Oral Presentations (Sun, 25 Sep, 09:00−10:30)
Personalized Medicine

800 ORAL

Comprehensive Next-Generation Sequencing (NGS) From Formalin-fixed NSCLC, CRC and Melanoma Cancer Tissues Identifies Novel Mutations With Potential Clinical Utility

G. Palmer[1], R. Yelensky[2], D. Lipson[2], M. Jarosz[2], A. Parker[2], C. Sheehan[3], S. Downing[2], J. Curran[2], M. Cronin[2], J. Ross[3]. [1]*Foundation Medicine, Clinical Affairs, Cambridge,* [2]*Foundation Medicine, Research and Development, Cambridge,* [3]*Albany Medical College, Pathology and Laboratory Medicine, Albany, USA*

Background: Rapid advancements in genomics paired with significant growth in the availability of targeted therapies offers clinicians expanding opportunities to provide increasingly effective cancer treatment. Currently, individual gene sequencing (e.g. *EGFR*) from formalin-fixed paraffin-embedded (FFPE) tissue is widely used in cancer diagnosis. Shifting this paradigm towards NGS-based, comprehensive mutation testing in routinely collected FFPE cancer specimens will enable more complete and accurate characterization of patients' cancers for individualized targeted therapy selection.
Materials and Methods: DNA was extracted from 2×20 micron sections of 83 specimens consisting of colorectal cancer, non-small cell lung cancer and melanoma. Hybridization-capture of 2574 exons across 176 oncogenes, tumour suppressor genes and ADME-related genes was performed to produce libraries appropriate for paired-end sequence analysis on the Illumina HiSeq2000 platform.
Results: In-depth sequence analysis of 176 genes in 50 CRC, 29 NSCLC, and 4 melanoma specimens with median coverage averaging 213-fold (range 8 to 461) detected a per-sample average of 2 previously-described mutations, 7 novel mutations and 2 CNAs in the colon specimens, including frequent alterations in *TP53* (33), *APC* (27), *KRAS* (12) and *BRAF* (6). The lung specimens averaged 1 previously described mutation, 8 novel mutations and 1 CNA per sample, most frequently *KRAS* (10), *TP53* (7), *JAK2* (3), *EGFR* (2) and *BRAF* (2). The melanoma cases exhibited on average 1 previously described mutation, 7 novel mutations and 3 CNAs including *TP53* (4) and *BRAF* (2). In addition to validated clinically actionable mutations in *EGFR*, *KRAS*, and *BRAF*, and multiple alterations in well-known cancer genes such as *TP53, STK11*, *APC*, *MLH1, BRCA2,* and *SMAD4*, we detected many other mutations that are plausibly clinically actionable. These included activating mutations in the PI3 kinase subunit gene *PIK3CA,* as well as mutations in *MET*, *KIT*, *ERBB2* and *CDKN2A*.
Conclusions: It is feasible to perform highly sensitive and specific sequence analysis of hundreds of genes from routinely collected FFPE tissues. This approach detects not only the "hot spot" mutations commonly tested for in CRC, NSCLC and melanoma but also many additional mutations that could plausibly inform therapeutic decision-making. We suggest that clinical-grade next-generation sequencing should become a routine part of all clinical trials, and increasingly, of clinical care.

801 ORAL

High Throughput Molecular Analyses to Select Patients for Targeted Agents

M. Arnedos[1], J. De La Cruz[2], B. Job[2], V. Scott[2], P. Dessen[3], D. Gentien[4], S. Roman-Roman[4], S. Delaloge[2], V. Lazar[2], F. Andre[2]. [1]*Institut Gustave Roussy, Department of Medical Oncology, Villejuif,* [2]*Institut Gustave Roussy, Department of Medical Oncology Unite INSERM U981, Villejuif,* [3]*Institut Gustave Roussy, CNRS FRE 2939 Bioinformatics Group, Villejuif,* [4]*Institut Curie, Department of Translational Research, Paris, France*

Background: High throughput analyses allow the identification of genomic alterations at the whole genome level. In the present study, we have evaluated whether the use of array CGH and a panel of hot spot mutations were feasible in daily practice and could allow for optimal selection of patients for targeted agents.
Materials and Methods: One hundred and six patients were prospectively included in a program of molecular screening. Samples were profiled on array CGH (Agilent 4*44K) and a panel of hot spot mutations detected by Sanger methods (PIK3CA and AKT). In addition, in order to better define optimal technologies to profile samples for daily practice, 30 pairs of samples were profiled both on Affymetrix SNP array 6.0 and on agilent platforms. In 19 of these samples, the profile obtained was compared between fine needle aspiration and biopsy. Samples were obtained from either the primary tumour or a metastatic site.
Results: Analyses showed that array CGH was suitable for daily practice even when biopsy had already been done. The use of biopsy and affymtrix platform provided a less amplified signal. The final cut-off to diagnose amplification was log2(ratio)>0.89 when biopsy was used (Agilent platform). This cut-off was decreased to 0.5 when using the affymtrix platform. A total of 106 patients were then profiled prospectively in order to drive them into targeted agents. This study showed that arrays CGH and hot spot mutations were feasible in 80% of cases. The optimal results were obtained with frozen samples. Array CGH done for daily practice was robust since the concordance with FISH for ERBB2 was of 98%. A total of 39 informative amplifications or deletions were observed in the population, and 21 mutations were found (14 PIK3CA and 7 AKT). Out of these 106 patients, 20 were treated with specific targeted agents given according to the genomic alteration observed. Outcome for these patients will be presented during the meeting.
Conclusions: This study shows that array CGH is feasible in the context of daily practice and allows for the enrichment of clinical trials in patients harboring specific genomic alterations.

802 ORAL

Epigenetic Markers Identify MGMT-methylated Glioblastoma Poorly Responding to Combined Radiotherapy-temozolomide (Stupp Regimen)

M. Aubry[1], A. Etcheverry[2], A. Idbaih[3], Y. Marie[3], M. Sanson[3], J.Y. Delattre[3], J. Mosser[4]. [1]*Université de Rennes 1, Genomic Platform, Rennes,* [2]*CHU Hôpital de Pontchaillou, Genomic Platform, Rennes,* [3]*Inserm − UPMC, U 975, Paris,* [4]*CNRS − Université de Rennes 1, UMR 6061, Rennes, France*

Background: Glioblastoma (GBM) is the most common and aggressive primary brain tumour in adult. At the time of diagnosis, median survival is around 15 months, despite the use of concomitant and adjuvant temozolomide to standard postoperative radiotherapy (standard treatment, Stupp regimen). It is a very heterogeneous tumour in terms of molecular alterations, aggressiveness, and response to treatment. Despite this strong molecular heterogeneity very few biomarkers have been identified so far in GBM. Among them, the methylation status of the *MGMT* promoter is currently the strongest predictive biomarker of outcome and benefit from standard treatment in GBM.
Materials and Methods: We used high-throughput quantitative DNA methylation screening (HumanMethylation27 beadchip, Illumina Inc.) to search for new relevant prognostic epigenetic biomarkers in 50 GBM patients homogeneously treated (standard treatment). Performance validation of these markers was performed with a mid-plex custom methylation profiling (VeraCode GoldenGate Methylation technology, Illumina Inc.) of 156 GBM patients treated according to the standard treatment.
Results: The screening study identified 61 CpG sites associated with the overall survival (OS) in GBM patients. The validation study isolates, in addition to *MGMT* promoter methylation, two highly robust and relevant prognostic markers for GBM patient stratification. These markers stratify *MGMT*-methylated patients into three survival groups of significantly different OS (p = 1e-06): a short-term survival group (n = 10, median OS=8 months), a mid-term survival group (n = 41, median OS=18.5 months), and a long-term survival group (n = 49, median OS = 30 months). The same stratification is observed for progression free survival (median PFS 7, 11, and 18 months, respectively).
Conclusions: We describe an original and robust experimental design that combines two complementary approaches. We identify two highly robust and relevant prognostic markers refining conventional *MGMT* stratification of GBM patients treated according to the standard treatment. Although there is no rationale so far to base treatment decisions on these markers, they might be useful for patient recruitment and results interpretation in clinical trials as they clearly capture a significant part of the GBM heterogeneity. Eventually, this enhanced stratification could yet influence treatment decision for elderly patients (more than 70 year-old) for whom there is no standard treatment.

803 ORAL

Analysis of Blood Plasma Factors in the AVITA Phase III Randomized Study of Bevacizumab (bev) With Gemcitabine-Erlotinib (GE) in Patients (pts) With Metastatic Pancreatic Cancer (mPC)

E. Van Cutsem[1], G. Jayson[2], C. Dive[3], P. Dilba[4], S. de Haas[4], N. Wild[5], P. Delmar[6], S.J. Scherer[7]. [1]*University Hospital Gasthuisberg, Digestive Oncology Unit, Leuven, Belgium;* [2]*Christie Hospital and University of Manchester, Medical Oncology, Manchester,* [2]*Paterson Institute for Cancer Research, Cancer Research, Manchester, United Kingdom;* [4]*F. Hoffmann-La Roche, Biomarker, Basel, Switzerland;* [5]*Roche Diagnostics GmbH, New Reagent Assessment, Penzberg, Germany;* [6]*F. Hoffmann-La Roche, Biomarker/Experimental Medicine, Basel, Switzerland;* [7]*Genentech Inc, Biomarker, South San Francisco, USA*

Background: Circulating factors involved in tumour angiogenesis might influence cancer therapy with anti-angiogenics. Recent data suggest that