Detection and Biomarkers #1

Association between dedicated breast PET and MR imaging textural features in primary invasive breast cancers

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Abstract

Introduction: Dedicated breast PET (dbPET) is an emerging PET imaging technology specially designed for imaging of the breast. DbPET imaging with [F-18]fluordeoxyglucose (FDG) is a direct measurement of active glucose metabolism that reflects tumor growth and aggressiveness. High FDG uptake is known to associate with aggressive tumors. In breast MRI, contrast kinetics with rapid early enhancement and delayed contrast washout reflects the robust angiogenic property of high-grade tumors. In the past, we observed complementary imaging patterns between DCE-MRI and FDG-dbPET of a breast cancer patient who presented with both an ER+/HER2- as well as a triple negative (TN) tumor. The concordance of MRI and PET measurements suggests that tumor angiogenic/metabolic properties are highly coupled. In this study, we performed textural analysis and evaluated the relationship between dbPET and MR imaging features in invasive breast cancers.

Materials and Methods: In an IRB-approved protocol, patients with biopsy-confirmed stage II/III locally advanced breast cancers were imaged with breast MRI (1.5 T Signa LX, GE Healthcare, WI) and dbPET (MAMMI, General Equipment and Medical Imaging SA (OncoVision), Valencia, Spain). Standard dynamic contrast-enhanced MRI was obtained using a dedicated breast coil. Patients also underwent dbPET imaging with 5 mCi of FDG at 45 min post-injection. Image texture analysis was performed using 3D Slicer with the Heterogeneity CAD plug-in module. Spearman correlation was used to assess the relationship between each dbPET and MR imaging feature. P-value <0.05 was considered statistically significant.

Results: Eight unique primary tumors from four patients with invasive breast cancers were analyzed. First-order statistics, based on a discrete pixel value and histogram analysis, yielded features such as mean intensity, uniformity, entropy, skewness and kurtosis. Second-order statistics using the gray-level (or intensity) co-occurrence matrix (GLCM) and gray-level run length method (GLRL) yielded features including contrast, energy (also known as angular second moment, pixel repetition/orderliness), entropy, homogeneity and correlation. Among the 57 imaging features obtained from first order (16) and second order statistics (32), and morphology and shapes (9), 30 features showed a statistically significant correlation between dbPET and MRI. Features measuring regional variations within the tumor (such as GLCM energy and entropy) have the strongest Spearman correlation (>0.95, p=0.003).

Conclusions: The high degree of concordance of tumor glucose metabolism and angiogenic properties allows more biologic synergy, and together confer a more invasive phenotype. Our findings support the hypothesis that angiogenic properties and glucose metabolism are highly coupled and when increased reflect greater tumor aggressiveness. This work warrants further studies with a larger cohort to verify the metabolism/angiogenicity concordance and to investigate the sensitivity of this relationship in response to treatment. Persistence of either or both may be an early marker of emerging drug resistance.

This retreat is supported in part by Puma Biotechnology, Inc., an educational grant from Daiichi Sankyo, Inc., and AstraZeneca.

Detection and Biomarkers #2 – BEST DETECTION POSTER

Initial experience of dedicated breast PET imaging of ER+ breast cancers using [F-18]fluoroestradiol

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Abstract
Introduction: Breast cancer is a heterogeneous disease encompassing distinct subtypes with variable treatment response, relapse risk and overall prognosis. The majority of breast cancers are estrogen receptor-positive (ER+). While neoadjuvant endocrine therapy trials have been proposed to better identify therapeutic approaches for ER+ breast cancer, accurate quantification of the ER biomarker is necessary to assess the primary tumor and its likelihood of response to treatment.
Dedicated breast positron emission tomography (dbPET) is an emerging technology with high spatial resolution that enables detection of sub-centimeter lesions and depiction of intratumoral heterogeneity. In this study, we report our initial experience with [F-18]fluoroestradiol (FES) dbPET in assessing ER+ primary breast cancers.

Materials and Methods: In an IRB-approved protocol, patients with biopsy-confirmed ER+ breast cancers were imaged with dbPET (MAMMI, OncoVision, Valencia, Spain) as a companion diagnostic tool to standard breast MRI. A dose of 5 mCi of FES was administered and patients were imaged in the prone position at 45 min post-injection. As part of routine clinical care, MR images were reviewed by a certified breast radiologist experienced in breast MRI. DbPET was reviewed by a radiologist specialized in nuclear imaging.

Results: Five patients with ER+ breast cancers were imaged. Patient ages ranged from 33 to 64. Two patients with infiltrating lobular carcinomas measuring up to 6.7 cm and 5.3 cm at MRI demonstrated corresponding FES tumor-to-normal maximum standard uptake value (SUVmax) ratio at 4.81 and 2.49 respectively. A third patient demonstrated multifocal FES uptake corresponding to multifocal invasive ductal carcinoma and ductal carcinoma in situ with disease foci ranging from 9-13 mm. In this patient, the more posterior disease foci seen on MRI were excluded from the field of view of dbPET. One patient demonstrated an absence of FES uptake in her 3.4 cm infiltrating ductal carcinoma, which was due to estrogen receptor blockade from the administration of tamoxifen for a fertility preservation procedure. The final patient had metastatic cervical and axillary lymphadenopathy secondary to a breast primary that was occult on mammography and MRI. FES-dbPET also showed no corresponding uptake in the ipsilateral breast, possibly due to the small size of the primary lesion and/or low tumor to background uptake ratio.

Conclusions: FES-dbPET imaging has potential as a diagnostic tool that is complementary to MRI in characterizing ER+ primary breast cancers. Limitations include variations of FES uptake in different ER+ breast cancer diseases and exclusion of posterior breast tissue near the chest wall and the axillary regions. However, FES-dbPET has high potentials for clinical utility, especially in measuring response to neoadjuvant endocrine treatment. Further development to improve the dbPET field of view and studies with a larger cohort of ER+ breast cancer patients are therefore warranted.

This retreat is supported in part by Puma Biotechnology, Inc., an educational grant from Daiichi Sankyo, Inc., and AstraZeneca.

Detection and Biomarkers #3

Diffusion-weighted MRI Improves Imaging Prediction of Response in the I-SPY 2 TRIAL

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Abstract
Diffusion weighted MRI (DWI) is a non-contrast method that characterizes water mobility and tissue cellularity by measuring the apparent diffusion coefficient (ADC), acquired during the same MRI exam as dynamic contrast-enhanced (DCE) MRI, can provide valuable distinct information about tumor response. A sub-cohort of 311 patients who had completed with investigational or control regimens in I-SPY 2 were included in this study. Each patient had four MRI exams: pre-treatment (T0), early treatment (after 3 weekly cycles of experimental drugs, T1), between regimen (T2), and pre-surgery (T3). Functional tumor volume (FTV) and ADC were measured for the whole tumor at T0, T1, and T2. Percent change of FTV (%ΔFTV) and ADC (%ΔADC) at T1 and T2 compared to T0 were analyzed as predictors of pCR. The predictive performance of %ΔFTV, %ΔADC and their combination was evaluated using a logistic regression model treating pCR as the binary outcome. Odds ratios were estimated for each 10% decrease of %ΔFTV and 10% increase of %ΔADC to reach pCR. The likelihood ratio test was used to evaluate the effect of variables in the logistic model. The statistical significance level for all testing was set at 0.05. The results of univariate analysis showed that FTV and ADC percent change from baseline are both strong predictors for pCR at both T1 and T2. However, their predictive performance varied in breast cancer subtypes defined by HR and HER2 status. In addition, area under the ROC curves (AUCs) are estimated higher at T2 than at T1. In multivariate analysis, AUC of the model combining FTV, ADC, and HR/HER2 subtype were highest at both T1 and T2. In conclusion, the addition of ADC to standard FTV may help refine the prediction of treatment response. Further improvement can be achieved by adjusting the model for breast cancer subtype. The effect of different novel agents should be considered in future studies on a larger cohort.

This retreat is supported in part by Puma Biotechnology, Inc., an educational grant from Daiichi Sankyo, Inc., and AstraZeneca.

Detection and Biomarkers #4 – BEST BIOMARKERS POSTER

Kinome rewiring reveals AURKA limits the efficacy of PI3K/mTOR-pathway inhibitors in breast cancer

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Abstract
Dysregulation of the PI3K-AKT-mTOR signaling network is a prominent feature of breast cancers. However, clinical responses to drugs targeting this pathway have been modest. We hypothesized that dynamic changes in signaling, including adaptation and feedback, limit drug efficacy. Using an unbiased quantitative chemoproteomics approach, we mapped kinome dynamics in response to inhibitors centered on this pathway and generated a kinome-response signature to identify signaling changes that correlate with drug sensitivity. By performing a systematic analysis of kinome activity profiles between drug-sensitive and resistant cells following drug treatment, we found that in multiple breast cancer cell lines spanning various subtypes and genotypes maintenance of AURKA activity was associated with drug resistance. Incomplete inhibition of AURKA was a common source of therapy failure, and combinations of PI3K, AKT or mTOR inhibitors with the AURKA inhibitor MLN8237 were highly synergistic leading to apoptosis and tumor regression in vivo. Moreover, targeting AURKA potentiates the activity of PI3K-pathway inhibitors by enabling durable and complete suppression of mTOR signaling via AKT. We identified a novel circuit whereby the PI3K-pathway regulates the abundance of its own activator through MYC-mediated transcription of AURKA, constituting a positive feedback loop that continuously activates the pathway and can drive resistance. Blocking AURKA in combination completely suppresses mTOR signaling to 4E-BP1 and S6, eliminating the feedback loop to AKT, and thus renders cells sensitive to PI3K-pathway inhibitors resulting in tumor cell death. This signaling map identifies survival factors whose presence limits the efficacy of targeted therapies and presents a new synthetic lethal opportunity to unlock the full potential of PI3K-AKT-mTOR pathway inhibitors in breast cancer.

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Detection and Biomarkers #5

Trajectory patterns of circulating tumor cells (CTC) in chemotherapy-treated metastatic breast cancer (MBC) patients predict poor clinical outcomes: CALGB 40502 (Alliance)/NCCTG N063H study

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Abstract
Background: Little is known about the dynamics of CTCs during treatment and its clinical significance. We examined the predictive utility of serial CTC analysis in ER+HER2- MBC patients (pts) treated with chemotherapy in the CALGB 40502/NCCTG N063H study, a randomized phase III trial of weekly paclitaxel compared to weekly nanoparticle albumin bound nab-paclitaxel or ixabepilone +/- bevacizumab as first-line therapy (ClinicalTrials.gov Identifier: NCT00785291, Support: U10CA180821, U10CA180882).

Methods: Of the 783 pts treated, 469 had ≥3 serial blood samples (including baseline) successfully analyzed for CTCs by CellSearchÂ® and were included in this analysis (n=2,202). Samples with ≥5 CTCs per 7.5 mLs of blood were considered CTC+. The prognostic and predictive performance of baseline CTCs (bCTC) and CTC status from baseline to cycle 2 (b2CTC) were compared to a novel latent mixture model classification based on trajectory of CTCs (tCTC). Akaike Information Criterion (AIC) was used to select the model (bCTC vs b2CTC vs tCTC) that best predicts overall survival (OS), progression-free survival (PFS), and time-to-treatment failure (TTF).

Results: 53% of the pts were CTC+ at baseline. b2CTC status changed in 36% of the pts, most of whom were CTC+CTC- (35%), and very few CTC-CTC+ (1%); the rest of the pts did not experience a change in b2CTC status (46% CTC-CTC- and 19% CTC+CTC+). Mixture model analysis revealed 4 groups of pts that show distinct tCTC patterns over the course of treatment: consitently very low/undectectable CTCs (tCTCneg, 56%), low (tCTClo, 24%), intermediate (tCTCmid, 15%), or high (tCTChi, 5%). bCTC, b2CTC, and tCTC were significantly correlated with tumor subtype (all p <0.0022) and presence of bone metastasis (all p <0.0001). Multivariate analysis showed that pts who were CTC+ at baseline, and those whose b2CTC status remained positive (CTC+CTC+) had significantly reduced OS, PFS and TTF. Pts with tCTClo, tCTCmid and tCTChi had significantly shorter OS, PFS and TTF compared to those with tCTCneg. After adjustment for potential confounders, AIC analysis revealed that the tCTC model best predicts OS and PFS, while b2CTC best predicts TTF.

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Conclusions: Analysis of CTC trajectory patterns identified pts with poor outcome who could potentially benefit from more effective treatment. Validation in independent cohorts is warranted to confirm the findings in this study.

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Detection and Biomarkers #6

Genomic and expression profiling reveals molecular heterogeneity of disseminated tumor cells in early breast cancer

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Abstract
BACKGROUND: The presence of disseminated tumor cells (DTCs) in bone marrow of early breast cancer (EBC) patients is a strong predictor of poor prognosis. We assessed heterogeneity in copy number, PIK3CA mutation, and gene expression in DTCs to shed light on the molecular biology of these cells.

METHODS: We isolated EPCAM-positive DTCs in 71 EBC patients using immunomagnetic enrichment combined with fluorescence-activated cell sorting (IE/FACS). Isolated DTCs, along with corresponding primary tumors (n=16), were subjected to genome-wide copy number profiling (n=47) by array comparative genomic hybridization, and mutation screening of the PIK3CA gene (n=53) by Sanger sequencing. The expression of 64 cancer-related genes in DTCs was analyzed by microfluidic-based multiplexed RT-QPCR (n=30). DTC expression profiles were compared with available gene expression data from circulating tumor cells (CTCs) of metastatic breast cancer patients.

RESULTS: Copy number profiles of DTCs were less aberrant and distinct from their corresponding primary tumors. PIK3CA mutations detected in 26% of DTCs were mutually exclusive to those found in matched primary tumors. Expression profiles of DTCs were distinguishable from marrow leukocytes, and displayed up-regulation of oncogenes MYC and CCNE1. Unsupervised hierarchical clustering analysis revealed two subtypes of DTCs: (1) luminal with dual epithelial-mesenchymal properties (high ESR1 and VIM/CAV1 expression), and (2) basal-like with proliferative/stem cell-like phenotype (low ESR1 and high MKI67/ALDH1A1 expression). DTCs possessed gene expression signatures that were unique from those of CTCs. ALDH1A1, CAV1 and VIM were up-regulated in DTCs relative to CTCs. ESR1/ER and ERBB2/HER2 status in DTCs vs. corresponding primary tumors showed high discordance (40% and 43%, respectively), suggesting shift in biomarker status in micrometastatic cells in the bone marrow.

CONCLUSIONS: We demonstrate the feasibility of isolation and comprehensive molecular characterization of DTCs from bone marrow of EBC patients. Comparative genomic analysis suggests that DTCs disseminate early and acquire genomic aberrations independently of the primary tumor. Expression profiling revealed two distinct subpopulations of DTCs. Validation in larger cohorts is needed to confirm the presence of these molecular subtypes and to evaluate their biological and clinical significance.

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Detection and Biomarkers #7

Detection of circulating tumor cells in blood and disseminated tumor cells in bone marrow at the time of definitive surgery identifies breast cancer patients with long-term risk of recurrence and death

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Abstract
Background: We examined the prognostic significance of circulating tumor cells (CTCs) and disseminated tumor cells (DTCs) detected at the time of definitive breast surgery in treatment-naive primary breast cancer patients.

Methods: Blood and bone marrow samples were collected immediately prior to surgery. DTCs (n=584) were enumerated via immunomagnetic enrichment and flow cytometry (IE/FC). CTCs were enumerated either by IE/FC (n=288) or CellSearch (n=380). Survival analyses were performed to determine associations between CTCs and DTCs with clinical endpoints: recurrence-free (RFS), distant recurrence-free (DRFS), breast cancer-specific (BCSS) and overall survival (OS). The median follow-up for the study was 8.9 years (range: 0.71-18.9).

Results: DTC detection rates ($p<0.001$) and mean concentration ($p<0.001$) were significantly higher than those of CTCs. CTC/DTC results did not show correlation with standard clinicopathologic features. CTCs detected by CellSearch showed significant association with DRFS (HR 3.13, p=0.0107). CTC detected by IE/FC were significantly associated with DRFS (HR 2.08, p=0.0255), BCSS (HR=2.72, p=0.0133), but not OS (HR=1.63, p=0.0592) and RFS (HR=1.18; p=0.5618). DTC detection was not significantly associated with survival.

Conclusions: We demonstrate the feasibility of IE/FC for quantitative evaluation of CTC and DTC levels in blood and bone marrow. Detection of CTCs by IE/FC at the time of definitive surgery is associated with increased long-term risk of distant recurrence and disease-specific death.

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Detection and Biomarkers #8

Chromosomal Instability as a Cancer Biomarker for Predicting Chemo and Radio-Sensitity in Cancer Patients

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Abstract
Chromosomal instability (CIN) is a cancer hallmark that contributes to various malignant properties. CIN presents a major challenge to cancer management due to its role in tumor heterogeneity and drug resistance. Aberrant centromere function causes CIN through chromosome missegregation in experimental systems. Centromere is regulated epigenetically by replenishing nucleosomes containing CENP-A histone variant at the centromere in each cell cycle. Currently, the role and mechanisms of centromere misregulation in tumorigenesis are unknown and limit the medical application. Here, I present evidence that key centromere proteins are progressively overexpressed during tumorigenesis. We hypothesize that overexpression of centromere proteins causes centromere misregulation and CIN. We developed a CES (Centromere and kinetochore gene Expression Score) signature that quantitates overall misregulation of 14 key centromere structural protein genes in cancers. High tumor CES values strongly correlate with increased copy number alterations and mutation frequencies, and prognosticate poor patient survival for breast cancers and other cancer types. High CES values also signify high levels of genomic instability that sensitize cancer cells to additional genotoxicity. Furthermore, the CES signature forecasts patient response to adjuvant chemo- or radiotherapy for breast and lung cancers. These findings are expected to help address the over-treatment problem prevalent in cancer treatments. Our approach validates the critical importance of incorporating basic knowledge of chromosome segregation pathways into cancer research and clinical applications.

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Detection and Biomarkers #9

A Microfabricated Fluorescence Imager for Intraoperative Margin Imaging

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Abstract
Cancer treatment faces the challenge of identifying small clusters of residual tumor cells in the resection cavity after lumpectomy or mastectomy. Despite the introduction of targeted fluorescent probes to guide cancer surgeries, large, bulky, optical components restrict the ability of fluorescence imaging devices to detect small clusters of tumor cells in the complex surgical cavity. This is particularly problematic in breast cancer, where microscopic residual disease can double the rate of cancer returning, from 15% to 30% over 15 years, affecting a striking 37,500 women annually. We have developed a small millimeter-scale contact fluorescence image sensor that does away with conventional optics in favor of a microfabricated optical structures and a 15 μm thin optical wavelength filter for detecting residual cancer tissue in vivo. The sensor has a customizable planar form factor, with a total thickness of only 0.8 mm, allowing it to be integrated with surgical instruments and used intraoperatively. Using a custom fluorescent probe combining a fluorescent dye, IR700DX, with a targeted antibody, Trastuzumab, we label and visualize breast tissue in *in vivo* mouse models of breast cancer. When imaging tumor-bearing mice injected with the probe, HER2+ breast cancer tissue intensity is 3.8±0.8 times brighter than other tissue. Excised cancer tumors and residual cancer attached to healthy tissue are imaged using the custom image sensor. Residual cancer tissue can be detected in real-time and is imaged with a high signal to noise ratio >100 using an integration time of only 40 ms. Future work will extend this to lymph node imaging.

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Detection and Biomarkers #10

Identifying Disease Signatures of Taxol Non-Responders In Breast Cancer

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Abstract
Aims: Currently, a large number of patients given neoadjuvant taxanes do not achieve pathological complete response (pCR) at the end of treatment. Given these low response rates, it is valuable to distinguish the molecular expressions between responders and non-responders in order to find therapies that target the abnormally regulated genes unique to taxane non-responders. In this study, we compared gene expression signatures of breast cancer patients treated with taxanes who did or did not achieve a pCR.

Methods: We identified breast tumor gene expression datasets from the Gene Expression Omnibus database that involved patients treated at least with taxane, paclitaxel, or docetaxel. The nine identified datasets were further stratified by estrogen receptor (ER), progesterone receptor (PR), and HER2 status into 11 groups. Using the Significance Analysis of Microarrays model, we compared tumor gene expression of responders and non-responders to taxol-based therapy. Heat maps were generated to visualize gene signatures within datasets and forest plots were generated to visualize expression of single genes across datasets.

Results: Of the 836 patients examined in this analysis, only 219 (26.2%) achieved a pCR after neoadjuvant chemotherapy with taxane. With or without stratification by hormone receptor status, there was no clear distinction between overall tumor expressions of responders and non-responders when visualized using histogram clustering. We then examined single gene expressions across datasets to find commonly overexpressed or under expressed genes. We found that eight genes (H1VEP1, VWF, DNAJB14, INHBA, CCDC25, RNG2, MAP2K7, and RNF126P1) were overexpressed in non-responder populations compared to responder populations in 4 out of 11 groups ($q<0.05$).

Conclusions: The difference in molecular signatures of responders vs. non-responders did not become more clear after stratification by hormone receptor status; this shows that more nuanced subtyping of breast cancer is needed to gain insight into molecular differences between tumors of responders and non-responders. The fact that we can find commonly overexpressed genes in 4 out of 11 groups is also consistent with our observation of the heterogeneity of tumor expression within the responder and non-responder groups. We will further explore the role of the 8 genes from our analysis in taxane resistance and investigate their potential as candidates for targeted therapy.

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Detection and Biomarkers #11

A chemical-genetic interaction map for precision therapies in breast and ovarian cancers

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Abstract
Nearly every cancer patient, especially those with highly lethal and aggressive tumor types, is treated with cytotoxic chemotherapy. Currently, the selection of appropriate chemotherapy is based on the average response over a large number of patients. This belies our understanding of DNA repair which has shown that the inability of a tumor cell to properly repair particular types of DNA damage has a dramatic influence on cell survival. Here, we report the generation of a quantitative chemical-genetic interaction map to chart the influence of knockdown of 625 genes on sensitivity to nearly all FDA approved chemotherapeutic agents in breast epithelial cells. This quantitative map is predictive of interactions maintained in cancer cell lines and can be used to identify new cancer-associated DNA repair factors, predict cancer cell line responses to therapy and prioritize drug combinations. We identify that GPBP1 loss in breast and ovarian cancer confers resistance to cisplatin and PARP inhibitors through the regulation of genes involved in homologous recombination. This map may help navigate patient genomic data and optimize chemotherapeutic regimens by delineating factors involved in the response to specific types of DNA damage.

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Detection and Biomarkers #12

Development of xCT specific antibodies by phage display

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Abstract
xCT is the sole transporter for cellular acquisition of environmental cystine. Expressed by few normal cells, xCT is transcriptionally induced during oxidative stress and amino acid deprivation. In preclinical studies using cell lines derived from multiple tumor types, xCT inhibition can severely attenuate growth or induce death via apoptosis, necrosis, and iron dependent Reactive Oxygen Species (ROS) increases (ferroptosis) - in vitro and in xenograft. Disruption of the trimerization partner protein, CD44, inhibits tumor metastasis and viability of tumor stem cells. Finally, inhibition can sensitize to chemotherapeutics and radiation therapy. These facts indicate that xCT should be treated as a serious therapeutic target in oncology, but publications using nonspecific reagents have muddied the literature and prevented significant progress towards this goal. A recently published and exhaustive analysis of all commercially available antisera concluded that in fact no truly xCT-specific reagents exist.

Our objective is to obtain xCT specific antibodies using the phage display technology. We use 3 different selection strategies depending on the future use of the isolated antibodies. These include: 1) Develop antibodies that specifically identify xCT in histochemical analyses of Formalin Fixed, Paraffin Embedded (FFPE) sections of normal and pathological human tissue specimens and immunofluorescence of cultured cell lines. 2) Generate antibodies that specifically react with extracellular portions of xCT in live cells for future use in live assays including Fluorescence Activated Cell Sorting (FACS), functional inhibition of (cystine:glutamate (C:E) exchange; and regulation of cell phenotypes such as proliferation and death. 3) Isolate antibodies that react with a specific region of xCT for future use in western blot.

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Detection and Biomarkers #13

Sequencing of exons 9 and 20 of PIK3CA gene reveal recurrent mutations in breast circulating tumor cells

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Abstract

Background: The phosphatidylinositol-3-kinases (PI3Ks) are essential for regulating various normal cellular functions, as well as the cancer process. Sequencing studies have shown that the catalytic alpha subunit in PI3K (or PIK3CA) is frequently mutated in many invasive carcinomas, including 36% of primary breast cancers. More than 80% of point mutations in PIK3CA are found in the three mutational hotspots, i.e., codons 542 and 545 in exon 9 and codon 1047 in exon 20.

Methods: Serial blood samples were drawn from 19 metastatic breast cancer patients enrolled in the Cancer and Leukemia Group B (CALGB) 40502 study. Blood was processed using a two-step process involving immunomagnetic enrichment followed by fluorescence-activated cell sorting (IE/FACS) to isolated circulating tumor cells (CTCs; n=139) along with matched leukocyte samples (n=20). Genomic DNA from small pool of isolated cells (~10-20) was subjected to whole genome amplification. PCR primers were designed to amplify the regions containing the complete exon 9 and exon 20 of the PIK3CA gene. The amplicons were sequenced using the Sanger method to screen for mutations not only in the hotspot regions but also across the whole exons. Forward and reverse sequencing reactions were performed to confirm the mutations found.

Results: Sequencing analysis revealed that 8.2% (8/97), 15.5% (15/97), 3.1% (3/97) of patients had mutations in PIK3CA exons 9, 20 and both, respectively. Among 139 CTC samples analyzed, the most common mutations observed were in the mutational hotspots (e.g., E545K/A in exon 9 and H1047R in exon 20). Novel mutations not previously reported in the Catalogue of Somatic Mutations in Cancer and by The Cancer Genome Atlas were also detected (e.g., exon 9: S553G; exon 20: Y1021D). In some patients, the same mutations were observed over time while others displayed different mutations across time points.

Conclusions: A noticeable mutation rate was detected in PIK3CA of breast CTCs. The clinical relevance of PIK3CA mutations found in CTCs has yet to demonstrate.

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Detection and Biomarkers #14

Correction of image distortion and gradient nonlinearity in DTI of breast cancer

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Abstract
Introduction: Diffusion tensor imaging (DTI) can provide information on tissue cellularity and microstructure, and a DTI-derived metric, the apparent diffusion coefficient (ADC), has shown promise as a biomarker of early breast cancer treatment response. However, one limitation of breast DTI is that standard echo-planar imaging (EPI) suffers from geometric distortions resulting from the susceptibility-induced variation in the B0 field and eddy-currents (ECs) from diffusion-encoding gradients. This distortion occurs in addition to gradient non-linearity (GN) effects, however the effects on the quantitative accuracy of breast DTI have not been characterized. In this work, a robust reverse phase gradient (RPG) method for correcting geometric distortions was implemented, and the individual and combined effects of geometric distortion, EC, distortion and bias from GN on two DTI-derived metrics, ADC and fractional anisotropy (FA), were evaluated in a phantom and in malignant breast tumors.

Methods: DTI and corresponding RPG data were acquired on a 1.5T GE MRI scanner: 1) from bilateral ice-water phantoms with known ADC and FA values and 2) in12 patients with biopsy-confirmed, locally-advanced breast cancer enrolled in an institutional review board-approved, HIPAA compliant, clinical trial. The effects of adding RPG and eddy current corrections to GN correction on ADC and FA values from the phantoms and from breast tumor regions of interest were calculated.

Results: The addition of RPG and EC correction to GN correction resulted in improved agreement between phantom ADC and FA measurements calculated from DTI and the expected known values. For in-vivo DTI measurements, the additional corrections had similar effects on tumor ADC and FA values as seen for the phantom, with a significant effect found on FA values.

Discussion: The changes in tumor FA and ADC after GN correction measured in this work were analogous to the phantom measurements and consistent with previous findings. This work showed that RPG and EC correction, when applied in addition to GN correction, yielded further improvement in accuracy of ADC and FA measurements in a phantom. The analogous effects of RPG and EC correction found for DTI breast tumor measurements suggest that these corrections may improve quantification of in-vivo DTI measurements.

This retreat is supported in part by Puma Biotechnology, Inc., an educational grant from Daiichi Sankyo, Inc., and AstraZeneca.

Detection and Biomarkers #15

Adjunctive Role of Quantitative Background Parenchymal Enhancement for Predicting Response to Chemotherapy

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Abstract
Purpose: We investigated how background parenchymal enhancement (BPE) may additively improve an MR tumor model for prediction of non-pathologic complete response (non-PCR) patients in the neoadjuvant setting. This abstract represents an update of our data from 2017, using an expanded set of patients.

Materials and Methods: 162 patients with Stage 2/3 breast cancer (46 PCR, 116 non-PCR) were evaluated with serial breast MRIs to assess neoadjuvant response. Dynamic contrast-enhanced (DCE) MRI scans with 80-100 second acquisition intervals were obtained for each patient over multiple time points: pre-treatment (V1), after 1 month of neoadjuvant therapy (V2), after 3 months (V3), and pre-surgery (V4). MRI segmentation and tissue classification was performed on the contralateral breast using fuzzy c-means clustering. BPE was calculated as the average enhancement of all tissue voxels, defined as (S1 – S0)/S0 (S0: signal intensity prior to injection; S1: signal intensity at first postcontrast acquisition). Logistic regression models were created using relative change of BPE as predictors at each time point. Diagnostic accuracy was assessed by focusing on the percentage of non-PCR patients identified (or sensitivity) while constraining to a low PCR misclassification rate (1-specificity) set to less than 10%. This constrained misclassification model is being developed for an eventual clinical strategy to redirect therapy in non-responsive patient.

Results: Using the univariate model, BPE change to month 3 (V3/V1) identified 26% of non-PCR patients (95% CI: 9 to 42%), and BPE change to pre-surgery (V4/V1) identified 18% of non-PCR patients (95% CI: 5 to 31%). Using a multivariate tumor model excluding BPE, 19% of non-PCR patients were identified (95% CI: 0 to 37%). Addition of BPE change at month 3 (V3/V1) identified 33% of non-PCR patients (95% CI: 7 to 58%), and BPE change at pre-surgery (V4/V1) identified 29% of non-PCR patients (95% CI: 8 to 49%).

Conclusion: BPE identified 18-26% of non-PCR patients independent of tumor factors while maintaining a low misclassification of PCR patients. Addition of BPE to tumor model improves prediction from 19% to 33% of non-PCR patients, although with overlap of confidence intervals. Future work will continue to verify results in a larger cohort.

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Therapeutics and Clinical Trials #1

xCT inhibition Sensitizes Tumors to gamma-Radiation via Glutathione Reduction

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Abstract
Approximately 1 million US cancer patients receive radiation therapy (RT) each year, with treatment duration limited by collateral damage to normal tissues. Tumor-specific sensitization could reduce this damage by allowing the use of lower radiation doses, vastly improving the quality of patient care. This remains a longstanding, largely unrealized therapeutic goal. The cystine:glutamate exchanger, xCT is expressed on poor prognosis subsets of most solid tumors, but not on most normal cells. xCT provides cells with environmental cysteine when the need for thiols outstrip a cell's endogenous synthetic resources. Glutathione is a key thiol used to control reactive oxygen species, which are therapeutic effectors of RT. We tested whether xCT inhibition would specifically sensitize xCT+ tumor cells to ionizing radiation. We found that pretreatment with the xCT inhibitor erastin reduced cellular glutathione levels, increased endogenous ROS, and potently sensitized xCT+ but not xCT- cells to subsequent ionizing radiation, in vitro and in xenograft. Similarly, targeted gene inactivation also sensitized cells, and both modes of sensitization were overcome by glutathione supplementation in vitro. Mechanistically, sensitization prolongs DNA damage signaling, enhances cell death, and increases genome instability, revealing an unforeseen role for cysteine access in the maintenance of genome integrity. We conclude that development of an xCT-specific therapeutic would provide tumor-specific sensitization to RT, sparing normal xCT- tissues, both quiescent and proliferating, producing far fewer side.

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Therapeutics and Clinical Trials #2

Myeloid reprogramming determines metastatic progression of breast cancer

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Abstract
Metastasis is the main cause of mortality related to breast cancer. However, metastatic behaviour varies significantly among breast cancer patients. While prior studies have focused on, and identified factors that promote metastatic progression, mechanisms explaining why some breast cancer patients never develop metastatic breast cancer are largely lacking. Here we show a crosstalk between the primary tumour and innate immune response, acting together to control metastatic progression. Tumour-derived CCL2 recruits CCR2+ monocytes. The CCR2+ monocytes then enhance the killing capacity of neutrophils. Transcriptomic analysis revealed that cellular pathways associated with tumoricidal capacity were upregulated in both CCR2+ monocytes and neutrophils infiltrating the metastatic site in a CCL2high context compared to a CCL2low context. These findings provide an explanation as to why the majority of patients do not develop metastatic breast cancer and could underlie the development of novel immunotherapeutic target molecules that activate and augment the function of CCR2+ monocytes and neutrophils.

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Therapeutics and Clinical Trials #3

Preference-Tolerant Randomized Trial of Risk-Based vs. Annual Breast Cancer screening: WISDOM study in progress

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Abstract
Purpose: Women Informed to Screen Depending on Measures of risk (WISDOM) trial is a pragmatic study comparing two real world approaches to clinical care for breast screening: annual screening versus personalized screening. The novelty of the personalized arm of the study is that we are combining known risk factors (age, family history, history of breast disease, ethnicity, BIRADS breast density, and genetics) into a single risk assessment model. All components of the model have been tested and established, but have never been used jointly. The goal of the WISDOM study is to examine the effectiveness of personalized breast cancer screening and to bring objective recommendations to the current mammography screening debate.
Methods: The WISDOM trial will enroll 100,000 women with a preference-tolerant design that will determine if risk-based screening vs. annual screening, is as safe, less morbid, enables prevention, and is preferred by women. Women 40 - 74 years of age with no history of breast cancer or DCIS, and no previous double mastectomy can join the study from the WISDOM Study website (wisdomstudy.org). All participants sign up, elect randomization or self-select the study arm, provide electronic consent using DocuSign (eConsent), and sign a Medical Release Form. For all participants, 5-year risk of developing breast cancer is calculated according to the Breast Cancer Screening Consortium (BCSC) model. For participants in the personalized arm, the overall 5-year risk BCSC score is combined with a Polygenic Risk Score, based on a genetic test including mutations in 9 genes (BRCA1, BRCA2, TP53, PTEN, STK11, CDH1, ATM, PALB2, and CHEK2) and a panel of 75 common single nucleotide polymorphisms known to increase breast cancer risk. Risk stratification will determine frequency of screening. The study is registered on ClinicalTrials.gov as NCT02620852.
Results: The WISDOM study is live at all UC medical centers and recruitment is open to all eligible women in California and at Sanford Health medical centers. We are partnering with health insurance companies and self-insured companies to reach our recruitment goal. Supported by the Patient-Centered Outcomes Research Institute (PCORI), PCS-1402-10749 to L.J.E.

Therapeutics and Clinical Trials #4 – BEST POSTER

BluePrint Luminal subtype predicts non-response to HER2-targeted therapies in HR+/HER2+ I-SPY 2 breast cancer patients

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Abstract

Background: Previous studies suggest that within the triple positive HR+HER2+ subtype, patients classified as BluePrint (BP) Luminal subtype are more responsive to pertuzumab and trastuzumab (P/H) as opposed to trastuzumab (H) alone. In the I-SPY2 TRIAL, HER2-targeted treatment arms include H, P/H, neratinib (N), and T-DM1/Pertuzumab (T-DM1/P); and patients were classified by BP molecular subtyping in addition to conventional receptors. We evaluated BP subtype as a predictor of response in HR+HER2+ patients and assessed pathway differences between BP molecular subtypes.

Methods: 125 HR+HER2+ patients (N: 42; P/H: 29, T-DM1/P: 35; H: 19) with pre-treatment Agilent microarrays and BP subtype assignments were considered. We assess association between BP subtypes and pCR using Fisher's exact test. To identify genes associated with BP Luminal vs. BP HER2 subtype, we (1) apply a Wilcoxon rank sum test and (2) fit a logistic model, with the Benjamini-Hochberg (BH) multiple testing correction (BH $p<0.05$ from both tests). We then perform pathway enrichment analysis using DAVID. Our study is exploratory and does not adjust for multiplicities of other biomarkers in the trial outside this study.

Results: Of the 125 HR+HER2+ patients, 71 were BP HER2-type and 50 were BP Luminal-type. The distribution of pCR rates in BP Luminal/ HER2 subtypes are as follows:

	N (n = 40)	P/H (n = 29)	T-DM1/P (n = 34)	H (n = 18)	All arms (n = 121)
BP Luminal (n = 50)	0 / 9 (0 %)	1 / 12 (8.3%)	2 / 16 (12.5%)	1 / 13 (7.7%)	4 / 50 (8%)
BP HER2 (n = 71)	12 / 31 (38.7%)	13 / 17 (76.5%)	15 / 18 (83.3%)	1 / 5 (20%)	41 / 71 (57.7%)
Odds Ratio	6.1 p = 0.127	30.2 p = 0.000484	29.6 p = 8.60E-05	2.8 p = 0.490	15.3 p = 1.04E-08

In a whole transcriptome analysis, 1725 genes were differentially expressed. By DAVID enrichment analysis, the most significantly enriched pathways were related to immune function, with the BP HER2 subgroup showing higher expression.

Conclusion: Our analysis suggests that HR+HER2+ BP Luminal subtype is associated with lower response rates to HER2-targeted agents, including P/H, and may need an alternative strategy. BP HER2 subtype appears associated with higher expression of immune-related genes, relative to BP Luminal; and suggests that immune signaling may contribute to HER2-targeted therapy sensitivity.

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Epidemiology and Population Science #1

Combining genetic variants and clinical risk factors for breast cancer risk stratification in a population-based trial (WISDOM Study)

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Abstract
Background: Genetic variants known as single nucleotide polymorphisms (SNPs) are promising markers of breast cancer risk, but their role in guiding screening and prevention decisions has not been established. The WISDOM (Women Informed to Screen Depending on Measures of Risk) Study is an ongoing trial comparing the safety, efficacy, cost, and patient acceptability of personalized versus annual breast cancer screening. Personalized screening recommendations are based on sequencing of hereditary breast cancer genes and a 5-year risk estimate from the Breast Cancer Surveillance Consortium (BCSC) risk model modified by a polygenic risk score (PRS). WISDOM represents the first use of a PRS to prospectively modify risk estimates and allows comparison of risk model performance in a population setting. Thus, we compared the risk estimates generated by the BCSC model alone and the BCSC model modified by the PRS (BCSC-PRS).

Methods: For participants in the personalized arm, 5-year risk estimates were generated using the BCSC model. The PRS was calculated using genotypes of 75 SNPs and used to modify the BCSC estimate in a Bayesian manner. Using paired statistical tests, we compared the distributions of BCSC and BCSC-PRS risk estimates around a low-risk (<1%) and moderately high-risk (>=3%) threshold, with the latter corresponding to the threshold above which some guidelines suggest considering chemoprevention.

Results: We analyzed 2,060 participants in the personalized arm of WISDOM who have completed risk assessment. The median 5-year risk was 1.5% (IQR1.0-2.1%) using the BCSC model and 1.4% (IQR 0.8-2.5%) using the BCSC-PRS model. Compared with the BCSC model, BCSC-PRS classified more women below the low-risk threshold, 34% vs 20% ($p < 0.001$). BCSC-PRS also classified more women above the moderately high-risk (>=3%) threshold, 18% vs 11% ($p < 0.001$).

Conclusions: Adding a PRS to the BCSC model categorized more women below the low-risk threshold and above the moderately high-risk threshold. Follow-up with incident breast cancer data is needed to determine whether the reclassification provided by the PRS improves risk stratification and clinical outcomes. However, our findings suggest that incorporating genetic variants into a validated clinical model is feasible and could enhance risk prediction.

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Epidemiology and Population Science #2 – BEST POSTER

Tailoring screening to individual risk decreases the cost and improves the value of screening

Kenneth Wimmer (UCSF), Martin Eklund (Karolinska Institutet), Karyn DiGiorgio (Quantum Leap Health Collaborative), Allison Stover Fiscalini (UCSF), Marcus Thygeson (Formerly Chief Health Officer, Blue Shield of California), Arash Naeim (UCLA), Diane Heditsian, Wisdom Study and Athena Breast Health Network Investigators and Advocate Partners, Laura Esserman (UCSF)

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Abstract
Introduction: Health care spending rose from 5% to 17.8% of GDP between 1960 and 2015. Clinicians and researchers must engage in increasing health care value – better outcomes at less cost. Personalized screening is one such opportunity. The Patient Centered Outcomes Research Institute recently funded WISDOM (Women Informed to Screen Depending On Measures of risk), a randomized trial to tests the safety and efficacy of basing starting age, stopping age, frequency and modality of breast cancer screening on individual risk (Clinical Trials Identifier NCT02620852). The personalized arm of WISDOM integrates genetic testing into the risk algorithm. Funding for the clinical services of WISDOM (genetic test, risk assessment, high-risk counseling) are expected to be covered (health plans, insurers). Risk determines the frequency, time to initiate screening and drives cost of downstream screening services. The cost of genetic testing is now less than $250, comparable to a mammogram. The WISDOM study model brings payers, policy makers, provider, technology, and advocate partners together to generate evidence to see if risk based screening is as safe, less morbid, preferred by women, promotes prevention, and has greater health care value. Health plans need to know the value proposition, thus we evaluated financial implications of coverage for risk-based screening.

Methods: A model was developed to compare costs and benefits of risk-based vs. current screening practices from the perspective of a health plan. Modeled cohorts resembled a screening population with risk determining screening interval for the risk-based model, and average time between mammograms determining the interval for the model of current screening practices. Model parameters were gathered from published literature, national databases, early findings from WISDOM and health plan claims data. Sensitivity analysis was performed on all parameters, including costs of clinical services, screening rates, and health plan turnover. The clinical services specific to WISDOM use a fixed-fee schedule, and not varied in the model. All other costs were conservative, based on Medicare rates and published literature.

Results: We estimated that over five years, risk-based screening is at worst cost neutral with potential for savings of up to $215 per participant. Based on current trial enrollment, we estimated that 30 per 1,000 health plan enrollees would join, resulting in an upfront cost of $6,000 for WISDOM-specific services, primarily the genetic test, and $600 in ongoing costs after Year 1. However, the health plan would save on mammogram and work up costs as participants would receive an average of 2-3 fewer mammograms over five years. Savings are sensitive to the age of participants, cost of mammograms, and savings increase over time. Per participant, five-year savings of $300 and $35 for those aged 40-49 and 65-74 respectively, and increased costs of $30 for those aged 50-64. Overall, an upfront investment of $6,000 per 1,000 health plan enrollees (30 participants) yields $3,800 in five-year savings.

Conclusion: Personalized screening could provide cost savings and has the potential to increase health care value. Enrollment in the Wisdom study is ongoing and results will be reported in 5 years.

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Epidemiology and Population Science #3

Catalyzing Advocate Engagement To Propel Convergent Innovation

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Abstract
What happens when you mix the foundations of mechanics with advocacy? Looking back, it turns out that in a shared quest for exciting scientific frontiers beyond genomics, Bay Area physical scientists, clinical researchers, and advocates worked in dynamic symbiotic relationships to accelerate innovative and needed paradigm shifts in cancer research. For example, focusing on the mechanobiology of tumor progression in breast cancer, advocates (Susan Samson, and Carole Baas) shared the convergence goals of researchers (Valerie Weaver, Jan Liphardt, Irene Acerbi) and clinicians (Hope Rugo) in determining how tumor microenvironments regulate cancer initiation and behavior through interactions among cell types (e.g., initiated cells, activated stromal cells, and components of the extracellular matrix).

We wish to emphasize that the role played by advocates in spurring innovation in physical sciences and oncology research did not happen by chance. This concept came from a guiding framework of Science Advocate Engagements (SAE's) focusing on organizational foundations, including leadership commitment to change and transdisciplinary levers for change, as well as strategic inputs promoting shared governance, bidirectional collaboration, advocacy inclusion, and the prioritization of the development, sharing, and translation of research addressing the questions of importance to patients. Welcoming advocacy inquiries regarding the testing of novel non-traditional physical sciences based approaches to conceptualize, identify, and study the heterogeneity of breast cancer, including the misregulation of multiple pathways related to cell differentiation, cell cycle control, apoptosis, angiogenesis, and the development of metastasis, the Weaver lab serves as a successful example of interdisciplinary and cross sector team engagement in mechanobiology settings.

Highlighting the advances, challenges, and acceptance of advocates as credible stakeholders, we offer recommendations for the ways advocates offer insights/perspectives regarding innovative mechanics-directed clinical interventions that emphasize the importance of the physical organization of cell-to-cell contacts, tissue architecture, tumor microenvironments, and mechanical properties in the response to therapy. Additionally, our specific focus on breast density, biomarker discovery, and evolving therapeutics suggests that catalyzing advocate engagement in convergent science settings may help propel and enhance research as well as translational opportunities.

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Epidemiology and Population Science #4

Deep learning methods aid in predicting risk of interval cancer

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Abstract
Purpose: The purpose of this study was to apply deep learning methods to a dataset of women who later experienced either screening detected or interval cancers and determine if it aids in classifying risk of interval cancer compared to using BI-RADS density.

Materials and Methods: Full-field digital screening mammograms acquired in our clinics were reviewed from 2006-2015 and patients were identified that were later diagnosed with screen detected and interval cancers. A deep learning model (ResNet50) was trained on this dataset with the goal to classify between interval and

screen detected cancers. The initial model was initially trained on ImageNet images (a large set of a variety of images used in computer vision) and the final fully connected layers were retrained. Prediction loss and accuracy were calculated using this deep learning architecture. An additional measure of prediction accuracy, the receiver operating characteristic (ROC), was computed and compared to predictions using BI-RADS density.

Results: 182 interval and 173 screening-detected cancers were found in our study group. The area under the ROC, where 0.5 is equal to accuracy of a coin flip and 1 is perfect accuracy), improved from 0.65 using only BI-RADS density to 0.84 using the ResNet50 deep learning model and BI-RADS density.

Conclusions: We conclude that deep learning methods are able to identify risk factors for interval cancer not contained in BI-RADS density alone. These deep learning methods may be useful in identifying individuals at risk of interval cancer and allow for development of automated methods that can aid radiologists in identifying women at risk of interval cancer and suggesting supplemental screening.

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Molecular and Cellular Biology #1

Mechanical cues potentiate hypoxic signaling and metastasis in breast cancer

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Abstract
Intratumoral stiffness and hypoxia are driving forces of tumor progression. Aberrant dense tumor stroma and increased mechanical signaling are known to affect oxygen availability and subsequent hypoxic signaling. However, underlying molecular mechanisms through which stromal mechanics regulate hypoxic signaling and tumor progression remain elusive. Here, we demonstrate that increased stromal stiffness modulates breast cancer aggression and metastasis, through reduction of vascularity and increased activity of HIF1a. Inhibition of Lysyl oxidase (LOX) using beta-aminopropionitrile (BAPN) attenuated tumor fibrosis, intratumoral stiffness, lung metastasis and the number of circulating tumor cells. Moreover, LOX inhibition increased tumor vascularity and reduced Hif1a activation. Enhanced clustering of a beta1-integrin mutant (V737N substitution) mimicked a stiff fibrotic phenotype by affecting metastasis and Hif1a stabilization. Finally, independent of LOX, stiffening of the tumor stroma increased tumor growth and aggression in a Hif1a-dependent fashion. Taken together, we demonstrated that tissue stiffness and fibrosis drive breast cancer aggression by hampering tumor vascularization and activation of Hif1a signaling.

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Molecular and Cellular Biology #2

Breast Cancer and the Human Microbiome

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Abstract
The human body harbors ten times more bacterial cells than human cells â€“ a stunning figure that suggests a likely dynamic between our bodies and the bacteria we carry, both in health and disease. In this study, we characterized and compared the gut and oral microbiomes from women with invasive breast cancer, women with ductal carcinoma in situ (DCIS), and healthy women. Samples were collected prior to any systemic therapy to avoid therapy-associated effects on the microbiomes studied. Kits containing materials for collecting oral and stool swab samples were distributed to patients for self-collection. DNA was isolated from these samples and bacterial 16S rRNA was PCR amplified and sequenced. Based on the sequencing results, bacterial taxa present in the samples were enumerated. In our analyses, we looked at microbial diversity and differential relative abundance of bacterial taxa across the three cohorts. We found distinct variation in microbial composition of the gut and oral microbiomes according to disease state. Oral and gut microbial diversity at various taxa levels were assessed using Shannon and Simpson diversity indices. The oral microbiome did not show any significant difference in microbial diversity across the three cohorts. In the gut microbiome, the invasive cohort showed a significant decrease in microbial diversity when compared to the healthy cohort. Differences in phylogenetic and relative abundance of bacterial taxa across the three cohorts were measured using a T-test analysis with a p value less than 0.05 considered significant. In the oral microbiome, there was no significant difference in the relative abundance of bacteria across the three cohorts. In the gut microbiome, there were significant differences in the relative abundance of bacteria within each cohort on the genus level. The genus Fusicanterbacter was significantly reduced while the genus Bacteroides was enhanced in the gut microbiomes of women with invasive breast cancer and DCIS when compared to the gut microbiomes of healthy women. Understanding how gut and oral microbiomes relate to breast cancer may open up new opportunities for the development of novel markers for early detection (or markers of susceptibility) as well as new strategies for prevention and/or treatment.

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Molecular and Cellular Biology #3

Functional imaging platform to monitor progression and response to radiation therapy in pre-clinical models of breast cancer brain metastases

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Abstract
Radiotherapy (RT) is administered to brain metastases using a high single dose (radio-surgery) or with whole-brain radiation, according to the number of metastatic foci at the time of discovery. We have previously shown that radiation induces activation of TGFbeta a cytokine whose activity is tightly regulated in normal tissue but deregulated in the tumor microenvironment. Our prior work shows that TGFbeta signaling compromises the efficacy of RT by both endorsing an effective DNA damage response, as well as through the suppression of immune response in different cancer models. Our ultimate goal is to develop a non-invasive functional imaging to monitor TGFbeta activity to determine whether activity in irradiated brain metastases correlates with outcome.

METHODS: For these studies, we used luciferase-expressing, brain-adapted 4T1 cells (BrA4T1), a model of triple-negative breast cancer, and stereotactic injection into syngeneic mouse brains. The Small Animal Radiation Research Platform (SARRP) at Mt Zion was used to perform image-guided dose delivery (1x14Gy, 3x8Gy, 5x6Gy)to intracranial tumors. A multi-modal functional imaging approach was used to monitor tumor growth and response to different radiotherapy protocols.

RESULTS: The growth of tumors was measured using bioluminescence. RT treatment planning was based on computerized tomography (CT) using the SARRP. The characteristics of BrA4T1 tumors were imaged using diffusion weight imaging magnetic resonance imaging. We determined metabolic changes within the tumor using FDG-PET-CT and hyper-polarized 13-C pyruvate. Fresolimumab, a humanized monoclonal antibody that recognizes the active form of TGFbeta, was labeled with 89Zr to monitor the activity of this cytokine in situ.

CONCLUSIONS: Pre-clinical use of multi-modal functional imaging platform will stimulate the development of agents to assess functional characteristics of cancers that may predict response to treatment with RT as well as combinations with immunotherapy.

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Molecular and Cellular Biology #4

The conditional requirement for HER3 in HER2-amplified breast cancers

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Abstract
The amplification and overexpression of HER2 underlies the pathogenesis of a large subset of breast, gastric, and esophageal cancers as well as smaller subsets of many other types of cancer. A substantial body of work has confirmed that overactive HER2 signaling is etiologically linked with the pathogenesis, progression and metastasis of these cancers.

HER3, the preferred heterodimerization partner of HER2, is known to be critical for HER2-amplified tumorigenesis, and this has been thought to be due to its unique ability to activate PI3K/Akt pathway. This has fueled efforts to develop HER3 targeting agents to combine with HER2 inhibitors for the treatments of these cancers and many such agents are in the preclinical and clinical pipelines.

We have been modeling HER2-HER3 signaling co-targeting in experimental models to foresee the activities and limitations of this clinical strategy and have identified potentially significant escape mechanisms that limit the therapeutic potential of this approach. This appears to be largely due to the mistaken attribution of PI3K/Akt signaling to HER3 and the dogma that HER2 cannot activate PI3K by itself. The functions of HER3 are not entirely understood and may be partly redundant with HER2, enabling HER2 to escape the requirement for HER3. In this regard, we found that HER3-knock out HER2-overexpressing cells form tumors and some of these tumors exhibit increased HER2 phosphorylation and Akt activation. We validated, in three different HER2-amplified cancer cell lines that phosphorylated HER2 drives PI3K-mediated Akt activation in a HER3-independent manner. Interestingly, we found that HER2 interacts with the regulatory subunit of PI3K in these cells. We characterized this interaction and identified a tyrosine in the C-terminal tail of HER2 involved in PI3K binding. Most importantly, in vivo work demonstrates that mutation of this tyrosine reduces the transforming and tumorigenic potential of HER2-amplified cancer cells.

This work highlights that the activation of PI3K/Akt signaling by HER3 may be an important but not essential function and may ultimately be redundant with the functional capabilities of massively overexpressed HER2. Therefore, building a better understanding of the molecular mechanisms that underlie HER2-HER3-induced tumorigenesis is essential for the development of more effective therapeutic strategies.

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Molecular and Cellular Biology #5

Just say NO! NO• is the message between healthy and cancerous breast tissue

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Abstract
Breast tissue health hangs in the balance between the positive and negative factors of the NO*- p53- ECM circuit.

How mammalian tissues maintain their architecture and tissue-specificity is poorly understood. Previously, we documented both the indispensable role of the extracellular matrix (ECM) protein, laminin-111, in the formation of normal breast acini, and the phenotypic reversion of cancer cells to acini-like structures in 3- dimensional (3D) gels with inhibitors of oncogenic pathways. Here, we asked how laminin proteins help to integrate the signaling pathways necessary for morphogenesis. We report a surprising reciprocal circuitry comprising positive players: laminin-5 (LN5), nitric oxide (NO*), p53, HOXD10 and three microRNAs (miRNAs) that are involved in the formation of mammary acini in 3D. Significantly, cancer cells grown on either 2-dimentional (2D) or 3D, and non-malignant cells on 2D plastic, do not produce NO*, and instead upregulate negative players: NFkB, EIF5A2, SCA1 and MMP-9 that disrupt the network. Introducing exogenous NO*, LN5 or individual miRNAs to cancer cells re-integrates these pathways and induces phenotypic reversion in 3D to a non-malignant state. These findings uncover the essential elements of breast epithelial architecture, where the balance between positive- and negative-players leads to homeostasis. Disruption of this circuitry or loop leads to cancerous transformation.

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Molecular and Cellular Biology #6 – BEST POSTER

Tissue tension promotes RANKL induced mammary stemness and breast cancer risk

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Abstract
To investigate the impact of elevated tissue tension on mammary tumorigenesis, we generated transgenic mice expressing a β-Integrin mutant (V737N) in the mammary epithelium and crossed them with a mouse model of HER2-positive breast cancer (MMTV-NEU). V737N expression stimulated integrin-mediated mechanosignaling in mammary epithelial cells (MECs), resulting in significantly augmented tumor incidence and lung metastasis despite having little effect on primary tumor outgrowth. Moreover, histological examination of tumors and gene expression analysis uncovered a phenotypic shift, such that V737N-expressing tumors displayed patterns resembling mesenchymal-like breast cancers. The observed increase in tumor incidence led us to explore V737N-induced alterations to the normal mammary epithelium that might enhance risk to malignancy. V737N expression promoted precocious ductal branching, end bud formation and MEC proliferation, which was accompanied by an increase in the ratio of basal/myoepithelial to luminal MECs. Examination of gene expression using sorted MECs showed that the presence of V737N resulted in amplified levels of gene transcripts associated with stemness, and subsequent colony formation and transplantation assays revealed a functional increase in progenitor/stem cell frequency in V737N-expressing basal/myoepithelial MECs when compared to their corresponding controls. Further analysis uncovered a V737N-mediated heightened MEC sensitivity to hormone-mediated expansion of stem/progenitor populations that was dependent on progesterone-induced expression of RANKL; a paracrine factor known to promote the proliferation of breast epithelial progenitor cells. Recent data revealed that patients with BRCA1-mt also have elevated RANKL signaling and our studies show that breast tissue from women with BRCA1-mt is stiffer. Mammographic density (MD) associates with increased fibroglandular tissue and our preliminary data also indicate that high MD reflects a stiffer extracellular matrix (ECM). MD and germline mutations in BRCA1 (BRCA1-mt) are two strong risk factors for breast cancer (BC) suggesting that a stiff ECM may promote BC risk by enhancing RANKL signaling. Indeed, our studies show that activated mechanosignaling causes an expansion of mammary epithelial progenitor cells, and human breast tissue with high MD and a stiff ECM appears to sensitize breast epithelium to produce more RANKL. Thus, high MD and a stiff ECM may predispose women to BC by enhancing stem/progenitor expansion through RANKL signaling.

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