

Con el aval científico de:



Sociedad Española de Anatomía Patológica

Madrid, 9 de mayo de 2012

USCAP & AACR HIGHLIGHTS

Avances tecnológicos en nuevos marcadores
en cáncer y en biología tumoral AACR

Dr. Federico Rojo – Fundación Jiménez Díaz, Madrid

USCAP
& AACR
HIGHLIGHTS
2012

#2806 BRAF and MEK inhibitor profiling across 240 tumor cell lines to correlate with sensitivity and resistant biomarkers

Karen Bernards, Yulia Ovechkina, Jushun Cheng, John Kushleika, Alison Angione, James Hnilo, Christine O'Day, Ricerca Biosciences, Bothell, WA.

Activated kinases that induce cell proliferation have been attractive targets for targeted cancer therapy development. Cancer cells could become dependent on tumor-specific activated kinases and this tendency has been coined oncogene addiction. Here, we investigated three activated kinase inhibitors: BRAF inhibitor, PLX-4032 analog, and the MEK inhibitors, CI1040 and PD0325901. We established a high throughput cellular approach to profile 240 human tumor cell lines identifying genotype-correlated sensitivity or resistance. Proliferative, apoptotic and cell cycle arrest responses were measured using multiplexed high content screening with automated fluorescence microscopy and image analysis based technology. Growth index was measured using nuclear dye. Activated caspase 3 antibodies were used for the apoptosis induction detection. Phospho-histone H3 antibodies were used to measure the cell cycle block. We generated cell line profiles to reveal drug sensitivity and resistance patterns that may correlate with the clinical genotype responses. Cell lines with BRAFV600E mutations showed overlapping sensitivity to all three MEK and BRAF inhibitors. RAS mutations confer resistance to the BRAF inhibitor and confer sensitivity to both MEK inhibitors. In addition, we used Alphascreen technology to measure phosphoERK across the 240 non-treated tumor cell line panel. We found that the majority of PLX-4032 sensitive cell lines expressed high levels of phosphoERK. In addition, we investigated BRAF, MEK and EGFR inhibitor combinations to evaluate potential synergies. This preclinical approach can be used to predict mechanisms of susceptibility or resistance to these agents which in turn could be used for the optimization of targeted cancer therapeutics.

#2156 Preclinical combinations of vemurafenib, a selective BRAF inhibitor, with other targeted therapies in BRAFV600E colorectal cancer models

Hong Yang¹, Brian Higgins¹, Kenneth Kolinsky¹, Jiakui Li¹, Richard Margolis¹, Zenaida Go¹, Aruna Railkar¹, Kathryn Packman¹, Gideon Bollag², Fei Su¹. ¹Hoffmann-La Roche, Nutley, NJ; ²Plexikon Inc., Berkeley, CA.

Mutations in BRAF at codon 600 promote catalytic activity and are associated with 8% of all human (solid) tumors, including about 10% of colorectal cancers (CRCs). Vemurafenib (RG7204, PLX4032) is a first-in-class, BRAF-specific small molecule inhibitor that dose-dependently inhibits tumor growth in BRAFV600E CRC xenografts. Despite these encouraging preclinical findings, unlike the remarkable responses observed in melanoma, single agent vemurafenib in a Phase I extension trial of 21 patients with previously treated metastatic CRC resulted in a modest 5% response rate. We therefore explored a range of combinations of vemurafenib with different novel targeted therapies including a MEK inhibitor, an AKT inhibitor, a PI3K inhibitor, an mTOR/PI3K dual inhibitor and an EGFR inhibitor in BRAFV600E CRC cell lines. The consequences of these combinations on proliferation were analyzed by MTT assay and the combined effect was determined by combination index (CI) calculated using CalcuSyn software. Western analysis and Annexin V staining were utilized to evaluate combination effects on downstream signaling events and apoptosis induction. Optimized doses of both vemurafenib and a MEK inhibitor were tested as single agents and in combination in CRC xenograft models in nude mice. Synergistic anti-proliferative effects were observed with combinations of vemurafenib and other targeted therapies in the BRAFV600E positive CRC cell lines tested. More effective signaling inhibition and apoptosis induction were observed with the combinations than either agent alone. Combining vemurafenib with a MEKi delivered greater anti-tumor activity and increased life span of animals in the LS411N CRC xenograft model. In a BRAF-mutant CRC xenograft model with inherent resistance to vemurafenib (RKO), tumor growth inhibition by vemurafenib was enhanced by combining with an AKT inhibitor. These in vitro and in vivo data suggest that the administration of vemurafenib in combination with novel targeted therapies may be effective in delivering enhanced and sustained clinical antitumor efficacy in colorectal cancers harboring the BRAFV600E mutation.

- ▶ **Targeting mutations in melanoma.** Smalley, KSM. Educational session Melanoma: Moving beyond BRAF.
- ▶ **NRAS mutations in melanoma: How can we develop targeted therapies for these patients?** Davies, MA. Educational session Melanoma: Moving beyond BRAF.

#5608 Functional genetic screens to find modulators of sensitivity to inhibitors of the PI3K/mTOR pathway

Michiel S. Van der Heijden, Katrien Berns, Michael O. Ports, Rene Bernards. Netherlands Cancer Institute, Amsterdam, Netherlands.

The PI3K/mTOR pathway is an important signaling pathway which is often hyperactivated in breast cancer by genetic events. Inhibitors of several PI3K pathway members have been developed in recent years. Clear improvements in survival have not been demonstrated in most cancer patients, including breast cancer patients. Clearly, additional targets for combination therapies and biomarkers of resistance are needed. We performed siRNA based screens, to find genes that can modulate the sensitivity of breast cancer cells to PI3K and mTOR inhibitors.

- ▶ **Resistance screens:** We screened the retroviral NKI hairpin library, consisting of 21,000 hairpins against 8,000 genes, in four breast cancer cell lines, using rapamycin (an inhibitor of mTORC1), AZ4 (a small molecule inhibitor of mTORC1 and 2) and the dual PI3K/mTOR inhibitors PI103 and NVP-BEZ235. Common hits identified in these resistance screens and validated in multiple cell lines were PTEN, GSK3A/B and ARID1A. PTEN and GSK3A/B are known members of the PI3K pathway; resistance by PTEN loss is most likely caused by hyperactivation of PI3K signaling, whereas GSK3A/B knockdown probably leads to decreased degradation of Cyclin D (published previously). Knockdown of the SWI/SNF complex component ARID1A could also induce resistance against PI3K and mTOR inhibition in various cell lines. Similar to PTEN knockdown, ARID1A downregulation caused hyperactivation of PI3K signaling. Further studies should elucidate which SWI/SNF transcriptional targets are responsible for this induction of PI3K signaling.
- ▶ **Synthetic lethal screens:** We screened the Dharmacon 'kinome' and 'druggable genome' siRNA libraries (over 8,000 genes) in HCC1954 breast cancer cells, to find genes whose knockdown enhances the toxicity of mTOR inhibitors. Few genes could reproducibly enhance the sensitivity of breast cancer cells to mTOR inhibition; **most of these genes, such as RHEB and PIK3CA**, are important members of the PI3K/mTOR pathway. We found knockdown of the anti-apoptotic MCL1 gene to increase sensitivity to PI3K and mTOR inhibition by causing an increase in apoptosis. **Although not clearly established as a specific PI3K pathway member**, MCL1 expression may be regulated by downstream proteins in the PI3K/mTOR pathway. Knockdown of the integrin α /vitronectin receptor ITGAV had a sensitizing effect on PI3K/mTOR inhibition in HCC1954 and SKBR3 breast cancer cells. The mechanism of this interaction is still under investigation but may involve upregulation of integrin function in response to PI3K/mTOR inhibition.

In conclusion, most of the genes that can modulate the sensitivity of breast cancer cells to PI3K/mTOR inhibition are known members of the PI3K pathway, however ARID1A and ITGAV are new modulators of sensitivity to PI3K pathway inhibition.

#8 Genomic analyses of heterogeneous HER2 3+ invasive ductal carcinomas of the breast

Arnaud Gauthier¹, Maryou Lambros², Daniel N. Rodrigues², Laurent Arnould³, Magali Lacroix-Tikri⁴, Frédéric Zdzienicka¹, Penault-Llorca⁵, Marie-Christine Baranzelli⁶, Xavier Sastre¹, Rachael Natrajan², Olivier Delattre¹, Paul Cottu¹, Jorge Sergio Reis-Filho², Anne Vincent-Salomon¹. 1Inst. CURIE, Paris, France; 2Institute of Cancer Research, London, United Kingdom; 3Centre Geroges Franiçois Leclerc, Paris, France; 4Institut Claudius Regaud, Toulouse, France; 5Centre Jean Perrin, Clermont-Ferrand, France; 6Centre Oscar Lambret, Lille, France.

- ▶ **Aims:** HER2 gene amplification is observed in 9% to 15% of breast carcinomas. According to current guidelines, cases with >10% but <30% of cells displaying complete membrane staining are considered of equivocal HER2 status. In a minority of HER2-positive cancers, however, HER2 overexpression and gene amplification are restricted to a subpopulation of cancer cells of the tumor bulk (i.e. HER2 intra-tumor heterogeneity). We investigated cases displaying heterogeneous HER2 overexpression and gene amplification (i.e. overexpression/ amplification in >10% but less than 100% of the cancer cells) i) to determine whether the HER2-positive and HER2-negative components are clonal and ii) to define the genomic differences between the HER2-positive and HER2-negative components.
- ▶ **Material and methods:** Forty-four cases were collected consecutively among five French hospitals and for which initial reports mentioned HER2 heterogeneity. Diagnosis of HER2 heterogeneity was re-assessed by immunohistochemistry and chromogenic and/ or fluorescence in situ hybridization. Each component was microdissected (laser capture microdissection) from representative tissue sections stained with an anti-HER2 antibody (Herceptest). After DNA extraction, the DNA samples from the HER2-positive and HER2-negative components of each case were subjected to microarray-based comparative genomic hybridization (aCGH), using a platform with 50Kb resolution.
- ▶ **Results:** Out of 44 cases, the HER2-positive and HER2-negative components from 13 cases yielded DNA of sufficient quantity and quality and were subjected to aCGH analysis. Tumors were preferentially ductal carcinomas of no special type (83%), of histological grade 3, ER positive (83%), and lymph node positive (70%). In 11 of the 13 pairs (85%), the only significant difference among the genomic profiles was the presence of the region of amplification located at the 17q12-q21 locus encompassing the HER2 gene in the HER2-positive component. Unsupervised clustering of aCGH data demonstrated strikingly similar patterns of copy number profiles in different components of each tumour. In cases that were ER-positive, both the HER2-positive and HER2-negative components harboured alterations associated with ER-positive (luminal) carcinomas, such as gains of 1q+, losses of 16q, and amplifications of 8p12 and 11q13. Conclusions: Albeit rare, our results demonstrate that HER2 can be

heterogeneously overexpressed and amplified in breast cancers, and this phenomenon appears to be more prevalent in ER-positive breast carcinomas. Based on the similarities of their genomic profiles, the HER2-positive and HER2-negative components from each case are clonal and are likely to have diverged relatively early in the tumor evolution.

#916 Role of delta16HER2 splice variant in HER2-driven tumor progression and response to targeted therapy

Serenella M. Pupa¹, Lorenzo Castagnoli¹, Gaia Cristina Ghedini¹, Roberta Zappasodi¹, Valentina Ciravolo¹, Giulia Marzano¹, Guido Santilli¹, Augusto Amici², Cristina Marchini², Massimo Di Nicola¹, Rossella Canese³, Egidio Iorio³, Manuela Iezzi⁴, Elda Tagliabue¹. 1Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy; 2University of Camerino, Camerino, Italy; 3Istituto Superiore di Sanità, Roma, Italy; 4Aging Research Centre, G. di Chieti, Italy.

We reported that the splice variant of human HER2 lacking exon 16 (delta16HER2) represents a highly penetrating HER2 oncogenic alteration identified in human primary breast tumor specimens and is able to influence the response to Trastuzumab. This HER2 variant forms covalent cysteine bonds that generate constitutively active homodimers, thereby activating multiple oncogenic downstream signaling pathways that we recently found to be mediated through activated Src kinase. To examine the ability of delta16HER2 to transform mammary epithelium in vivo and to monitor delta16HER2-driven tumorigenesis in live mice, we generated a FVB transgenic mouse model for the human delta16HER2 isoform. Transgenic female mice developed multifocal mammary tumors with a rapid onset starting at about 12 weeks of age and progressively thereafter, clearly pointing to the candidacy of the delta16HER2 isoform as the transforming form of the human HER2 oncoprotein. Histological and immunohistochemical analysis (IHC) of primary mammary nodules revealed a population of polygonal cells with classical epithelia-like aspects distinctly expressing HER2 and also a population of smaller spindle-shaped cells arranged in fascicles with lower levels of HER2 expression, suggesting the onset of the epithelial-to-mesenchymal transition (EMT). Consistent with these findings, FACS analysis of delta16HER2-positive tumor cells immunomagnetically purified from disaggregated transgenic primary tumors indicated the increased mean fluorescence intensity of HER2 staining with increasing tumor cell size. IHC analysis of the lung metastases that had formed in the majority of female mice revealed monomorphic and classical epithelial tumor cells homogeneously expressing high levels of delta16HER2. FACS and IHC analyses confirmed the lower binding efficacy of Trastuzumab to delta16HER2-overexpressing primary tumor cells cultured both under bidimensional (2D) and tridimensional (3D) conditions as compared to monoclonal reagents directed to different HER2 extracellular domain epitopes. Experiments in both primary and metastatic in vitro and in vivo delta16HER2-positive models are in progress to determine whether delta16HER2-driven tumor aggressiveness and Trastuzumab susceptibility depend not only on genetic changes intrinsic to the tumor cell, i.e., the EMT process, but also on extrinsic tumor surrounding microenvironment-related factors such as an imbalance between extracellular and intracellular pH, redox state and hypoxia. Preliminary FACS and IHC analyses indicate that delta16HER2-positive primary tumor cells are reactive for known epithelial markers as EpCAM, E-cadherin- and ck-18 and, a small subset of these mammary tumor cells, also stain positive for the mesenchymal differentiation markers vimentin, N-cadherin and ck14 significantly indicating an active EMT program. **Conclusions:** A subset of cutaneous and soft tissue ME tumors appear genetically linked to their salivary gland counterparts, displaying frequent PLAG1 gene rearrangements and occasionally PLAG1-LIFR fusion. Category: Bone & Soft Tissue

#4835 Genetic determinants of mTOR inhibitor response in breast and endometrial cancer

Britta Weigelt¹, Patricia H. Warne¹, Maryou Lambros², Jorge S. Reis-Filho², Julian Downward¹. 1Cancer Research UK London Research Institute, London, United Kingdom; 2The Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, United Kingdom.

- ▶ **Background:** Breast and endometrial cancers frequently harbour genetic aberrations that result in increased activation of the PI3K-AKT-mTOR signalling pathway including amplification and overexpression of receptor tyrosine kinases, activating PIK3CA mutations, and inactivating PTEN mutations or loss of PTEN function. Given the pivotal role of the PI3K-AKT-mTOR pathway in cancer cell growth and survival, inhibitors of several of its components are currently being tested in clinical trials. The aim of this study was to define the genetic determinants of mTOR inhibitor response in breast and endometrial cancer cells.
- ▶ **Methods:** A panel of 31 breast and 26 endometrial cancer cell lines was treated with serial dilutions of 'rapalogs' (i.e. allosteric inhibitors of mTOR complex 1 (mTORC1)) and mTOR kinase inhibitors targeting mTORC1 and mTORC2. Cell viability was determined using CellTiter Blue. PTEN protein levels and mutations in PTEN transcripts were assessed in all cell lines using western blotting and Sanger sequencing, respectively. PIK3CA mutations were assessed using Sanger sequencing in the breast cancer cells, and hot-spot mutations in PIK3CA and 18 additional oncogenes using the Sequenom OncoCarta Panel v1.0 in the endometrial cancer cells.
- ▶ **Results:** Response of breast cancer cells to both the rapalog everolimus and the mTOR kinase inhibitor PP242 was determined by the presence of activating PIK3CA mutations but not PTEN loss of function. In addition, PP242 treatment induced a more efficient response in HER2-amplified breast cancer cell lines, irrespective of the PIK3CA mutation status. By contrast, endometrial cancer cell lines not only harbouring mutations in PIK3CA but also in PTEN were sensitive to treatment with the rapalog temsirolimus. The mTOR kinase inhibitor AZD8055 induced a strong reduction in cell viability in all endometrial cancer cell lines tested.
- ▶ **Conclusions:** Our results suggest that response to mTOR inhibitors in breast and endometrial cancer cells may be determined by the presence of alterations affecting PIK3CA and PTEN. Our data further indicate, however, that the epistatic interactions between different components of the PI3K-AKT-mTOR pathway are distinct in cancers of the breast and endometrium.

#691 Estrogen receptor mRNA level in breast cancer predicts response to tamoxifen

Jennifer Bordeaux¹, Huan Cheng¹, Allison Welsh¹, Bruce Haffty¹, Donald Lannin¹, Xingyong Wu², Nan Su², Xiao-Jun Ma², Yuling Luo², David Rimm¹. ¹Yale University, New Haven, CT; ²Advanced Cell Diagnostics, Hayward, CA.

- ▶ **Purpose:** Quantification of mRNA has historically been done by reverse transcription polymerase chain reaction (RT-PCR). Recently, a robust method of detection of mRNA utilizing in situ hybridization has been described that is linear and shows high specificity with low background. Here we describe the use of the AQUA method of quantitative immunofluorescence (QIF) for measuring mRNA in situ using ESR1 (the estrogen receptor alpha gene) in breast cancer to determine its predictive value compared to Estrogen Receptor α (ER) protein.
- ▶ **Methods:** Messenger RNA for ER (ESR1) and Ubiquitin C (UbC) were visualized using RNAscope probes and levels were quantified by quantitative in situ hybridization (qISH) on two Yale breast cancer cohorts on tissue microarrays. ESR1 levels were compared to ER protein levels measured by QIF using the SP1 antibody.
- ▶ **Results:** ESR1 mRNA is reproducibly and specifically measurable by qISH on tissue collected from 1993 or later. ESR1 levels were correlated to ER protein levels in a non-linear manner on two Yale cohorts. High levels of ESR1 were found to be predictive of response to tamoxifen.
- ▶ **Conclusion:** Quantification of mRNA using qISH may allow assessment of large cohorts with minimal formalin fixed, paraffin embedded tissue. Exploratory data using this method suggests that measurement of ESR1 mRNA levels may be predictive of response to endocrine therapy in a manner that is different from the predictive value of ER. Further studies are underway using tissue microarrays from other institutions to determine how tissue age affects this assay.

#3 HER2 gene-amplified human breast cancer cells harboring a gatekeeper T768M mutation in HER2 overexpress EGFR ligands and are sensitive to dual therapeutic blockade of EGFR and HER2

Ritwik Ghosh¹, Archana Narasanna¹, Anindita Chakrabarty¹, Jeffrey A. Engelman², Carlos L. Arteaga¹. ¹Vanderbilt University, Nashville, TN; ²Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA.

Intrinsic or acquired mutations in receptor tyrosine kinases (RTKs) can confer resistance to RTK-targeted therapies. A previous study reported that a 'gatekeeper' T768M mutation in HER2 confers resistance to the tyrosine kinase inhibitor (TKI) lapatinib. We generated BT474 cells stably expressing either T768M HER2 or GFP. Treatment with lapatinib disrupted HER3-p85 complex and blocked phosphorylation of HER2, HER3, AKT and Erk1/2 in BT474-GFP but not in BT474-T768M cells.

BT474-T768M cells were also resistant to other anti-HER2 therapies such as trastuzumab. BT474-T768M cells were sensitive to the PI3K inhibitor BKM120 but not the MEK inhibitor CI1040, suggesting they still depend on the PI3K pathway for survival. Treatment with the irreversible HER2/EGFR inhibitor BIBW2992 (afatinib) eliminated P-HER2 and inhibited growth of cells expressing the mutant, suggesting they still depend on the ErbB receptor network to activate PI3K. Indeed, like in BT474-GFP (control) cells, BIBW2992 disrupted the association of p85 (PI3K) with HER3 and blocked phosphorylation of AKT.

A co-culture experiment with varying proportions of BT474-GFP and BT474-T768M cells revealed that lapatinib-resistant acini emerge when as low as 5% of the population has T768M HER2. Moreover, we found that only 3.2% of the HER2 alleles had the T768M mutation in the BT474-T768M cells. This result suggests that allelic dilution will render it difficult to identify this drug resistant mutation with conventional sequencing techniques (i.e., Sanger) in patients with HER2 gene-amplified breast cancer.

Next, we stably expressed either wild-type or T768M HER2 in MCF10A cells. Both WT and T768M HER2 resulted in increased phosphorylation of HER3 and AKT. MCF10A-HER2 but not MCF10A-T768M cells were sensitive to growth inhibition by lapatinib. Lapatinib blocked HER2, HER3 and tyrosine phosphorylation in MCF10A-HER2 but not in MCF10A-T768M cells. Quantitative PCR indicated that the BT474-T768M cells produce more EGFR ligands (EGF, TGF α , amphiregulin and HB-EGF) compared to BT474-GFP cells. Treatment with cetuximab, an EGFR antibody that blocks ligand binding, restored sensitivity of BT474-T768M cells to trastuzumab. Further, the BT474-T768M cells were sensitive to a combination of trastuzumab and lapatinib both in vitro and in vivo. Hence, dual blockade of HER2 and EGFR might be effective at trumping the development of HER2-overexpressing cancers bearing a gatekeeper mutation in T768M.

#23 Sensitivity of EGFR exon 20 insertion mutations to EGFR inhibitors is determined by their location within the tyrosine kinase domain of EGFR

Hiroyuki Yasuda, Natasha J. Sng, Wee-Lee Yeo, Lorena L. Figueiredo-Pontes, Susumu Kobayashi, Daniel B. Costa. Beth Israel Deaconess Medical Ctr., Boston, MA.

- ▶ **Background:** Epidermal growth factor receptor (EGFR) mutations define an important subgroup of non-small-cell lung cancer (NSCLC). Most patients whose tumors harbor exon 19 deletions or L858R EGFR mutations have responses to reversible ATP-mimetic EGFR tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib. Exon 20 inframe insertion mutations comprise ~4% of all EGFR mutations, occur at the N-lobe of EGFR after its C-helix (after AA M766; notwithstanding some C-helix exon 20 insertions have been reported affecting E762 to Y764), and most NSCLCs with EGFR exon 20 insertion mutations display lack of responses to EGFR TKIs. The reported response rate is below 5% and most patients have short intervals of disease control (Yasuda et al. Lancet Oncol 2011). Up to now, very few EGFR exon 20 insertion mutations had been studied using in vitro models and there was no available NSCLC cell line with such a mutation.
- ▶ **Objectives:** In the present study, we have attempted to: 1) study the most comprehensive panel of EGFR exon 20 insertion mutations, 2) evaluate their pattern of response/resistance to EGFR TKIs, and 3) derive a novel NSCLC cell line with an exon 20 insertion mutation.
- ▶ **Methods:** Response to EGFR TKIs of EGFR exon 20 insertion mutated NSCLCs was compiled. We generated a panel of representative exon 20 EGFR mutant constructs using site-directed mutagenesis. Vectors were introduced into Cos-7 or Ba/F3 cells for in vitro analysis. In addition, NSCLC cell lines with EGFR mutations were evaluated and compared to a novel malignant pleural effusion-derived cell line.
- ▶ **Results:** The NSCLC with EGFR-A763_Y764insFQEA (located within the C-helix of EGFR) achieved a partial response to erlotinib that lasted 18 months. Most other exon 20 insertion mutation-positive NSCLCs (15/16, p=0.11) did not respond. The cell line with EGFR-A763_Y764insFQEA had phosphorylated EGFR, ERK and AKT inhibited by nanomolar concentrations of erlotinib; and subsequently underwent upregulation of the pro-apoptotic BH3-only BIM and apoptosis. Eight different exon 20 insertion mutations were studied (including EGFR-A763_Y764insFQEA, A767_V769dupASV, D770_N771insNPG, H773_V774insH). All, but A763_Y764insFQEA, were resistant to micromolar concentrations of erlotinib/other TKIs. Ba/F3 cells with EGFR-A763_Y764insFQEA underwent apoptosis upon exposure to nanomolar concentrations of erlotinib. Detailed analysis of these exon 20 insertion mutations is ongoing.
- ▶ **Conclusions:** Not all EGFR exon 20 insertion mutations are resistant to EGFR TKIs, and in specific EGFR-A763_Y764insFQEA can be labeled an EGFR TKI-sensitive mutation. This finding has clinical implications for the care of the 10,000 cases of EGFR exon 20 insertion mutated NSCLC diagnosed yearly and points towards the need to define the molecular mechanisms that underlie the differential response to EGFR TKIs in EGFR mutated NSCLC.

BIOLOGÍA DEL CÁNCER, NUEVAS TECNOLOGÍAS

#3178 Can DNA from archived formalin-fixed paraffin embedded (FFPE) cancer tissues be used for somatic mutation analysis in next generation sequencing?

Emanuele Palescandolo, Robert Jones, Alina Raza, Ashwini Sunkavalli, Priscilla K. Brastianos, Matthew Ducar, Christina Go, Christine Roden, Charles Hatton, Megan Hanna, Adri Mills, Ravali Adusumilli, Prateek Kumar, Matthew L. Meyerson, Hahn C. William, Laura MacConaill, Paul Van Hummelen. DFCI, Boston, MA.

Formalin-fixed paraffin embedded (FFPE) tissues are far more abundant in most tissue banks and pathology departments than fresh or fresh frozen (FF) samples, but typically yield varying degrees of degraded DNA as a result of the fixation process. The quality of FFPE tissues may vary based on a number of factors such as: age of the block, fixation time, storage and handling conditions. Being able to harness the power of next generation sequencing technologies to genomically characterize these abundant and diverse archived samples would be tremendously valuable to the cancer research community and would enable the use of this material for clinical purposes. The goal of this study was to assess the performance of FFPE samples in next generation sequencing applications. We monitored close to 100 samples from tumor and normal tissues of FFPE, FF and blood origin. These samples underwent several processes for whole-exome or targeted sequencing, including DNA fragmentation, size selection, library preparation, and hybrid-capture enrichment. We observed variable performance across these samples at several of the above steps, which correlated predominantly with FFPE tissues and the age of block. Other quality metrics that showed relative lower performance of FFPE DNA were the cluster density, duplication rate or library complexity. Most FFPE samples still generated good quality sequence, however, older FFPE blocks, over 10 years, may need more input DNA or higher sequence depth to reach minimum coverage if somatic mutation analysis is the goal. There was no difference in performance between DNA from tumor and normal tissues.

#5543 Hetmap: Evaluating tumor heterogeneity in immunohistochemistry-stained breast cancer tissue.

Steven J. Potts¹, Nicholas Landis¹, David Eberhard², Stephen C. Schmechel³, David Young¹, Joseph S. Krueger¹, Holger Lange¹. ¹Flagship Biosciences, Flagstaff, AZ; ²University of North Carolina, Raleigh, NC; ³University of Minnesota, Minneapolis, MN.

Immunohistochemistry assays examining the HER2 receptor in breast cancer is the most widely adopted example of a companion diagnostic approach, which seeks to dictate therapeutic strategy based on a molecular description of a patient's disease. There are well-established guidelines for selecting patients for anti HER2 adjuvant therapies in breast cancer treatment, yet the current HER2 companion diagnostic approach is qualitative, does not sufficiently account for intratumor heterogeneity, and does not utilize any additional information about tumor cells that score beyond a specific threshold level.

A major contributing factor for the failure of both treatment and diagnostic paradigm is thought to be intratumor heterogeneity. This lack of information in the scoring paradigm may contribute to inappropriate patient selection and explain why the disease in many trastuzumab treated patients progresses or becomes recurrent. Thus, a quantitative clinical measurement of heterogeneity in immunohistochemistry staining would be useful in better predicting patient therapeutic response.

To answer this, we created a heterogeneity scoring approach (HetMap) that allows the visualization of an individual patient's IHC heterogeneity in the context of a cell population. We combined HER2 semi-quantitative analysis with the use of ecology diversity statistics to evaluate cell-level heterogeneity (consistency of protein expression within neighboring cells in a tumor nest) and tumor-level heterogeneity (differences of protein expression across a tumor as represented by a tissue section). We evaluated the approach on HER2 immunohistochemistry stained breast cancer samples, using 200 specimens across two different CLIA laboratories, with three pathologists at each laboratory each outlining regions of tumor for scoring by automatic cell-based image analysis. HetMap was evaluated using three different scoring schemes: HER2 scoring according to ASCO/CAP guidelines, H-Score and a new continuous HER2 score (HER2cont).

Cell-level heterogeneity, reported either as an average or the maximum area of heterogeneity across a slide, had low levels of dependency on the pathologist choice of region. Tumor-level heterogeneity measurements had more dependence on the pathologist choice of regions. Significantly, discordant pathologist assessments of IHC scores in the +2, or equivocal score, range occurred most in tumors with high heterogeneity, leading to potentially major clinical impact. Thus, HetMap is a measure of heterogeneity, by which pathologists, oncologists, and drug development organizations can view cell-level and tumor-level heterogeneity for a patient for a given marker in the context of an entire patient cohort. Including such measures of heterogeneity in diagnostic approaches can help establish better thresholds for patient selection, thereby improving patient response.

#964 Intra-tumor heterogeneity and Darwinian selection revealed by multi-region exome sequencing of renal cell carcinomas

Marco Gerlinger¹, Andrew Rowan², Stuart Horswell², James Larkin³, David Endesfelder², Eva Gronroos², Pierre Martinez², Nicholas Matthews², Aengus Stewart², Patrick Tarpey⁴, Ignacio Varela⁴, Benjamin Phillimore², Sharmin Begum², Neil McDonald², Adam Butler⁴, David Jones⁴, Keiran Raine⁴, Calli Latimer⁴, Claudio Santos², Mahrokh Nohadani⁵, Aron Eklund⁶, Bradley Spencer-Dene², Graham Clark², Lisa Pickering³, Gordon Stamp², Martin Gore³, Zoltan Szallasi⁷, Julian Downward², P. Andrew Futreal⁴, Charles Swanton⁸. ¹Cancer Research UK London Research Institute and Barts Cancer Institute, London, United Kingdom; ²Cancer Research UK London Research Institute, London, United Kingdom; ³Royal Marsden Hospital, London, United Kingdom; ⁴Wellcome Trust Sanger Institute, Hinxton, United Kingdom; ⁵Institute of Cancer Research, London, United Kingdom; ⁶Denmark Technical University, Lyngby, Denmark; ⁷Harvard Medical School, Boston, MA; ⁸Cancer Research UK London Research Institute and UCL Cancer Institute, London, United Kingdom.

- ▶ **Background:** Genetic intra-tumor heterogeneity (ITH) may foster tumor adaptation by providing selectable phenotypes for Darwinian evolution. Extensive genetic heterogeneity may also hinder personalized medicine strategies that rely on the portrayal of the mutational landscape from single tumor biopsies.
- ▶ **Methods:** To examine ITH, we have subjected multiple biopsies from primary renal cell carcinomas and associated metastatic sites to exome capture sequencing (n=2), SNP-array based chromosomal aberration and ploidy profiling analysis (n=4). Phylogenetic relationships of tumor regions were reconstructed by clonal ordering of non-synonymous somatic mutations. The phenotypic consequences of genetic ITH were characterised by immunohistochemistry, mutation functional analysis and mRNA expression profiling.
- ▶ **Results:** Phylogenetic reconstruction revealed branched evolutionary tumor growth with 63-69% of somatic mutations identified from single biopsies not detectable across all sequenced tumor regions. Independent tumor suppressor gene loss of function mutations with distinct regional distributions were detected within individual tumors: SETD2 harbored 5 different mutations in 2 tumors and PTEN and KDM5C two different mutations in one tumor, each. Thus, despite genetic divergence during tumor progression, phenotypic convergent evolution occurred, indicating a high degree of early mutational diversity. ITH was observed for a mutation in the kinase domain of mTOR, correlating with S6 and 4EBP phosphorylation specifically in cancer regions carrying the mutation and constitutive activation of mTOR kinase activity in vitro. Expression of a renal

cancer-specific prognostic signature differed between tumor regions. Chromosomal aberration analysis revealed extensive ITH with 26/30 tumor biopsies from four tumors harboring divergent allelic imbalance profiles. Ploidy profiling revealed heterogeneity in two out of four tumors and identified an aneuploid tumor cell population in a metastasis that probably evolved from a tetraploid intermediate detectable in the primary tumor.

- ▶ **Conclusions:** Genetic ITH was present in all tumors and occurs through spatially separated heterogeneous somatic mutations and chromosomal and ploidy aberrations leading to both, phenotypic intra-tumor diversity (mTOR activating mutation) and convergent loss of function (SETD2, PTEN and KDM5C). ITH can lead to underestimation of the tumor genomic landscape portrayed from single biopsies and may present significant challenges to personalized medicine and biomarker development. ITH, associated with heterogeneous protein function, may foster tumor adaptation and therapeutic failure through Darwinian selection. Targeting mutations commonly located on the trunks, rather than the branches of phylogenetic trees, may improve therapeutic outcomes.
- ▶ **A bad marker is as bad as a bad drug: applying scientific principles to tumor biomarker research.** Hayes DF, Meshane L, Ransohoff D.

Educational session Current Challenges in Molecular Marker Research

