

Book of poster abstracts



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No	Last name	First name	Poster title	Page
1	Ahmed	Israa	Investigation of αSMA expression in p16 and p53 positive Oral Cancer	4
2	Alam	Jahedul	Generation and Characterization of integrin ITGA11-Cre mouse strain	5
3	Anandan	Shamun- deeswari	Preclinical Imaging in High Grade Serous Ovarian Cancer Cell Line Based Mouse Models: A Comparative Study	6
4	Birkeland	Even	Proteomic profiling of breast cancer patient samples, cell lines and conditioned media	7
5	Bjørnstad Furevik	Ronja Sarah	The impact of tumor interstitial fluid pressure on nanoparticle accumulation by cAMP receptors modulation	8
6	Bokil Alhourani	Ansooya Abdelnour	The effects of metformin treatment in colorectal cancer cells of opposing metabolic phenotype	9
7	Bredholt	Geir	Establishment of humanised immune system mice for evaluation of immunotherapies in patient derived xenograft tumour models	10
8	Dyrstad	Sissel	The mitochondrial succinate dehydrogenase complex is an important regulator of epithelial to mesenchymal transition	11
9	Fasmer	Kristine	Functional MRI predicts aggressive disease in endometrial cancer	12
10	Fonnes	Tina	Validation of Asparaginase like protein 1 (ASRGL1) as Prognostic Biomarker in Endometrial Carcinoma	13
11	Halle	Mari	FOXA1 predicts good outcome in HER2+ endometrial cancer patients by inhibiting EGFR/HER2 signaling	14
12	Hinz	Stefan	AKT3 subcellular localization and signaling modulates tumorigenicity	15
13	Hua	Yaping	Novel STAT3 inhibitors targeting the STAT3 dimerization	16
14	Hugdahl	Emilia	Prognostic impact and concordance of TERT promoter mutation and protein expression in primary and matched secondary cutaneous melanomas	17
15	Jacob	Havjin	MicroRNA signature as a potential biomarker for predicting recurrence in colon cancer	18
16	Jebsen	Nina Louise	Trimodal pilot study of neo-adjuvant regional hyperthermia, chemotherapy and radiotherapy in locally advanced, high-grade soft tissue sarcoma	19
17	Kang	Jing	The Role of Axl Signaling in Tumor Metastasis and Anti-tumor Immune Evasion	20
18	Karlsen	Ida	Exploration of Novel Therapeutic Options in Mantle Cell Lymphoma	21
19	Kjølle	Silje	Angiogenic proteins are overrepresented in the hypoxic secretome from basal-like breast cancer cell lines	22

20	Kleinmanns	Katrin	Optimization of human tumor dissociation to enhance engraftment rates in ovarian cancer patient derived xenograft models	23
21	Lapin	Morten	Single–Cell mRNA profiling reveals Transcriptional Heterogeneity among Pancreatic Circulating Tumour Cells	24
22	Lie	Maria	Inhibition of Axl abrogates the autophagic flux in NSCLC cells with acquired erlotinib resistance and induces immunogenic cell death	25
23	Mohamed	Nuha	Optimization of bacterial DNA extraction protocol from saliva of Sudanese oral squamous cell carcinoma patients	26
24	Mohamed	Nazar Gafar	Head and neck cancer detection using an electronic nose device: results from a pilot study on Sudanese patients	27
25	Morera Mojonero	Erika	Comparison of two isogenic non-tumorigenic and tumorigenic breast epithelial-derived cell lines with mesenchymal phenotype	28
26	Myklebost	Ola	The NOSARC project, a national, prospective and population- based study of mutations and mechanisms in sarcomas, and preclinical validation of novel targeted therapies, with the Intention to lead clinical trials	29
27	Nymark Aasen	Synnøve	The good drug, the bad barrier and the handy peptide: K16ApoE improves the treatment of experimental brain metastases	30
28	Pilskog	Martin	Predictive biomarkers in sunitinib treatment of metastatic renal cell carcinoma	31
29	Ramnefjell	Maria	Microvascular proliferation is associated with aggressive tumor features and reduced survival in lung adenocarcinomas	32
30	Rolland Jacobsen	Martha	Development of a molecular diagnostic tool for more precise diagnosis of oral squamous cell carcinoma	33
31	Sembajwe	Lawrence Fred	Indirect role of fibroblast Ext1 in regulating P311 expression in A549 carcinoma cells	34
32	Straume	Oddbjørn	A Phase Ib/II randomised open label study of BGB324 in combination with pembrolizumab or dabrafenib/trametinib compared to pembrolizumab or dabrafenib/trametinib alone, in patients with advanced non-resectable (Stage IIIc) or metastatic (Stage IV) melanoma	35
33	Steinhäuser	Sophie	Heterotypic interactions between endothelial cells and normal- and cancerous breast epithelial cells	36
34	Sørlie	Therese	Molecular characterization of MPA/DMBA induced mouse mammary tumors	37
35	Tangen	Ingvild Løberg	Expression of L1CAM in curettage and high L1CAM level in pre- operative blood samples predicts lymph node metastases and poor outcome in endometrial cancer patients	38
36	Tveitarås	Maria	Oxygen-dependent regulation of tumour growth and metastasis in human breast cancer xenografts	39
37	Wik	Elisabeth	Axonogenesis and vascular proliferation are associated gene expression programs in hormone receptor negative breast cancer	40
38	Wnuk-Lipinska	Katarzyna	BGB324, a selective small molecule inhibitor of receptor tyrosine kinase AXL, abrogates tumor intrinsic and microenvironmental immune suppression and enhances immune checkpoint inhibitor efficacy in lung and mammary adenocarcinoma models	41

Investigation of aSMA expression in p16 and p53 positive Oral Cancer

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BACKGROUND:

Head and neck cancer (HNC) is the 10th most common malignancy worldwide. The incidence rate is especially increasing in low income countries. With standard therapy 50% of patients survive up to 5-years due to late diagnosis and aggressive treatment. The expression of cancer stroma-associated biomarkers could be used to identify aggressive tumors at an early stage.

AIM:

To investigate the correlation between activated stroma (α SMA positive) and p16/ p53 expression.

METHODS:

A cohort of 120 patients diagnosed with Oral Squamous Cell Carcinoma (OSCC) at Khartoum Dental Teaching Hospital during 2012-2015 were included in the study. Immunohistochemistry was performed on paraffin embedded, formalin fixed tissue samples to assess the expression of p16, p53 and stromal expression of α SMA at the tumor front. The staining was evaluated semi-quantitatively manually and the data was analyzed using SPSS program.

RESULTS:

Out of 120 cases, only 3 showed an intense positive staining for p16, which was used as a surrogate marker for HPV positivity, therefore, the data were analyzed further only on p16 negative samples. A strong correlation between p53 expression and a strong α SMA expression at the tumor front was found (p=0.006). Strong expression of α SMA at the tumor invasion front was also significantly associated to tumor occurrence (p=0.004).

CONCLUSIONS:

The results of this study suggest that minor cases of OSCC in the Sudanese population seem to be correlated with HPV infection, and that the activation of fibroblasts at the tumor front is correlated with more aggressive tumors in the Sudanese OSCC patient, as it has been previously indicated by studies on cohorts on Caucasian patients. A novel finding is the correlation between alterations in p53 in the tumor cells and activation of the tumor stroma.

Generation and Characterization of integrin ITGA11-Cre mouse strain

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Abstract

Fibroblasts are increasingly being seen as an interesting target cell population in tissue and tumor fibrosis. To assess the importance of fibroblasts in experimental fibrosis models mouse strains claimed to express Cre recombinase in a fibroblastspecific pattern have been used. Careful analyses have however revealed that Cre expression is not limited to fibroblasts in the strains used so far.

Integrin $\alpha 11\beta 1$ is a collagen-binding integrin, which is receiving increased attention in the context of wound healing and fibrosis. Using a $\phi C31$ -integrase-based approach 3 kb of the regulatory promoter elements of the human ITGA11 gene was used to generate a novel transgenic mouse strain with Cre recombinase expression restricted to fibroblasts.

Characterization of β-galactosidase staining of embryonic tissues obtained from one transgenic ITGA11-Cre mouse line crossed with Rosa 26R reporter mice revealed

 α 11-driven Cre expression and activity in fibroblastic cells in a variety of mesenchymal tissues in a pattern highly reminiscent of endogenous α 11 protein expression. No expression was noted in non-fibroblastic cells.

We predict that the ITGA11-Cre transgenic mice described in this report will be a highly useful reagent for the ablation of genes whose transcripts are restricted to fibroblasts in the developing mouse and further studies are needed to determine if the strain will also be useful for conditional deletion of fibroblast genes in tissue and tumor fibrosis.

Preclinical Imaging in High Grade Serous Ovarian Cancer Cell Line Based Mouse Models: A Comparative Study

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Of all the gynecological malignancies, epithelial ovarian cancer (EOC) is the 6th most common malignant neoplasm and the leading cause of death. High-grade serous ovarian cancer (HGSOC) is the most common (>70%) and most lethal among the multiple histological subtypes of EOC. Diagnosis of the disease in an advanced state is one of the major drawbacks, which reduces its overall survival rate to <45%. Debulking surgery remains to be the cornerstone in terms of treatment, followed by adjuvant chemotherapy with platinum-taxane regimens. However, HGSOC is a complex disease which has ways to circumvent chemosensitivity, leading to recurrences of incurable chemoresistant forms in >70% of patients with advanced stage disease.

Although the degree of cytoreduction matters the current imaging techniques (CT, PET-CT and MRI) can only be used preoperatively to identify the anatomical position of the tumour. In contrast, fluorescence imaging is an optical technique with superior resolution and high sensitivity that allows surgical inspection by enabling localization of lesions that are difficult or impossible to detect visually.

By combining optical imaging and surgical technique we can develop a procedure for optimization of debulking surgery. Developing a biologically relevant ovarian cancer model holding the aspects of both a humanized and orthotopic ovarian patient derived xenograft mice could be highly useful in examining this combination technique. As the first step, in this study we aim to identify the most promising strain for better engraftment and growth of HGSOC.

The established mouse model was from a well characterized HGSOC cell line named OV90. NSG and NSGS mice were injected intraperitoneally and orthotopically with OV90 cells. The level of tumour engraftment and growth were measured using both fluorescence and bioluminescence imaging. We used fluorophore (Dye Alexa 680) conjugated CD24 antibody for detecting the primary tumour, as CD24 was found to be the most promising molecular target for HGSOC cell lines. We observed comparable results from both fluorescence and bioluminescence imaging showing equal sensitivity and specificity in tumour detection. From the results, we observed that the NSG mice showed better tumour engraftment and growth rate when compared with the NSGS mice.

Proteomic profiling of breast cancer patient samples, cell lines and conditioned media

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Omics profiling of breast cancer patient samples has almost entirely been focusing on mRNA and DNA. With the advent of new proteomic technologies, deep profiling of FFPE patient material is possible. We analyzed 42 basal-like and luminal A and B FFPE (formalin fixed paraffin embedded) patient samples. We also analyzed cell lysate and conditioned media from 12 breast cancer (6 luminal and 6 basal-like) cell lines. These proteomic profiles identified functional differences between the subgroups both in the patient samples and cell lines. By integrating data from cell lines and the patient material we derived a 14 protein signature that predicts basal-like subgroup affiliation. Our work shows the potential in retrospective proteomic analysis of archive breast cancer tissue and may in the future be used in subgroup specific treatment of breast cancer.

The impact of tumor interstitial fluid pressure on nanoparticle accumulation by cAMP receptors modulation

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Nanomedicine has emerged as a promising drug formulation in cancer chemotherapy, due to their ability to protect normal tissues by preferential accumulation in tumors, and the opportunity to attach targeting moieties to the nanoparticle surface. The tumor endothelium is an important barrier to pass for successful nanoparticle drug delivery. To deliver drugs to the cancer cells, the nanoparticles must pass the endothelium against a pressure gradient.

We investigated whether nanoparticle accumulation could be modulated by the two cAMP receptors PKA and Epac1. PKA is known to lower the interstitial fluid pressure (PIF) by fibroblast relaxation, while Epac1 can tighten the endothelial barrier by narrowing the gap between the endothelial cells.

Here, we present data on PIF in syngeneic breast tumors implanted in mice with functional or deleted Epac1 (Epac1KO), and the role of stimulation of cAMP signaling on the PIF profile. Further, we looked at how nanoparticles distribute in tumors and other tissues in the mouse models and how a transient increase in cAMP affects tissue distribution of nanoparticles.

This study gains new insight into the barrier for nano sized drug carrier penetration into the tumor interstitium and its regulation by cAMP.

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The effects of metformin treatment in colorectal cancer cells of opposing metabolic phenotype

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Background

Metformin is widely used to treat type 2 diabetes (T2DM), and studies have shown that it could have an effect in both cancer prevention and treatment (1-6). However, conflicting results have recently shown no improvement in survival or cancer risk upon metformin treatment (7), which is further supported in the many inconclusive clinical trials using metformin as a drug in cancer treatment. The conflicting results may be a result of dosage, target effects of metformin and how to best select cancer patients who will respond to this treatment. Since metformin is administred orally it has been suggested that the highest achievable dosage is found in the gastrointestinal tract (GI) (8) and that only tumors residing in GI could be responsive to metformin treatment due to dosage. In addition, it has been suggested that only tumors with an OXPHOS (mitochondrial dependent) metabolic profile are sensitive to the effects of metformin (9). To address some of these issues, we used a colorectal cancer cell model system with cells of opposing metabolic phenotype (glycolytic vs. OXPHOS) to assess the effects of physiological relevant dosages of metformin.

Method

The colorectal cancer cell line SW948 and SW1116 were used to model a "glycolytic" (less dependent on their mitochondria for ATP production) versus an "OXPHOS" dependent tumor, respectively. Proliferation, protein and gene expression of key players of metabolism (i.e. GLUT1) were assessed after metformin treatment in medium of high glucose (25mM) and low glucose (5mM).

Results

SW1116 and SW948 show reduced proliferation upon metformin treatement in high and low glucose medium. SW1116 shows a more marked reduction in proliferation in response to metformin treatement, than the glycolytic SW948 cell line. Protein expression of the glucose receptor GLUT1 was increased after low glucose treatment in SW948, whereas metformin had little additional effect. In SW1116, GLUT1 expression did not change significantly under low glucose conditions, but increased in expression under treatment of metformin. Gene expression analysis of regulators involved in metabolism and metformin import showed that metformin treatment in high glucose gave an increase in *GLUT1* in SW1116 whereas in SW948 there was only an increase in *GLUT1* expression under low glucose conditions. Both cell lines responded by decreased expression of the organic cation transporter 1 (*OCT1*) involved in import of metformin after prolonged drug treatment (48h). The mitochondrial uncoupling protein 2 (*UCP2*) was downregulated upon low glucose and metformin treatment in both cell lines, but did not change under high glucose conditions in SW948.

Conclusion

Preliminary results show that in addition to the glucose levels in the medium, the metabolic phenotype of cells plays a role when testing for the effects of metformin treatment. GLUT1 expression levels demonstrate that the "glycolytic" SW948 is not as responsive to metformin treatment as the "OXPHOS" SW1116. *OCT1* is involved in import of metformin, and here showed a marked decrease in expression upon prolonged drug treatment, which could indicate that it would be a valuable biomarker for evaluating the effects of metformin in cancer.

References

- 1. Bayraktar S, Hernadez-Aya LF, Lei X, Meric-Bernstam F, Litton JK, Hsu L, et al. Effect of metformin on survival outcomes in diabetic patients with triple receptor-negative breast cancer. Cancer. 2011;118:1202-11.
- 2. Bodmer M, Becker C, Meier C, Jick SS, Meier CR. Use of metformin and the risk of ovarian cancer: a case-control analysis. Gynecol Oncol. 2011:123:200-4
- 3. Cardel M, Jensen SM, Pottegård A, Jørgensen TL, Hallas J. Long-term use of metformin and colorectal cancer risk in type II diabetics: a population-based case-control study. Cancer Med. 2014;3(5):1458-66.
- 4. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila). 2010;3:1451-61.
- 5. Garrett CR, Hassabo HM, Bhadkamkar NA, Wen S, Baladandayuthapani V, Kee BK, et al. Survival advantage observed with the use of metformin in patients with type II diabetes and colorectal cancer. Br J Cancer. 2012;106(8):1374-8.
- 6. Sehdev A, Shih Y-CT, Vekhter B, Bissonnette MB, Olopade OI, Polite BN. Metformin for primary colorectal cancer prevention in patients with diabetes: A case-control study in a US population. Cancer. 2015;121(7):1071-8.
- 7. Tsilidis KK, Capothanassi Ď, Allen NE, Rizos EC, Lopez DS, van Veldhoven K, et al. Metformin Does Not Affect Cancer Risk: A Cohort Study in the U.K. Clinical Practice Research Datalink Analyzed Like an Intention-to-Treat Trial. Diabetes care. 2014;37(9):2522-32.
- 8. Buse JB, DeFronzo RA, Rosenstock J, Kim T, Burns C, Skare S, et al. The Primary Glucose-Lowering Effect of Metformin Resides in the Gut, Not the Circulation: Results From Short-term Pharmacokinetic and 12-Week Dose-Ranging Studies. Diabetes care. 2016;39(2):198-205.
- 9. Sadeghi N, Abbruzzese JL, Yeung SJ, Hassan M, Li D. Effect of metformin on survival of diabetic patients with pancreatic adenocarcinoma. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2011;29.

Establishment of humanised immune system mice for evaluation of immunotherapies in patient derived xenograft tumour models

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Background

Patient derived xenografts (PDX) in mice with a reconstituted human immune system may give greater insight into the clinical potential of novel immunotherapies and combination therapies. Subsequently, one must identify the ideal host that will, on the one hand, permit engraftment of patient-derived tumour samples and, on the other hand, have a functional human immune system. Here, we describe the initial development of humanised immune system PDX (iPDX) model for evaluation of immunotherapies to acute myeloid leukaemia.

Methods

To define the ideal murine host and pre-conditioning that permits engraftment of human immune system we injected six NSG (NOD-scid IL2Rgnull) and six NSGS (NOD-scid IL2Rgnull Tg(CMV-IL3,GM-CSF,SCF)) mice intravenously with umbilical cord blood CD34+ hematopoietic stem cells (1x105/mouse). To enhance HSC engraftment by increasing homing of injected cells to stem cell niches in the bone marrow, three mice of each strain were pre-conditioned with the myeloablative agent busulfan. Mice were bled every four weeks and analyzed by flow cytometry to monitor the development of human CD45+ cells.

Results

We found that busulfan drastically enhanced CD34+ hematopoietic stem cell engraftment development of human CD45+ cells, particularly in NSG mice. At 4 and 8 weeks after CD34+ hematopoietic stem cell injection the NSGS strain had considerably more peripheral human CD45+ cells compared to the NSG strain. However, at week 12 the busulfan treated NSG mice had the highest percentages of human CD45+ cells. At 12 weeks, NSGS mice and busulfan treated NSG mice had high proportions of hCD45+CD19+ B cells, but barely detectable hCD45+CD3+ T cells.

Conclusion

At 12 weeks, the NSG strain seems to be the most promising strain for reconstitution of a human immune system. However, reconstitution success will also depend on subpopulations of the human CD45+ cells such as human T cells, B cells, NK cells, dendritic cells and myeloid cells. We will further dissect immune cell development at later time points by flow cytometry and mass cytometry in peripheral blood, spleen and bone marrow.

The mitochondrial succinate dehydrogenase complex is an important regulator of epithelial to mesenchymal transition.

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Epithelial to mesenchymal transition (EMT) represents a well-characterized change in cell phenotype that has important physiological implications. EMT is a major area in research fields of stem cell and cancer biology, as it illuminates important aspects of phenotypic plasticity that occur in cells as a consequence of contextual influences. The dramatic changes in cellular features and functions involved in EMT are driven by multiple regulatory cues that coordinate the transition into the new phenotype. However, relatively little is known about how metabolism is remodeled to accommodate the new cellular requirements brought by the shift in cell phenotype.

Based on previous findings, we hypothesized that the functional suppression of succinate dehydrogenase (SDH) is important to fulfill the phenotypic plasticity involved in EMT. To address this we used the SDH enzyme inhibitor malonate and the CRISPR/Cas9 system to knock down SDH subunits using MCF10A and MCF7 cells, respectively. In addition, we investigated the mRNA expression of SDH subunits in a patient cohort of endometrial cancer and breast cancer.

In several cell models of EMT, we have previously shown that repression of mitochondrial respiration was consistently associated with the mesenchymal phenotype. Here, we show that the regulation of the SDH complex may be crucial in respect to this. Inhibition of the SDH enzyme activity with malonate reduced the mitochondrial respiration in MCF10A cells and upregulated markers of EMT. Furthermore, CRISPR/Cas9 mediated knock down of the SDHB subunit caused similar effects in MCF7 cells. Upon analyzing patient cohorts, we found SDHC expression to correlate with EMT signature expression in breast tumors, and there was a similar trend for SDHB. In endometrial tumors, expression of four SDH subunits (SDHA-D) was found to be associated with induction of EMT.

In summary, these data demonstrate that the SDH complex, also referred to as mitochondrial respiratory complex II, may be an important regulator of EMT and cell plasticity. As well as reviling the importance of altered metabolic signature during EMT as a cellular phenomenon, our findings may open new avenues in the search for novel therapeutic targets in cancer.

Functional MRI predicts aggressive disease in endometrial cancer

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Purpose

To explore the value of preoperative quantitative dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and diffusion weighted imaging (DWI) for the prediction of histological subtype and aggressive disease in a large patient series with endometrial cancer.

Materials and Methods

From June 2009 to November 2013, preoperative DCE-MRI and DWI (1.5 T) was performed in 183 consented endometrial carcinoma patients under institutional review board-approved protocols. This allowed calculation of physiological parameters *in vivo* reflecting tumor microvasculature (e.g. blood flow, transfer rates between the extravascular extracellular space (EES) and blood plasma) and tumor microstructure (e.g. apparent diffusion coefficient (ADC) value). The Mann-Whitney U test was used to explore the relation between the calculated imaging parameters including tumor volume (based on MRI) and histological subtype/grade. The Mantel-cox test and the Cox proportional hazards models were used to assess any association between imaging parameters and patient outcome.

Results

Low tumor blood flow (Fb) and low rate constant for contrast agent intravasation (kep) were associated with high-risk histological subtype ($P \le 0.04$ for both) and tended to be associated with poor prognosis ($P \le 0.09$). Low tumor ADC value and large tumor volume were also unfavorable prognostic factors (P = 0.05 and P < 0.001, respectively).

Conclusion

DCE-MRI and DWI may represent valuable supplements to preoperative conventional MRI by providing novel imaging biomarkers enabling improved risk stratification in endometrial cancer patients.

Validation of Asparaginase like protein 1 (ASRGL1) as Prognostic Biomarker in Endometrial Carcinoma

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Abstract

Endometrial carcinoma (EC) is the most common pelvic gynecological malignancy in western countries, and novel biomarkers that identify high risk patients are needed. Loss of the enzyme Asparaginase-like protein 1 (ASRGL1) was recently reported to be an independent biomarker for disease specific survival in endometrioid endometrial carcinoma patients, and our aim was to validate this in a large, prospectively collected patient cohort. Curettage specimens from patients diagnosed with endometrial carcinoma were collected, and formalin fixed and paraffin embedded tissue was used to generate tissue microarrays. Automated immunhistochemical staining with the anti-ASRGL1 antibody AMAb90907 was performed on curettage samples from 1159 patients, and a total staining index (0-9) was calculated for each patient based on staining intensity and tumor staining areal. ASRGL1 loss was defined as staining index 0-1, corresponding to the lower quartile of cases. In our cohort loss of ASRGL1 was found to be significantly associated with established clinicopathological features of aggressive disease in EC patients, such as high age, high FIGO stage, non-endometrioid histology, and poor tumor differentiation (P < 0.001 in all). In addition ASRGL1 loss predicted poor survival, even within the endometrioid subgroup which is otherwise believed to have a more favorable prognosis. Interestingly, we also found loss of ASRGL1 in curettage samples to be significantly associated with lymph node metastasis, potentially adding important information whether or not to perform lymphadenectomy in patients with intermediate or low risk disease.

FOXA1 predicts good outcome in HER2+ endometrial cancer patients by inhibiting EGFR/HER2 signaling

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ABSTRACT

HER2 is overexpressed in a significant number of endometrial cancer (EC) tumors. Yet, the prognostic and therapeutic implications of HER2 expression have not been clearly elucidated. Here we identify that FOXA1 expression stratifies tumors with HER2-high expression into two subgroups, significantly and independently associated to prognosis. Our study suggests that HER2-high tumors with low levels of FOXA1 possess innate resistance against HER therapies. The comparison of genes differentially expressed between FOXA1-high *vs.* FOXA1-low within the HER2-high subgroup of EC patients revealed an enrichment of a gene signature associated with response to the anti-EGFR drug Gefitinib. In EC cells, FOXA1 binds towards a significant number of these genes associated with response, and the increased expression of FOXA1 attenuates expression of these genes by recruiting polycomb-associated proteins to FOXA1 binding sites. Moreover, the ectopic expression of FOXA1 results in a full response to anti-EGFR/HER2 therapies. These results suggest that, in HER2-high tumors, FOXA1 represses the expression of genes key for EGFR-HER2 signaling and might explain why women with high FOXA1 and HER2 overexpressing EC show improved outcome. Moreover, these results might explain why women with HER2-high EC in general respond poorly to current HER2-directed therapies

AKT3 subcellular localization and signaling modulates tumorigenicity

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Abstract: will be added.

Novel STAT3 inhibitors targeting the STAT3 dimerization

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Background Prostate cancer recurs and leads to incurable castration recurrent prostate cancer (CRPC) after the failure of androgen deprivation therapies. It is reported that some prostate cancer cell populations harbor cancer stem cells (CSCs), which can both mediate and propagate CRPC. STAT3 pathway is of interest in CSCs and we have found in our experimental model of stepwise prostate tumorogenesis that an autocrine II6-STAT3 loop charactarizes the tumor initiating cells in animal experiments. In a drug discovery and development program we have screened nearly 600 natural compounds for STAT3 inhibiting activity. Two small molecules named compound 323-1 and 323-2 were identified as STAT3 pathway inhibitors in a series of experiments.

Material and methods: The antitumor activity of compounds 323-1 and 323-2 was determined by clonogenic assays in different prostate tumor cells. Effects of compound 323-1 and 323-2 on STAT3 transcriptional activity were determined by the luciferase assay. Western blotting was used to examine phosphorylation of STAT3 (Y705) and STAT1 (Y701) proteins. Drug affinity responsive target stability (DARTS) assays were performed to examine whether compound 323-1 and 323-2 could bind to the STAT3 protein. DNA binding ELISA kit was used to verify if 323-1 and 323-2 block STAT3 DNA-binding activity. Co-immunoprecipitations were utilized to test whether 323-1 and 323-2 disrupt STAT3 dimerization.

Results Clonogenicity of four prostate cancer cell lines was reduced in a dose dependent manner after treatment with compound 323-1 and 323-2 for two weeks. The two compounds inhibited STAT3 transcriptional activity and prohibited the expression of phoshorylation of STAT3 (Y705) over phoshorylation of STAT1 (Y701). 323-1 and 323-2 were identified as direct STAT3 inhibitors by prohibiting STAT3 dimerization, without altering STAT3 DNA-binding activity.

Conclusions Evidence demonstrated that compound 323-1 and 323-2 may have potential capacity of modulation of the IL6/STAT3 pathways, thereby indicating a promising candidate in prostate cancer treatment.

Prognostic impact and concordance of TERT promoter mutation and protein expression in primary and matched secondary cutaneous melanomas

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Background: Although *TERT* promoter mutations are frequent in melanoma, reports on their prognostic impact are contradictive. Here, we analyzed for the first time the concordance and prognostic impact of *TERT* mutation and TERT protein expression in primary and matched secondary cutaneous melanomas.

Methods: In a series of 255 primary nodular melanomas and 78 matched loco-regional metastases, *TERT* promoter mutation status was assessed by Sanger sequencing, and TERT protein expression was examined by immunohistochemistry.

Results: *TERT* promoter mutations were found in 68 % of primary melanomas and 64 % of the metastatic lesions. There was sufficient tissue from both the primary and metastatic tumor in 58 cases. The *TERT* promoter mutation status was discordant between primary and metastatic tumors in 24 % of the cases. *TERT* mutated cases tended to be thicker, have a higher mitotic count and higher patient age than *TERT* wildtype cases, but there was no significant association with reduced survival. TERT protein expression did not correlate with *TERT* promoter mutation status, but showed a similar intertumoral discordancy and was associated with reduced survival.

Conclusion: *TERT* promoter mutations was highly frequent in both primary melanoma and metastases, and the mutation status changed during tumor progression. We found no prognostic impact of *TERT* mutations. TERT protein expression was associated with reduced survival, but was not related to *TERT* mutation status.

MicroRNA signature as a potential biomarker for predicting recurrence in colon cancer

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Background

Colon cancer is one of the most common cancers with increasing incidence and high mortality worldwide. Estimation of prognosis and choice of treatment are largely based on the TNM tumor stage at presentation. Thus, finding novel biomarkers for predicting survival and recurrence is highly desirable. Lately, several studies have been looking at microRNAs (miRNAs) in several cancers, including colon cancer. MicroRNAs are conserved, non-coding RNA molecules that play an important role in the regulation of post-transcriptional gene expression. Our aim was to identify miRNAs that predict recurrence in colon cancer.

Material and Methods

We profiled microRNA in 172 TNM stage I-IV colon cancer patients. Total RNA was extracted from fresh frozen tissue samples using the Qiagen miRNeasy-kit. The miRNA expression was profiled by RT-qPCR in a custom Pick & Mix Panel from Exiqon including 84 different cancer related miRNAs. Binary logistic regression and Lasso-analysis were used to identify miRNA signatures associated with recurrence in 111 TNM-stage II and III patients. Diseases-free survival (DFS) was calculated as time from operation to first recurrence of primary colon cancer. We calculated a risk score for each patient to classify patients into "low" and "high" risk of recurrence.

Results

Lasso-analysis in Cox-mode identified a signature of 16 miRNAs that classified patients into "low" or "high" risk of recurrence. The miRNAs in the signature were miR-143-5p, miR-27a-3p, miR-31-5p, miR-181a-5p, miR-30b-5p, miR-30d-5p, miR-146a-5p, miR-23a-3p, miR-150-5p, miR-210-3p, miR-25-3p, miR-196a-5p, miR-148a-3p, miR-222-3p, miR-30c-5p and miR-223-3p. Sensitivity and specificity was both 93 %. Patients with low and high 16-miRNA signature had 3-year DFS of 98 % and 27%, respectively. The signature was further reduced to a 4-miRNA signature (miR-23a-3p, miR-25-3p, miR-30d-5p, and miR-31-5p) with sensitivity 65 % and specificity 74 %. The 3 -year DFS for patients with low versus high 4-miRNA signature was 88 % versus 60%, respectively.

Conclusion

The present study has identified a 16-miRNA signature and a 4-miRNA signature predicting recurrence in colon cancer stage II and III patients.

Trimodal pilot study of neo-adjuvant regional hyperthermia, chemotherapy and radiotherapy in locally advanced, high-grade soft tissue sarcoma

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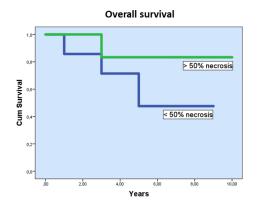
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Introduction: Long term results presented from the EORTC/ESHO 62961 study randomizing between neo-adjuvant and adjuvant chemotherapy with etoposide, Ifosfamide and Adriamycin (EIA) +/- regional hyperthermia (RHT), demonstrated 11% improved 9-year overall survival in the hyperthermia-arm compared with EIA alone. The Bergen hyperthermia oncology group contributed with 20 out of 341 patients. Stimulating a systemic immune response by induction of heat shock proteins has been postulated as an explanatory mechanism for the improved overall survival in the hyperthermia arm. After conclusion of the EORTC/ESHO 62961, we initiated a trimodal pilot study with RHT as a method to enhance the effects of both radiotherapy and chemotherapy in patients with locally advanced, high-risk sarcoma.

Material and methods: RHT was initially administered concomitantly with chemotherapy (Ifosfamide and Adriamycin) twice weekly every 3 weeks, followed by the trimodal phase with radiotherapy concomitant with weekly thermochemotherapy (Ifosfamide) over 5 weeks. In total 15 patients diagnosed 2006 - 2014 with high-risk, deep extra-compartmental, poorly resectable sarcoma of the extremities, pelvis, retroperitoneum and trunk wall were included (60% non-extremity site). RHT was applied with a BSD Sigma Eye/60 applicator aiming at a tumour temperature of > 42 °C for 60 minutes. The patients were younger (mean age 48 y) and presented with larger tumours (mean size 11 cm) than in population-based data from Scandinavian sarcoma cohorts.

Results: The majority completed planned chemotherapy (14/15) and RHT (12/15). Radiation dose ranged from 45 to 50.4 Gy in 1.8-2 Gy fractions. All but two of the patients underwent surgery after thermochemoradiotherapy. The two non-resected patients either experienced disease progression before surgery, or suffered fatal hemofagocytic lymphohistiocytosis. Microscopic negative surgical margins were obtained in 10 of 13 specimens. Grade 3-4 neutropenia was reported in 87% of the patients and 67% were hospitalised due to infections. Six patients (46 %) experienced postoperative wound complications. Severe late morbidity was recorded as follows: grade 3-4 fibrosis in 2 cases; pathological fracture in 2, erectile dysfunction in 3 (pelvic or prostate tumours) and gut fistulation in 1. The 5-year metastasis-free and overall survival was 66% and 56%, respectively. Survival was markedly better among the 6/13 patients reported to have > 50% necrosis in the specimen compared with < 50% necrosis (Fig. 1).

Conclusions: In locally advanced soft tissue sarcoma, neo-adjuvant regional hyperthermia, chemotherapy and radiotherapy is feasible, although acute toxicity of chemotherapy and preoperative radiotherapy is noticeable. Trimodal pre-treatment including RHT is associated with a high frequency of complete/R0 surgical resection. Extent of necrosis in the surgical specimen seems to correlate with survival outcome.



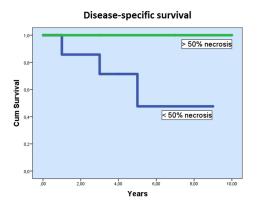


Figure 1.
Overall and
diseasespecific survival in relation to extent
of tumour necrosis in the
surgical specimen.

The Role of Axl Signaling in Tumor Metastasis and Anti-tumor Immune Evasion

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Abstract

The Axl receptor tyrosine kinase is a key regulator of phenotypic plasticity, metastasis and drug resistance, which is also required by carcinoma cells to escape the anti-tumor immune response. Thus understanding the role of Axl signaling in tumor metastasis and anti-tumor immune evasion is vital. In our study, we elucidated how AxI signaling contributed to metastasis via Akt3 using CRISPR to knock out AxI or Akt3 in melanoma cell lines; we also developed a CRISPR-based complementation approach for functional in vivo mapping of AxI signal transduction derived from combined retroviral expression system. We demonstrated that knocking out Axl increased Akt3 expression but reduced the abilities of migration and invasion; we established 11 Axlcomplemented cell lines to evaluate for anti-tumor immune evasion. Axl receptor mutations blocking anti-tumor immune evasion will be assessed by Western blot analysis of cancer cell extracts using standard phospho-epitope antibodies probed against specific tyrosine residues. We are also investigating the interaction between Axl and its ligand Gas 6, and particularly how Vitamin K influences this interaction. We hypothesize that Axl signaling is dependent on the manner in which Gas6 is presented to the receptor. Vitamin K dependent carboxylation of glutamic acid residues of the Gla domain facilitates Gas 6 attachment to extracellular apoptotic vesicles creating a cluttering effect that encourages Axl receptor dimerization. We are currently investigating this mechanism along with determining the expression and functional consequences of genes such as GGCX, VKORC1, VKORCL1and EPHX1 encoding various components of the Vitamin K pathway. Collectively, our results support a therapeutic rationale for clinical Axl pathway inhibitors in tumor metastasis and immunotherapy resistance treatment.

Exploration of Novel Therapeutic Options in Mantle Cell Lymphoma

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Mantle cell lymphoma (MCL) is an aggressive non-Hodgkin lymphoma that originates from mature B-cells. The disease is characterised by t(11;14)(q13;q32), a translocation that is virtually ubiquitous in MCL. MCL is a heterogeneous disease, exhibiting various phenotypic forms. Furthermore, the disease has demonstrated a high level of tumor clonality. Although mutations in p53 is a poor prognosis factor, it is rarely observed in MCL. Patients are typically given various combinations of cytostatics, often in combination with rituximab. While treatment results in initial responses, ultimately all patients relapse and succumb to their disease.

Small molecule inhibitors targeting tyrosine kinases have recently emerged as a promising approach to treat MCL. Ibrutinib is a Bruton's tyrosine kinase (BTK) inhibitor. BTK is an important signal transductor for the B-cell antigen receptor-signalling pathway, which is important for survival and proliferation of B-cells. Inhibition of this pathway through ibrutinib will cause growth inhibition and apoptosis of MCL cells. BTK is also an indirect downregulator of p53, through activation of the PI3K-Atk-MDM2 pathway. It is hypothesised that ibrutinib inhibition of BTK will upregulate p53 in MCL cells. Ibrutinib is currently FDA approved for use in patients with recurrent or refractory MCL. Axl is a tyrosine kinase that is upregulated in some cases of NHL according to the human protein atlas. Axl is a stimulant of cell growth and survival, and is – similarly to BTK – an indirect downregulator of p53 through activation of the PI3K-Akt-MDM2 pathway. BGB324 is a selective inhibitor of Axl. It is hypothesised that treating MCL with BGB324 may have an anticancer effect.

Dehydroorotate dehydrogenase (DHODH) is an enzyme that catalyses the fourth step of the *de novo* biosynthesis of prytimidines, an essential pathway. Published studies have shown that inhibition of DHODH can cause tumor growth inhibition, apoptosis and S-phase arrest. (*R*)-HZ05 is a highly potent DHODH inhibitor (DHODHi) and was newly discovered by performing a screening for molecules that upregulate p53. We hypothesised that the anti-cancer effects of DHODH inhibition is caused by upregulation of p53.

Here we present that very low doses of (R)-HZ05 cause cell growth inhibition, apoptosis and S-phase arrest, in addition to p53 upregulation in MCL cell lines, irrespectively of p53 status. (R)-HZ05 was tested in combination with ibrutinib and BGB324, and especially the second combination shows a powerful synergistic effect. This preliminary data is very promising. We have developed an animal model of MCL by transducing a MCL cell line with a luciferase-containing vector. In the near future, we intend to study the preclinical efficacy of (R)-HZ05 as a single treatment and in combination with BGB324. We also wish to further study the (R)-HZ05 mechanism of action further, as it seems like the mechanism is indeed not through p53 upregulation.

Angiogenic proteins are overrepresented in the hypoxic secretome from basal-like breast cancer cell lines

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Approximately 15 % of genes, codes for proteins that are secreted. We wanted to investigate the effect of hypoxia on secreted proteins from breast cancer cell lines, and the differences in secretion of angiogenesis-related proteins between the luminal and basal-like subtypes of breast cancer. To test this, we used conditioned medium from hypoxic and normoxic conditions from 2 luminal and 2 basal-like breast cancer cell lines. 1782 proteins were detected in a label-free mass spectrometry analysis, 64 and 66 proteins with significantly increased secretion in response to hypoxia for luminal and basal-like cell lines, respectively. A protein network of these proteins and gene ontology analysis revealed that angiogenic proteins were overrepresented in this network. Among these proteins were VEGF-A and ANGPTL4, and angiogenic proteins were seen especially in the hypoxic secretome from the basal-like cell lines.

Optimization of human tumor dissociation to enhance engraftment rates in ovarian cancer patient derived xenograft models

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In 70% of all cases, high grade serous ovarian cancer (HGSOC) is diagnosed at an advanced stage with spread into the peritoneal cavity. At present, successful debulking surgery followed by adjuvant chemotherapy, consisting of a combination of taxane and a platinum based drugs serves as the most efficient treatment options and enhances survival chances for patients. However, most of the patients relapse with 70% of them being chemoresistant. Despite development of surgical techniques and chemotherapeutic regimens, the overall survival is still below 45%. The tumors are heterogeneous and they can be subclassified both based on morphological features and molecular signatures.

The last years have used much efforts to establish relevant mouse models for ovarian cancer, and a bioluminescent orthotopic combined surgical/chemotherapeutic xenograft model in immunosuppressed mice has been developed. New knowledge and demands necessitate improvements.

Immune-competent patient-derived xenografts (iPDX) models are suggested to be the most suitable preclinical models. These models contain both a functional human immune system as well as an orthotopically implanted tumor from the same individual. As the iPDX models conserve genetic, phenotypic and functional characteristics of the primary tumor, they represent a precise and predictive preclinical model to improve image-guided surgery as well as they will serve as a tool for identification of new biomarkers and for examination of the effect of different immunotherapeutics.

There are still several problems hindering the use of PDX models, the insufficient engraftment rate being one. Therefore, we have developed an enzyme dissociation protocol for soft and solid ovarian cancer tissues. After debulking surgery, tissues from seven ovarian cancer patients were processed with 8 differential enzymatic cocktails. All enzymes were tested for optimal incubation time and the suitability of employing calcium chloride as activity stabilizer.

Our findings can be summarized in four major points: i) the gentle enzymatic method increases cell viability and cell fitness compared to the conventional mechanical dissociation; ii) the dissociation effectiveness strongly depends on the cytomorphological characteristics of the tumor; iii) calcium chloride and TrypLE enhances the dissociation efficacy of Collagenase II; and iv) 3D culture of the heterogeneous cell suspension shows that the tumor cell suspension forms 3D architecture over up to 20 days which may increase engraftment rates after orthotopic injection.

Single-Cell mRNA profiling reveals Transcriptional Heterogeneity among Pancreatic Circulating Tumour Cells

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Background: Single-cell mRNA profiling of circulating tumour cells (CTCs) may contribute to a better understanding of the biology of these cells and their role in the metastatic process. In addition, such analyses may reveal new knowledge about the mechanisms underlying chemotherapy resistance and tumour progression in patients with cancer.

Methods: Single CTCs were enriched from blood samples (n = 56) from patients with advanced pancreatic cancer using our previously established negative depletion strategy MINDEC, and detected by immuno–fluorescence microscopy. The single cells were isolated by micromanipulation, reverse transcribed, pre-amplified, and analysed using single cell multiplex mRNA profiling to reveal transcriptional heterogeneity.

Results: CTCs were detected in 33 of 56 (59%) examined blood samples. A total of 48 morphologically intact CTCs and 3 morphologically intact CTC clusters (containing ≤3 CTCs) were isolated, in which 18 cells had detectable mRNA levels and were identified as CTCs by expression of epithelial (KRT8, KRT19, EPCAM, E-Cadherin) or mesenchymal (Vimentin, N-Cadherin, ZEB1) markers. Hierarchical clustering and principal component analyses revealed both epithelial–like and mesenchymal–like CTC subpopulations, which were distinct from leucocytes. The profiled CTCs also expressed elevated levels of cancer stem cell (CD24, CD44, ALDH1A1) markers and the extracellular matrix marker SPARC. The expression of SPARC might correspond to an epithelial-mesenchymal transition in pancreatic circulating tumour cells.

Conclusion: The analysis of single pancreatic CTCs identified distinct subpopulations and revealed elevated expression of transcripts relevant to the dissemination of CTCs to distant sites.

Inhibition of AxI abrogates the autophagic flux in NSCLC cells with acquired erlotinib resistance and induces immunogenic cell death

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Lung cancer is responsible for most cancer-related deaths worldwide. Mutations activating the epidermal growth factor receptor (EGFR) are prevalent in non-small cell lung cancer (NSCLC), and EGFR inhibitors like erlotinib are standard-of-care for these patients. In spite of initial patient responses, acquired drug resistance invariably occurs, in part attributed to activation of the Axl receptor tyrosine kinase. BGB324 is a first-in-class selective small molecule Axl inhibitor currently in Phase I/II clinical trial in combination with erlotinib for advanced stage NSCLC patients (NCT02424617). Preliminary results from the Run-in Arm to establish the safety and tolerability of BGB324 showed that 2 out of 8 (25%) of the heavily pre-treated NSCLC patients experienced prolonged disease stabilization upon BGB324 mono treatment (Byers et al. ENA, 2016), suggesting a unique role for Axl signaling in NSCLC. The aim of the present study is to evaluate the relationship between Axl expression and autophagy in erlotinib-resistant NSCLC cells, and further explore the molecular basis for targeting Axl signaling in therapy resistant NSCLC. As a model of acquired drug resistance, we used the human NSCLC cell line HCC827 and erlotinib-resistant sub-clones derived from this cell line. HCC827 cells harbor an EGFR mutation (E746-A750 deletion) rendering EGFR constitutively active. The erlotinib sensitive HCC827 cells display an epithelial morphology, while the erlotinib-resistant sub-clones (HCC827 ER3 and ER10) display mesenchymal phenotypes and increased expression of Axl. Furthermore, an increase in the lipidated membrane-associated form of LC3 (LC3-II) in these cells indicates a higher autophagic flux in the erlotinib-resistant clones compared to the parental cell line. Upon Axl inhibition by the selective Axl kinase inhibitor BGB324 we observed major changes in cellular morphology and prominent cytoplasmic vacuolization. This effect was investigated by transmission electron microscopy (TEM), and in order to further examine the dynamic alterations in the autophagic degradation pathway upon Axl inhibition, we performed Western Blot and confocal imaging analysis of autophagy-related proteins. Importantly, we also observed release of damage associated molecular patterns (DAMPs), including increased extracellular calreticulin exposure, increased ATP release and increased secretion of High mobility group box 1 (HMGB1) upon AxI inhibition, which is consistent with an immunogenic cell death. In conclusion, our results highlight a unique Axl signaling-dependent autophagy-mediated drug

References:

Byers *et al.*, A phase I/II pharmacokinetic study of BGB324, a selective Axl inhibitor as monotherapy and in combination with erlotinib in patients with advanced stage Non-Small Cell Lung Cancer (NSCLC). Abstract presented at the 28th EORTC-NCI-AACR Molecular Targets And Cancer Therapeutics Symposium 2016 (Munich), 11/16.

BGB324 abrogates the autophagic flux in erlotinib-resistant NSCLC cells and induces an immunogenic form of cell death. These findings further support AxI as a therapeutic target in NSCLC.

resistance mechanism in NSCLC, and we demonstrate that inhibition of AxI signaling with

Optimization of bacterial DNA extraction protocol from saliva of Sudanese oral squamous cell carcinoma patients

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Background:

Oral squamous cell carcinoma (OSCC) is a high morbidity and mortality disease in Sudan. Several studies show that microbiome plays a role in carcinogenesis. More precise diagnostic tool using oral microbiome parameter is necessary.

Aim:

To optimize a protocol for efficient and robust bacterial DNA extraction from saliva of OSCC patients.

Methodology:

DNA extraction from different volumes (600 μl, 300 μl and 150 μl) of 12 pure saliva samples and 8 samples of 200 μl of pure saliva diluted in 1000 μl of sputasol was performed using the FastDNA® SPIN Purification kit (mpbio) per the manufacturer's recommendations. An enzyme-cocktail (consisting of lysozyme, mutanolysin and lysostaphin) was used for cells lysis. DNA quality and quantity were assessed (260/280 and 260/230 ratios, NanoDrop 1000 Spectrophotometer, and Qubit® 3.0 Fluorometer; Thermo Fisher Scientific). A modified protocol was used for 16S rRNA amplicon sequencing library preparation (Illumina protocol). The variable region V3–V4 of the 16S rRNA gene was PCR amplified (Klindworth et al. 2013). The amplicon size was approximately 460 bp. Different dilutions of purified DNA have been used to amplify V3-V4 region in 16s ribosomal gene using PCR analysis for 30 and 40 cycles.

Results:

All samples yielded DNA in various quantities and qualities, with the aliquots of 300 μ l of saliva diluted in 1000 μ l sputasol showing the highest DNA concentrations. The same samples showed the most robust and consistent DNA yield. Thirty cycles of PCR produced brighter bands with all dilutions when compared with the 40 cycles PCR.

Conclusion:

A robust protocol for extracting bacterial and genomic DNA from saliva of OSCC Sudanese patients has been optimized for further use for microbiome characterization.

Head and neck cancer detection using an electronic nose device: results from a pilot study on Sudanese patients

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Keywords: VOCs, electronic nose, oral cancer in Sudan

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Background: Many metabolic pathways such as glycolysis are altered in the case of cancer. Metabolic-related changes in the body of cancer patients may alter the production of volatile organic compounds (VOCs) in the body and can be reflected in the exhaled breathing air. The exhaled breath of breast, lung and head and neck cancer (HNC) patients has been shown to display a distinct VOCs profile. Utilization of an electronic nose for measuring VOC is promising as a rapid and inexpensive screening tool in the field of cancer, and several devices able to measure VOCs in the exhaled air of cancer patients have been developed by several companies.

This study aims to explore the validity of clinical application of an electronic nose device, as a rapid and cost-effective screening tool for head and neck cancer.

Methods: An e-nose device (Aenose Company, Zutphen, Nederlands) was used to collect samples of exhaled breath from HNC patients (n=40) and clinically healthy volunteers matched for age and gender. The samples were analyzed using an artificial neural network for generation of an HNC-specific profile for Sudanese patients.

Results: A specific VOC profile for Sudanese patients has been developed. HNC patients could be differentiated from normal clinically healthy volunteers with a diagnostic accuracy of 81 %.

Conclusions: The e-nose technique using double cross-validation is able to discriminate between HNC patients and healthy controls.

Comparison of two isogenic non-tumorigenic and tumorigenic breast epithelial-derived cell lines with mesenchymal phenotype

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D492 is a breast epithelial cell line with stem cell properties that can generate both luminal and myoepithelial cells and in 3D culture it forms branching terminal ductal lobular units TDLU-like structures. D492M was generated by co-culture of D492 and breast endothelial cells (BRENCs) that resulted in subpopulation of cells undergoing epithelial to mesenchymal transition giving rise to D492M. D492HER2 was generated by overexpression of HER2 in D492 and this also resulted in a cell line showing EMT phenotype.

D492M is non-tumorigenic while D492HER2 is highly tumorigenic. The objective of this project is to compare these two cell lines because even though both cell lines show EMT phenotype, they differ in tumorigenicity.

Regarding in the procedures that could influence this difference in tumorigenecity, we have found significant differences between the cell lines in migration and invasion *in vitro*, proliferation and glucose metabolism. D492HER2 migrates, invades and proliferates more than D492M, and consumes more glucose as well.

Furthermore, from our following the transcriptome data we have confirmed by qPCR the expression of the most differently expressed genes. From this list, we have selected one gene, YKL-40 as a candidate. YKL-40 has previously been linked to cancer progression, and is higher expressed in D492HER2 than D492M both at RNA and protein level. Using a blocking antibody against YKL-40 reduces the ability of conditioned media from D492HER2 to stimulate angiogenesis in vitro. The siRNA of YKL-40 reduces migration and invasion. We are currently analyzing if knock down of YKL-40 inhibits tumorigenicity in vivo.

In conclusion, we have identified YKL-40 as a molecule that could be involved in the tumorigenicity of D492HER2 cells.

The NOSARC project, a national, prospective and population-based study of mutations and mechanisms in sarcomas, and preclinical validation of novel targeted therapies, with the intention to lead clinical trials

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Objective: The Norwegian Sarcoma Consortium, NoSarC, is a subproject of the Norwegian Cancer Genomics Consortium (see <u>cancergenomics.no</u> and <u>nosarc.no</u>), and is a clinical scale model for the introduction of personalized strategies for orphan cancers. One objective is to understand better sarcoma biology. However, we also hope to identify biomarkers indicating sensitivity to therapies already developed and approved for treatment of more common cancers. A subproject is to validate candidate therapies in preclinical sarcoma models, to support their clinical use. This unique project is based on the collaboration with the clinical sarcoma centres in all health regions of Norway, and is intended to feed new candidate approaches to trial designs adapted for small patient groups, hopefully as part of international networks like SSG and WSN.

Methods: We have initiated collection of a prospective, population-based biobank of samples from all patients treated at the Norwegian university hospitals with sarcoma centres. Current funding allows collection of 2-3 annual cohorts, but the intention is 5, i.e. up to 1000 patients, although we do not get fresh tumour tissue from all. We are also developing new in vivo and in vitro models representing the most aggressive sarcomas, to obtain improved models with known germline and somatic variants. As a complementary strategy, we are doing medium-scale drug screens to discover new therapies not predictable from genome data.

Results: Currently we have samples from almost 400 patients, and frozen tumour tissue from the majority. NGS exome analysis is on-going, and the first batch of germ line data has been used as a validation cohor for the International Sarcoma Kindred Study (Ballinger et al, Lancet Oncology, in press). Several targets, including FGFR and PARP inhibitors, are being investigated in sarcoma models, and with in vivo pdx results, clinical investigations may be initiated.

Conclusion: A population-based sarcoma cohort is an important resource for investigation on mechanisms and new therapeutic targets, new biomarkers, and genetic predisposition. A panel of preclinical models complements these discoveries by allowing mechanistic studies and preclinical validation of drug activity in mesenchymal cells and tissues. We hope this will lead to new treatment options that may be evaluated in clinical trials.

The good drug, the bad barrier and the handy peptide: K16ApoE improves the treatment of experimental brain metastases

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Abstract

Patients with metastatic disease in the brain usually await a poor prognosis. The blood brain barrier (BBB) excludes almost all therapeutic compounds, as highly charged, hydrophilic or large compounds are unable to penetrate this barrier. Thus, regardless of the ongoing development of novel drugs, a significant challenge is the delivery of drugs across the BBB and into the metastatic neoplasms. Different strategies to transiently open the BBB have been studied previously. Our approach involves using a peptide transporter encompassing 16 lysine residues and the 20 amino acid residues corresponding to the low-density lipoprotein receptor binding domain of apolipoprotein E (ApoE). We aimed to study and subsequently quantify the therapeutic window provided by K16ApoE in a well-established animal model of experimental melanoma brain metastases.

The ability of the peptide to open the BBB was studied *in vivo* using dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) in nonobese diabetic/severe combined (NOD/SCID) mice. Further, cellular effects after treatment with the peptide were investigated *in vitro* using electron microscopy and flow cytometry. The biodistribution of the peptide was studied in blood plasma and several organs using K16ApoE labeled with 125I. Finally, we treated NOD/SCID mice with experimental brain metastases with the peptide in combination with the B-RAF inhibitor Dabrafenib, only Dabrafenib or vehicle.

After intravenously administering K16ApoE into the NOD/SCID mice, a transient opening of the BBB of at least 30 minutes was demonstrated by DCE-MRI. Electron microscopy revealed intact intercellular tight junctions in MDCK II canine kidney endothelial cells after treatment with the peptide *in vitro*, and a dose-dependent cell death was seen by viability experiments. Flow cytometry displayed a clathrin-mediated uptake of albumin into RBE 4 rat brain endothelial cells. The biodistribution study showed that the peptide was eliminated from blood plasma in less than five minutes through the kidneys. In the treatment study, we demonstrated that the group of animals receiving K16ApoE followed by Dabrafenib had less number of tumors and smaller tumor volumes than in the other two experimental groups.

In summary, K16ApoE combined with Dabrafenib decreased the numbers and volumes of experimental brain metastases, likely due to increased penetrance of Dabrafenib into the brain. The use of K16ApoE may thus have a general, clinical potential, by improving the treatment of brain metastasis patients with Dabrafenib as well as other therapeutic drugs.

Predictive biomarkers in sunitinib treatment of metastatic renal cell carcinoma

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Introduction/background:

Clear cell renal cell carcinoma (ccRCC) is the most common among renal cell carcinomas. Antiangiogenic treatment is currently first line therapy in metastatic ccRCC. Response rates and duration of response show considerable variation between patients, and adverse events have major influence on quality of life. The need of a predictive biomarker to select responders to antiangiogenic receptor tyrosine kinase inhibitors (rTKI) upfront is urgent. We have investigated the role of systemic inflammation in metastatic ccRCC using immunohistochemistry.

Material and methods:

Forty-six patients with metastatic or non-resectable ccRCC were included for sunitinib treatment. Primary and metastatic tumor paraffin embedded tissue was available for immunohistochemically staining for selected markers related to angiogenesis (VEGFR2, PDGFRB, VEGFA and HSP27) and immunology (IL6, IL6R, JAG1). Tissue lesions were available in 41 of 46 patients. The most recent biopsy, the metastatic lesion or the non-resectable primary tumor diagnosed closest to the date of clinical trial inclusion, was chosen. The predictive potential of these markers was assessed based on the objective response, which was evaluated according to the RECIST criteria at base line and every 12 weeks of sunitinib treatment. Additionally, progression free survival (PFS) and overall survival (OS) were investigated.

Results:

Absent/low immunostaining of IL6, IL6Rα and VEGFR2 was significantly associated with sunitinib response. Furthermore, absent/low expression of IL6 showed significant association with PFS and OS. Absent/low expression of IL6 showed also significant association with absent/low expression of JAG1, VEGFR2 and PDGFRB. Median/high expression of IL-6 showed significant association with median/high expression of VEGFA. Median/high expression of IL6R showed significant association with median/high expression of VEGFA and HSP27.

Conclusion:

Absent/weak immunoexpression of IL6 and VEGFR2 in primary tumor alone and IL6R in most recent biopsies in tumors of metastatic ccRCC patients predicts response to sunitinib treatment.

Microvascular proliferation is associated with aggressive tumor features and reduced survival in lung adenocarcinomas

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Objective: Despite new treatment options in lung cancer, there is still need for better prognostic factors to assist in treatment decisions. Angiogenesis has been associated with tumor growth and dissemination, and studies in other tumors show that vascular proliferation index (VPI) is a valuable prognostic marker. We examined the prevalence and prognostic impact of VPI on cancer specific survival (LCSS) in lung adenocarcinomas (AC).

Methods: Selected tumor slides from a cohort of 210 patients surgically treated for AC at Haukeland University Hospital (Bergen, Norway) during 1993-2010, were stained for Nestin-Ki67. VPI was evaluated as the ratio between proliferating vessels (pMVD) and the microvessel density (MVD), and cut-off value was set at 4.4% (upper quartile).

Results: High VPI was associated with presence of blood vessel invasion (p=0.007) and tumor necrosis (p=0.004). There was a trend towards an association with higher tumor grade (p=0.094). Further, high VPI was significantly associated with reduced LCSS (p=0.020). In a multivariate model, VPI remained an independent prognostic factor for reduced cancer specific survival (HR 1.7; 95% CI 1.04-2.68; p=0.033) when other prognostic clinico-pathologic variables were adjusted for.

Conclusion: Angiogenesis by VPI can be evaluated by Nestin-Ki67. High VPI is associated with aggressive tumor features such as blood vessel invasion and reduced cancer specific survival, in lung adenocarcinoma.

Development of a molecular diagnostic tool for more precise diagnosis of oral squamous cell carcinoma

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The five year survival rate of oral squamous cell carcinoma after diagnosis is significantly lower than other cancers like breast, cervix and colorectal. This is due to late presentation, failure of pre-malignant lesions to respond to treatment, and lack of biomarkers for risk stratification of OSCC lesions in order to guide further management. Expression of cancer-associated biomarkers represents a great potential for a more precise diagnosis for patient stratification and more personalized treatment of OSCC. However, due to heterogeneous nature of oral cancer, a single biomarker was unable to accomplish risk stratification.

The aim of this study was to develop an immunohistochemistry-based malignancy index diagnostic system (IHC-MIDS) based on the expression of a panel of several molecules known to have diagnostic and prognostic value for progression and survival in OSCC.

IHC was performed on paraffin embedded formalin fixed tissue samples from 102 patients for the following biomarkers p16, p53, ki67, p75NTR, LoxL4, αSMA, Foxp3, CD68, CD 163, CD80, FVIII and D2-40. The stained tissues were scanned and quantified manually or by using ImageJ open source software. For the development of IHC-MIDS, statistical analysis was performed to estimate the correlation between expression profile of biomarkers and clinicopathological parameters along with tumour invasion pattern. Further, a novel method of machine learning algorithm was implemented to validate the performance of the prediction models and to evaluate their generalization ability.

IHC-MIDS was able to stratify OSCC lesions for several clinical pathological features, including the risk of recurrence. Preliminary analysis shows that a scoring system based on expression of a panel of biomarkers is more precise than the use of a single biomarker.

Eventually, we plan to build a nomogram based on the scoring system that can guide additional management for risk stratification of OSCC lesions.

Indirect role of fibroblast *Ext1* in regulating *P311* expression in A549 carcinoma cells

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Cancers require an appropriate microenvironment to survive and thrive in a patient's body. This microenvironment is created and modified by both the tumor and stromal cells. Fibroblasts and the extracellular matrix constitute the majority of this stromal milieu. The communications between the tumor cells and the neighboring stromal fibroblasts are important in ensuring tumor growth and ability to spread to other organs. This involves both physical and molecular interactions between the different cell types, which makes the cell surface characteristics crucial. In this study we hypothesize that fibroblast *Ext1* levels and hence cell surface heparan sulfate (HS) amounts influence gene expression in neighboring A549 carcinoma cells.

The *Ext1* encodes a glycosyltransferase involved in polymerization of HS chains. *P311* encodes an 8 kDa protein that influences cell differentiation and migration both in normal physiology and in tumorigenesis. It has been demonstrated that mouse embryonic fibroblasts (MEFs) with a gene trap mutation in *Ext1* (*Ext1*^{Gt/Gt}) and short HS chains affect tumor cell migration when co-cultured with A549 cells in a spheroid model. Our spheroid model consists of a mixture of human A549 cells mixed with either *Ext1* Gt/Gt or wild type (*Ext1* Mt/Wt) MEFs.

A human specific-microarray analysis showed that P311 was down-regulated in A549 cells co-cultured with Ext1-mutated fibroblasts. The $Ext1^{Gt/Gt}$ cells displayed reduced Tgf- $\beta1$ mRNA levels and lower levels of secreted active TGF- $\beta1$. Re-introduction of Ext1 into $Ext1^{Gt/Gt}$ MEFs rescued tgf- $\beta1$ mRNA levels and the amounts of soluble active TGF- $\beta1$ as well as P311 mRNA expression levels in A549 cells in co-culture with the fibroblasts.

Our results suggest that in a heterogeneous cellular co-culture model, fibroblast *Ext1* levels influence *P311* expression in A549 cells. This could be through its effect on $TGF-\beta1$ expression and the amount of active TGF- $\beta1$ molecules secreted by the fibroblasts.

A Phase Ib/II randomised open label study of BGB324 in combination with pembrolizumab or dabrafenib/trametinib compared to pembrolizumab or dabrafenib/trametinib alone, in patients with advanced non-resectable (Stage IIIc) or metastatic (Stage IV) melanoma

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Background. MAPK inhibitors and immune checkpoint inhibitors are major breakthroughs in metastatic melanoma showing high response rates and durable responses. To further improve patient outcome, and to overcome treatment resistance, combination strategies have been suggested. We have initiated a phase Ib/II randomized clinical trial with the Axl inhibitor BGB324 with or without immune check point inhibition (pembrolizumab) or MAKP inhibitors (dabrafenib+trametinib) (NCT02872259).

Upregulation of the Axl kinase has been associated with reduced response to anti-PD-1 therapy. The drug resistant low MITF/ high Axl melanoma signature has been associated with an immune suppressive micro-environment. Axl is a key negative feedback regulator of the innate immune response and attenuates macrophage, dendritic and natural killer (NK) cell activity. Hence, AXL signaling contributes to both tumor intrinsic and microenvironmental immune suppression mechanisms. Further we have recently shown that BGB324 sensitizes the highly metastatic K1736 murine melanoma tumors to anti-PD-1 treatment. This provides a rationale for targeting AXL to enhance the anti-cancer immune response.

Axl dependent cell plasticity signaling pathways confer resistance to inhibitors of BRAF/MEK. Melanomas display either a high E-cadherin/high MITF-M expression on the one hand, or high N-cadherin/high Axl expression on the other. The low MITF/high AXL phenotype is linked to drug-resistance and common among mutant BRAF and NRAS melanoma cell lines. Interestingly, Axl-mediated resistance to BRAF and MEK targeting agents could be predicted by soluble Axl receptor in patient blood samples and Axl targeting with BGB324 reversed acquired resistance to BRAF/MEK inhibitors.

BGB324(R428) is a highly potent and selective ATP-competitive Axl kinase inhibitor that blocks receptor autophosphorylation and downstream signal transduction pathways (IC50=14 nM). BGB324 is being developed by BerGenBio AS, and is currently in several clinical trials.

Methods. This is a Phase Ib/II, multicentre, open label, parallel group study in patients with metastatic melanoma. Part 1 is a dose selection phase in up to 12 patients to evaluate the safety of BGB324 when administered in combination with dabrafenib+trametinib and to determine the BGB324 dose to be administered in the combination in Part 2 and Part 3.

Part 2 is an open label, multiple arm, randomised treatment phase evaluating efficacy and safety of pembrolizumab and dabrafenib+trametinib in combination with BGB324 as first line treatment in 80 patients with advanced melanoma compared to pembrolizumab or dabrafenib/trametinib alone. Patients will be stratified according to BRAF status and tumor load to receive pembrolizumab or dabrafenib+trametinib with or without BGB324, randomised in a 2:1 ratio.

Part 3 investigates second line treatment for BRAF mutated patients continuing from Part 1 or Part 2. This is an open label, multiple arm, non-randomised treatment phase evaluating efficacy and safety of pembrolizumab and dabrafenib/trametinib in combination with BGB324 or alone. Patients treated with pembrolizumab will be switched to dabrafenib+trametinib and the other way around. BGB324 will be continued when given/implemented in Part 1 or Part 2. Co-primary endpoints are response rates according to RECIST 1.1. Enrollment began in January 2017.

Heterotypic interactions between endothelial cells and normal- and cancerous breast epithelial cells

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Investigating heterotypic interactions between cancer cells and the microvasculature is important for improving our understanding of how the microenvironment supports tumor growth and facilitates metastasis.

Here, we analyze how isogenic non-tumorigenic vs. tumorigenic breast epithelial cell lines affect their vascular niche in 2D and 3D co-culture and how endothelial cells respond back to the epithelial cells. D492, D492M and D492HER2 are isogenic breast epithelial cells. D492 is a breast epithelial cell line with stem cell properties that in co-culture with endothelial cells undergoes epithelial to mesenchymal transition (EMT) that gave rise to D492M. Both D492 and D492M are non-tumorigenic D492HER2 was generated by overexpressing the HER2/ErbB2 oncogene in D492, is mesenchymal and shows tumorigenic properties.

In both 2D and 3D co-culture with HUVEC endothelial cells, D492HER2 showed a greater physical interaction with HUVEC compared to D492. Migration assay confirmed increased migration of D492HER2 towards HUVECs. To assess crosstalk between epithelial cells and the HUVECs, conditioned media (CM) was collected from all three cell-lines. Treatment of HUVECs with CM from D492HER2 demonstrated increased 3D capillary network formation. Also CM from endothelial cells that had been pre-treated with CM from D492HER2 increased migration of D492HER2 cells.

These data, although still descriptive, suggest that cancer cells do affect their endothelial niche and that cross-interaction with endothelial cells might be beneficial for cancer cells. Identification of candidate molecules mediating the cross-talk between endothelium and breast cancer cells could give valuable insights into a cancer-specific response of the vascular niche. To identify cancer cell-secreted factors and responsive endothelial target genes, secretome analysis and RNA sequencing of conditioned endothelial cells will be performed.

Molecular characterization of MPA/DMBA induced mouse mammary tumors

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Chemically induced mammary tumor mouse models are used for characterizing breast cancers, but their value as model systems is debated. We aim to characterize medroxyprogesterone acetate (MPA) and 7,12-dimethylbenz[a]anthracene (DMBA) induced mouse mammary tumors as a model for breast cancer. Whole exome sequencing of 18 tumors from 14 mice revealed a mean frequency of 12.20 mutations per megabase (12.20/MB); significantly higher than in human breast cancers (1.31/MB). Mutations in known driver genes were found in all tumors, including several recurrently mutated genes such as Trp53 (5/18), Atr (5/18), Nf1 (5/18), Fat1 (5/18), Kras (4/18), Kmt2c (4/18) and Fat4 (4/18). Mutation spectrum analysis showed a strong disposition towards T>A transversions with a 3' guanine. Three mutational signatures were evident in the tumors, in particular signature 22, which has been reported to be associated with aristocholic acid exposure. Gene expression analysis revealed high intertumor heterogeneity. One subgroup of tumors exhibited properties similar to the claudin-low subtype, including high expression of genes related to immune response. Results from immunohistochemical staining for keratins 5 and 18 correspond well with the gene expression subtypes. MPA-DMBA induce a heterogeneous set of mammary tumors that may serve as relevant models to study human breast tumorigenesis.

Expression of L1CAM in curettage and high L1CAM level in preoperative blood samples predicts lymph node metastases and poor outcome in endometrial cancer patients

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Purpose: Several studies have identified L1CAM as a strong prognostic marker in endometrial cancer. To further underline the clinical usefulness of this biomarker, we here investigated L1CAM as a predictive marker for lymph node metastases and its prognostic impact in curettage specimens and preoperative plasma samples.

Experimental design: Immunohistochemical staining of L1CAM was performed for 1134 curet-tage specimen from endometrial cancer patients. In addition L1CAM level in preoperative blood samples from 372 patients was determined using ELISA. Association between L1CAM level and clinicopathologic variables including lymph node status and survival was investigated.

Results: Expression of L1CAM in curettage specimen was significantly correlated to L1CAM level in corresponding hysterectomy specimen. Both in curettage specimen and preoperative plasma samples was L1CAM upregulation significantly associated with features of aggressive disease and poor outcome. L1CAM was an independent predictor of lymph node metastases, after correction for curettage histology, both in curettage specimen and plasma samples.

Conclusions: We demonstrate that preoperative evaluation of L1CAM levels, both in curettage or plasma samples, predicts lymph node metastases and adds valuable information on patient prognosis. Our results strongly support the usefulness of L1CAM as a biomarker in endometrial cancer.

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Oxygen-dependent regulation of tumour growth and metastasis in human breast cancer xenografts

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Background: Hypoxia in tumours affects tumour growth, metabolism, resistance to chemotherapy and metastasis. Previously, we have shown that hyperoxia, using hyperbaric oxygen treatment (HBOT), supressed tumour growth and shifted the phenotype from mesenchymal to epithelial (MET) in the DMBAinduced mammary tumour model. This study describes the effect of HBOT on tumour growth, angiogenesis, chemotherapy efficacy and metastasis in a triple negative MDA-MB-231 breast cancer model, and evaluates tumour growth using a triple positive BT-474 breast cancer model.

Materials and methods: 5 x 105 cancer cells were injected s.c. in the groin area of NOD/SCID female mice. 2-days prior to tumour cell injection, the BT-474 group was supplied with Progesterone and Estradiol pellets. Mice were divided into two groups; controls (1 bar, pO2=0.2 bar) or HBOT (2.5 bar, pO2=2.5 bar, 90 min, every third day until termination of the experiments). The effects of the treatment were evaluated by assessment of tumour growth, metastasis (immunostaining), collagen type I (immunostaining), angiogenesis (CD31 staining), EMT markers (western blot) and chemotherapeutic efficacy (5-FU).

Results: Tumour growth was significantly supressed by HBOT in both MDA-MB-231 and BT-474 tumour models. Both number of metastasis and total area of metastatic lesions were significantly reduced by HBOT, as well as reduced N-cadherin, Axl and collagen type I in the MDA-MB-231 model. No differences were found in angiogenesis or 5FU efficacy between HBOT and controls in the MDA-MB-231 model.

Conclusion: Despite the fact that triple-positive and triple-negative subtypes of cancer behave differently and have different prognosis, HBOT had a similar suppressive effect on tumour growth, indicating that they share a common oxygen dependent anti-tumour mechanism. HBOT also significantly reduced the number and area of metastasic lesions in the triple negative model, as well as a significant reduction in the EMT markers N-cadherin, Axl and density of collagen type I.

Axonogenesis and vascular proliferation are associated gene expression programs in hormone receptor negative breast cancer

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Objective: Interactions between cancer cells, vasculature and nerves have been suggested as important for tumor progress. We aimed to explore these relations in subtypes of breast cancer (BC), with particular attention to novel treatment targets.

Methods: We analyzed multiple BC mRNA cohorts and signatures reflecting vascular proliferation and axonal sprouting were explored. A cohort of primary BC tissue (n=461) was studied by IHC for validation (Factor VIII-Ki67; Neurofilament).

Results: High angio- and axonogenesis signature scores associated with ER/PR negativity, a basal-like phenotype and shorter survival. Notably, the angio- and axonogenesis scores were significantly associated, and a jointly activated neuro-angiogenic profile strongly associated with the basal-like phenotype and gene sets reflecting hypoxia and immune responses. An association between vascular proliferation and axon density by IHC was found. Through a drug signature database (Connectivity-Map), compounds with dopaminergic action was identified as negatively correlated with the expression profile of VP-high tumors.

Conclusions: Our findings indicate vascular proliferation and axonogenesis as coordinated programs in aggressive breast cancer. Dopaminergic drugs are suggested as potentially relevant, especially for the basal-like subtype with few treatment options.

BGB324, a selective small molecule inhibitor of receptor tyrosine kinase AXL, abrogates tumor intrinsic and microenvironmental immune suppression and enhances immune checkpoint inhibitor efficacy in lung and mammary adenocarcinoma models

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Abstract

The AXL receptor tyrosine kinase is associated with poor overall survival in a wide spectrum of cancers including lung and breast adenocarcinomas. AXL signaling is an important regulator of tumor plasticity related to epithelial-to-mesenchymal transition (EMT) and stem cell traits that drive metastasis and drug resistance. Signaling via AXL is also a key suppressor of the antitumor innate immune response, and AXL is expressed on several cells associated with the tumor immune microenvironment including natural killer (NK) cells and tumor-associated macrophages. Hence AXL resides uniquely at the nexus between tumor and microenvironmental anti-tumor immune suppression mechanisms. We report that BGB324, a selective clinical-stage small molecule Axl kinase inhibitor, enhances the effect of immune checkpoint blockade in aggressive adenocarcinoma models with limited immunogenicity by targeting both tumor intrinsic and microenvironmental immune suppression. Immune therapy with anti-CTLA4/PD1 in the 4T1 model increased AXL and EMT-marker expression correlating with a lack of response. Combination with BGB324 resulted in durable primary tumor clearance versus anti-CTLA4/PD1 alone. In a separate study, BGB324 + anti-CTLA4 treatment resulted in significant long-term primary tumor clearance while no response was observed with anti-CTLA4 treatment alone. The extensive metastasis to the lung, liver and spleen characteristic of the 4T1 model was not detected in animals responding to the combination treatment. Importantly, responding animals rejected orthotopic 4T1 tumor cell re-challenge, demonstrating sustained tumor immunity. In the LL2 Lewis Lung model. BGB324 in combination with anti-PD1/PDL1 significantly prevented tumor growth compared to treatment with anti-PD1/PDL1. Tumors from mice treated with BGB324 in combination with immune checkpoint inhibitors displayed reduced EMT traits, altered cytokine expression, enhanced tumor infiltration of effector cells and decreased number of mMDSC. Also, BGB324 significantly reduced IL10 secretion by isolated human macrophages and enhanced human NK-cell mediated NSCLC tumor cell lysis. Collectively these results support a prominent role for AXL in resistance to immune therapy and support clinical translation of combining BGB324 with immune checkpoint inhibitors to improve cancer treatment.

