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ORAL PRESENTATIONS

BIOMARKERS

O1 The sialyl-glycolipid SSEA4 marks a subpopulation of chemotherapy resistant breast cancer patients with mesenchymal features

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Tribe negative breast cancer (TNBC) is an aggressive breast cancer subtype associated with high risk of early relapse and metastasis [1]. At the moment chemotherapy remains the main option for systemic therapy of TNBC patients but complete remission occurs only in 20% of the patients [2]. In order to identify biomarker for chemotherapy-resistant TNBC cells, we performed a cell surface marker screen in 4 TNBC patient-derived xenografts (PDX) models that respond well to adriamycin/cyclophosphamide-based (A/C) chemotherapy but fail to reach complete pathological response. We used multi-parameter flow cytometry to screen the expression of 45 cell surface markers during the course of chemotherapy. We identified the sialyl-glycolipid SSEA4 as a marker of chemotherapy-resistant cancer cells in all 4 models. In addition, 3 out of 4 TNBC PDXs showed higher percentage of SSEA4-positive cells compared to all A/C-sensitive TNBC PDXs analysed. Gene expression comparison between SSEA4-positive and SSEA4-negative tumor cells from 3 TNBC PDXs highlighted an overexpression of mesenchymal-associated genes and a deregulation of drug resistance pathway-associated genes and miRNAs in SSEA4+ breast cancer cells. In addition, high expression of ST3 beta-galactoside alpha-2,3-sialyltransferase 2 (ST3Gal2), the enzyme catalyzing the last step of SSEA4 synthesis, was found associated with poor outcome in ER-, PR- breast cancer patients treated with chemotherapy (p < 0.01, HR 3.08).

Thus, we propose SSEA4 as a novel marker of mesenchymal and chemoresistant breast cancer cells, and ST3Gal2 as an effective marker for chemoresistance associated with poor outcome in breast cancer patients.

References

O2 Foxp3 expression in breast cancer patient from Qatar: survival analysis

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In breast cancer, the presence of Foxp3 (Tregs) [1] within the tumor milieu has been a matter of debate. Some studies have determined that infiltration of Tregs was associated with poor survival, while others revealed no impact on survival, however this depends on their type, type of cells expressing Foxp3 and the density of the Tregs population [2]. The goal of our study was to quantify Foxp3 in breast cancer patients from Qatar and correlate with their survival.

Methodology: Expression of Foxp3 was studied in 132 FFPE samples with known clinico-pathological data by immunohistochemistry technique and quantified by modified H-score system by pathologist. Results were analyzed via SPSS.

Results: Analysis was carried for 132 patients. Age at time of diagnosis was 49 ±10.4 years. 76.2% of the patients showed positive expression of Foxp3. Foxp3 expression was not correlated with patient age or hormone receptors. Expression of Foxp3 positively correlate with better patient survival when compared to negative expression (94.1, 95% CI 85.6 - 102.6 versus 83.6, 95% CI 71.8 - 95.5, P = 0.60).

Conclusion: Foxp3 is expressed on lymphocytes that are present in the tumor microenvironment regardless of breast cancer subtype. Foxp3 is correlated with better survival.

References

O3 Clínico-pathological and transcriptomic determinants of SLFN11 expression in invasive breast carcinoma

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SLFN11 is a putative DNA/RNA helicase we discovered as causally associated with sensitivity to DNA damaging agents, such as platinum salts, topoisomerase I and II inhibitors, and other alkylators in the NCI-60 panel of cancer cell lines [1]. Later, SLFN11 was identified as an early interferon response gene, in association with HIV infection [2]. Here we assessed SLFN11 determinants in a gene expression meta-set of 5,061 breast cancer patients annotated with clinical data and multigene signatures obtained with the package genefu [3]. By correlation analysis, we found 537 transcripts above the 95th percentile of Pearson’s coefficients with SLFN11, identifying “immune response,” “lymphocyte activation,” and “T cell activation” as top Gene Ontology enriched processes [4]. Through multiple correspondence analysis, we discovered a subgroup of patients characterized by high SLFN11 levels, ER negativity, basal phenotype, elevated CD3D, STAT1 signature [5], and young age. Fitting a penalized maximum likelihood lasso regression model [6], we found a strong multivariable association of SLFN11 with the stroma 1 and stroma 2 signatures [7,8], associated with basal cancer and response to chemotherapy in ER- tumors. Finally, using Cox proportional hazard regression, ER-, high proliferation, high SLFN11 patients undergoing chemotherapy treatment showed a significantly longer disease-free interval than other patient categories included in our model.

**References**


### PRECLINICAL/CLINICAL TRIALS

**O4**

**CTLA-4 checkpoint blockade in breast cancer, a case in point report**

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A patient concomitantly affected by breast cancer and melanoma is presented in order to contribute to the ongoing debate on breast cancer immunotherapy. In 1996, the patient was operated on for left early breast cancer and treated with adjuvant radiotherapy and 5 years Tamoxifen. In 2009 biochemical progression and bone metastases appeared so Letrozole hormonal therapy was carried out, ensuing in clinical stabilization. In 2010, a left arm cutaneous melanoma was excised (pT4a Breslow 4.5 mm), and axillary nodal dissection detected an involved node. Because of subsequent in-transit metastases, the patient underwent chemo-hyperthermic perfusion, reaching a dermic complete response (CR). Suddenly melanoma progressed in the lung and liver, as confirmed by ultrasound driven biopsies. Chemotherapy (DTIC) was given, achieving visceral partial response. At this point ipilimumab [3] was available and CR of liver, lung, and dermic metastases was obtained after 4 cycles. In 2012, while bone lesions remained stable and considered breast cancer. Four months later novel liver metastases appeared and biopsy unexpectedly confirmed their breast origin. The patient after unsuccessful chemotherapy, finally died of liver failure, without evidence of melanoma metastases. This very special case shows the impressive discrepancy in response to CTLA4 check-point therapy between melanoma and breast cancer.

**References**


**05**

**Long-term in vivo expression of trastuzumab following intramuscular electrotransfer of the encoding DNA in mice**

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In vivo antibody expression is at crossroads between monoclonal antibody (mAb) and gene therapy. Following a single intramuscular injection of the DNA that encodes a therapeutic mAb, the muscle is turned into a ‘bioreactor’, resulting in prolonged mAb secretion in circulation [1-3]. This innovative approach addresses several challenges associated with conventional mAb proteins. In R&D, in vivo mAb expression allows production and evaluation of leads directly into animal models. In the clinic, it can improve treatment efficacy and patient comfort by avoiding repeated high-dose injections, and provide a cost-effective answer to the increasing need for mAb combination therapies - in the field of cancer immunotherapy and beyond. This study outlines the development and delivery of a trastuzumab-encoding plasmid for in vivo mAb expression. To improve intramuscular DNA delivery in mice, we first established an optimal electrotransfer protocol using novel reporter plasmids. Following the optimized intramuscular electrotransfer of the trastuzumab-encoding plasmid, trastuzumab was detected with a commercial ELISA at therapeutically relevant concentrations (1-15 μg/ml) in the sera of athymic nude mice (n=16) for the full duration of the ongoing follow-up (>3 months). A cell viability assay demonstrated similar activity of the expressed versus commercial trastuzumab in the BT-4747 breast cancer cell line. In conclusion, we achieved proof of concept for the long-term in vivo expression of biologically active trastuzumab in mice. Ongoing work focuses on optimizing in vivo mAb expression for clinical application and evaluation for combination therapy.

**References**

BACKGROUND: In mice, cryo plus checkpoint blockade facilitates tumor antigen release, T-cell priming, and improved survival [1]. Here, we assess immune response in ESBC patients using biomarkers that have been attributed to clinical benefit following checkpoint blockade [2-5].

METHODS: Women with ESBC were treated 7-10 days preceding mastectomy with either cryo (n=6), single-dose ipi at 10mg/kg (n=6), or cryo+ipi (n=6) [6]. From serial blood (baseline & 1-month post-mastectomy) and tumor (biopsy & mastectomy), fold-changes following cryo+ipi versus monotherapy were compared (Wilcoxon rank-sum) across the following measures: Ki67+ or ICOShi T-cells [2] and intratumoral T-effector/T-regulatory [3] cells by flow cytometry, plasma Th1/Th2 cytokines [4] (Meso Scale Discovery), and intratumoral T-cell expansion by immunohistochemistry [5] and T-cell receptor (TCR) deep sequencing (ImmunoSEQ®) [5].

RESULTS: Cryo+ipi generated greater increases in peripheral Ki67+CD4+ (p=0.05), Ki67+CD8+ (p=0.05), ICOShiCD4+ (p=0.005), and ICOShiCD8+ (p=0.005) cells. The intratumoral T-effector/regulatory ratio was higher following cryo+ipi, but only when Ki67-gated (p=0.01). Cryo+ipi generated greater increases in IL-2 (p=0.01), IFNγ (p=0.06), and IL-5 (p=0.09). Despite negligible intratumoral changes by immunohistochemistry, cryo+ipi generated more high-magnitude (~1000 amplicon) clonal expansions by TCR sequencing (medians: 52 v. 3 clones).

CONCLUSIONS: Cryo+ipi is associated with potentially favorable immunologic effects. Ki67-gating and TCR sequencing may identify intratumoral changes otherwise undetectable by flow or IHC.

References
immunophenotypes. Therefore, another method to promote anti-tumor immunity is to prime T cells against tumor-associated stromal cells. We have reported [1] that IL-12 gene therapy of established HLA-A2neg B16 melanomas in HLA-A2+ transgenic mice resulted in CD8+ T cell-mediated immunity against the host HLA-A2+ stromal cells within the tumor microenvironment (TME). We have also shown [2] that vaccines based on a subset of tumor blood vessel antigen (TBVA)-derived peptides (DLK1, EphA2, HB8, NRP1, PDGFRI, RGS5 or TEM1) prevented HLA-A2neg MC38 tumor establishment and promoted the regression of tumors in HLA-A2+ mice by CD8+ T cell targeting of HLA-A2+ pericytes and vascular endothelial cells in the TME. Based on this pre-clinical data, we propose to undertake a Susan G. Komen-funded trial (IR13261822) clinical trial of chemo-immunotherapy using the immunomodulatory drug gemcitabine with a dendritic cell vaccine pulsed with six with HLA-A2-presented TBVA-derived peptides (DLK1310-318, EphA2883-891, HB831-39, NRP1433-441, RGS555-13 and TEM1691-700) in 30 HLA-A2+ patients with metastatic breast cancer. The specific aims of this study are to determine vaccine-induced generation of TBVA-Tc1 immunity and clinical response.

References

P4
Synergistic antitumor interaction between valproic acid and capcitabine in breast cancer
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Caucitabine, commonly used in different settings for metastatic breast cancer, is an inactive prodrug that take advantage of the tumor elevated levels of thymidine phosphorylase (TP), a key enzyme for its conversion to 5-flourouracil. Potentiation of anticancer activity of caucitabine is required to improve its therapeutic index.

We demonstrated that histone deacetylase inhibitors (HDACi), including the anti-epileptic valproic acid (VPA), induced dose and time-dependent upregulation of TP transcript and protein in breast cancer cells but not in non-tumorigenic MCF-10A cell line. By using siRNA or isomorphic specific HDACi we demonstrated that HDAC3 is the main isoform whose inhibition is involved in TP modulation. Combined treatment of HDACi, including VPA, and caucitabine resulted in synergistic/additive antiproliferative and proapoptotic effects in all cell lines tested. TP knockdown experiments demonstrated the crucial role of TP modulation in the synergism observed. The synergistic antitumor effect between VPA and capcitabine was also demonstrated in vivo in a breast cancer xenograft model, but not in xenografts from TP-knocked cells, confirming in vitro data. Overall, this study suggests that the combination of an HDACi, such as valproic acid, and capcitabine, is an innovative antitumor strategy and warrants further clinical evaluation for the treatment of metastatic breast cancer.

P5
Carbon nanomaterials as contrast agents for breast cancer diagnosis and therapy
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Nanotechnology is the promise to fight breast cancer (BC) more specifically and effectively [1]. In this context carbon nanomaterials (CNs) have attracted the scientific community and the public interest [2]. Common modalities for BC diagnosis are ultrasonography (US) and magnetic resonance imaging (MRI). US is the most useful modality in the evaluation of palpable BC masses that are mammographically occult in women younger than 30’s. Here we show CNs as highly and long lasting echogenic materials. Experiments on swine models confirmed that CNs are clearly visible under US and didn’t exert toxicity. In the current market dual-imaging agents are missed; here we also demonstrate the immune-compatibility and high echogenic properties in water and in whole blood of cystine functionalized super paramagnetic nanoparticles (CY-SPION), well-known MRI agents [3]. Thanks to these findings, and the ability to load CNs to many moieties [4-6], we propose dual-contrast agents, CNs-CY-SPION conjugates, to improve BC diagnosis. Future perspectives is to conjugate CN-SPION to targeted drugs against BC. In summary, we lay the foundations for novel contrast agents, for therapy and multimodal diagnosis of BC, combining high imaging performances with unique potential therapeutic applications, such as specific targeting capabilities, drug delivery, immunotherapy and hyperthermia.

P6
Systems biology analysis of gene expression data and gene network reverse-engineering approaches reveal NFAT5 as a candidate biomarker in Inflammatory Breast Cancer
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Inflammatory Breast Cancer (IBC) is the most aggressive and highly metastatic form of breast cancer [1-3]. In a recent study [4], we analysed breast cancer with peritumoral neoplastic lymphovascular invasion (ePVI) in comparison with inflammatory breast cancer, showing that ePVI breast cancer have more clinicopathologic affinity than differences with the most aggressive cancer in the breast. Here, we aim to identify potential master regulators (MRs) that drive the expression pattern in IBC. Transcriptomic (i.e., mRNA) data from 197 breast tumours were used for this analysis (GEO GSE23720) [5]. All tumours were classified as "IBC" (n=63) or "MBC" (n=134). To identify novel MRs that drive the IBC phenotype, all expression data were analysed using a network-based...
strategy (ARACNe [6]) and Master Regulator Analysis (MRA) [7]. We chose to perform in-vivo IHC analysis, in two independent cohorts of IBCs (n = 39), nIBC (n = 82) and normal breast tissues (n = 15), for the top significant Master Regulators: MGA, CTTNBN1 and NFATS. Biological validation confirmed that NFATS expression was higher in IBC than in nIBC (70% vs. 20%) and that the majority of NFATS-positive IBC samples displayed NFATS nuclear expression in comparison with nIBC samples (89% vs. 12%). We provide evidence that NFATS expression could constitute a novel IBC biomarker that could help to identify the most aggressive forms of BC into routine clinical practice.

**References**


**P9**

**Haplotypes of SNPs associated with COX-2 and their comparison with histopathological features of breast cancer patients**

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**Introduction:** In breast cancer, increased cyclooxygenase-2 expressions is associated with poor prognosis. COX-2 expression influences production of pro-inflammatory prostaglandins. The present study investigated association between COX-2 promoter polymorphisms (rs689465, rs689466, rs20417) and histopathological features of breast cancer patients.

**Methods:** We selected 150 HER-2 amplification positive breast cancer patients from our previous case-control study. The participants were evaluated for histopathological features and genotyped for COX-2 SNPs. Comparisons of genotype data with histopathological characteristics were performed by chi square test. Logistic regression was applied for estimation of odd ratios. COX-2 protein level and other markers were assessed by immunohistochemical staining. Statistical analyses were performed using SPSS version 19.0 and p value was set at <0.05.

**Results:** Our data showed that elevated COX-2 expression was significantly associated with HER-2 amplified tumours. In addition, a positive association between rs20417 (GC+CC) and estrogens receptor (OR: 0.383, 0.161-0.913, P: 0.030) and IDC tumour (OR: 0.264, 0.070-0.993, P: 0.049) was noted. Eight haplotypes were deduced and associations with tumour size (P: 0.030), HER-2 amplification (P: < 0.0001), ER positivity (P: 0.017) were observed.

**Conclusion:** Present study suggests COX-2 expression and haplotypes of its associated SNPs should be considered for characterizing breast cancer prognosis.

**P10**

**Activity of the dietary flavonoid, apigenin, against multidrug-resistant tumor cells as determined by pharmacogenomics and molecular docking**

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**Introduction:** Natural products have been extensively studied and involved in cancer therapy field [1], apigenin has considerable cytotoxic activity in vitro and in vivo. Despite many mechanistic studies, less is known about resistance factors hampering apigenin’s activity. We investigated the ATP-binding cassette (ABC) transporters BCRP/ABCG2, P-glycoprotein/ABCB1 and its close relative ABCBS. Apigenin inhibited not only P-glycoprotein, but also BCRP by increasing cellular uptake of doxorubicin and showed synergistic inhibitory effect in combination with doxorubicin or docetaxel against multidrug-resistant cells. To perform in silico studies, we first generated homology models for human P-glycoprotein and ABCBS based on the
crystal structure of murine P-glycoprotein. Their nucleotide binding domains (NBDs) revealed the highest degrees of sequence homologies (89%-100%), indicating that ATP binding and cleavage is of crucial importance for ABC transporters. In silico studies showed a pigenin bound to the NBDs of P-glycoprotein and ABCB5. Hence, apigenin may compete with ATP for NBD-binding leading to energy depletion to fuel the transport of ABC transporter substrates. Furthermore, we performed COMPARE and hierarchical cluster analyses of transcriptome-wide mRNA expression profiles of the National Cancer Institute tumor cell line panel. Microarray-based mRNA expressions of genes of diverse biological functions significantly predicted responsiveness of tumor cells to apigenin [2].

References