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?

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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

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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

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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

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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.