risk tumors *KIT* exon 11 deletions were more frequently found than point mutations (p = 0.017). On the other hand mutations in *PDGFRA* were more often observed in very low-/low- than high risk GISTs as compared to *KIT* exon 11 (p = 0.0026). There was no statistically significant correlation between disease-free survival and the spectrum or frequency of mutations.
Conclusions: Spectrum and frequency of *KIT* and *PDGFRA* mutations in Polish GIST population are similar to the Spectrum and frequency of and mutations in Polish GIST population are similar to the previously described groups. No significance of mutations for disease outcome after surgery of primary tumors was found.

9406 ORAL

Can a surgical classification provide information on the necessity of adjunctive medical treatment for resected GIST?

U. Ronellenfitsch[1], J. Jakob[1], P. Ströbel[2], D. Pink[3], C. Krefter[1], P. Reichardt[3], E. Wardelmann[4], P. Hohenberger[1]. [1]*University Hospital Mannheim, Division of Surgical Oncology, Mannheim, Germany;* [2]*University Hospital Mannheim, Institute of Pathology, Mannheim, Germany;* [3]*Sarcoma Center Berlin-Brandenburg, Department of Internal Medicine, Bad Saarow, Germany;* [4]*University Medical Center Bonn, Department of Pathology, Bonn, Germany*

Background: Gastrointestinal stromal tumors (GIST) differ in their risk to recur or metastasize after resection of the primary. It has been tried to define criteria for the usefulness of adjunctive medical therapy. So far, most classifications draw on size, mitotic count and location and do not regard surgical aspects. We evaluate if a classification of the surgical procedure used to resect the primary proves useful in defining candidates for adjunctive treatment.
Patients and Methods: 457 pts with confirmed GIST were retrieved from a prospective database. Primary location was: oesophagus n = 9 (2%), stomach n = 199 (44%), duodenum n = 21(4%), small bowel n = 141(31%), rectum n = 31(7%), others and metastatic n = 56 (12%). Resections were classified into: enucleation (class1), limited (segmental small bowel, Billroth I; class2), standard organ removal (gastrectomy, anterior resection; class 3), and multivisceral (class 4). Median tumor size was 7.5 (range 0.5−37) cm. All tumors were classified according to Consensus (Hum Pathol 2002). Median follow-up was 31 months. Pts treated with imatinib pre- or postoperatively were excluded from the analysis of recurrence.
Results: Operations were class 1: n = 57, class 2: n = 158, class 3: n = 59, class 4: n = 96. N = 51 M1 pts did not undergo resection of the primary, and for n = 36 pts data were incomplete. R0 resection rate was 80%, R1 rate 11%, and R2 rate 9%. Pre- or intraoperative tumor rupture occurred in n = 24 pts. Tumors were classified in 4.4% as very low, 11.5% low, 19% intermediate, and 65% high risk for aggressive behaviour. 44% of pts eligible for analysis recurred. After multivisceral resection (class 4), 52/68 eligible pts (77%) developed recurrence after a median of 10 months. In groups 3, 2, and 1, the recurrence rate was 59%, 50% and 37% respectively (p < 0.01). 18/19 pts with tumor rupture suffered from recurrence.
Conclusions: For GIST, the risk of postoperative tumor recurrence increases with the extent of the surgical procedure. Patients who require multivisceral resection or have tumor rupture show a significantly adverse course with early recurrence even after R0 resection. They must be considered having metastatic disease and adjunctive medical therapy is strongly recommended regardless of tumour size or mitotic count. In other cases, a classification of the surgical procedure can provide complementary information to estimate the risk of tumor recurrence and thus the necessity for adjunctive treatment.

Poster presentations (Wed, 23 Sep, 09:00−12:00)
Sarcoma

9407 POSTER

Ewing's family (EFT) tumours: biomolecular characterization on paraffine-embedded samples

S. Del Bianco[1], A. Parafioriti[1], E. Armiraglio[1], P. Daolio[2], S. Mapelli[2]. [1]*Orthopaedic Institute Gaetano Pini, Pathology, Milan, Italy;* [2]*Orthopaedic Institute Gaetano Pini, Oncologic Orthopaedic Surgery Center, Milan, Italy*

Background: Ewing sarcoma is a malignant bone tumour characterized, in 90% of the cases, by the balanced chromosomal translocation t(11;22) which generates a chimeric oncogene that acts as a transcriptional activator. The detection of translocation can be fundamental in cases with an extraosseous or unusual location which are difficult to diagnose histologically and it is also helpful in evaluation of residual disease. We joined immunohistochemical analysis with a routine RT-PCR method which allows the detection of the more common fusion transcript EWS-FLI1 in archival paraffine-embedded tissues of EFT patients. We used a pair of primers which allowed us to discriminate between two subtypes of EWS-FLI1 transcript. We selected some samples for EWS-FLI1 typing using a Real.Time PCR assay.
Material and Methods: We analysed 54 EFT patients. RNA was extracted from paraffine-embedded sections and reverse transcribed into cDNA. On every sample we performed RT-PCR and immunohistochemistry for the marker CD99; we also selected 5 samples for Real-Time PCR analysis.
Results: Fourty-nive out of 54 samples had a RNA suitable for analysis. Thirthy-six patients had EWS-FLI1 type I fusion transcript while 6 patients EWS-FLI1 type II; in 7 samples we couldn't find any fusion transcript although their RNA was good. We tested 5 of these negative samples with Real-Time PCR and we found 2 patients who were carriers of EWS-FLI1 type I fusion transcript. CD99 resulted positive in 34 samples out of 54.
Conclusions: The detection of fusion transcripts using RT-PCR methods can be useful as a support to EFT diagnosis. Moreover the possibility to assess a Real-Time PCR assay enhances analysis sensibility and minimizes false positives risk. EFT cytogenetic characterization completes morphologic and immunophenotipic data owing a more careful classification and an identification of subgroups with different prognosis.

9408 POSTER

Synovial sarcoma: molecular characterization from paraffine-embedded samples

S. Del Bianco[1], A. Parafioriti[1], E. Armiraglio[1], P. Daolio[2], S. Mapelli[2]. [1]*Orthopaedic Institute Gaetano Pini, Pathology, Milan, Italy;* [2]*Orthopaedic Institute Gaetano Pini, Oncologic Orthopaedic Surgery Center, Milan, Italy*

Background: Synovial sarcomas are mesenchymal tumours with undefined histogenesis which represent 5−10% of soft tissues tumours; they are divided into different subtypes according to morphology and epithelial differentiation. From a molecular point of view, synovial sarcoma is characterized by t(X;18)(p11; q11) translocation which joins SYT gene with a member of SSX gene family. We developed an efficient method to detect the two main fusion transcripts SYT-SSX1 and SYT-SSX2 based on RT-PCR or Real-Time PCR applied to archival paraffine-embedded samples.
Material and Methods: This study includes 51 patients surgically treated for synovial sarcoma and analyzed with routine immunohistochemical analysis. We used alternatively nested-PCR or Real-Time PCR, with SYBR green method, to detect SYT-SSX transcripts: these techniques allowed us to discriminate between the two transcripts.
Results: In 44 subjects out of 51 we could find a specific fusion transcript and, in particular, 32 patients were carriers of SYT-SSX1 translocation. Interestingly we could find 7 patients who were carriers of both SYT-SSX1 and SYT-SSX2 transcripts. In 5 patients we didn't detect any fusion transcript. We selected 12 samples for Real-Time PCR analysis and we could quantify the reciprocal ratio between the two fusion transcripts when they were both present in the same sample.
Conclusions: The use of molecular techniques such as RT-PCR represents a sensitive and reliable tool as an aid to histopathologic diagnosis of synovial sarcoma. Moreover, Real-Time PCR enormously enhances sensibility and enables to dose single transcript quantity when both SYT-SSX1 and SYT-SSX2 are present in the same sample. This method can also be used to reclassify those cases whom diagnosis is still undefined after routine analysis.

9409 POSTER

Inhibition of Notch pathway prevents osteosarcoma growth by regulation of cell cycle

T. Setoguchi[1], M. Tanaka[1], S. Komiya[1]. [1]*Graduate School of Medical and Dental Sciences Kagoshima University, Department of Orthopaedic Surgery, Kagoshima, Japan*

Background: It was demonstrated constitutive activation of the Notch pathway in various types of malignancies. However, it remains unclear how the Notch pathway is involved in the pathogenesis of osteosarcoma. We investigated the expression of the Notch pathway molecules in osteosarcoma biopsy specimens' and examined the effect of Notch pathway inhibition.
Materials and Methods: Real-time PCR was performed with specific primers. Immunohistochemistry was performed using human osteosarcoma cell lines and human osteosarcoma samples. Cells were treated with increasing concentrations of various GSI (Notch signal inhibitor). *CBF1* siRNA was used to confirm the effect of Notch signal inhibition. Cell proliferation was quantitated using a MTT assay. Nude mice were inoculated with osteosarcoma cells. Cell cycle was analyzed by flow cytometry. The expression of the components of cell cycle machinery was analyzed by real-time PCR and western blot.

Results: Real-time PCR revealed over-expression of *Notch2, Jagged1, HEY1*, and *HEY2*. On the other hand, *Notch1* and *DLL1* were down-regulated in biopsy specimens'. Notch pathway inhibition using g-secretase inhibitor and *CBF1* siRNA slowed the growth of osteosarcomas in vitro. In addition, g-secretase inhibitor -treated xenograft models exhibited significantly slower osteosarcoma growth. Cell cycle analysis revealed that g-secretase inhibitor promoted G1 arrest. Real-time PCR and western blot revealed that g-secretase inhibitor reduced the expression of accelerators of the cell cycle including cyclin D1, cyclin E1, E2, and SKP2. On the other hand, $p21^{cip1}$ protein, a cell cycle suppressor, was up-regulated by g-secretase inhibitor treatment.
Conclusion: These findings suggest that inhibition of Notch pathway suppresses osteosarcoma growth by regulation of cell cycle regulator expression, and that inactivation of the Notch pathway may be a useful approach to the treatment of patients with osteosarcoma.

9410 POSTER

IGF1R expression in Ewing's sarcoma

Y.M.H. Versleijen-Jonkers[1], A.C.M. van de Luijtgaarden[1], M.H.S. Roeffen[1], H.W.B. Schreuder[2], U.E. Flucke[3], W.T.A. van der Graaf[1]. [1]*Radboud University Nijmegen Medical Center, Medical Oncology, Nijmegen, The Netherlands;* [2]*Radboud University Nijmegen Medical Center, Orthopaedics, Nijmegen, The Netherlands;* [3]*Radboud University Nijmegen Medical Center, Pathology, Nijmegen, The Netherlands*

Background: Survival in bone sarcoma patients is still limited and new therapeutic options are awaited. Early clinical trials in Ewing's sarcoma (ES) targeting the transmembrane Insulin-Like Growth Factor 1 Receptor (IGF1R) show promising results. Theoretically, IGF1R targeted therapy would be effective against tumors with membranous expression of the receptor. However, data on IGF1R expression in human ES tumor tissue and on possible change of expression after neoadjuvant chemotherapy are lacking. Therefore, the aim of this study was to analyze the expression of IGF1R in a panel of clinically annotated human ES samples.
Patients and Methods: Tissue and clinical data from primary ES patients treated at the Radboud University Nijmegen Medical Center between 1985 and 2006 were retrieved. Immunohistochemical staining for the IGF1R was performed using a polyclonal antibody (Cell Signaling Technology, #3027) on the therapy-naïve biopsy specimen and the resection specimen after neoadjuvant chemotherapy, whenever available. Only molecularly confirmed ES cases with complete follow-up data were used. Intensity of membranous and cytoplasmic positivity were separately scored on a three-point scale. Intrapatient change of IGF1R expression was evaluated using the Wilcoxon signed rank test.
Results: Thirty-two samples (21 therapy-naïve biopsy specimens and 11 resection specimens after neoadjuvant chemotherapy) from 24 patients (42% male, median age 14.5 years [1−53], median follow-up 44.5 months [2−252], 22 patients EWS-FLI1 and 2 EWS-ERG translocation) were available for this study. Membranous IGF1R staining was found in 11/21 biopsy specimens (52%) and 4/11 tumor resections (36%). Cytoplasmic positivity was encountered in 19/21 biopsies (90%) and 10/11 resections (90%). There was no systematic change in IGF1R expression, neither membranous nor cytoplasmic, between biopsies and resection material post chemotherapy (Wilcoxon signed rank $p = 1.0$ resp. $p = 0.75$).
Conclusions: Based on membranous staining alone, about half of ES patients would benefit from IGF1R targeted therapy. There was no apparent change in IGF1R expression after exposure to chemotherapy. As yet it is unknown which biological factor correlates best with response to IGF1R targeted treatment. At this moment downstream pathways are being studied in these tumor samples to get better insight into the biology of the IGF1R pathway in ES.

9411 POSTER

The novel HSP90 inhibitor NVP-AUY922 demonstrates activity in rhabdomyosarcoma cell lines

O. Slater[1], J. Renshaw[1], P. Workman[2], K. Pritchard-Jones[1]. [1]*The Institute of Cancer Research, Paediatric Oncology, Sutton, United Kingdom;* [2]*The Institute of Cancer Research, Cancer Therapeutics, Sutton, United Kingdom*

Background: Rhabdomyosarcoma is the most common soft tissue sarcoma in children. HSP90 (heat shock protein 90) inhibitors are novel anticancer drugs targeting multiple signalling pathways, many of which are altered in rhabdomyosarcoma. This study assessed the effect of HSP90 inhibitors in rhabdomyosarcoma cell lines.
Materials and Methods: Rhabdomyosarcoma cell lines were treated with the novel small molecule HSP90 inhibitor NVP-AUY922. The GI_{50} concentrations (compound concentration inhibiting cell proliferation by 50% compared with vehicle control) were determined using an MTS assay (colorimetric assay which uses (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium and measures the formation of its soluble formazan product which is directly proportional to the number of live cells). Effects of the drug on downstream signalling were evaluated using Western blotting. Progression through the cell cycle was determined using propidium iodide staining and FACS (Fluorescence Activated Cell Sorting) analysis.
Results: NVP-AUY922 showed activity in 10 rhabdomyosarcoma cell lines with GI_{50} concentrations in nanomolar units, comparable to other tumours and cancer cell lines. Depletion of molecular targets of HSP90 inhibition was analysed and evident in two rhabdomyosarcoma cell lines. Cell cycle analysis after treatment with NVP-AUY922 demonstrated G_2/M arrest suggesting that the drug has a cytostatic effect.
Summary: NVP-AUY922 showed activity in rhabdomyosarcoma cell lines with GI_{50} values at nanomolar concentrations and depleted HSP90 client proteins in a concentration and time dependent manner, causing induction of HSP70. Cell cycle analysis showed transient G_2/M arrest. NVP-AUY922 is currently being tested in the rhabdomyosarcoma cell lines in combination with other novel agents, particularly Met kinase inhibitors.

9412 POSTER

Cone-beam CT guidance for set-up verification in extremity soft tissue sarcomas patients

R. Haas[1], A. Betgen[1], J.J. Sonke[1]. [1]*The Netherlands Cancer Institute, Radiotherapy, Amsterdam, The Netherlands*

Introduction: Standard of care for most extremity soft tissue sarcomas patients (ESTS) is surgery, followed by radiotherapy (RT). Several studies show benefit of preoperative RT, especially concerning late morbidity like fibrosis and joint stiffness. Smaller fields seem to be an important prognostic factor for these endpoints. To achieve smaller fields, geometrical uncertainties have to be reduced. This study quantifies volume and positional changes of the Gross Target Volume (GTV) during preoperative RT and investigates the potential of advanced correction strategies.
Methods: Twenty-seven ESTS patients were investigated. To quantify volume changes the planning CT-scan and CBCT-scans acquired for setup verification were used for delineation of the GTV. Two methods were used: 1) registration of bony anatomy (used as a surrogate for tumor position during treatment), 2) registration of soft tissue near air interfaces. GTV volume was calculated from each CBCT-scan, and the changes in volumes were evaluated during the five-week treatment interval. For method 1 chamfer matching was used. For method 2, a multi clipbox approach was employed to improve on method 1 in the presence of volume changes. Multiple clipboxes were placed for each patient at air-tissue interfaces. Both methods were analyzed separately. Systematic and random errors were calculated in left-right (LR), cranio-caudal (CC) and anterior-posterior (AP) direction with respect to the table. Anisotropic CTV-to-PTV margins were calculated to account for residual setup errors and for setup errors combined with tissue deformations.
Results: In nine patients an increase of the GTV up to 26% was seen, eleven patients showed no change and in seven patients a decrease of the GTV up to 58% was observed. All tumours in the latter group were diagnosed as myxoid liposarcoma (MLS). For MLS and tumour boundaries adjacent to bony anatomy, required margins were 0.75 cm in each direction. For tumour boundaries adjacent to normal tissue or air, required margins were 1.2 cm in LR and CC direction and 1.4 cm in AP direction.
Conclusions: Considerable changes in GTV were seen during the overall treatment time. A reduction in GTV was only seen for MLS patients. For these patients a 0.25 cm CTV-to-PTV margin reduction compared to our clinically prescribed margins of 1 cm is possible, using online CBCT verification. For all other sarcoma subtypes the clinically used PTV margin was too small at the tumour boundaries at normal tissue and air interfaces.