observed decreased $p16^{INK4a}$. Detailed analysis showed that HLX1 affects *INK4a* expression at the mRNA level and HLX1 binds to the *INK4a* promoter as observed by ChIP. We also observed increased levels of the repressive H3K27me3 mark and recruitment of PRC2 components at the *Ink4a*/*Arf* locus correlating with HLX1 expression. Co-immunoprecipitation studies showed that HLX1 associates with the PRC2, in particular with Suz12. RNAi studies showed that the repression of $p16^{Ink4a}$ by HLX1 is dependent of PRCs. In an attempt to understand if the repression of HLX1 was a property shared by other homeobox genes, we tested 20 homeobox-containing genes and identified that multiple homeobox can also repress $p16^{INK4a}$.

Conclusions: We identified the homeobox protein HLX1 as a novel $p16^{INK4a}$ repressor. HLX1 binds to the *INK4a* promoter region and recruits Polycomb repressive complexes. Multiple homeobox proteins can also regulate $p16^{INK4a}$ expression, which implies a conserved role for this family oftranscription factors in regulating the *Ink4a*/*Arf* locus, highlighting its potential physiological relevance for both senescence and carcinogenesis.

1004 ORAL

Lactate Influx and Efflux Through Monocarboxylate Transporters Bridge Cancer Cell Metabolism and Angiogenesis

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Background: Tumour cells fuel their metabolism with a variety of nutrients to meet the bioenergetic and biosynthetic demands of proliferation. In particular, conversion of pyruvate into lactate allows the production of intracellular NAD^+ to maintain high glycolytic flux in tumours. Here, we investigated whether the consecutive lactate accumulation in the tumour microenvironment could indirectly modulate the endothelial cell phenotype and thereby promote angiogenesis.

Materials and Methods: Microfluidic low-density arrays were used to examine the influence of lactate on the endothelial expression profile of angiogenesis-related genes. The consecutive identification of IL-8/CXCL8 mRNA as the major upregulated transcript in response to lactate led us to study the signaling pathway bridging lactate and IL-8-driven angiogenesis using dedicated gene silencing and pharmacological strategies. We also addressed the *in vivo* relevance of this pathway in different mouse tumour models combining the injection of shRNA-transduced tumour and endothelial cells into extracellular matrix plugs.

Results: We found that lactate could enter endothelial cells through the monocarboxylate transporter MCT-1 and then stimulate an autocrine NFkB/IL-8 (CXCL8) pathway driving endothelial cell migration and tube formation. We further identified the capacity of lactate to activate NFkB through the phosphorylation and consecutive degradation of IkBa. These effects were prevented by 2-oxoglutarate and reactive oxygen species (ROS) inhibitors, pointing to a role for prolyl-hydroxylase and ROS in the integration of lactate signaling in endothelial cells. Prolyl-hydroxylase PHD2 silencing in glucose-fuelled endothelial cells recapitulated the pro-angiogenic effects of lactate, whereas a blocking IL-8 antibody or IL-8-targeting siRNA prevented them. Finally, we documented in mouse xenograft models of human colorectal and breast cancers that lactate release from tumour cells through the MCT4 (and not MCT1) transporter was sufficient to stimulate IL-8-dependent angiogenesis and tumour growth.

Conclusions: Our findings establish the existence of a lactate-driven feed-forward IL-8 autocrine loop driving angiogenesis in tumours and the key roles of monocarboxylate transporters MCT1 and MCT4 in this lactate-based dialog between cancer cells and endothelial cells. More generally, our study provides a new rationale for associating elevated lactate concentrations in tumours and negative outcomes for patients, and further supports the current enthusiasm for new cancer treatments targeting metabolic pathways.

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HER-3, IGF-1, NF K-B and EGFR Gene Copy Number in the Prediction of Clinical Outcome for Colorectal Cancer Patients Receiving Irinotecan-cetuximab

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Background: A large proportion of colorectal cancer patients does not benefit from the use of anti-EGFR treatment although in the absence of a mutation of the K-RAS gene. Preliminary observations suggested that HER-3, IGF-1, NF-kB and EGFR GCN might identify patients not likely to benefit from anti-EGFR therapy. We tested the interaction between HER-3, IGF-1, NF-KB, EGFR GCN and K-RAS mutational analysis to verify the relative ability of these variables to identify a sub-group of patients more likely to benefit from EGFR-targeted treatment among those harbouring a K-RAS wild type status.

Materials and Methods: We retrospectively collected tumours from 168 patients with metastatic colorectal cancer patients treated with irinotecan-cetuximab. KRAS was assessed with direct sequencing, EGFR amplification was assessed by chromogenic in situ hybridization and HER-3, IGF-1 and NF-kB were assessed by immunoistochemistry.

Results: In patients with K-RAS wild type tumours, the following molecular factors resulted independently associated with response rate: HER-3 (OR = 4.6, 95% CI: 1.8–13.6, p = 0.02), IGF-1 (OR = 4.2, 95% CI: 2–10.2, p = 0.003) and EGFR GCN (OR = 4.1, 95% CI: 1.9–26.2, p = 0.04). These factors also independently correlated with overall survival as follows: HER-3 (HR = 0.4, 95% CI: 0.28–0.85, p = 0.008), IGF-1 (HR = 0.47, 95% CI: 0.24–0.76, p < 0.0001) and EGFR GCN (HR = 0.59, 95% CI: 0.22–0.89, p = 0.04) (table 1).

Conclusions: We believe that our data may help further composing the molecular mosaic of EGFR resistant tumours. HER-3, IGF-1 and CISH EGFR GCN proved to possess a relevant role in defining subgroups of colorectal cancer patients more likely to benefit from anti-EGFR treatment. Interestingly HER-3 and the IGF-1 driven pathway have also been demonstrated to be possible molecular targets as part of a treatment protocol focused on control of either HER receptors or the PI3K/AKT pathway. The possibility to use HER-3 and IGF-1 inhibitors in biologically-selected anti-EGFR resistant tumours promise then to be a crucial challenge for the future development of targeted therapy in colorectal cancer patients.

Table 1

	HER-3		IGF-1		EGFR	
	Positive (n = 46)	Negative (n = 44)	Positive (n = 59)	Negative (n = 31)	<2.12 (n = 47)	⩾2.12 (n = 43)
Response Rate (%)	25%	50%	22%	65%	6%	37%
Multivariate OR (95% CI	4.6 (1.8–13.6)		4.2 (2–10.2)		4.1 (1.9–26.2)	
Logistic regression *p* value	0.02		0.003		0.04	
Median Overall Survival (months)	11.3	25	8.3	25	10.4	18
Multivariate HR (95% CI)	0.4 (0.28–0.85)		0.47 (0.24–0.76)		0.59 (0.22–0.89)	
Cox regression *p* value	0.008		<0.0001		0.04	

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Prognostic Factors for Progression-free Survival (PFS), Overall Survival (OS), and Long-term OS (LT-OS) With Sunitinib in 1,059 Patients, Treated on Clinical Trials, With Metastatic Renal Cell Carcinoma (mRCC)

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Background: With the advent of multiple targeted therapies for mRCC, further information on factors affecting prognosis facilitates both clinical decision making and trial design for evaluation of new therapies. Here, we report a retrospective analysis of prognostic factors for PFS, OS and LT-OS (⩾30 months) in patients (pts) with mRCC treated with sunitinib in 6 clinical trials (NCT00054886, NCT00077974, NCT00083889, NCT00338884, NCT00137423, NCT00267748; Pfizer).

Methods: Analyses used pooled data from 1,059 pts treated with single-agent sunitinib on the approved 50 mg/day 4-week-on/2-week-off schedule (n = 689; 65%) or 37.5 mg continuous once-daily dosing (n = 370; 35%), in the first- (n = 783; 74%) or second-line (n = 276; 26%) setting. Baseline variables were analyzed for prognostic significance using a Cox proportional hazards model, with each factor investigated in univariate and then multivariate analyses using a stepwise algorithm.

Results: Multivariate analysis of PFS and OS identified 9 and 10 independent predictors, respectively (Table). Overall, 215 pts (20%) survived at least 30 months. An analysis of baseline characteristics of these long-term survivors showed characteristics differed between these pts and non-long-term survivors, including risk status based on the published Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic criteria (Motzer, 2002; P < 0.0001). For example, 70% of the long-term survivors had favorable risk features compared with 31% of non-long-term