025 Poster Discussion, Sun, 2:00 PM - 6:00 PM

rculating tumor cells monitored over time in lung cancer patients. *M. S. ttgen, D. Marrinucci, D. Lazar, M. Malchiodi, P. Clark, E. Huynh, K. ethel, L. Bazhenova, J. Nieva, P. Kuhn; The Scripps Research Institute, Jolla, CA; Scripps Clinic, La Jolla, CA; University of California, San ego, La Jolla, CA; Billings Clinic, Billings, MT*

ckground: Circulating tumor cell (CTC) detection and enumeration is a luable tool for monitoring cancer patient status and outcome. While any current techniques employ immunomagnetic-enrichment based otocols focused on the importance of a particular CTC number as the dicator of patient status or outcome, we employ a cytometric, enrichment ee approach using an immunofluorescent protocol to monitor CTC counts patients with non-small cell lung cancer (NSCLC) over the course of eatment. **Methods:** Eligible patients had progressive stage IV NSCLC. The stological subtypes in the 42 cases for which the data was available cluded adenocarcinoma (22/42), squamous cell carcinoma (6/42), large ll undifferentiated carcinoma (3/42), and non-small cell lung carcinoma ot further described, poorly differentiated, or with a mixed pattern 1/42). Blood samples were collected 3 wks, 3 mo, 6 mo, 9 mo, and 1 yr ter the initial sample. CTCs were identified via immunofluorescence and tometric analysis. Patient response to therapy was determined by ECIST every 3 months between time 0 and time 12 mo. **Results:** 80 of 109 atient samples have CTCs (73%) and all of the 52 patients tested have TCs. 13 of 52 patients have CTC data for time 0 and 3 wks. Only 4 of these atients (30.8%) show a correlation linking CTC count change between me 0 and 3 wks and clinical assessment. 13 patients have CTC data for me 0 and 3 mo, 10 of whom show a correlation linking CTC count change etween time 0 and 3 mo and clinical assessment. 7 of the 8 patients 37.5%) showing stable or partial response at 3 mo show a decrease in CTC ount between time 0 and 3 mo. Five of the 6 patients (83.3%) clinically owing progressive disease at the 3 mo time point show an increase in CTC ount between time 0 and 3 mo. The patients that do not show a correlation nking CTC count change between time 0 and 3 mo and clinical ssessment at 3 mo show a correlation at the 6 mo time point. **Conclusions:** TCs can be effectively enumerated in metastatic NSCLC patients, with the ajority demonstrating CTCs in the setting of progressive disease. The hange in CTC count at 3 mo, but not at 3 wks, correlates with radiographic esponse to chemotherapy. Further follow-up will determine the predictive alue of CTC enumeration on survival.

11026 Poster Discussion, Sun, 2:00 PM - 6:00 PM

Gauging the response of circulating epithelial tumor cells (CETC) and tumor stem cell subpopulations to therapy of early-stage cancer in the individual patient. *K. Pachmann, O. Camara, I. B. Runnebaum, K. Hoeffken; Clinic for Internal Medicine II, Jena, Germany; University Hospital Friedrich Schiller University, Jena, Germany*

Background: Cells released from the primary tumor persisting and recirculating in the host can lead to the formation of distant metastases. It was claimed that such cells are detectable only in a minor fraction of early-stage cancer patients but we can show that CETC are detectable and can be quantified in the peripheral blood of almost all cancer patients including early-stage solid malignancies. **Methods:** Using anticoagulated peripheral blood, red blood cell lysis as the only enrichment step, one centrifugation step, staining live cells with fluorochrome labelled anti-epithelial antigen as a search antibody, automated image analysis for detection of positive events and evaluation of exclusively surface located epithelial antigen on vital unfixed cells, CETC were detected in most patients with early stage cancer. Subsequently cells could be stained with anti-ALDH-antibody and in situ hybridized for HER2/neu amplification and quantified repeatedly during neo/adjuvant chemotherapy and during maintenance therapy with hormones or trastuzumab. **Results:** We here report the results from 497 breast cancer patients analyzed more than three times during the course of disease, 248 during neoadjuvant/adjuvant chemotherapy, 249 during trastuzumab and or hormone therapy. Different pattern of therapy response were obtained with rapidly responding CETC changes over several logs in response to chemotherapy and slow and long-lasting changes extending over several years in response to hormone therapy and trastuzumab. Stem cell like staining was seen in a minor fraction of cells (1%) in about 10% of patients. An increase in cell numbers and in the fraction of HER2/neu amplified cells was under all treatment conditions unequivocally significantly correlated to highly increased risk of relapse. **Conclusions:** CETC and subpopulation monitoring provides an invaluable tool for prompt gauging of systemic therapy in early stage solid tumors as a tool for therapy guidance and optimal personalized therapies to improve therapy results and spare unnecessary treatments.

11029

Epidermal growth factor (EGF) g (GERD), and esophageal adeno *Kulke, R. Heist, K. Asomanin University of Toronto, Toronto Boston, MA; Dana-Farber Can Hospital, Boston, MA; Princes*

Background: Single nucleotide as *EGF A61G*, are associated penetrance indicates that the esis are modified by additiona is an established risk factor between *EGF* polymorphism a GERD. **Methods:** *EGF* genotyp history was collected for 309 Associations between genoty logistic regression. Genotype- stratified by GERD history and duration of GERD symptoms. between cases and controls common (p=0.02) and GERD controls. When compared to th associated with an increased Stratified analyses revealed t increase in EAC risk among in for GERD-free individuals (se EAC was also highest for *G/G* more than once per week (OR GERD for longer than 15 year was a highly significant intera of GERD (p<0.001). **Conclus** EAC susceptibility through an ing for patients with severe or at the greatest risk of EAC.

Odds of EAC stratified by EGF A61G polym

	Number of cases/controls
Overall study cohort	309/275
GERD subset	150/62
GERD-free subset	159/213

EAC = esophageal adenocarcinoma; GER

11027 Poster Discussion, Sun, 2:00 PM - 6:00 PM

ffects of G-CSF on circulating tumor cells (CTC) and CA 27.29 in breast cancer atients. *P. Hepp, B. Rack, A. Schneider, M. Rezai, H. Tesch, T. Beck, U. öling, W. Lichtenegger, M. W. Beckmann, W. Janni, SUCCESS Study Group; leinrich Heine University, Düsseldorf, Germany; Ludwig-Maximilians-Univer- ity, Munich, Germany; Charité, Berlin, Germany; Luisenkrankenhaus, Düssel- orf, Germany; Praxis Prof. Tesch, Frankfurt, Germany; Städtisches Klinikum osenheim , Rosenheim, Germany; Gemeinschaftspraxis Siehl & Söling , Kassel, ermany; University of Erlangen, Erlangen, Germany; SUCCESS Study Group*

ackground: Some recent publications indicated that the use of G-CSF could be onnected to an increase in CTC as well as elevated levels of tumor markers such s CA 27.29. In the SUCCESS Trial CTC and CA27.29 are examined before and fter adjuvant chemotherapy (CHT) in 3754 breast cancer patients (pts). **lethods:** The SUCCESS Trial is a phase III trial comparing FEC-Docetaxel vs. EC-Doc-Gemcitabine regime and 2 vs. 5 years of treatment with zoledronate in atients with primary breast cancer (BC) (N+ or high risk). Blood samples are aken before and after CHT. CTC were assessed with the CellSearchSystem Veridex, Warren, USA). After immunomagnetic enrichment with an anti-Epcam- ntibody, cells were labeled with anti-cytokeratin (8,18,19) and anti-CD45 ntibodies to distinguish epithelial cells and leukocytes. CA27.29 has been neasured with ST AIA-PACK Ca27.29 reagent using MUC-1 for AIA-600II Tosoh Bioscience, Tessenderlo, Belgium). The cutoff for CA27.29 is 32 U/ml nd >1 cell for the CTC analysis. Patients were grouped to CTC/CA27.29 raise or o raise and 1 to 6 cycles with G-CSF or no G-CSF at all. **Results:** Data on 1510 ts are available for CTC analysis. 745 pts (49%) received at least one course of -CSF. 117 pts (8%) showed an increase in CTC after CHT. In this group 52 3%) pts received G-CSF and 65 (4%) did not. 693 pts with stable or decreased TC received G-CSF (46%) and 700 did not (46%). There was no significant difference (p=0.29). The analysis of CA27.29 is based on the data of 2556 pts. 252 pts (49%) received at least one course of G-CSF. 338 pts (13%) exceeded he threshold for CA27.29 only after CHT. In this group 209 pts (8%) received -CSF and 129 (5%) did not. 1043 pts with stable or decreased CA27.29 eceived G-CSF (41%) and 1175 did not (46%). This difference was highly ignificant (p<0.0001). **Conclusions:** No evidence can be provided for a ignificant correlation between an increase in the number of CTC and the pplication of G-CSF over CHT. Nevertheless the results on CA27.29 showed a ighly significant correlation between the administration of G-CSF and elevated A27.29 levels directly after CHT. This could be a possible explanation for the ften observed increase of tumor markers after CHT.

11028 Poster Discussion, Sun, 2:00 PM - 6:00 PM

Preclinical testing of the PARP inhibitor ABT-888 in microsatellite instable colorectal cancer. *E. Vilar Sanchez, A. Chow, L. Raskin, M. D. Iniesta, B. Mukherjee, S. B. Gruber; University of Michigan, Ann Arbor, MI*

Background: Microsatellite instability (MSI) represents approximately 15% of colorectal cancer (CRC) cases. MSI due to hypermethylation or mutation in DNA mismatch repair genes leads to genetic instability and a mutator phenotype. Genetic instability is particularly high at repetitive sequences such as those located in MRE11, RAD50, CtIP and MBC. Each of these genes are implicated in the double strand break (DSB) repair pathway. PARP inhibitors induce single strand breaks that remain unrepaired and then will be converted to DSB during DNA replication. Our objective was to assess the preclinical activity of a novel PARP inhibitor ABT-888 in MSI cell lines deficient in the DSB repairing pathway and compare it to Microsatellite Stable (MSS) lines. **Methods:** We used the systems biology tool "Connectivity Map" to synthesize data from 5 different published studies of expression profiling of MSI CRC phenotype and to identify target compounds. We assessed the mutational status of MRE11, RAD50, CtIP and MBC in a panel of 10 CRC cell lines displaying either MSI or MSS, and measured the expression of MRE11 by quantitative RT-PCR. We tested the cytotoxic activity of single-agent ABT-888 for 6 days in MSS and MSI cell lines, stratified by mutational status. Flow cytometry was performed after 24 hours. **Results:** Systems biology studies identified PARP inhibitors as a candidate compound relevant for MSI CRC. Mutational status of MRE11 was perfectly correlated with MSI status. ABT-888 shows a preferential activity on those MSI cell lines harboring mutations in both MRE11 and RAD50 genes compared to MSS cell lines (wild-type for both genes). A significant correlation exists between MRE11 expression levels and cytotoxicity to ABT-888 at 10 μM (R^2=0.915, P<0.001). Flow cytometry analyses show a G1 arrest following to the treatment with ABT-888 that is higher in MSI cell lines with mutations in MRE11 and RAD50 compared to MSS cell lines. **Conclusions:** This is the first report of the preclinical activity of a PARP inhibitor in CRC models. MSI colorectal tumors deficient in DSB repair show a higher sensitivity to PARP inhibition. Further clinical investigation of ABT-888 as a single agent or in combination with other chemotherapy drugs inducing DSB is warranted in MSI CRC with mutations in MRE11 and RAD50.

11031

Detection of BRAF kinase carcinomas: Evidence for t *Litterman, M. Yancovitz, R. S. Blank, P. Lee, I. Osma Medicine, New York, NY*

Background: Several studie polyclonal malignancies, a ties in achieving durable t larly targeted therapies ma tumor subclones. In this st with a fluorescent-based n the BRAF hotspot mutatio including paired primary a **Methods:** BRAF MS-PCR ar DNA from 304 tumors (1 determine the presence of melanomas were 18 match metastatic specimens fro metastases. **Results:** DNA s ovarian tumors, 1/82 (1.2 mas. In contrast, the MS-P ovarian tumors, 15/82 (18 mas. The presence of con melanoma sample, but exc using either methodology. were also detected by MSP and metastatic melanoma, patients with mutant prima 19 patients with multiple wild-type and mutant) tum mutation detection method neity within clinical tumor noma samples, where mult with respect to the prese suggest that failures of directed against mutant B among the tumors under tr