survivors. In contrast, 42% and 5% of the non-long-term survivors had intermediate and poor risk features compared with 28% and 0% of long-term survivors, respectively. Additional characteristics associated with LT-OS will be presented.

Variable	PFS		OS	
	HR (95% CI)	P-value*	HR (95% CI)	P-value*
Ethnic origin (white vs non-white)	0.598 (0.459, 0.781)	0.0002	0.730 (0.535, 0.996)	0.0474
ECOG PS† (⩾1 vs 0)	1.250 (1.043, 1.498)	0.0159	1.505 (1.218, 1.859)	0.0002
Time from diagnosis to treatment† (⩾1 vs <1 year)	0.814 (0.680, 0.975)	0.0252	0.666 (0.541, 0.820)	0.0001
Bone metastases (yes vs no)	–	–	1.535 (1.250, 1.886)	<0.0001
Baseline hemoglobin† (⩽LLN vs >LLN)	1.384 (1.144, 1.675)	0.0008	1.548 (1.245, 1.925)	<0.0001
Baseline lactate dehydrogenase† (>1.5 × ULN vs ⩽1.5 × ULN)	1.664 (1.201, 2.305)	0.0022	1.571 (1.103, 2.238)	0.0123
Baseline corrected calcium† (>10 vs ⩽10 mg/dL)	1.374 (1.080, 1.747)	0.0096	2.208 (1.722, 2.832)	<0.0001
Baseline neutrophils (⩽ULN vs >ULN)	0.629 (0.483, 0.821)	0.0006	0.681 (0.508, 0.915)	0.0107
Baseline platelets (⩽ULN vs >ULN)	0.607 (0.469, 0.785)	0.0001	0.670 (0.505, 0.889)	0.0055
Prior cytokine (yes vs no)	1.342 (1.085, 1.659)	0.0066	1.387 (1.094, 1.759)	0.0068

*Wald Chi-Square Test; †Factor included in MSKCC prognostic model

Conclusions: These analyses validated use of clinical risk factors previously reported from MSKCC (J Clin Oncol 20: 286, 2002) and by Heng et al (J Clin Oncol 27: 5794, 2009). These factors were predictive for shorter PFS as well. In addition, pts with bone metastases had shorter OS to sunitinib. Favorable MSKCC risk status was associated with higher likelihood of achieving LT-OS. Continued progress requires incorporation of RCC tumour-specific biology.

1007 ORAL

Efficacy of DNA Vaccination Against Anaplastic Lymphoma Kinase (ALK) in Non Small Cell Lung Carcinoma (NSCLC)

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Background: Lung cancer is the leading cause of cancer-related mortality worldwide. Recently, NSCLC harbouring ALK translocations have been described. Although standard chemotherapy or molecularly targeted therapies are effective in NSCLC, tumour recurrence and metastatic dissemination still remain a frequent event. Our previous findings show that ALK is an effective oncoantigen for ALK positive lymphoma vaccination and thus it could, as well, represent a feasible target for ALK positive NSCLC therapy.
Materials and Methods: We generated transgenic (Tg) mice ectopically expressing human TFG- or EML4-ALK protein in lung epithelium under the murine lung specific SP-C promoter. For DNA vaccination, we injected 50 ug of plasmid DNA in the femoral muscle of anesthetized mice for a total of at least 3 immunization, as previously described (*Chiarle et al., Nature Medicine 2008*). To evaluate the generation of an immune response, we performed an *in vivo* citotoxicity assay with CSFE-labelled cells one month after vaccination. Histology and immunohistochemistry were performed on different specimens. Tumour growth and progression was monitored overtime by Nuclear Magnetic Resonance (NMR).
Results: ALK Tg mice developed multifocal adenocarcinomas similar to human tumours, starting from 1 month after birth. A strong ALK specific CTL response was elicited in ALK positive vaccinated mice, thus demonstrating that ALK vaccination could overcome the immune tolerance to the ALK protein. By MRI analysis, vaccinated mice showed a reduced number of neoplastic foci and a smaller tumour mass as compared to mice vaccinated with a mock plasmid. The efficacy of DNA vaccination was dependent on mice age as the specific CTL activity against ALK and the ability to limit the tumour expansion decreased proportionally to the mice age. The number of T lymphocytes infiltrating both the tumours and the spared lung was significantly increased in vaccinated mice.
Conclusions: Our Tg mice represent a suitable model to dissect the role of ALK in lung tumour pathogenesis and for the development of innovative treatment strategies. Our findings indicate that ALK-DNA vaccination is able to elicit a specific cytotoxic response and to delay tumour progression in ALK+ Tg mice. Therefore, in ALK positive NSCLC a strategy that combines DNA vaccination combined with standard chemotherapy or specific ALK inhibitors could represent an alternative treatment to prevent tumour relapse or metastasis.

Poster Presentations (Sat, 24 Sep, 14:00–16:30)
Basic Science

1008 POSTER

The Effects of Telomerase Inhibitor GRN163L(Imetelstat) on Cell Cytoskeleton, Cell Cycle and Matrix Metalloproteinases

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Background: As telomerase activity can not be determined in somatic tissues but can be determined in 90% of human tumours, it is an attractive target for cancer therapy to telomerase. GRN163L is an N3′ → P5′-*thio-phosphoramidate* oligonucleotite which is complementary to the template region of telomerase RNA. We have previously reported that A549-luc cells treated before cell attachment with a single dose of GRN163L weakly attached to the substrate and remained rounded, whereas control cells exhibited typical epitheloid appearance and adhesion properties. In this study, we aimed to determine whether cell cytockeleton and adhesion proteins are relative with rapid morphologic alterations and loss of adhesion in GRN163L treated A549 cells. In addition, we investigated the potential decrease in MMP levels in GRN163L treated cells and also performed cell cycle analyses.
Material and Methods: A549 cells were plated in the presence of GRN163L (1 mM) and incubated for 24hrs. The untreated control cells and treated cells were collected following 24 hr of GRN163L incubation, then actin, tubulin and e-cadherin expressions were analysed by both Western Blot and immunohistochemistry. Real-Time PCR assay was used for cell cycle analyses and determination of MMP mRNA expressions of A549 lung cancer cells treated with GRN163L.
Results: We observed that actin, tubulin and e-cadherin expressions of GRN163L treated cells were significantly decreased within 24 hrs compared with the untreated control cells. Immunohistochemistry results also showed that all the actin and tubulin filaments were displaced and concentrated along the cell membrane. Interestingly, all of the effects were reversible after 72 hrs due to the the cessation of treatment. Additionally, according to Real-time PCR results, it was obvious that Cdk 6, cdk 4 and cyclin D1 mRNA levels that regulate the G1 phase of the cell cycle decreased following 1 week of GRN163L treatment when compared with the controls. Besides these results, MMP-2 expression of A549 cancer cells decreased following 24hrs of GRN163L treatment, but there was no significant change in MMP1 expression.
Conclusions: We can conclude that the morphological changes in cell cytoskeleton and loss of adhesion occur which ocur within 24 hr in GRN163L treated cells. These target-off effects besides telomerase inhibition decrease the adhesion, proliferation and metastatic potential of A549 cancer cells. For this reason, it may be possible to inhibit metastasis of residuel cancer cells by combining GRN163L with other chemotherapeutics or surgery.

1009 POSTER

Mammary Gland Tumour Formation in Conditional Transgenic Mice Expressing GLI1

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Background: Up regulation of the Hedgehog pathway effector GLI1 in breast cancer correlates with unfavourable overall survival. The Hedgehog pathway has a role in the regulation and maintenance of CD44 positive breast cancer stem cells. Skin and intestinal stem cells express the orphan G protein coupled receptor (GPCR) LGR5. Previously, we have shown that multiparous conditional transgenic mice (MMTVrtTA;TREGLI1) expressing GLI1 develop hyperplastic lesions and tumours.
Materials and Methods: GLI1 expression was induced in female transgenic mice expressing GLI1 in the mammary gland. The mice were monitored for the occurrence of tumours. Palpable tumours and hyperplastic lesions developed in the mice with induced GLI1 expression. Normal and tumour tissue were analysed.
Results: We show that the cells of the basal cell layer of the large mammary ducts are Lgr5 positive. Lgr5 is also expressed in mammary gland tumours induced in conditionally transgenic mice expressing GLI1 in the mammary gland. Hyperplastic lesions and palpable mammary gland tumours also develop in nulliparous transgenic mice, after long term low level GLI1 expression. Both solid and acinar adenocarcinomas develop in GLI1 expressing nulliparous mice, even within the same mammary gland. The expression of the stem cell marker CD44 is increased in the mammary ducts as well as the tumours in the GLI1 expressing mice. The GLI1