Materials and Methods: We retrospectively investigated associations between A/F and efficacy endpoints using data from 416 patients (pts) with GIST from four trials (RTKC-0511–013, NCT00075218, NCT00137449 and NCT00372567; Pfizer). Pts received sunitinib either on an intermittent schedule (n = 325; 283 of whom received sunitinib at a starting dose of 50 mg/day on a 4-week-on/2-week-off schedule) or a continuous daily dosing schedule (37.5 mg/day; n = 91). Adverse events were recorded regularly using CTCAE version 3.0. Median time to tumour progression (TTP), progression-free survival (PFS) and overall survival (OS) were estimated using Kaplan–Meier (KM) methods and compared between pts with and without A/F using the log-rank test. Multivariate analysis was performed using age, gender, race, baseline Eastern Cooperative Oncology Group performance status, time from diagnosis, relative dose intensity, duration of prior imatinib treatment, baseline tumour volume, baseline granulocyte count, baseline hemoglobin, and baseline blood pressure as covariates. Time-dependent covariate analysis was performed to address potential bias from longer drug exposure, and landmark analyses were used to compare outcomes in pts with or without A/F after 6 and 12 weeks of treatment.
Results: Of 416 pts, 311 (75%) developed A/F of any grade, compared with 105 (25%) who did not. TTP and PFS were significantly longer in pts who developed A/F on sunitinib: median TTP was 7.8 vs 5.8 months and median PFS was 7.7 vs 5.1 months for pts with vs without A/F, respectively ($P \leqslant 0.004$). There was a trend for improved OS in pts with A/F (median OS: 20.1 vs 18.1 months; P = 0.135). Multivariate analysis showed that sunitinib-associated A/F was a significant predictor of improved outcome for all endpoints ($P \leqslant 0.013$). However, these results were not confirmed statistically in time-dependent covariate and landmark analyses. Analyses investigating the impact of A/F severity on outcome are in progress.
Conclusions: In pts with GIST, sunitinib-related A/F was significantly associated with improved TTP and PFS in KM analysis and was a significant predictor of TTP, PFS and OS in multivariate analysis. Since time-dependent covariate and landmark analyses supported the hypothesis that A/F may develop in pts who have longer drug exposure, the value of A/F as an early predictor of efficacy requires further analysis. This is the first reported link between drug-associated A/F and efficacy, and prospective studies are needed to validate these results.

1141 POSTER

Neutropenia and Thrombocytopenia During Treatment as Biomarkers of Sunitinib Efficacy in Patients With Metastatic Renal Cell Carcinoma (mRCC)

F. Donskov[1], A. Carus[1], C.H. Barrios[2], B. Escudier[3], S. Li[4], J. Perkins[5], R.J. Motzer[6]. [1]*Aarhus University Hospital, Department of Oncology, Aarhus, Denmark;* [2]*PUCRS School of Medicine, Oncology Research, Porto Alegre, Brazil;* [3]*Institut Gustave Roussy, Immunotherapy Unit, Villejuif, France;* [4]*Pfizer Oncology, Clinical Statistics, Shanghai, China;* [5]*Pfizer Oncology, Oncology, New York,* [6]*Memorial Sloan-Kettering Cancer Center, Medicine, New York, USA*

Background: Baseline neutrophils, thrombocytes and hemoglobin have been validated as prognostic factors in mRCC. We retrospectively studied the correlation between these hematologic variables during treatment and efficacy endpoints in sunitinib-treated mRCC patients from 5 clinical trials (NCT00054886, NCT00077974, NCT00083889, NCT00338884, NCT00137423; Pfizer).
Materials and Methods: Analyses included pooled data from 770 mRCC patients who received sunitinib 50 mg/d on a 4-wk-on-2-wk-off schedule (n = 544; 71%) or 37.5 mg/d continuous dosing (n = 226; 29%). Median PFS, TTP and OS were estimated by Kaplan–Meier methods and compared between subgroups using log-rank test. Multivariate and time-dependent covariate analyses were performed, the latter to address potential bias from longer drug exposure. Myelosuppression was graded using CTCAE v 3.0.
Results: In multivariate analyses, neutropenia grade $\geqslant$2 and thrombocytopenia grade >1 were associated with significantly longer TTP, PFS and OS (Table). Within the time-dependent covariate analysis, neutropenia grade $\geqslant$2 was significantly associated with all three efficacy endpoints; there was a trend for improvement in the endpoints with thrombocytopenia grade >1. Baseline neutrophil count $\leqslant$ULN and baseline thrombocyte count $\leqslant$ULN were associated with significantly longer TTP, PFS and OS in multivariate analysis. Baseline and on-treatment hemoglobin data will be presented at the meeting.
Conclusions: Neutropenia and thrombocytopenia during treatment may be previously unrecognized biomarkers of sunitinib efficacy, significantly associated with improved TTP, PFS and OS in mRCC patients. These data require validation in prospective trials. Hematologic parameters should be monitored closely with sunitinib treatment.

Table: Association between myelosuppression and efficacy outcomes

Efficacy endpoint	Median time to progression/survival event (mo)		P	Multivariate analysis, HR (P*)	Time-dependent covariate analysis, HR (P*)
Neutropenia during treatment (AE data)					
	Gr $\geqslant$2 (n = 366)	Gr <2 (n = 404)	<0.0001	$\geqslant$/< Gr 2	$\geqslant$/< Gr 2
TTP	13.7	7.8	<0.0001	0.553 (<0.0001)	0.775 (0.0073)
PFS	13.6	7.1	<0.0001	0.520 (<0.0001)	0.759 (0.0032)
OS	35.6	15.8	<0.0001	0.415 (<0.0001)	0.467 (<0.0001)
Baseline neutrophil count (lab data)					
	$\leqslant$ULN (n = 69)	>ULN (n = 74)		$\leqslant$/> ULN	$\leqslant$/> ULN
TTP	10.8	3.9	<0.0001	0.469 (<0.0001)	NA
PFS	10.7	3.2	<0.0001	0.482 (<0.0001)	NA
OS	24.9	9.1	<0.000	0.664 (0.0048)	NA
Thrombocytopenia during treatment (AE data)					
	Gr >1 (n = 101)	Gr $\leqslant$1 (n = 669)		>/$\leqslant$ Gr 1	>/$\leqslant$ Gr 1
TTP	13.8	10.1	0.001	0.660 (0.004)	0.765 (0.058)
PFS	13.7	8.8	0.001	0.658 (0.003)	0.767 (0.056)
OS	31.1	21.4	0.014	0.724 (0.038)	0.776 (0.088)
Baseline thrombocyte count (lab data)					
	$\leqslant$ LN (n = 649)	>ULN (n = 117)		$\leqslant$/> ULN	$\leqslant$/> ULN
TTP	11.0	4.7	<0.0001	0.481 (<0.0001)	NA
PFS	10.8	4.6	<0.0001	0.525 (<0.0001)	NA
OS	24.9	9.1	<0.0001	0.664 (0.0048)	NA

Gr: grade; NA: not applicable; *Wald chi-square test.

1142 POSTER

Clinical Significance of Macrodissection in Two Different KRAS Tests for Colorectal Cancer: Results From a Multi-center Clinical Trial

H. Bando[1], T. Yoshino[1], A. Ochiai[2], N. Mizunuma[3], E. Shinozaki[3], T. Denda[4], H. Soda[4], H. Shimada[5], S. Mori[6], K. Hatake[3]. [1]*National Cancer Center East, Department of Endoscopy and Gastrointestinal Oncology, Kashiwa,* [2]*National Cancer Center East, Pathology Division Research Center for Innovative Oncology, Kashiwa,* [3]*Cancer Institute Hospital of JFCR, Department of Medical Oncology, Tokyo,* [4]*Chiba Cancer Center, Department of Gastroenterology, Chiba,* [5]*Toho University Omori Medical Center, Department of Gastrointestinal Surgery, Tokyo,* [6]*Kotobiken Medical Laboratories Inc., Pathlogy Division, Tokyo, Japan*

Background: The *KRAS* mutation is a predictive marker for non-responsiveness to anti-EGFR antibodies for metastatic colorectal cancer. Macrodissection (MD) is currently recommended to enrich tumour cellularity when the ratio of tumour area is less than either 50 or 70%. However, evidence supporting the importance of MD is inadequate. We previously reported that a Luminex® *KRAS* detection kit showed a high concordance rate (99.1%) with direct sequencing (DS) and met our primary endpoint.
Methods: Formalin-fixed paraffin-embedded (FFPE) tissue specimens from 227 patients with colorectal cancer were registered. In total, 212 samples were analyzed as the full analysis set. The percentages of tumour area were blindly determined by independent pathological review and tumours were classified according to the percentages of tumour area (<50% versus $\geqslant$50%, <70% versus $\geqslant$70%). DNA from FFPE tumour tissues, with and without MD, were analyzed by both DS and the Luminex method. The results from DS with MD were used as a standard. We investigated the concordance of *KRAS* status according to the percentages of tumour area. Statistical analysis was performed by binominal tests.
Results: The *KRAS* mutation ratio detected by DS, with and without MD, was 34.9% and 32.1%, respectively. The *KRAS* mutation ratio detected by Luminex, with and without MD, was 35.8% and 33.5%, respectively. In the 165 samples with <70% of tumour area, 6 samples showed a discordance between DS with and without MD, which was statistically significant (P = 0.016). In the 47 samples with $\geqslant$70% tumour area, the concordance rate was 100%. On the other hand, 3 samples with <70% tumour area showed discordance between Luminex without MD and DS with MD (P = 0.125), while in samples with $\geqslant$70% tumour area, the concordance rate was 100%. For samples classified with tumour areas between <50% and $\geqslant$50%, the same trend was observed, but one sample with $\geqslant$50% area showed discordance between DS with and without MD.

Tumour area (%)	Luminex method					Direct-sequencing method				
	True positive	False positive	False negative	True negative	P value	True positive	False positive	False negative	True negative	P value
<50	38	0	3	81	0.125	36	0	5	81	0.031
50–100	33	0	0	57	1	32	0	1	57	0.5
<70	54	0	3	108	0.125	51	0	6	108	0.016
70–100	17	0	0	30	1	17	0	0	30	0.5

Conclusion: We showed that MD is an essential procedure for *KRAS* testing by DS when samples show tumour areas less than 50 or 70%; in contrast, MD may not be necessary for the Luminex method.

1143 POSTER

KRAS Mutational Status Strongly Impact Progression Free Survival of Patients Treated With Platinum Based Chemotherapy in NSCLC − Final Results of a Multicenter Prospective Study

M. Marabese[1], E. Rulli[1], A. Bettini[2], G. Farina[3], F. Longo[4], L. Moscetti[5], I. Pavese[6], C. Lauricella[7], M. Broggini[1], M.C. Garassino[3]. [1]*Mario Negri Institute for Pharmacological Research, Oncology, Milan,* [2]*Ospedali Riuniti di Bergamo, Medical Oncology, Bergamo,* [3]*Azienda Ospedaliera Fatebenefratelli Oftalmico, Oncology, Milan,* [4]*Policlinico Umberto I, Oncology, Rome,* [5]*Ospedale Belcolle, Oncology, Viterbo,* [6]*Ospedale San Pietro Fatebenefratelli, Oncology, Rome,* [7]*Ospedale Niguarda Ca' Granda, Pathology, Milan, Italy*

Background: KRAS mutations in NSCLC are supposed to indicate a poor prognosis and poor response to anticancer treatment. However, such evidence is only drawn from retrospective series giving controversial results. Moreover, it is possible that the various KRAS mutations differently affects prognosis, carcinogenesis and drug response as demonstrated in preclinical setting.
Aim of this study is to prospectively assess the prognostic value of KRAS mutations in NSCLC patients treated with a first line platinum containing regimen. This is a properly planned ancillary study within the TAILOR trial (NCT00637910) which is mainly focused on the second line.
Methods: Tissue and blood samples were collected at diagnosis in the whole cohort of registered patients. KRAS status was centrally determined with standard direct sequencing and KRAS genotype was assessed by real time PCR. The primary hypothesis is a difference in PFS according to KRAS mutational status; the impact of the three more frequent KRAS substitutions (G12C, G12V, and G12D) was also explored. The analysis was planned at occurrence of 200 events (HR $\geqslant$ 1.49, power 80%, 2-tailed alpha 10%),in a Cox model adjusting for Performance Status and radical surgery.
Results: Out of 565 patients registered, 341 (60.5%) were evaluable for KRAS and 85(25%) were mutated.
At a median follow-up of 17 months KRAS mutated patients showed a statistically significant worse PFS (HR 1.42 95% CI 1.06–1.94; p = 0.02). No differences among doublets were observed in KRAS mutated patients. The most frequent KRAS mutations were: G12C (36.4%), G12V (21.1%), G12D (16.4%), others (25.9%). Prognostic differences among variants are observed. Final genotype analyses are ongoing.
Conclusions: This is the first prospective, pre-planned and adequately sized evaluation of KRAS in NSCLC. Patients mutated for KRAS seem to have a higher risk of progressing. These results suggest that KRAS mutation epidemiology in this setting highly differs from that of colon cancer. Clinical data suggest that tailored strategies for these patients are warranted and our preclinical studies will help in clarifying the molecular mechanisms.

1144 POSTER

Using Single-cell Network Profiling (SCNP) Signatures to Predict Response to Induction Therapy and Relapse Risk in Pediatric Patients With Acute Myeloid Leukemia (AML)

A. Cesano[1], M. Westfall[1], D. Rosen[1], B. Louie[1], S. Meshinchi[2], T. Alonzo[2], R. Arceci[2], Y. Ravindranath[2], G. Dahl[2], N. Lacayo[2]. [1]*Nodality, Research and Development, South San Francisco,* [2]*Childrens Oncology Group, Paediatric Oncology, Arcadia, USA*

Background: In pediatric AML, cytarabine-based combination regimens result in 80−90% complete remission (CR) rate but ultimately only half of the patients achieve long term remissions. The need for accurate prediction of two separate outcomes is of interest: 1. response to induction therapy, which offers guidance on patient specific induction therapy and 2: early relapse (CR-Rel), which allows for consolidation therapy decisions. Current prognostic factors (e.g., cytogenetics, FLT3 ITD) are not completely predictive of response or outcome for individual patients. SCNP is a functional evaluation measuring the effects of multiple modulators (including drugs) on signaling pathways at the single-cell level.
Methods: SCNP assays were analyzed for 67 BM samples from pediatric AML patients enrolled in POG (now COG) trial 9421 (46 CR and 21 NR). 80 signaling nodes (i.e., the combinations of modulators and intracellular activated proteins) were investigated including the PI3K, JAK/STAT, DNA damage response and apoptosis pathways. Basal and modulated protein levels in leukemic blasts were measured, and nodes were examined by univariate and multivariate analyses.
Results: DNA Damage and Apoptosis nodes (e.g., Etoposide or AraC+Daunorubicin $\rightarrow$ c-PARP and p-Chk2, p = 0.001) and induced phosphorylation (p-) levels of PI3K/MAPK pathway members S6 and ERK (Flt3 $\rightarrow$ p-S6, p = 0.04) showed higher levels in CR samples. Induced apoptosis was also associated with risk of relapse. Thapsigargin, a calcium modulator, induced higher levels of p-Erk, p-CREB and p-S6 in patients with CCR as compared to CR-Rel samples (p = 0.02). More importantly, in multivariate analysis, combination of 2−5 nodes (representing apoptosis, Jak/Stat and PI3K pathways) resulted in classifiers with good performance characteristics (bootstrap adjusted AUC 0.80–0.86) in predicting response to induction therapy and risk of relapse. The model predictions remain significant (p < 0.04 for both models) after adjusting for any one of the clinical covariates e.g., cytogenetics, FLT3-ITD, WBC, cytogenetics and age.
Conclusion: This study showed that performing quantitative SCNP under modulated conditions could serve as the basis for developing improved predictive tests for response to induction chemotherapy in pediatric patients with newly diagnosed AML. Additionally, the biology revealed could prove useful in determining alternative therapeutic strategies. Independent validation is ongoing.

1145 POSTER

Drug Resistance Induced by Plasmatic Concentrations of Paclitaxel and Carboplatin in Cancer Cell Lines

R. Luque[1], J. Delgado[1], E. González[1], J. Prados[2], C. Melguizo[2], J. Valdivia[1], J. Martínez[1], J. Ortega[1], R. Ortiz[2], A. Aránega[2]. [1]*Hospital Universitario Virgen de las Nieves, Oncología Médica, Granada,* [2]*Universidad de Granada, Anatomía y Embriología Humana, Granada, Spain*

Background: Several proteins, as PgP and MRP family, are involved in the resistance to chemotherapy of the tumour cells. PgP (mdr1) and MRP family are members of the ATP-binding-cassette (ABC) transporters family. ABC transporters are a protein family able to transport a wide variety of substrates such as lipids, bile salts, toxins, and antigen-presenting peptides. This transport process is carried out across the membrane against a concentration gradient and gained from ATP hydrolysis. Antineoplastic drugs from natural sources such as taxanes, vinca-alkaloids, antracyclines, and epipodophyllotoxins are some of the ABC transporters substrates.
Material and Methods: We have studied the MDR1 and MRP3 expression in 6 cell lines, 3 of non small cell lung cancer (NSCLC), 1 of breast cancer, 1 of gastric cancer and other one of seminoma, after being exposed to plasma concentrations of Paclitaxel, Carboplatin, and the combination of both.
Results: After chemotherapy, we observed that paclitaxel induced MDR1 and carboplatin induced MRP3 in NSCLC cell lines. The association of both drugs increased significantly the expression of MDR1, and very few the expression of MRP3. Paclitaxel induced MDR1 in all cell lines derived from other tumours. Carboplatin did not induce MDR1 as previously, nor MRP3 in gastric cancer cell line.
Conclusions: Plasma concentrations of paclitaxel induced MDR1 expression but not MRP3 in NSCLC and other tumours derived cell lines. However, carboplatin produced overexpression of MRP3 but not MDR1 in the same cell lines.
The combination of both drugs was not able to activate a new resistance mechanism in the studied cell lines, but it was able to improve the resistance mechanism induced by each one of the drugs individually. This fact resulted in an increase of MDR1 expression with paclitaxel and MRP3 expression with carboplatin.

1146 POSTER

Pro-angiogenic Factor Cyr61 is Linked to Colorectal Cancer Development and Prognosis

M. Baek[1], T. Ahn[1], D. Jung[2], S. Park[2], H. Kim[2], W. Lee[2], D. Park[2]. [1]*Soonchunhyang University Cheonan, Surgery, Cheonan,* [2]*Soonchunhyang University College of Medicine, Pathology, Cheonan, Korea*

Background: Angiogenic factor Cysteine-rich 61 (Cyr61) is a member of the CCN protein family that has been implicated in diverse biological processes such as cell adhesion, proliferation, angiogenesis, and tumorigenesis. An altered expression of Cyr61 is found to be associated with several human cancers. However, the correlation of expression of Cyr61 protein and clinical features of colorectal cancer remains unknown.
Material and Methods: Cyr61 expression in colorectal cancer and normal tissues was evaluated by Western blot analysis. Immunohistochemical staining was carried out using Tissue Microassay(TMA) to examine the