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

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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

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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

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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.