these administration dependent effects of TAA-specific Th1 cells during immunotherapy.

GLYCOLYTIC PHENOTYPE INFLUENCES CHANGES IN CELL PROLIFERATION AND TUMOR HYPOXIA AFTER ANTI-ANGIOGENIC THERAPY

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Introduction: Anti-angiogenic therapy is increasingly used in cancer patients but therapeutic response is heterogeneous and predictive biomarkers for patients stratification are currently lacking. In a previous study, it has been observed that levels of “glucose addiction” modulate the pathologic response of tumor xenografts to VEGF neutralization. Here we investigated whether the glycolytic phenotype affects therapeutic responses to anti-angiogenic therapy by evaluating cell proliferation and regional hypoxia changes at early and late time-points by in vivo [^{18}F]FLT and [^{18}F]FAZA PET imaging.

Methods: SCID female mice were injected in the upper right back part with 4 x 10^5 poorly glycolytic IGROV-1 cells (n=20) or 3 x 10^5 highly glycolytic OC316 cells (n=20) mixed with liquid Matrigel. Following generation of tumor xenografts, mice were randomized into two groups: control (n=10) and treated (n=10). Bevacizumab (Avastin; i.p. 5 mg/kg) administration started when tumors reached 90-100 mm^3 volume and lasted for 4 weeks (9 doses). Animals underwent PET with [^{18}F]FLT and [^{18}F]FAZA at the baseline and 7 and 28 days from the beginning of therapy. Quantification analysis was performed with pmod 2.7 software and max radiotracers uptake values (SUVmax), tumor to background ratio and metabolic volumes were obtained. Finally, histological and immunohistochemical analysis were performed.

Results: We found that at 7 days highly glycolytic treated tumors volume was smaller than that of controls but there were no differences in radiotracers uptake. Moreover, treated tumors became necrotic but rapidly resistant to anti-VEGF therapy. In contrast, in poorly glycolytic xenografts we observed an early but transient reduction in [^{18}F]FLT uptake (7 days; $p<0.05$) and a long-term cytostatic effect as revealed by the lack of tumour volume increase up to 28 days. However, despite their small size, using [^{18}F]FLT and [^{18}F]FAZA PET imaging we found that tumors treated with bevacizumab were composed by highly proliferative and hypoxic cells. Finally, we also observed that protracted anti-VEGF therapy selects for highly glycolytic tumors with large necrotic areas similar to OC316.

Conclusions: Bevacizumab arrests growth of poorly glycolytic tumours although it selects a more aggressive phenotype, as indicated by [^{18}F]FLT and [^{18}F]FAZA PET images. Moreover, at the end of therapy, the lack of reduction in [^{18}F]FLT uptake might be predictive of anti-VEGF resistance associated with the highly glycolytic phenotype, despite the transient reduction in tumour volume found at early times.

Acknowledgement: This research has been supported by AIRC Project Molecular and Cellular Imaging of Cancer and by PIO Project PET Molecular Imaging.

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Layout: Doris Kracht
printing: Hundt Druck GmbH, Cologne