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Molecular Breast Cancer Subtypes: Relationship With Angiogenesis Genes Polymorphism, Efficacy of Neoadjuvant Chemotherapy and Survival

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Background: Breast cancer heterogeneity suggests that specific molecular subtypes will have distinct biological characteristics and clinical behavior. The purpose of this study was to analyze the polymorphisms of angiogenesis genes and their impact on the response to treatment and outcome of breast cancer patients according to molecular cancer subtypes.
Material and Methods: Our subjects comprised 272 breast cancer patients who received 2−4 cycles of neoadjuvant chemotherapy in the Tomsk Cancer Research Institute. The patients were classified into two subtypes: triple negative (TN) and non-TN. The VEGF-2578C/A (rs699947), FGFR2A/G (rs1219648) and TGFB1−509C>T (rs1800469) TGFB1−29T>C (rs1982073) genotypes were determined by polymerase chain reaction and restriction fragment length polymorphism method.
Results: A marginal trend towards significant association of TGFB1−29(CT/TT) genotypes with positive lymph nodes status was found among non-TN patients compared to TN cases (P = 0.057). In addition, we observed that this non-TN group with TGFB1−29 (CT/TT) genotypes tended to have shorter overall survival after neoadjuvant chemotherapy than TN patients (P = 0.08). A similar pattern was found between non-TN and TN patients carrying VEGF-2578 (CC) genotype (P = 0.08). However, the survival curves of wildtype VEGF genotype patients were very similar between the non-TN and TN groups (P = 0.18). Overall survival of women with the TGFB1−509 (CT/TT) genotypes was significantly worse than for TGFB1−509 (CC) carriers independent of patients molecular subtype (P = 0.009). Only TN women carrying FGFR2 (AA) genotype showed an association with better response to neoadjuvant chemotherapy in comparison non-TN (P = 0.02). In the logistic regression analysis, the FGFR2A/G genotype and molecular subtypes were the independent factors that predicted the probability of pathological regression (P = 0.013).
Conclusion: Our study indicates that the analysis of angiogenesis-related gene polymorphisms may help to identify patient subgroups at high risk for the progression of breast cancer. FGFR2A/G may be considered as a prognostic factor for efficacy to neoadjuvant chemotherapy in TN patients.

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Immunohistochemical Profiling of Signalling Pathways in Cancer of Unknown Primary (CUP)

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Background: The molecular biology of Cancer of Unknown Primary (CUP) has not been studied, resulting in a lack of candidate biomolecules for therapeutic targeting.
Methods: 15 mm tissue cores from 100 CUP tumours were loaded in tissue microarrays in duplicate and studied for immunohistochemical (IHC) expression of transmembrane receptors and associated ligands (Notch1, 2, 3, Jagged1, cMET), signal transducers and regulators (PTEN, phosphoAKT1, 2, 3 at Thr308, phospho-p44/42 MAPK at Thr202/Tyr204), cell cycle controllers (Cyclin D1, p21), effectors of protein synthesis (phospho-S6Rp at Ser235/236). The percentage of staining tumour cells was recorded and IHC cut-off values were based on distributional analyses and the medical literature. Complete clinicopathologic and management data were electronically recorded for analysis. Cox regression and Breslow testing were applied for prognostic analysis.

Results: The study population consisted of 100 patients with CUP (47 males, 53 females), of a median age of 65 and fit performance status (PS 0−1 in 75%). Histological diagnosis was adenocarcinoma in 60%, squamous ca in 22% and undifferentiated carcinoma in 17% of cases, with high grade seen in 50% of CUP. The clinicopathologic subgroups were visceral 30%, axillary nodal 8%, peritoneal carcinomatosis 22%, nodal disease 40%. Therapy consisted of palliative chemotherapy (platinum-based combination regimens in 55%). The median progression-free survival and overall survival (OS) were 7 and 12 months respectively. Biomolecules with frequent expression or activation (median % of staining tumour cells across all cases) were Notch3 (95%), p44/42 MAPK (52%), PTEN (60%), pS6Rp (72%), pAKT1/2/3 (97%). At univariate analysis, high IHC tumoural expression of p44/42 MAPK Thr202/Tyr204, pAKT Thr308, pS6Rp Ser235/236 and low cMET were associated with adverse outcome (Table 1). In fact, the adverse prognostic impact was enhanced in tumours with concurrent high IHC expression of both p44/42 MAPK Thr202/Tyr204 and pAKT Thr308. In multivariate analysis, high p21 and high cMET IHC tumoural expression were associated with reduced risk of death (RR of death 0.34, p = 0.005 for p21, RR 0.48, p = 0.025 for cMET), while high expression of pAKT Thr308 (RR 2.39, p = 0.01) and high expression of pS6Rp Ser235/236 (RR 2.76, p = 0.008) were linked to increased risk.
Conclusions: The major intracellular AKT and MAPK axes are frequently activated in CUP and carry adverse prognostic significance. Further findings incriminate the AKT axis, activating pS6Rp and inhibiting the tumour suppressor p21, as biologically important and worth exploration for therapeutic targeting.

Table 1. Biomolecules with significant prognostic utility in CUP

Biomolecule	IHC cut-off	Median OS (months)	95% CI	Breslow P
cMET	⩾20%	15 9	9.1−20.9	0.05
	<20%		4.5−13.5	
phospho p44/42 MAPK Thr202/Tyr20	⩾40%	9	3.7−14.3	0.016
	<40%	17	10−24	
phospho AKT1,2,3 Thr308	⩾85%	11	7.3−14.7	0.047
	<85%	15	8.7−21.2	
Both p44/42 MAPK and pAKT positive	yes	8	5.2−10.7	0.001
	no	17	13.1−20.8	
phospho S6Rp Ser235/236	⩾60%	9	6−12	0.03
	<60%	17	8−26	

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Breast Cancer Molecular Subtypes in Omani Patients: Correlation With Age, Histology, Stage Distribution and Outcome − an Anaylsis of 542 Cases

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Background: Breast cancer is the commonest malignancy and a major health issue globally. There is initial evidence and high possibility of molecular differences across the ethnic and geographic groups. This may be responsible for variation in presentation, responses to treatment, and outcome.
Patients and Methods: Breast cancer data from 2006–2010 at National Oncology Center − The Royal Hospital Oman was retrospectively retrieved from electronic patient record system. It was analyzed with respect to ER, PR, and Her-2 status and tumours were classified on molecular basis. Molecular subtypes with correlated with age, histology, and treatment out come. The results were compared with published international and regional data.

Parameter	Study			
	Southern Switzerland (Ticino)	The Polish Breast Cancer Study	The Carolina Breast Cancer Study	National Oncology Center Royal Hospital, Oman
No. of patients/IHC markers done	1214 (91%)	804 (34%)	496 (43%)	452 (100%)
Time period	2003–2007	2000–2003	1993–1996	2006–2010
Age	All ages	20−74 years	20−74 years	All ages
Race	Caucasian	Caucasian	African and Non-African Americans	Omani
Luminal A	73.2%	69%	54.8%	34.7%
Luminal B	13.8%	6.0%	16.6%	15.9%
Her2 +ve	5.6%	12.0%	7.1%	24.1%
BCL (TNBC)	7.4%	8.0%	21.5%	25.3%

Results: A total of 542 cases were available for evaluation (7 male and 535 female: 452 Omani and 83 Non-Omani). Right, Left, and bilateral tumours were 42.6%, 51.4% and 6%. IDC were 79.6%. G1 were 7.7%, while other grades were equally distributed. Luminal A, Luminal B, Basal like (TNBC), and Her-2 positive were 35.9%, 15.8%, 25.5% and 22.8% respectively.

There age (cut off 50 years) was a significant factor in Basal Like (63.8 vs. 36.2%) and Her2 tumours (60.9 vs. 39%). High grade tumours were highest (41%) in basal tumours and lowest in Luminal A (19%). Higher stage at presentation (Stage 3 and 4) was highest in Her2 tumours (59%). Mortality was recorded higher 22.4% in basal like/TN tumours. Table shows comparison with other selected published data.

Conclusions: The molecular classification and sub typing have shown ethnic and geographic variation in taxonomy. These differences may have diagnostic, therapeutic and prognostic implications. Large scale and multi-centre studies may confirm these findings. They can be translated and incorporated to management strategies wherever applicable.

1452　POSTER
Efficacy of DNA Repair Mechanisms in Blood Lymphocytes of Melanoma Patients as Prognostic Factor of Chemotherapy

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Background: Melanoma is characterized by primary or acquired resistance to multiple cytotoxic drugs. Alkylating agents are considered to be the most active drugs for the treatment of this cancer, with a response rate of 15−20%. It is the urgent aim to predict the response of patients to chemotherapy using non-invasive molecular markers.

Methods: In our work we investigated the response to standard chemotherapy of blood lymphocytes of patients suffering with melanoma. studied patients were treated according to the next protocol − dacarbazine with cisplatin, lomustine, and interferon alfa-2b. DNA single and double strand breaks were determined using comet assay; intracellular levels of marker proteins (APE1, PARP, FasR, hMSH2, hMLH1) were detected using immunocytochemistry. Ultimately this set of parameters allows to characterize two mechanisms of DNA repair (base excision repair, BER and mismatch repair, MMR) which together with apoptosis proneness underlie response of tumour cells to chemotherapy.

Results: We found AP sites and single strand breaks to be the most numerous lesions produced by N-methylated bases in response to chemotherapy. Single strand breaks were formed as intermediates and eliminated through the course of repair of AP sites by BER mechanism. Despite of interindividual variability of BER efficiency in lymphocytes of patients there was no damage in single strand DNA (ssDNA) 30 days after the 1-st cycle of chemotherapy. Less abundant but highly cytotoxic DNA lesion O^6-methylguanine (O^6meG) induced apoptosis in stimulated lymphocytes. Cell death caused by O^6meG adducts is promoted by MMR system by inducing unrepaired double strand breaks in DNA. There was a linear correlation between the level of dsDNA breaks in lymphocytes after 1-st cycle of chemotherapy and MMR efficiency in them. We observed a correlation between the level of dsDNA damage after 1-st cycle of therapy and the response of patients to the full course of chemotherapy: lymphocytes of patients exhibiting stable disease and progressive disease contained less dsDNA damage (less effective MMR) compared with lymphocytes of patients exhibiting partial remission of the disease.

Conclusion: The level of double strand breaks in DNA after 1-st cycle of chemotherapy is predictive of clinical outcome. Otherwise damage at the level of ssDNA (AP-sites and single strand breaks) and BER mechanism associated with it couldn't be a good prognostic factor of this protocol of chemotherapy. High level of double strand breaks in DNA in blood lymphocytes of melanoma patients 48 hours after 1-st cycle of chemotherapy appears to be a marker of a good prognosis.

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BRCA1/2 Genes − Psychological Side Effects of Unknown Mutation Result

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Background: Genetic test for the BRCA1/2 genes mutations can be: negative (N-no mutation found), pathogenetic (P- mutation correlated to an higher risk to develop breast and/or ovarian cancer), or unknown mutation (UM-the effect of the mutation on cancer risk is unknown). When an UM is found, the consultant usually do not follow the same risk-reducing program addressed to the BRCA+ mutation carriers, but a surveillance strategy

for the general population could be however inadequate. We assume that being an UM carrier could result in a stress condition that can arises anxiety in the carriers and their families and doubts about the future and the risk management.

The aim of the study was to compare the psychological effects of being a not-informative mutation subject with respect to the effects developed in subjects with P or N mutation.

Methods: After genetic test result, 103 patients were administered psycho-logical questionnaires: Minnesota Multhipasic Personality Inventory-2 short form (Personality Characteristics), Hospital Anxiety and Depression Scale (Situational anxiety and Depression), Family Adaptability and Cohesion Scale (Familial Satisfaction).

Results: Sample = 103 subjects, 7 male/96 female, mean age 54 years (range 32−77). Thirty of 103 were healthy and 73 were cancer affected. The mean age at the diagnosis was 46 years (range 29−76). 60 subjects were N, 21 P and 22 had an UM. The situational anxiety and depression levels result higher for P mutation carriers although they do not reach statistical significance (mean anxiety N = 7.9; P = 8;UM = 6.6. Mean depression N = 4.7; P = 5.5; UM = 3,5; p > 0.05). The P mutation carriers also feel lower satisfaction about their emotional bound with family members and worse adaptability skills (Mean satisfaction for cohesion: N = 5.7; P = 6.8; UM = 4. Mean satisfaction for Adaptability: N = 6; P = 7.6; UM = 5. P > 0.05). The value of Psychastenia is significantly higher only for P mutation carriers (mean psychastenia N = 48; P = 53; UM = 47. P = 0.05).

Conclusions: Other than the initial assumption, the data don't seem to indicate that a worse psychological condition is linked to the uncertainty of molecular test. Major psychological attention remains to be paid to the pathogenetic mutation carriers in order to help them to elaborate anxiety, depression and the feeling of unsatisfied family functioning. Anyway, more data on these aspects of genetic counseling are still needed.

1454　POSTER
Total Cell Divison, the Ultimate Biomarker for Personalized Medicine in Cancer? Serum Mediated Thymidine Incorporation Filling a Gap in Clinical Chemistry?

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Aim: To invent and document a method for measurement of total cell division in humans useful with serum and other bodyfluids in order to fill a gap in the current clinical chemistry arsenal.

Method and Materials: The nucleotidetriphosphate in a standardised assay for SIV Reverse Transcriptase, using immobilised template, was exchanged for nucleoside and complemented with yeast extract. Therafter optimal conditions for combined enzyme activity directly incorporating the distal nucleoside phosphorylated by thymidine kinase (TK) present in serum was experimentally determined. Material used for developing, characterising and documentation of the assay comprises human sera from blood donors and sera collected in connection with trauma and various kinds of cancers. For FPLC fractioning of sera a Superose 6 column was used.

Results: Using the novel DiviTumO procedure the sensitivity to measure thymidine kinase (TK) increased a 100-fold and the clinical sensitivity (signal/reference) a 10-fold compared to other techniques.

Separation of sera from patients with various diseases showed that the major TK activity resided in 700 kD complex active only with ATP as substrate. Serum samples collected serially after trauma reveal a transient increase TK which normalised upon healing. In cancer, the s-TK level upon detection of disease related to stage and especially to parameters related to aggressivity, which will be illustrated for breast-,renal- and prostatic carcinoma. Alteration in the s-TK level followed the effect of therapy given, as long as no folate inhibitors were included. Depending on type and spread of the cancer up more than 1000 times reduction in growing mass can be followed. Compared to traditional tumour markers mostly differentiation and volume related, the s-TK activity differed by relating to known risk factors indicating dedifferentiation.

Conclusion: Considering the high sensitivity of the novel assay it is concluded that a standardised method for measurement of cell division is now available for clinical chemistry. The reason for its sensitivity to pick up malignant cell divison compared to natural benign cell division will be discussed with reference to the ubiquitine system responsible for its mitotic exit and with reference to the reexpression of TK1 in G2 for cells with much DNA damage. Considering the rapid increase in cancer treatment modlities it is concluded that DiviTum is optimal for personalised medicine as the cell division is essential for the progress of cancer. Further, as increases in s-TK occurs before detection of methastases by imaging the test may be well used for stratification of patients to costly investigations.