without tumor than that of non-metastatic patients and metastatic patients ($p = 0.009$ and $p = 0.003$, respectively; with c2 test). However, no significant associations were found between the presence of LOH/MSI in plasma DNA and clinicopathologic parameters except poor prognosis. As a result, it was suggested that grade I markers could be an early marker for carcinogenesis and predicting outcome of breast cancer. In addition, the results of real time quantitative PCR showed that MYCN amplification was detected in plasma DNA from in 2 of 16 (12.5%) metastatic patients, but deletions were observed in 10 of 16 metastatic patients (62.5%). However, 2 of 13 (15.4%) non-metastatic patients revealed MYCN deletion, and 7 of 13(53.8%) non-metastatic patients showed MYCN amplification. We also assessed plasma from non-metastatic, metastatic patients, and anonymous individuals without tumor to evaluate the quantity of plasma DNA by generating a standard curve for reference gene, b-actin. Low amounts of plasma DNA were found in the plasma samples from anonymous individuals without tumor.

Conclusion: As a conclusion, increasing tumor DNA was detectable in plasma, especially in breast cancer patients. Here we provided plasma DNA-based noninvasive methods, which, we hope, could be applied for clinical detection for breast cancer in the future.

322 Poster

An ERbeta isoform that lacks exon 5: correlation with ERalpha, PR and classical prognostic indicators in breast cancer

D. Nikolic-Vukosavljevic[1], V. Mandusic[2], K. Kanjer[1], B. Dimitrijevic[2]. [1]*Institute of Oncology and Radiology of Serbia, Belgrade, Serbia;* [2]*Vinca Institute of Nuclear Sciences, Belgrade, Serbia*

In this study, the relationship of ERbeta1 (wt) mRNA and ERbeta-delta5 mRNA expression to ER and PR levels was investigated in breast carcinomas. Samples and RNA Isolation: 60 samples of operable breast carcinoma were analyzed. All samples were examined histologically and only regions of tumor with more than 60% of malignant epithelial cells were used for RNA isolation. Levels of ERalpha and PR were measured by biochemical assay as recommended by EORTC. Total RNA was isolated with Trizol reagent and reverse transcribed with random hexamer. Quantitative real-time PCR: All PCR reactions were performed on a 7000 Sequence Detection System. Real time PCR was carried out with TaqMan Pre-Designed Gene Expression Assay specific for target transcript sequence. The target ERbeta1 (wt) mRNA and ERbeta-delta5 mRNA relative expression levels was normalized on beta-actin as endogenous control. Level of expression is expressed in relative units as N-fold differences in target gene expression normalized to the beta-actin gene and the relative to calibrator (sample with smallest amount of target gene mRNA). Regarding ERbeta1 (wt) mRNA and ERbeta-delta5 mRNA, samples were considered positive where the Ct value was above 32 (Ct – cycle threshold value). Analysis of correlation between ERbeta1 (wt) mRNA or ERbeta-delta5 mRNA and ERalpha or PR levels showed:

- absence of any correlation between ERbeta1 (wt) mRNA and ERalpha or PR expression;
- statistically significant negative correlation between ERbeta-delta5 mRNA and ERalpha expression;
- statistically significant negative correlation between ERbeta-delta5 mRNA and PR expression.

We also examined the relationship between ERbeta-delta5 mRNA expression status and classical clinical (age and menopausal status) and pathological parameters (nodal status, tumor size and type and histologic grade of tumor). There were no statistically significant correlations. Our findings indicate that ERbeta-delta5 mRNA expression, opposite to ERbeta1 (wt) mRNA expression, blocks ERalpha signaling pathways. In addition, ERbeta-delta5 mRNA expression could be useful biomarker, in its own right, of progression and endocrine response of breast cancer.

323 Poster

Large genomic alterations of the BRCA1 gene in Iranian breast cancer patients

R. Salehi, M. Salehi, R. Moradian, G. Amini. *Isfahan University of Medical Sciences, Genetic and Molecular Biology, Isfahan, Iran*

Breast cancer (BC) is the most frequent carcinoma in women. Some 5–10% of all cases of BC under the age of 35 years have a hereditary origin. BRCA1/BRCA2 mutations are responsible for 3–8% of all cases of BC and 30–40% of familial cases.

The lifetime risk of breast cancer in female carriers of a *BRCA1* mutation is 60–80% *BRCA1* is a large gene with 22 coding exons encoding a 220 kD protein that functions in maintaining genomic integrity and in transcriptional regulation.

In countries with mixed populations, such as Iran, point mutations of *BRCA1* are scattered throughout the coding sequence without any hot spots or prominent founder mutations. Conventional methods used to screen for mutations focus on genomic DNA and are usually PCR-based, enabling the detection of sequence alterations such as point mutations, and small deletions and insertions. However, during recent years an increasing number of large deletions and amplifications have been described in the *BRCA1* gene.

In this study we used multiplex ligation-dependent probe amplification (MLPA) method for detecting gross copy number changes and large deletion/amplification in *BRCA1* gene. MLPA is a method developed especially for detecting gross copy number changes in genomic sequences. Remarkably, many of the known genomic alterations of *BRCA1* have been found by this screening method.

Briefly, 100 ng of target DNA in a total volume of 5 µL TE was used for the ligation reaction. After the ligation step, multiplex polymerase chain reaction (PCR) amplification (33 cycles) was performed by adding 5 µL of the ligation mixture to 2 µL 10* SALSA PCR buffer and 13 µL aqua. Five microliters of polymerase mix containing 1 µL SALSA PCR primers, 1 µL SALSA enzyme dilution buffer, 0.25 µL SALSA polymerase, and 2.8 µL aqua was added to the tubes on ice, and then the amplification step was started in a PCR machine at the denaturation temperature of 95ºC. After PCR amplification, the DNA fragments were analyzed on a ALFexpress DNA sequencer (Pharmacia Biotech) using AlleliLink software. The SALSA BRCA1-MLPA kit contains probes for each of the 24 exons of the *BRCA1* gene, including two probes for the large exon 11. For quality control, we used samples from known *BRCA1* gene mutation carriers.

Using MLPA, we have screened 20 families and detected 5 families with aberrant exon copy numbers. Three deletions and two duplications were identified.

Our results show that MLPA is a rapid, reliable, and sensitive technique, which allows high-throughput screening.

324 Poster

Preclinical studies of the combination of RAD001 with tamoxifen or letrozole in breast cancer

I. Farmer[1], D.B. Evans[2], H.A. Lane[2], A.E. Lykkesfeldt[3], M. Dowsett[1], L. Martin[1]. [1]*Institute of Cancer Research, Academic Department of Biochemistry, Breakthrough Breast Cancer Centre, London, United Kingdom;* [2]*Novartis Pharma AG, Basel, Switzerland;* [3]*Institute of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark*

Resistance to endocrine therapies remains a major problem in breast cancer. Improved understanding of mechanisms underlying this is important for the development of new clinical strategies. We have characterized two MCF7 cell lines modeling relapse after long-term oestrogen deprivation (LTED) and resistance to tamoxifen (TamR). Cross-talk between HER-2 and ER pathways appears to contribute to endocrine-resistance in both settings leading to elevations in pMAPK and AKT, respectively. One strategy to improve the efficacy of current endocrine agents as well as delaying the onset of resistance is to target concomitantly ER and pertinent signal transduction pathways. One target is mTOR, a downstream member of the PI-kinase related kinase family, which regulates cell cycle progression, by enhancing translation initiation. We investigated the use of RAD001 (everolimus), a specific inhibitor of mTOR in combination with tamoxifen or letrozole on human breast cancer cell lines modelling endocrine-sensitive (MCF7 cells, wild-type (wt) and stably transfected with aromatase, Arom-1) and -resistant disease. The LTED and TamR cell lines revealed enhanced expression of proteins downstream of mTOR. Treatment of the cell lines with increasing concentrations of RAD001 resulted in a dose-dependent decrease in both proliferation and ER alpha-mediated transcription together with a concomitant decrease in phosphorylated p70S6 and S6. The TamR cells were highly sensitive to the anti-proliferative effects of RAD001: no additive effect was noted with the addition of tamoxifen. However, treatment of the 2% FBS MCF7 cells (parental line for TamR) with RAD001 in combination with 1uM tamoxifen resulted in an increase in sensitivity to RAD001. Similarly treatment of the Arom-1 cells with letrozole (0.1uM) in combination with a sub-optimal dose of RAD001 resulted in a further 50% reduction in proliferation compared to letrozole alone.

These data provide evidence that combinations of RAD001 with tamoxifen or letrozole may provide enhanced anti-tumour activity in endocrine-resistant disease and may delay the onset of resistance in the treatment of primary disease.

325 Poster

The mTOR inhibitor rapamycin stabilizes p27 by downregulating the expression of its ubiquitin ligase Skp2 in breast cancer

D. Hershko, E. Kakiashvili, Z. Rosenberg, M. Shapira. *Rambam Medical Center, Surgery A, Haifa, Israel*

Introduction: Recent studies have shown that overexpression of the ubiquitin ligase Skp2 results in rapid ubiquitin-mediated degradation of the