Results: Results across the cohort indicate Her2 MRM results correlate precisely with IHC and FISH demonstrating Her2 quantitation in Liquid Tissue lysates obtained by microdissecting multiple cancerous regions across the same tissue section and processing them together to produce a single lysate. Results of this assay across different regions within the same tumor tissue indicate different levels of Her2 are detected in different cancerous and stromal regions within the same tissue section that correlate precisely with IHC analysis in these various regions.
Conclusion: These results indicate the need for a sampling strategy for measuring quantitative levels of specific proteins directly in patient tissue that requires tissue microdissection with implications for the molecular analysis of tumor tissue for advanced diagnostic applications.

PP116

Lycopene and prostate cancer

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Background: The purpose of this Phase II randomized-controlled trial was to evaluate the safety and effect of administering several doses of lycopene to men with clinically localized prostate cancer, on intermediate endpoint biomarkers implicated in prostate carcinogenesis.
Materials and Methods: Forty-five eligible men with clinically localized prostate cancer were supplemented with 15, 30 or 45 mg of lycopene or no supplement from biopsy to prostatectomy. Compliance to study agent, toxicity, changes in plasma lycopene, serum steroid hormones, PSA and tissue Ki-67 were analyzed from baseline to completion of intervention
Results: Forty-two of forty-five five subjects completed the intervention for approximately 30 days from the time of biopsy until prostatectomy. Plasma lycopene increased from baseline to post treatment in all treatment groups with greatest increase observed in the 45 mg lycopene-supplemented arm compared to the control arm without producing any toxicity. Overall, subjects with prostate cancer had lower baseline levels of plasma lycopene similar to those observed in previous studies in men with prostate cancer. Serum free testosterone decreased with 30 mg lycopene supplementation and total estradiol increased significantly with 30 mg and 45 mg supplementation from baseline to end of treatment, with no significant increases in serum PSA or tissue Ki-67. These changes were not significant compared to the control arm for this sample size and duration of intervention.
Conclusion: Although antioxidant properties of lycopene have been hypothesized to be primarily responsible for its beneficial effects, our study suggests that other mechanisms mediated by steroid hormones may also be involved

PP60

Correlation of absolute lymphocyte count with clinical benefit and overall survival: results of compassionate-use trial of ipilimumab in advanced melanoma at Memorial Sloan-Kettering Cancer Center

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Background: Ipilimumab (ipi) is a monoclonal antibody which antagonizes cytotoxic T lymphocyte antigen (CTLA)-4, a negative regulator of the immune system. We report on advanced refractory melanoma Pts treated on a trial of compassionate-use ipi at the Memorial Sloan-Kettering Cancer Center.
Materials and Methods: Eligibility criteria included stage III (unresectable) or stage IV melanoma. Pts had experienced progressive disease to at least one prior systemic therapy (except for those with ocular primary tumors, who were required to have local control of their disease). Pts with primary ocular or mucosal melanomas were eligible, as were those with brain metastases. Pts received ipi 10 mg/kg every three weeks for four induction doses. Those Pts with evidence of clinical benefit (CB) at Week 24 − complete or partial response (CR or PR) or stable disease (SD) as defined by modified WHO criteria − then received maintenance ipi every 12 weeks.
Results: 53 Pts were enrolled, with 51 evaluable (one was lost to follow-up after one ipi treatment while the other received chemotherapy between ipi treatments). The median age of Pts was 62 years (range, 38−86 years). 64% of Pts were male and most had an excellent performance status (85% with ECOG status 0−1). 25% of Pts had an abnormally elevated lactate dehydrogenase (LDH) level $\leqslant 2\times$ the upper limit of normal (ULN) and 32% had a baseline LDH $> 2 \times$ ULN. Grade 3/4 immune-related adverse events (irAEs) were noted in 29% of Pts, with the most common irAEs being pruritus (43%), rash (37%) and diarrhea (33%). The response rate (CR+PR) was 12% (95% CI: 5%, 25%) while 29% had SD (95% CI: 18%, 44%). Median progression-free survival was 2.5 months while median overall survival (OS) was 7.2 months (95% CI: 4.0, 13.3). Pts with grade 3/4 irAEs appeared to have improved Week 24 CB rate. Pts with an absolute lymphocyte count (ALC) $\geqslant$1,000/μL (33/41 Pts) after two ipi treatments (week 7) had significantly improved CB rate (45% versus 0%, $p = 0.02$) and median OS (11.9 versus 1.4 months, $p < 0.001$) compared to those with an ALC < 1,000/μL (8/41 Pts). Six and 12 month OS were 75% vs. 0% and 47% vs. 0% when stratified by Week 7 ALC. This association remained significant when controlled for baseline LDH level.
Conclusion: Our results confirm that ipi is clinically active in Pts with advanced refractory melanoma. The ALC after two ipi treatments appears to strongly correlate with CB and OS and should be prospectively validated.

PP52

Amplification of the chromosome 17 q22 amplicon containing TOP2A gene is correlated with better survival in HER2 amplified/ hormone receptors negative breast cancers

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Background: HER2 amplified breast cancers are considered as a homogeneous sub-group of breast cancers. It has been described that amplifications in the chromosome 17 (ch17) could affect different genes implicated in cancer development. The aim of the study was to determine, by quantitative PCR, the levels of amplification of different genes located on ch17q (on the centromeric side and distal side of the chromosome in regards of HER2 localization), and their relation with patient's survival.
Materials and Methods: We determined MED1 (centromeric side) and TOP2A IGFBP4, CCR7, KRT20, KRT19 and GAS (distal side) gene copy numbers by quantitative PCR in 87 HER2 amplified breast tumors. Patients were included between 2002 and 2006 (median follow-up = 40.3 month). They received radiotherapy (100%), anthracyclin based regimen (78%), taxanes (2%), herceptin (24%) and hormonotherapy (40%) as adjuvant therapy according to therapeutic recommandations used at the time of surgery.
Results: Gene amplification occurs in 65.6% for MED1 (57/87), 23% for TOP2A (22/65), 19.5% for IGFBP4 (17/87), 18.4% for CCR7 (16/87), 13.8% for KRT20 (12/87), 11.5% for KRT19 (10/87), and 6.9% for GAS (6/87). The level of amplification of HER2 is correlated with the level of amplification of MED1 ($p < 0.0001$) but not with others gene amplification's levels. HER2-MED1 amplicon is associated mostly with hormone receptors positive breast tumor ($p < 0.01$). Hormone receptors negative patients have the worst overall survival (OS) (HR 0.356; 95% CI 0.16−0.783; $p < 0.01$), median progression-free survival (PFS) after recurrence was 36.5 compare to 43 months for positive hormone receptors patients. In hormone negative patients, amplifications of HER 2 +/− MED1 genes have a worse OS (HR 0.277; 95% CI 0.131−0.588; $p < 0.0008$) than patient with amplification of genes located on the distal side; PFS after recurrence was 35 compared to 43 months, respectively.
Conclusion: HER2 and MED1 are located on the same amplicons. The Amplification of genes located on the distal side of ch17 determines a profile with a better survival in hormone receptors negative breast cancer. This could be explained by a better response to anthracyclin based regimen for hormone-independent -TOP2A amplified breast cancer.

PP114

Non-coding mitochondrial RNA differential expression: a new biomarker for noninvasive cancer diagnosis

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Background: Recently, we described the existence in human cells of a family of non-coding mitochondrial RNAs (ncmtRNAs) formed by the sense or antisense 16S mitochondrial rRNA, and an inverted repeat (IR) of variable size covalently linked to its 5′ end. These transcripts were named sense ncmtRNA (SncmtRNA) and antisense ncmtRNA (ASncmtRNA) respectively. The expression of these transcripts varies depending of the proliferative status of the cell. In resting cells, the sense and antisense transcripts are down-regulated. In normal proliferating cells, both transcripts are highly represented. In tumor cells, however, only the antisense transcript is selectively repressed. Based on the universal representation and the differential expression of these ncmtRNAs, the goal of this research is to demonstrate the enormous potential in cancer diagnostic of these molecules and their use as a new biomarker in human neoplasias. By double in situ fluorescent hybridization (FISH), we are developing a non invasive approach for the diagnosis of prostate and bladder cancer

Materials and Methods: In order to identify tumor cells in urine, we loaded tumoral or normal proliferating cells in a urine sample of healthy volunteers. The cells were recovered by centrifugation, fixed and attached to positively charged slides. We performed a double FISH technique using specific ODN probes for each one of the ncmtRNAs; the SncmtRNA was identified with a specific probe labeled with Alexa fluor 488, and the ASncmtRNA with a Texas red labeled probe. The same set of experiments was performed in suspension cells, and analyzed by flow cytometry. Finally, patients' urine samples were analyzed in the same way.
Results: The double FISH approach developed here showed high sensitivity and specificity in the differentiation of tumor from normal cells in a mixture obtained from human fluids, constituting a promising new and highly accurate cancer diagnostic method.
Conclusion: We developed a double FISH approach with specific ODN probes complementary to SncmtRNA and ASncmtRNA which can identify normal or tumoral status in single cells. The same results were corroborated by flow cytometry. This approach, based on visualization of the expression of these new cancer biomarkers can be used as a novel approach for the non invasive diagnostis of bladder and prostate cancer.

PP23

Immunohistochemical marker profile in colorectal cancer: Our experience

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Background: Our aim was to examine whether certain immunohistohemical molecular markers, specifically PCNA, Ki-67 and p53, could be used to predict the tumor response of rectal cancer to determine the overall and disease-free survival rates of patients following adjuvant therapy.
Materials and Methods: In "Sveti Vracevi" Hospital in Bijeljina 301 patients suffering from colon cancer received treatment from 1st January 2000 to 31st December 2008. We analyzed the prognostic value of PCNA, Ki-67, and p53 by immunohistochemistry on formalin-fixed, wax-embedded sections in a series (n = 153) of stage III (Dukes C) colorectal cancers. An immunohistochemical score based on the intensity of immunoreactivity and, where relevant, the proportion of immunoreactive cells was established for each marker. We elected to investigate PCNA, Ki-67 as a marker of cell proliferation indices and p53 oncogenes/tumor suppressor gen because these markers have been demonstrated in a number of studies to have potential value in defining populations of individuals who either may or may not benefit from the use of adjuvant chemotherapy.
Results: Using 9 years of follow-up data, our retrospective analysis demonstrated an association between PCNA intensity (relapse-free survival [RFS]: risk ratio [RR] = 1.47, P = 0.01; overall survival [OS]: RR = 1.49, P = 0.002), Ki-67 (RFS: RR = 0.71, P = 0.05; OS: RR = 0.6, P = 0.05), and p53 (RFS: RR = 1.42, P = 0.01; OS: RR = 1.19, P = 0.0013) for RFS and OS. High PCNA intensity levels and positive p53 staining were associated with a worse outcome. Tumors containing a high percentage of Ki-67-positive cells enjoyed an improved outcome compared with those patients whose tumors contained relatively few positive cells. An interaction with treatment was not identified for any of the markers.
Conclusion: Immunohistochemical analysis is not used in the routine analysis of colon cancer. This retrospective investigation demonstrated that PCNA, and p53 staining each had significant prognostic value for patients colon carcinoma. There was not statistically significant difference in the survival rate of patients with positive immunohistochemical Ki-67 values in relation to the patients with the negative values.

PP84

Na+/H+ exchanger regulatory factor 1 (NHERF1) and angiogenesis in familial breast cancer

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Background: NHERF1 is a scaffolding protein that recruits membrane and cytoplasmic proteins into functional complexes. Our recent evidences demonstrate that in breast cancer NHERF1 overexpression is associated with increased tumor hypoxia and poor prognosis. Hypoxia is implicated in tumor proliferation and angiogenesis that interests neoplastic regions. In fact, the hypoxia-inducible factor-1 (HIF-1α), mediating trascriptional activation of vascular endothelial growth factor (VEGF) gene, is considerated the central initiator of angiogenic activity in tumor. Our aim was to determine NHERF1 expression on a series of familial and sporadic breast cancer patients and examine the relationship with other progression markers (HIF-1α, VEGFR 1 and HER2/neu).
Materials and Methods: NHERF1, VEGFR1, HIF-1α and HER2/neu proteins expression were analysed by immunohistochemistry on a tissue microarray, including 94 familial and 93 sporadic breast tumors. Cytoplasmic, membrane and nuclear NHERF1 reactivity was analysed.
Results: Membrane NHERF1 expression was significantly higher in sporadic than familial patients (p = 0.000). Familial cancers showed high levels not statistically significant of cytoplasmic NHERF1 expression compared with sporadic cancers. In familial breast patients, cytoplasmic NHERF1 overexpression was related with VEGFR1 positivity, in 48.3% of cases (p = 0.009). Furthermore, high levels of nuclear NHERF1 in familial cancers were associated with positive HIF1α tumors (p = 0.003). No significant correlation was found between NHERF1 and HER2/neu. In contrast, 48% of overexpressing HER2/neu sporadic tumors, showed a significant association with high cytoplasmic NHERF1 levels (p = 0.007). Moreover, in these tumors, nuclear NHERF1 protein is significantly correlated with HIF1α expression (p = 0.019). Any NHERF1 significant association between both VEGFR1 and HIF-1α was found.
Conclusion: In familial breast cancer, NHERF1 resulted strongly related with VEGFR1 and HIF-1α proteins with respect to sporadic tumors. In this context, we suggest an emerging role of NHERF1 in angiogenesis.

PP19

Randomised phase III clinical trial of 5 different arms of treatment for patients with cancer-related anorexia/cachexia syndrome (CACS)

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Background: Cancer-related anorexia/cachexia syndrome (CACS) is a multifactorial syndrome characterized by tissue wasting, loss of body weight, particularly of lean body mass (LBM), metabolic alterations, fatigue, reduced performance status, very often accompanied by anorexia.
Materials and Methods: In April 2005 we started a phase III randomised study to establish the most effective and safest treatment of CACS addressing as primary endpoints: LBM, resting energy expenditure (REE), total daily physical activity, serum IL-6, TNF-α, and fatigue evaluated by the Multidimensional Fatigue Symptom Inventory − Short Form (MFSI-SF). The sample size was 475 patients (pts). Eligibility criteria: hystologically confirmed tumors of any site; weight loss $\geqslant$5% in the last 3 months and/or abnormal values of proinflammatory cytokines and oxidative stress parameters predictive of the onset of CACS; life expectancy >4 months. Patients could be treated with either antineoplastic therapy with palliative intent or supportive care. All pts enrolled received as basic oral treatment: polyphenols + alpha lipoic acid + carbocysteine + Vitamins ACE. Pts were then randomised to one of the following 5 arms: (1) Medroxyprogesterone Acetate (MPA)/Megestrol Acetate (MA); (2) Pharmaco-nutritional support containing EPA; (3) L-carnitine; (4) Thalidomide; (5) MPA/MA + Pharmaco-nutritional support + L-carnitine + Thalidomide. Treatment duration was 4 months. Interim analyses were planned after every 100 randomized pts.
Results: At April 2009, 332 pts were randomized and 310 were evaluable: M/F 180/130, mean age 62 yrs (range 30−84), 96% were stage IV. A first interim analysis on all 125 pts enrolled showed a significant worsening of LBM, REE and fatigue in arm 2 (Pharmaco-nutritional support containing EPA) in comparison to the others and it was withdrawn from the study. A second interim analysis after the enrolment of 204 pts showed arm 1 (MPA/MA) significantly less effective than the others for primary efficacy endpoints: it was withdrawn from the study.
Statistical analysis at April 2009 showed a significant increase of LBM (by DEXA) and decrease of REE, IL-6 and fatigue in arm 5. As for safety, the treatment was overall well tolerated and patient compliance was very good.
Conclusion: The results so far seem to suggest that the most effective treatment for cancer pts with CACS should be the combination regimen.

PP106

DNA promoter methylation in breast cancer as possible biomarkers for screening breast cancer and association with molecular breast cancer subtypes

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Background: Aberrant DNA methylation has been found in breast cancers associated with the loss of expression of regulatory genes for growth.
Purpose: To investigate the association between DNA methylation as possible biomarkers for screening breast cancer and association with clinico-pathological and molecular breast cancer subtypes.
Materials and Methods: We quantified methylation levels of genes; APC, RAR-Beta, E-Cadherin, ESR1 and 14-3-3σ gene in 107 women with breast cancer and 108 control subjects. A sensitive PCR quantitative technique was used to analyze the utility of hypermethylation gene promoter regions.