has not been established yet. The aim of this study was to determine that pyrosequencing (PSQ) might be used to achieve MGMT promoter methylation using one to thirteen year-old archival tissue samples as a clinical biomarker in routine practice.
Material and Methods: The study set included 141 formalin-fixed paraffin-embedded (FFPE) glial tumours from the archives of the pathology department from 1997 to 2010. Quantitative measurement of MGMT gene promoter DNA methylation employed PSQ of PCR products amplified from bisulfite converted DNA.
Results: PSQ data were obtained from all 141 samples. The mean of all cases was 14.0±16.8%, and methylated cases were 32.3±14.9%. A value of percentage of methylation (PM) of each year was not significantly different ($p = 0.771$) and didn't show any linear increasing or decreasing pattern according to the age of the FFPE block. Thirty one (41.3%) out of 75 GBM were methylated. Average PM of methylated and unmethylated cases were 35.8±14.7% and 3.2±1.8% respectively ($p < 0.001$). Eight (36.4%) out of 22 anaplastic astrocytoma were methylated,with 31.8±15.5% average PM. Eight (42.1%) out of 19 astrocytoma were methylated with 22.4±15.1% average PM. A tendency was observed toward an increasing pattern of average PM with WHO grade ($p = 0.063$) in astrocytic tumours. Anaplastic oligodendroglioma showed that 4 out of 7 cases (57.4%) were methylated and average PMs were 30.0±8.5% and 4.7±1.1% in methylated and unmethylated cases, respectively. A total of five out of eight cases (62.5%) of oligodendroglial tumour were methylated, and 28.0±8.1% and 4.7±1.2% were the respective average PM of methylated and unmethylated oligodendroglial tumours ($p = 0.024$). A correlation was observed between average PM and WHO grade ($p = 0.038$) and bimodal distribution between methylated and unmethylated cases, using 9% cut-off value ($p < 0.001$).
Conclusions: The study showed that a quantitative approach for MGMT promoter methylation gave better results than the classical gel-based methylation specific polymerase chain reaction reported by various researchers on FFPE tissue samples from old archives. The PSQ method can be used for a large scale retrospective trial, but cut-off value and calculation method of the PM should be validated.

Table 1. Methylation status of glial tumours

	Methylated		Unmethylated		Total(%)	p value
	Cases, n (%)	Mean±SD	Cases, n (%)	Mean±SD		
Glioblastoma	31(41.3)	35.8±14.7	44(58.7)	3.2±1.8	75(100)	<0.001
Anaplastic astrocytoma	8(36.4)	31.8±15.5	14(63.6)	3.3±2.2	22(100)	<0.001
Astrocytoma	8(42.1)	22.4±15.1	11(57.9)	3.4±2.6	19(100)	0.001
Anaplastic oligodendroglioma	4(57.1)	30.0±8.5	3(42.9)	4.7±1.1	7(100)	0.005
Oligodendroglioma	1(100)	20.0±0.0	0	0	1(100)	
Pilocytic astrocytoma	0	0	9(100)	3.0±1.3	9(100)	
ETC	0	0	7(100)	2.7±1.1	7(100)	
Total	52	32.3±14.9	89	3.3±1.9		

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Observational Cohort Study of Plasma Levels of Biomarkers in Patients With Non-small Cell Lung Cancer Treated With Bevacizumab

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Background: Antiangiogenic drugs have shown increased response rate and survival in NSCLC. No biomarkers have been described predictors of response in these patients (Pt). The aim of this study is to investigate the usefulness of the quantification of soluble VEGF, bFGF, E-selectin, and ICAM1 as biomarkers in advanced NSCLC Pt treated with Bevacizumab (BV).
Material and Method: This is an observational cohort study, concurrently, consecutively selected Pt. We included NSCLC Pt (squamous excluded), stageIV, treated with BV plus chemotherapy and without any previous antiangiogenic therapy. Plasma samples were collected before cycle 1 and after completion of cycle 2 (week 7). Levels of selected biomarkers were analyzed using commercially available ELISA kits (R&D Systems and Biolegend). We collected clinical and radiological data at the beginning, after the second cycle and at the end of treatment.
Results: We present preliminary results of 15Pt: media age was 56.9 (range 45−75; 8 male, 7 female). Basal ECOG: 0 = 9Pt; 1 = 5Pt; 2 = 1Pt. Histological subtypes were 7 adenocarcinomas, 5 large cell carcinomas and 3 not otherwise specified. Chemotherapy regimens used with BV were platinum-based doublet or monotherapy (Pemetrexed, Docetaxel) or Erlotinib. Only 4Pt presented mild adverse effects (2 hypertension; 1 bleeding; 1 thrombosis). Causes of end of BV were progression of disease (11Pt), adverse effect (2Pt), minor surgery (1Pt) and transfer to another hospital (1Pt). Measures of VEGF in the week 7 samples show consistently higher levels than baseline (207.2±52.5 vs 74.2±86.5 pg/mL). ICAM1 values also were slightly higher in the second sample (230.9±81.6 vs 209.8±63.4 ng/mL). However bFGF levels were lower in the second samples (32.3±16.5 vs 78.0±23.8 pg/mL). VEGF levels were different between Pt with clinical stability and clinical weakness (220.9±49.7 vs 152.2±7.1 pg/mL). These differences were also found in the radiological evaluation (214.5±53.1, stable vs 192.5±53.9 pg/mL, clinical weakness). At the end of the treatment, we also found differences in sICAM1 levels in both the clinical response (263.1±58.1, stable vs 142.6±75.2 ng/mL, weakness), and in the radiological evaluation (238.2±80.2, disease control vs 227.3±86.4 ng/mL, progression).
Conclusions: Changes in both VEGF and ICAM-1 levels could be used to monitor response to antiangiogenic treatment in NSCLC patients; in addition to standard clinical and radiological evaluations.

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Polymorphism Analysis in the AVADO Randomised Phase III Trial of First-line Bevacizumab (BEV) Combined With Docetaxel in HER2-negative Metastatic Breast Cancer (mBC)

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Background: In the E2100 trial, single nucleotide polymorphisms (SNPs) in the promoter region of VEGF (VEGF −2578, VEGF −1154) were reported to correlate with overall survival (OS) in BEV-treated patients (pts) with mBC [Schneider, JCO 2008]. In a retrospective analysis of the AViTA trial of BEV in pancreatic cancer, SNPs in the VEGFR-1 gene appeared to correlate with efficacy [Lambrechts, ESMO 2009]. We retrospectively analysed data from the BEV–docetaxel mBC AVADO trial [Miles, JCO 2010] to explore potential relationships between genetic variability in the VEGF signalling pathway and efficacy.
Methods: In AVADO, 736 pts with HER2-negative mBC were randomised to BEV 7.5 mg/kg, BEV 15 mg/kg or placebo (PLA), each combined with docetaxel 100 mg/m^2. The primary endpoint was progression-free survival (PFS). A panel of 26 candidate SNPs in genes involved in angiogenesis and tumourigenesis was evaluated in germ line DNA using kinetic PCR. Simple Cox regression analysis (no adjustment for multiple testing) was performed to correlate genotypes with PFS and OS.
Results: Demographics and efficacy in the genetics population (n = 336) were consistent with the overall study population. In the PLA group, the VEGF −2578 C/A polymorphism correlated with PFS: each additional C allele was associated with a 23% decrease in risk of progression or death (HR = 0.727, $p = 0.043$). With BEV 7.5 mg/kg, there was an indication of potential treatment by genotype interaction ($p = 0.02$). VEGF −1154 A/G also correlated with PFS (HR = 1.4, $p = 0.015$) but only in the BEV 7.5 mg/kg arm, with a non-significant treatment interaction. No other correlations were seen between efficacy and VEGF −2578, VEGF −1154, VEGFR-1 SNP rs9582036 or other SNPs.
Discussion: Although correlations between several SNPs and efficacy have been proposed in previous studies of BEV, we observed only a weak correlation between the VEGF −2578 SNP and PFS, driven by an effect in the PLA arm. Thus, our analysis of germ line DNA samples did not confirm findings from E2100. Likewise, our data do not confirm previous findings for VEGF and VEGFR-1 SNPs. Further biomarker research to identify pts most likely to benefit from BEV continues. AVADO (NCT00333775, sponsored by Roche) has completed accrual.

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Methods of Identification and Diagnosis of Lung Cancer Using Classification Systems

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Background: The goal of the study was to develop a blood test to detect non-small cell lung cancer (NSCLC) along with a robust statistical method for multimarker data mining.