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

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

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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.

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High Throughput Molecular Analyses to Select Patients for Targeted Agents

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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.

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

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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.

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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)

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

high plasma VEGFA (pVEGFA) and VEGFR2 (pVEGFR2) levels might predict progression-free survival (PFS) benefit in metastatic breast cancer (mBC) pts treated with bev (Avastin®) [Miles et al. SABCS 2010]. An exploratory, retrospective analysis of the AVITA phase III study in mPC was performed to determine the relationship between baseline levels of different angiogenic markers and clinical outcome.

Methods: In AVITA, 607 pts with mPC were randomized to GE+bev 5 mg/kg/2w or placebo until disease progression (PD). The primary endpoint of overall survival (OS) was not met (HR 0.89, 95% CI 0.76–1.06), but the secondary endpoint of PFS was highly significant (HR 0.74, 95% CI 0.63–0.87). Plasma samples from consenting pts (n = 225) were collected at baseline, at cycle 2 and at time of PD and used for analysis of 4 biomarkers (BMs) using a novel multiplex ELISA assay, including VEGFA and VEGFR2. Ten additional angiogenic markers were analysed using SearchLight®. Median baseline levels of BMs were pre-specified as a cut point to categorize pts as low or high; correlation to PFS and OS was explored using simple/multiple regression approaches and subgroup analyses.

Results: Baseline characteristics of pts with BM samples were balanced between treatment groups, although some small differences in demographics were present in the BM vs the overall population. Furthermore, slightly faster PD and shorter OS were seen in the placebo group of the BM vs the overall population. High baseline levels of pVEGFA correlated with better PFS and OS in bev-treated pts vs those receiving placebo (table). For pVEGFR2 a similar correlation was found for OS only. No correlation was found for any other BM analysed.

	HR for PFS	HR for OS
pVEGFA low vs high (n = 222)	0.77 vs 0.52	1.02 vs 0.56
Interaction p value	$p = 0.06$	$p = 0.03$
pVEGFR2 low vs high (n = 224)	0.76 vs 0.56	1.04 vs 0.60
Interaction p value	$p = 0.47$	$p = 0.06$

Conclusions: In this subset analysis, pVEGFA and pVEGFR2 were identified as promising BM candidates for predicting PFS and OS with bev in pts with mPC. These data confirm the potential predictive value of pVEGFA and pVEGFR2 already observed in mBC. Similar findings for pVEGFA were also seen in advanced gastric cancer [Shah et al. submitted ECCO 2011]. Further evaluation of these BM candidates in bev studies across different cancer types should be considered.

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Evaluation of Plasma VEGFA as a Potential Predictive Pan-tumour Biomarker for Bevacizumab

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Background: Identifying patients (pts) who benefit from anti-angiogenics is an unmet need. The majority of bevacizumab (bev) trials include biomarker (BM) sampling, with a focus on plasma VEGFA (pVEGFA), which has shown prognostic rather than predictive value [Bernaards et al. ASCO 2010]. However, recent findings in breast (BC, AVADO), pancreatic (PC, AVITA) and gastric cancer (GC, AVAGAST), with a novel ELISA-based assay favouring shorter isoforms ($VEGFA_{121}$ and $VEGFA_{110}$), suggested potential predictive value [Miles et al. SABCS 2010; Carmeliet et al.; Shah et al. both submitted ECCO 2011]. We used this novel assay to retest residual baseline samples from trials in mCRC (AVF2107g), NSCLC (AVAiL) and RCC (AVOREN).

Methods: Aliquots from plasma citrate baseline samples were re-analysed from 398 (AVF2107g), 859 (AVAiL) and 404 (AVOREN) pts. Median levels of pVEGFA were pre-specified as a cut point to categorize pts as low or high; correlation to PFS and OS was explored using simple/multiple regression approaches and subgroup analyses.

Results: The prognostic value of pVEGFA was confirmed in all 3 trials; pts in the control group with high pVEGFA levels had shorter OS than pts with low levels. However, potential predictive value for pVEGFA was not seen. Table 1 shows key pVEGFA BM results for newly analysed studies plus data from AVADO, AVITA and AVAGAST.

	HR PFS pVEGFA low vs high *Interaction p-value*	HR OS pVEGFA low vs high *Interaction p-value*
AVADO (15 mg/kg)	0.86 vs 0.49 *0.08*	1.07 vs 1.02 *0.55*
AVADO (7.5 mg/kg)	0.96 vs 0.52 *0.01*	1.34 vs 0.87 *0.044*
AVITA	0.76 vs 0.56 *0.06*	1.02 vs 0.56 *0.03*
AVAGAST	0.89 vs 0.64 *0.06*	1.0 vs 0.73 *0.08*
AVF2107g	0.64 vs 0.52 *0.61*	0.70 vs 0.68 *0.95*
AVOREN	0.49 vs 0.67 *0.42*	0.62 vs 0.86 *0.55*
AVAiL (15 mg/kg)	0.96 vs 0.76 *0.13*	0.97 vs 0.98 *0.67*
AVAiL (7.5 mg/kg)	0.77 vs 0.75 *0.77*	0.92 vs 0.89 *0.99*

Conclusions: Results with the novel assay showed potential predictive value for pVEGFA in BC, PC and GC; high baseline pVEGFA levels correlate with improved PFS and/or OS following bev treatment. However, these findings were not replicated in mCRC, NSCLC and RCC. These differences might have been confounded by variations in sample handling (citrate instead of EDTA and increased number of freeze/thaw cycles). As the novel assay has increased sensitivity for shorter VEGFA isoforms, it could be hypothesised that $VEGFA_{121}$ and/or $VEGFA_{110}$ are driving predictive value and might be diverse in different tumour types. Investigation with regard to this hypothesis is ongoing.

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Evaluation of Anti-angiogenic Treatments With DCE-US in 539 Patients − Results After 2 Years Median Follow-up

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Background: A prospective evaluation of dynamic contrast-enhanced ultrasound (DCE-US) with quantification for the evaluation of antiangiogenic treatments was launched in 2007 in 19 French centers, supported by the French National Cancer Institute. The objectives were the diffusion of the standardized method, a cost evaluation and the identification of perfusion parameters predicting tumour response to different anti-angiogenic treatments.

Materials and Methods: All patients had an examination just before the start of the antiangiogenic treatment (D-1) and at D7, D14, D30, D60 and every two months. Each examination included a bolus injection of sonovue (Bracco®) and registration of 3 minutes of raw linear data with an Aplio (Toshiba). Raw data were analyzed with a mathematical model (patent PCT/IB2006/003742) to evaluate 7 parameters characterizing the tumour perfusion curve. The quantification of DCE-US raw data was done without any knowledge of the clinical data. Response to treatment was evaluated every 2 months with RECIST criteria. Complete or partial responses and stabilization were classified as successes, progressions as treatment failures. Patients were considered not evaluable after treatment stop followed by a progression or increase in drug dose followed by a success. Inclusions were closed in March 2010. In order to have sufficient follow-up data, the statistical analysis was performed more than 12 months after the inclusion of the last analyzed patient.

Results: Since October 2007, 539 patients have been included (mainly with renal cell carcinomas (157) and hepatocellular carcinoma (107)). 2368 DCE-US examinations and 1414 scanographic evaluations have been performed. After a median follow-up of 712 days, we confirm that the variation between day 0 and day 30 is significantly ($P < 0.05$) related to DFS for 5 parameters.

Conclusions: Our results confirm the validity of this tool for monitoring antiangiogenic treatments. Decision rules will be proposed to optimize treatment according to the risk of relapse.