to compare the effects of ZOL on bone resorption, angiogenesis, tumor markers and time to disease progression between a weekly low dose (the metronomic regimen) versus a conventional dosage.
**Materials and Methods:** Sixty breast cancer patients with bone metastases were recruited to a randomized phase II trial. They were randomized to either ZOL 1 mg IV weekly for 4 doses or a single dose of ZOL 4 mg IV. No other antitumor treatments were administered during the first month after randomization. Serial blood samples were collected on day 1, 15 and 29 to measure markers for bone resorption (N-telopeptide), angiogenesis (VEGF) and tumor burden (CEA and CA15-3).
**Results:** Compared to a single-dose administration, weekly low-dose of ZOL resulted within the first 4 weeks in significantly greater reductions in serum levels of VEGF and N-telopeptide, with more reduction towards the end of the first month of treatment. Compared with baseline serum VEGF levels, the percentages of more than 25% reduction with the metronomic regimen were 50% and 96.6% on day 15 and day 29, respectively, while the corresponding values with conventional dosing were 23.3% and 17.2%, respectively. Patients who received metronomic ZOL had a substantially longer median TTP (7.0 months, 95% CI, 6.1−7.9 months) than those who had a single dose of ZOL (2.8 months, 95% CI, 0−5.7 months; P = 0.076).
**Conclusion:** Metronomic use of low-dose ZOL appeared to be more effective than conventional regimen in sustained reduction of circulating VEGF and N-telopeptide levels, and in prolonging TTP. This dosing schedule should be further assessed in phase III trials.
ClinicalTrials.gov number: NCT00524849.

**PP65**

**PIK3CA mutations in patients with advanced cancers treated in a phase I clinic**

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**Background:** Phosphatidylinositol 3-kinase (PI3K) is thought to play an important role in tumorigenesis. Activating mutations of the p110á subunit of PI3K (PIK3CA) have been identified in a broad spectrum of tumors.
**Materials and Methods:** A mutational analysis (a PCR-based DNA sequencing) of exon 9 (helical domain) and exon 20 (kinase domain) of the PIK3CA was performed using DNA obtained from tumors of patients referred for clinical trials using targeted therapy. Patients with PIK3CA were preferably treated whenever possible with regimens containing inhibitors of PI3K-AKT-mTOR signaling pathway.
**Results:** To date 105 samples from patients with various advanced cancers have been collected. At the time of submission 80 results of mutational analysis were available (ovarian cancer, n = 17; colon cancer, n = 9; cervical cancer, n = 10; endometrial cancer, n = 7; breast cancer, n = 7; melanoma, n = 6; head and neck cancer, n = 4; soft tissue sarcoma, n = 4; renal cancer, n = 3; and other tumor types, n = 13). PIK3CA mutations were detected in 11 (14%) patients (2 in exon 9-helical domain, 9 in exon 20-kinase domain). In tumor types with more than 5 patients tested, PIK3CA mutations were most frequent in endometrial cancer (43%, 3 out of 7 patients), ovarian cancer (24%, 4 out of 17 patients), head and neck cancer (25%, 1 out of 4 patients), breast cancer 14% (1 out of 7 patients), and colon cancer (11%, 1 out of 9 patients). No mutations were identified in patients with melanoma or cervical cancer. The small number of patients at this point precludes statistical comparisons. Of the 11 patients with PIK3CA mutations, 9 were treated on a protocol that included a drug targeting the PI3K-AKT-mTOR pathway, and 4 (44%) responded (partial responses). Although numbers are small, in individual disease there were 2 (67%) responses in 3 endometrial cancers, 1 (33%) in 3 ovarian cancers, 1 (100%) in 1 breast cancer, and no response in 1 colorectal cancer patient.
**Conclusion:** PIK3CA mutations were detected in 14% of patients with various solid tumors. Patients with PIK3CA mutations had high response rates when treated with PI3K-AKT-mTOR inhibitors.

**PP104**

**TYMS and DPYD polymorphisms and toxicity of 5-fluorouracil and capecitabine chemotherapy in colon cancer patients**

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**Background:** Thymidylate synthase (TS) is key enzyme in the synthesis of thymidylate and is a target for 5-FU which is mainly catabolised by dihydropyrimidine dehydrogenase (DPD). VNTR and C>T SNP in the enhancer region of TYMS gene (TSER) as well as DPYD gene mutation (DPYD*2A) may influence toxicity of 5FU/Capecitabine-based chemotherapy. Aim of this study was to evaluate the correlation between TSER VNTR and the susceptibility to sporadic colon cancer and to correlate the genotype frequencies of TSER VNTR and DPYD*2A between Croatian and other european populations. We also aimed to correlate TSER polymorphisms and DPYD*2A and toxicity of 5FU/Capecitabine-based chemotherapy in colon cancer patients.
**Materials and Methods:** Genotyping was performed on 100 healthy unrelated Croatians and 100 colon cancer patients using PCR-RFLP method.
**Results:** Genotype frequencies of TSER VNTR did not differ statistically between controls and colon cancer patients. 49 patients were assesed for toxicity and two patients with worst toxicities were heterozygous for DPYD*2A. Among the remaining 47 patients, 33 were assigned into a 'low expression TSER genotype' group [13 (39.4%) with 2R/2R, 12 (36.4%) with 2R/3RC and 8 (24.2%) with 3RC/3RC TSER genotype] and 14 into a 'high expression TSER genotype' group [7 (50.0%) with 2R/3RG, 1 (7.14%) with 3RG/3RG and 6 (42.86%) with 3RG/3RC TSER genotype]. 25 patients (75.76%) from the 'low expression TSER genotype' group experienced a total of 65 toxicities. 6 patients (42.86%) from the 'high expression' group experienced total of 10 toxicities.
**Conclusion:** No correlation was found between TSER VNTR and the susceptibility to sporadic colon cancer. Genotype and allele frequencies were similar to other European populations. We assume that the worst toxicities experienced by two patients with DPYD*2A mutation were a consequence of that mutation but due to a small patient number, the impact of this mutation on risk of toxicity could not be proven to be statistically significant. The remaining forty-seven colon cancer patients were divided into two groups based on TSER genotype. 'Low expression TSER genotype' group of patients suffered from more and worse toxicities (grade III and IV) of 5FU/Capecitabine-based chemotherapy compared to a 'high expression TSER genotype' group ($p = 0.020606$, $p < 0.0001$, respectively). These results might have a prognostic role in the prediction of toxicity of 5FU/Capecitabine-based chemotherapy in colon cancer patients in Croatia.

**PP51**

**Heat shock of tumor cells at sublethal temperatures causes an immediate phosphorylation of the C-terminal hydrophobic motive of Akt**

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**Background:** Regional hyperthermia (RHT) combined with chemotherapy is proven to provide a new treatment option for high-risk soft-tissue sarcomas. The cellular signalling responses of heat exposed tumor cells at clinical relevant temperatures are of special interest. Here we investigate Akt signalling pathways of different human tumor cells in vitro. The protein kinase Akt is involved in major signalling events including important anti-apoptotic programs. Accordingly, mutations of the Akt pathway are frequently found in malignant transformed cells
**Materials and Methods:** For heat exposure, cells (FG-pancreatic carcinoma, MG63-osteosarcoma; A673-rhabdomyosarcoma cell lines) were incubated for 1−6 hrs at 41.8ºC (sublethal heat shock). Concentrations of phorphorylated and non-phosphorylated intracellular signalling molecules (PI3K, PIP3-DK, Akt, mTor, p70 S6K) and HSP70 induction were measured by SDS-PAGE and immunostaining.
**Results:** The inducible heat shock protein HSP70 is upregulated over this time-temperature exposure. In parallel, immediate activation of Akt signalling was detected after 1 hr heat exposure in all investigated tumor cell lines. Preliminary results indicate that sublethal heat exposure does not influence the activities of PI3K and PIP3-DK by changing their phosphorylation status. Accordingly − as shown by our results − the phosphorylation of Akt on its activation loop is not influenced. Usually this is the initial activation event after transmembrane RTK-activation and subsequent PIP3 synthesis by the PI3-kinase. In contrast, we found that Akt phosphorylation is selectively induced on its C-terminal hydrophobic motive as a consequence of sublethal heat shock. Furthermore, the phosphorylation of downstream targets (mTor, p70 S6K) indicates an increase of Akt activity.
**Conclusion:** The data suggest a specific and fast mechanism for heat stress related Akt activation. It is known that phosphorylation of the C-terminal hydrophobic motive − as observed under heat exposure − enhances Akt activity. Several protein kinases for example mTorC2, DNA-PK or PKCβII are discussed to be responsible for this phosphorylation event. In current experiments using different inhibitors and siRNAs we try to reveal further the mechanism of heat-induced Akt phosphorylation. Targeted modification of heat-induced Akt activation might enhance the efficacy of RHT without harming the tissues beyong the heated region.
*Supported by HGF Clinical Corporation Group Hyperthermia