mice. Treatment of mice with sphingosine (25 mg/kg) resulted in a time-dependent rise in 5-HIAA.
Conclusions: The results, together with those from work in progress, are consistent with the hypothesis that ASA404 activates the ceramide pathway. Ceramides and/or sphingosine may activate downstream targets including p38 MAP kinase, leading to vascular damage and consequent release of 5-HT from platelets. In addition to its role in ASA404 action, sphingosine may be a potentially useful biomarker for the assessment of patients treated with ASA404 in combination therapy.

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Analysis of EGFR and KRAS mutations in circulating tumor DNA (cTDNA) from plasma of NSCLC patients in phase 2 trials of XL647

F. Schimmoller[1], N.A. Rizvi[2], V.S. Vysotskaia[3], R.P. Funke[4], S. Dixon[5], R.E. Buller[1]. *[1]Exelixis Inc., Translational Medicine, South San Francisco, CA, USA; [2]Memorial Sloan-Kettering Cancer Center, Department of Medicine, New York, USA; [3]Exelixis Inc., Genome Biochemistry, South San Francisco, CA, USA; [4]Exelixis Inc., Clinical Development, South San Francisco, CA, USA; [5]Exelixis Inc., Biostatistics & Clinical Data Management, South San Francisco, CA, USA*

Background: EGFR and KRAS mutations play a critical role in the response to EGFR tyrosine kinase inhibitors (TKI) in NSCLC. Obtaining adequate tumor tissue in patients (pts) with non-operable disease, especially prior to initiation of a later line of therapy when the molecular profile of a cancer may have evolved, is a key element for the development of targeted anticancer agents and remains challenging. Sensitive assay methodology, eg allele specific PCR, to analyze cTDNA may facilitate development of targeted agents such as XL647, a TKI which potently inhibits EGFR (including the T790M resistance mutation), HER2 and VEGFR2 in vitro.
Methods: EGFR and KRAS mutations were determined in plasma cTDNA by the sensitive Scorpions ARMS technology (DxS Ltd, Manchester, UK). Samples were collected from pts enrolled in NSCLC clinical trials of XL647 either in a first line or late line of therapy after previous EGFR TKI benefit. EGFR and KRAS mutations in plasma were compared to those in available tumor samples.
Results: Plasma samples (n = 67) from 55 pts were analyzed in the DxS EGFR29 panel. A subset of 46 samples was analyzed with the DxS KRAS kit. EGFR ($p < 0.0001$) and KRAS ($p = 0.0003$) DNA levels were lower in plasma from late line pts compared to those with newly diagnosed disease. The spectrum of EGFR mutations detected included Exon19 deletions, L858R, T790M, L861Q, and G719X. Assay failure was more likely when testing KRAS (19/46) than EGFR (3/67), $p < 0.0001$ and more common when testing KRAS in the late line setting (16/23 vs 3/23), $p < 0.0001$, thought secondary to lower PCR performance specific to the KRAS sequence. When testing an individual patient over time, variability was noted. The significance of this observation is being explored. Data from 11 paired plasma and tumor samples in first line pts showed concordance for EGFR status at 82%, specificity at 100%, while the sensitivity to detect EGFR mutations was limited at 50%. In the late line, EGFR mutations were detected in plasma from 11/39 pts. Correlation of these results relative to direct analysis of archival tumor is ongoing.
Conclusions: cTDNA can be informative for EGFR and KRAS mutation testing; however, the current technology performs better on samples from newly diagnosed patients than on those with disease recurrence/persistence. Optimal timing for plasma sampling and larger studies are required to better understand the limitations of mutational analysis conducted exclusively in plasma.

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DUSP4 expression level in colorectal primaries predicts overall survival benefit in Kras wild-type and Kras mutant colorectal cancer after treatment with cetuximab for metastatic disease

W. De Roock[1], M. Janssens[2], B. Biesmans[1], B. Jacobs[1], J. De Schutter[1], Y. Humblet[3], M. Peeters[4], E. Van Cutsem[5], P. Marynen[6], S. Tejpar[7]. *[1]K.U.Leuven, Center For Human Genetics, Leuven, Belgium; [2]Independent Biostatician, Mechelen, Belgium; [3]Cliniques Universitaires Saint-Luc, Clinique des Pathologies Tumorales du Côlon et du Rectum Centre du Cancer, Brussels, Belgium; [4]Ghent University Hospital, Digestive Oncology Unit, Ghent, Belgium; [5]Gasthuisberg University Hospital, Digestive Oncology Unit, Leuven, Belgium; [6]K.U.Leuven – Flanders Interuniversity Institute for Biotechnology, Human Genome Laboratory – Department of Human Genetics, Leuven, Belgium; [7]K.U.Leuven – Gasthuisberg University Hospital, Center for Human Genetics – Digestive Oncology Unit, Leuven, Belgium*

Background: DUSP4 dephosphorylates and inactivates ERK and is upregulated by Kras mutations (MUT). Higher expression of DUSP4 was found in epithelial cell cultures with activated ras (Bild et al. Nature 2005). Microarray analysis on metastases of colorectal cancers (CRC) treated with cetuximab (CTX) (Khambata-Ford S et al. JCO 2007) identified DUSP4 as a top resistance marker. We correlated Kras MUT state and DUSP4 mRNA expression level in 197 primary CRC of patients (pts) treated with CTX +/− irinotecan (IRI) with objective response (OR), progression-free survival (PFS) and overall survival (OS).
Material and Methods: Tumor areas were manually dissected on FFPE samples. Kras codon 12&13 MUT were analyzed by an allelic discrimination assay. We measured DUSP4 mRNA expression by real-time qRT-PCR. Relative expression levels were calculated comparing average values of duplicate reactions with a calibrator and normalizing them to 3 housekeeping genes: GAPDH, RPL13, UBC. Levels were expressed as calibrated normalized relative quantities. We used the median of these values as cut-off to distinguish high from low expression.
Results: 45% had a Kras MUT. Kras wild type (WT) was associated with OR ($p < 0.0001$), PFS ($p = 0.0005$) and OS ($p = 0.0001$). Low DUSP4 was associated with Kras WT ($p < 0.0001$). DUSP4 was associated with OR: 72% (33/46) of responders had a low DUSP4 compared to 44% (66/151) of non-responders ($p < 0.0012$). Overall, there was no association between DUSP4 and PFS ($p = 0.33$). In Kras WT, there was a trend towards longer median PFS in low DUSP4 ($p = 0.10$). Overall, OS was associated with low DUSP4 [41w (95%CI [36−49]) vs 33w high DUSP4 (95%CI [27−46]) ($p = 0.06$)]. In Kras WT, OS was associated with low DUSP4 [54w (95%CI [430−72]) vs 34.5w high DUSP4 (95%CI [25−54]) ($p = 0.01$)]. In Kras MUT, OS was associated with high DUSP4 [32w (95%CI [15−33]) vs 18w low DUSP4 (95%CI [21−39]) ($p = 0.02$)]. A Cox regression model for PFS and OS was built using Kras, DUSP4 and skin toxicity (table).

	PFS				OS			
	Sig.	HR	95% CI		Sig.	HR	95% CI	
			Lower	Upper			Lower	Upper
Kras WT								
DUSP4 (< or > median)	0.0308	0.614	0.395	0.956	0.0024	0.507	0.327	0.786
Skin tox (gr 2−3 vs 0−1)	<0.0001	0.345	0.223	0.533	<0.0001	0.407	0.265	0.623
Kras MUT								
DUSP4 (< or > median)	0.1030	1.514	0.920	2.492	0.0772	1.582	0.951	2.631
Skin tox (gr 2−3 vs 0−1)	0.1576	0.678	0.395	1.162	0.0060	0.443	0.248	0.792

Conclusions: DUSP4 expression levels influence OS in both Kras WT and Kras MUT CRC treated with CTX +/− IRI. In Kras WT low DUSP4 levels are favourable, while in Kras MUT high DUSP4 levels are. Kras WT with high DUSP4 may have other oncogenic MUT e.g. Kras codon 61. The extent of ERK signalling is shown not to be the same in all Kras MUT. The prognostic effect of DUSP4 levels needs to be looked into, but the use of DUSP4 expression level as a substrate for the extent of ERK signalling may make it possible to select CRC likely to benefit from ERK-inhibitors.

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Models for response to the MEK inhibitor GSK1120212 confirm RAS and BRAF mutations as predictive biomarkers and suggest other, unexpected tumor types for clinical evaluation

R. Wooster[1], W. Cornwell[1], J. Jing[1], J. Greshock[1], A. Gilmartin[1], D. Sutton[1], J. Jackson[1], S. Laquerre[1], B. Weber[1], Y. Degenhardt[1]. *[1]GlaxoSmithKline, Oncology, King of Prussia, USA*

The growth factor stimulated RAS/RAF/MEK/ERK signaling pathway is involved in many aspects of cancer progression, and is activated in a large percentage of tumors evidenced by frequently occurring RAF and RAS mutations in cancer. BRAF mutations occur in ~45% of melanomas and KRAS in ~90% of pancreatic adenocarcinomas. GSK1120212 is a potent and selective allosteric inhibitor of the MEK1/2 enzymes that exhibits antiproliferative effect in cell lines and xenograft models. To identify predictive biomarkers to facilitate the clinical development of this compound, sensitivity profiling was carried out for over 300 cancer cell lines. The cell lines were from diverse tumor types to confirm existing hypotheses and explore the sensitivity of other tumor types that were not initially considered to be sensitive to MEK inhibition. As expected, BRAF/RAS mutation status emerged as a strong predictor of response. Cell lines from tumor types that have high occurrence of BRAF/RAS mutations (e.g., melanoma, pancreas and colon cancer) showed a higher rate of sensitivity. We further refined the predictors by grouping cell lines into cytotoxic and cytostatic groups. GSK1120212 has a cytostatic effect in cell lines that are RAF/RAS mutant with co-occurring PI3K/PTEN mutations but cytotoxic in the absence of PI3K/PTEN mutations, suggesting mutant PI3K/PTEN are cytotoxicity resistance markers for GSK1120212. However, for certain tumor types that do not carry BRAF or RAS mutations, sensitivity