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C1* GENETIC MODULATION OF THE *LET-7* MICRO-RNA BINDING TO *KRAS* 3'-UNTRANSLATED REGION (3'-UTR) IMPACTS ON SURVIVAL OF METASTATIC COLORECTAL CANCER (MCRC) PATIENTS (PTS) WITH UNRESPONSIVE DISEASE TO SALVAGE CETUXIMAB-IRINOTECAN

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Background: The *Let-7* micro-RNA targets *KRAS* mRNA playing a tumor suppressor function. The T>G variant in the *Let-7* complementary site (LCS6) in *KRAS* 3'-UTR alters *Let-7* binding and it is associated with KRAS overexpression and *Let-7* down-regulation. Clinical investigation in *Let-7* post-transcriptional control of the RAS pathway may lead to novel treatment strategies for MCRC pts who do not benefit from anti-EGFR therapy, especially if carriers of activating *KRAS* mutations.

Materials and Methods: LCS6 *KRAS* 3'-UTR genotypes have not been studied in colorectal cancer yet, therefore we first determined their frequencies and distribution, together with *BRAF* V600E and *KRAS* (codons 12,13,61) mutations in the tumor DNA of pts with irinotecan-refractory, MCRC who underwent salvage cetuximab-irinotecan. Subsequently, pts with stable disease or disease progression (unresponsive) and without the *BRAF* V600E mutation were studied for addressing the survival end-points. Overall survival (OS) and progression-free survival (PFS) times were compared between carriers of the LCS6 G-allele genotypes (T/G+G/G) and carriers of the wild-type T/T genotype.

Results: In 134 assessable pts, there were 34 carriers of the G-allele genotypes (25%) and 100 carriers of the T/T genotype (75%). G-allele genotypes carriers were more frequent in the *KRAS* mutation group (65%), while T/T genotype carriers were prevalent (64%) in pts with *KRAS* wild-type status (p=.004). Survival analyses were performed in 95 unresponsive pts to cetuximab-irinotecan. LCS6 G-allele carriers showed significantly worse PFS (p=.002) and OS (p=.001) than carriers of the wild-type T/T genotype. This association was maintained in the 55 pts with *KRAS* mutation. In this group, G-allele carriers and T/T carriers showed median PFS of 2.5 and 3.4 months, respectively (p=.01) and median OS of 5.9 and 9.7 months, respectively (p=.04).

Conclusions: These findings support additional investigations in the mechanisms of post-transcriptional *KRAS* regulation, as they may lead to the discovery of additive/alternative treatment strategy to current anti-EGFR therapy in MCRC, such as micro-RNA interference in the RAS pathway.

C2* ACQUISITION OF HER2/NEU OVER-EXPRESSION ON CIRCULATING TUMOR CELLS (CTCS) IN PATIENTS (PTS) WITH ADVANCED BREAST CANCER (ABC) DURING CHEMOTHERAPY

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Background: CTCs can be detected and isolated from the peripheral blood of women with ABC. In addition to enumeration, an exciting area of research involves the phenotyping and expression profiling of CTCs. The purpose of our investigation was to evaluate the *concordance* of HER-2/neu status between the pathological primary tumor analysis and CTCs and the *acquisition* of HER-2/neu overexpression on CTCs during the course of chemotherapy in peripheral blood of intensively treated breast cancer patients.

Methods: We collected 7.5 ml blood samples from each of 76 pts with ABC. CTCs were isolated with the CellSearch System® by means of immunomagnetic separation, using ferrofluid nanoparticles binding anti-epithelial cell adhesion molecules (EpCAM), and fluorescently stained with Epithelial Cell Kit®. CTCs were defined as nucleated (DAPI positive), epithelial (CK 8, 18, 19 positive) and negative for CD45. Concomitantly, we used Tumor Phenotyping Reagent® to investigate HER2/neu expression. Moreover, CTCs were isolated using the Profile Kit®.

Results: 59 pts had at least 1 CTC in 7.5ml of blood (prevalence 76%)., Median CTCs number was 7/7.5ml (range 1-4988). The concordance between the primary tumor and CTCs was 83% (49/59 pts). Sixteen pts had HER2/neu overexpression on primary tumor: 13 pts (81%) maintained HER2/neu overexpression on CTCs and 3 pts (19 %) were found HER2/neu negative upon CTCs assessment. Forty-three pts had a primary HER2/neu negative tumor and were followed every two months in order to evaluate the possible acquisition of HER2/neu on CTCs. Six of these pts (14%) acquired HER2/neu overexpression on CTCs prior to start the new chemotherapy regimen. Three patients acquired HER-2 overexpression on CTCs during the treatment started after study enrolment. Nine patients overall (21%) in this population acquired HER-2 overexpression from baseline tissue evaluation, during the course of their disease.

Conclusions: HER2/neu overexpression can be acquired during the course of the disease in pts with ABC. Our future intent is to evaluate the *efficacy* of a trastuzumab based therapy in this sub-group of pts within a clinical trial.

C3* EVALUATION OF THE OPTIMAL TIME INTERVAL FOR DEOXYCYTIDINE KINASE (DCK) AND HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER-1 (HENT1) GENE EXPRESSION INDUCTION FOLLOWING ADMINISTRATION OF PEMETREXED IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC): FINAL RESULTS FROM A PILOT STUDY

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Background: As shown by preclinical and clinical evidence, downstream signalling induced by pemetrexed promotes a synergic interaction pemetrexed-gemcitabine when administered in sequence. The molecular mechanisms underlying the optimal sequence are yet unclear, although preclinical studies argue that this synergistic activity might result from pemetrexed's induction of hENT1 and dCK gene expression, two determinants for the transport and activation of gemcitabine, following pemetrexed administration. This study aims to better evaluate the modulation of expression of these genes for gemcitabine activation, following pemetrexed administration, in NSCLC patients.

Patients and Methods: The dCK and hENT1 expression was examined by quantitative real-time PCR(qRT-PCR)on RNA extracted from peripheral blood mononuclear cells from patients with locally advanced(stage IIIb)and advanced(stage IV)NSCLC, before and after administration of pemetrexed(3 cycles of 500mg/m2,q2wks). The expression of dCK and hENT1 genes was measured during each cycle at various intervals(1, 2, 4, 6, 24 and 48h),after pemetrexed administration. Statistics were calculated for original dCK and hENT1 values, for the relative differences from baseline to each planned time,for peak-values and for the relative-difference-at-peak. Multivariate repeated-measures ANOVA on dCK and hENT1 values was performed using two-repeated within-subjects factors:cycle and time.

Results: Nineteen patients(11 males/8 females; ECOG PS0=5,PS1=11,PS2=3 patients, respectively;not pre-treated patients=6,pre-treated patients=13)were enrolled. Patients experienced grade 3 and/or 4 haematological toxicities(15.8% neutropenia, 15.8% leukopenia)and non-haematological toxicities(15.8%gastrointestinal disorders,26.4% general disorders,10.5% transaminases increase and 26.3% respiratory, thoracic and mediastinal disorders). The mean(± standard deviation)dCK and hENT1 peak values were 0.99±0.03; 0.93±0.03, respectively, while the relative differences at dCK and hENT1 peak were 7.82±2.15; 8.54±3.12, respectively. qRT-PCR analysis revealed a statistically significant($p<0.001$)biphasic increase(confirmed by standard curve or the $2\Delta\Delta CT$ method)in both hENT1 and dCK genes, at early(1-2 hours)and late(24-48 hours)time points(irrespective of cycle), following pemetrexed administration.

Conclusion: This is the first evidence of dCK and hENT induction by pemetrexed in humans. The kinetics of gene expression induction points out a peak at both early and late time points, suggesting a suitable administration time for gemcitabine at 1-2 hours following pemetrexed administration.

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C4* SYNERGISTIC ANTITUMOR ACTIVITY OF SORAFENIB, A MULTIKINASE INHIBITOR OF RAF, VEGF AND PDGF RECEPTORS, WITH ANTI-EGFR INHIBITORS (CETUXIMAB AND ERLOTINIB) IN A PANEL OF COLORECTAL AND LUNG CANCER CELL LINES

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EGFR and VEGF signalling pathways play a crucial role in tumor cell survival, growth, invasion, proliferation and metastasis. The purpose of this study is targeting both these pathways by using the combination of sorafenib, and cetuximab or erlotinib. This could provide a better anticancer therapeutic strategy.

A panel of human lung (A549, GLC-82, Calu3) and colon (GEO, HCT-15, HCT-116, HT-29, SW480) cancer cells were screened for EGFR and VEGFR expression by Real Time PCR and Western Blot; their ligands have been evaluated by ELISA. These cells lines are characterized for different expression of gene status for p53, K-ras and BRAF (Table 1).

The antiproliferative effects of sorafenib in combination with gefitinib or cetuximab were determined by using a soft agar anchorage-independent growth assay. Combination effects were analyzed by using the isobolographic model according to the Chou and Talalay method. Expression of proteins involved in intracellular cell signaling were assessed by Western Blot. The migration capabilities have been investigated by wound-healing assay.

The EGFR, VEGFR are expressed in almost all cancer cell lines as well as their ligands which were detected in the supernatant. A dose-dependent synergistic effect in growth inhibition was observed by the combined treatment with sorafenib 1μM and erlotinib 2 μM or with sorafenib and cetuximab 2.5 μM; colorectal cancer cell lines seem to be more sensitive to inhibition. The expression of active phosphorylated EGFR, MAPK and AKT,evaluated after 10, 20, 60 and 120 minutes of treatment, is markedly decreased by both combined drugs especially with sorafenib and erlotinib treatment as well as the downstream pathway of mTOR such as ppS6K and p4EBPI. Moreover the migratory activity was decreased by using sorafenib as single agent and in combination in H1299 NSCLC cell line.

Combination treatment with sorafenib and erlotinib or sorafenib and cetuximab has synergistic antiproliferative properties in human colorectal and lung cancer cell lines providing a rationale for further clinical studies.

Human cancer cell lines	K-ras	BRAF	p53
GLC-82	-	-	WT
H1299	WT	Mut	Mut
A549	Mut	WT	WT
Calu3	Mut	WT	Mut
H460	Mut	WT	WT
HT29	WT	Mut	Mut
HCT15	Mut	WT	Mut
HCT116	Mut	WT	WT
SW480	Mut	WT	Mut
GEO	Mut	WT	WT

C5* DOWN-REGULATION OF HYPOXIA INDUCIBILE FACTOR-1α (HIF-1α) EXPRESSION, THROUGH mTOR PATHWAY, BY LETROZOLE (FEMARA®) IN ER+ve BREAST CANCER PATIENTS: CLINICAL IMPLICATIONS

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Introduction: the HIF-1α pathway is involved in the selection of more aggressive phenotype being associated with resistance in breast cancer. Letrozole is a selective aromatase inhibitor (AI) used for the treatment of estrogen positive (ER+*ve*) post-menopausal breast cancer (BC). Whether HIF-1α expression could be modulated by AI is the subject of this investigation. HIF-1α expression was evaluated in primary letrozole therapy in elderly ER+*ve* patients enrolled in a randomized prospective trial (Bottini et al JCO 2006 24(22): 3623-8).

Patients and methods: 114 women with T2-4 N0-1 and ER+*ve* breast cancer were randomly assigned to 6 months of primary Letrozole (2,5 mg/daily) (L) plus/minus oral ''metronomic'' cyclophosphamide (50 mg/daily) (LC). Tumour response was assessed clinically with a calliper. HIF-1*a* and mTOR expression (an upstream regulator of HIF-1*a*) were evaluated in tumour specimens collected before and after treatment (6 months). The intensity of the staining for was scored 0 (no staining), 1 (weak/moderate staining), 2 (strong staining) for HIF-1α expression and mTOR.

Results: One hundred and seven patients had HIF-1*a* assessed at baseline, 80 patients had HIF-1α assessed both at baseline and after treatment. At baseline, there was a significant association between HIF-1*a* and mTOR (p=0.01) and HIF-1*a* expression inversely correlated with tumour grade (p<0.01) and N positivity (p<0.01) but not with stage, p53, bcl2, Ki67, progesterone or estrogen receptor alpha expression. Letrozole treatment led to a significant reduction in HIF-1α expression (p <0.004, Wilcoxon Rank sum test), with no difference between the arms. Reduction in HIF-1α did not show any relationship with disease response. As previously observed (Generali et al Clin Cancer Res 2008,14(9): 2673-80) letrozole-based therapy was able to down-regulate mTOR pathway. Interestingly, there was a significant association between mTOR and HIF-1α reduction after treatment (p=<0.03).

Conclusions: Letrozole therapy concomitantly reduced HIF-1*a* expression and mTOR. These results should be taken into account when planning new therapies targeting either HIF-1*a* and/or mTOR in association with Letrozole.

C6* THE PROGNOSTIC ROLE OF PI3K/p-AKT PATHWAYS ALTERATIONS AND THEIR CORRELATION WITH OTHER BIOMARKERS AS INDICATOR FOR UNFAVOURABLE BIOLOGIC PROFILE (UBP) IN EARLY STAGE BREAST CANCER (BC) PATIENTS (PTS) TREATED WITH CMF (CYCLOPHOSPHAMIDE/METOTREXATE/ 5-FLUOROURACIL)-BASED CHEMOTHERAPY

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Background: Akt activation through PI3K, leads to the phosphorylation of p27 protein thus avoiding nuclear entry and inducing mislocation to the cytoplasm of the protein with consequent inhibition of p27 cell cycle inhibitory function. The prognostic role of PI3K/Akt pathway alterations correlating phenotypic profiles with bio-pathologic variables of known clinical importance have been investigated in a retrospective series of early stage of BC pts.

Materials and methods: p-Akt, PI3K and p27 expression were evaluated by immunohistochemistry in a retrospective series of stage I/II BC pts who underwent conservative surgery and were candidates to receive CMF adjuvant therapy plus radiotherapy. Multiple Correspondence Analysis (MCA) was used to identify sub-groups of pts with different prognosis, while uni- and multivariate Cox regression analyses were applied to determine the impact of parameters identified by MCA on 10-yrs Disease Free Survival (DFS), together with clinico-pathological features. Receiver Operative Curve (ROC) analysis was adopted for optimal cut-off values.

Results: In a series of 133 pts, with a median follow-up of 107 months (range 40 to 141), those pts characterized by high Ki67 index, p53+, p-Akt+, PI3K+ and HER2+ (Adverse Biologic Factors, ABP) were associated with tumor relapse at the MCA analysis. ROC analysis dicotomized between pts with a Favourable Biological Profile (FBP, <3 ABF) and UBP (≥ 3 ABF). UBF pts showed a significantly shorter 10- yrs DFS than FBF pts at Kaplan-Meier analysis (67.0% vs 91.3%, p=0.0006). According to the multivariate analysis, the biologic profile was the only significant prognostic indicator for longer DFS (p=0.002). Significant factors at uni- and multivariate analysis are shown in the table.

Factors	Univariate (HR, 95% CI)	*p-value*	Multivariate (HR, 95% CI)	*p-value*
Grading	3.32 (1.31-8.43)	*0.012*	—	—
PgR	2.37 (1.03-5.48)	*0.043*	—	—
Biologic Profile	4.24 (1.73-10.40)	*0.002*	4.24 (1.73-10.40)	*0.002*

Conclusions: These data suggest that the activation of Akt may contribute together with high Ki67 index, p53+, p-Akt+, PI3K+ and HER2+ in predicting recurrence in early stage BC pts homogeneously treated with CMF based therapy.

C7 EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) EXPRESSION ON CIRCULATING ENDOTHELIAL CELLS FROM EGFR-POSITIVE COLORECTAL CANCER (mCRC) PATIENTS

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Background: The vascular endothelial growth factor receptor (VEGFR) and the EGFR play a key role in the growth and metastatic spread of tumors. Due to multiple cross-talks with respect to angiogenesis, a simultaneous blockade of VEGFR and EGFR pathways could enhance antitumor efficacy and this aspect is currently being evaluated in clinical trials. During our research program on cellular biomarkers of response to

antiangiogenetic treatment, we have focused on the EGFR expression on circulating endothelial cells (CECs) from mCRC pts.

Methods: The specificity of EGFR phenotyping on CECs was verified, by spiking with colorectal cancer cell lines (SNU-C2B, SW-480) with different levels of EGFR expression. For the in vivo study, 32 serial PB samples were obtained from 5 pts with mCRC (M/F:1/4, median age 58 yrs, all pre-treated for their EGFR-positive advanced disease) at baseline and during a Cetuximab-based treatment. The EGFR immunostaining was performed with the anti-EGFR MoAb following high resolution flow cytometric analysis of total CEC absolute number, identified as CD45-, CD106-, CD34+,CD146+ cells or CD31+ cells.

Results: At baseline, with respect to a group of 50 healthy donors utilized as control, pts showed an higher number of total CECs, that, in a proportion ranging from 70 to 90%, expressed consistent levels of EGFR. After 5 - 7 treatment courses, all pts showed a progressive decrease of total CEC absolute number while maintaining elevated levels of EGFR expression on these cells. In particular, none of the pts switched from positive to negative EGFR phenotype during the course of treatment.

Conclusions: Our study provides the first evidence that the measurement of EGFR expression on the surface of total CECs derived from pretreated mCRC pts is feasible and that this expression is reproducible over time and during treatment with an anti-EGFR agent. The described approach allows a better biological characterization of tumor-derived CECs and could be utilized in prospective clinical studies with dual inhibitors of VEGFR and EGFR signalling pathways.

C8 ENHANCED SENSITIVITY TO BORTEZOMIB PRO APOPTOTIC EFFECTS IN HUMAN CANCER CELLS WITH ACQUIRED RESISTANCE TO ANTI-EGFR TKIS

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Despite great clinical promise the majority of cancer patients show either intrinsic resistance or acquired resistance to EGFR inhibitor therapies. Bortezomib (PS-341; Velcade) is an approved drug for the treatment of haematological neoplasms and is being currently evaluated for the treatment of solid cancers. Recent works showed that bortezomib may play a role as sensitizer for the EGFR inhibitor demonstrating a rationale for the combined use of bortezomib with EGFR inhibitors and thus in cancer cells not anymore responding to the EGFR blockade. We developed gefitinib- and erlotinib-resistant non small cell lung cancer (Calu 3) and colon cancer (HCT116) cell lines.

These resistant cell line showed iperactivation of Akt and survivin if compared to parental lines. Bortezomib treatment induced a strong inhibition of cell proliferation and inhibition of Akt and survivin and induction of apoptosis, but in addition to the inhibitory effect on Akt signalling, bortezomib showed a strong ability to induce the expression of GADD153, a well-recognized ER stress-inducible transcription factor, and DR5, in all resistant cell lines, but not in wild type cells. Furthermore, bortezomib induced significant PARP and bid cleavage by caspase 8 activation. Conclusions: Together, these findings support a mechanistic framework for the induction of apoptosis in resistant cells by bortezomib in which the ER stress–inducible transcription factor, GADD153, is induced, leading to up-regulated DR5 expression and stimulation of the extrinsic apoptotic pathway.

C9 EXPRESSION OF THE CDK INHIBITOR P27^{KIP1} AND OXIDATIVE DNA DAMAGE IN RENAL CELL CARCINOMA

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Background: Deregulation of the normal cell cycle is a frequent event in human tumors and plays a pivotal role in carcinogenesis and cancer progression. p27^{kip1} is a cell-cycle inhibitor acting on the G1 phase, is frequently lost in tumor cells and, in some cases, its alteration is coupled with oxidative DNA damage.

Patients and methods: Nuclear expression of p27^{kip1} and the extent of endogenous oxidative DNA damage (by means of 8-hydroxydeoxyguanosine [8-OHdG] levels) was evaluated by immunostaining in 125 (median age 64[range 23-86]yrs) renal cell carcinomas (RCCs) and tested for prognostic significance. Median values of expression were used as cut-off. p27^{kip1} expression was also evaluated by Western Blot in a second series of 34 fresh-frozen RCCs.

Results: at report time median follow-up is 29 [range 4 - 104] months. p27^{kip1} expression was lost in the majority of tumors (55%) with a median percentage of positive cells of 20% [range 0-60%]. Loss of p27^{kip1} nuclear staining correlated with higher tumor grade (p=0.049). Recurrence (p=0.007) and death (p=0.006) from RCCs were significantly more frequent in patients p27^{kip1} negative compared with positive ones. Kaplan-Meier analysis showed a significant separation between high vs low p27^{kip1} expression groups for both disease-free (p=0.011) and overall (p=0.002) survival. At multivariate analysis, loss of p27^{kip1} expression was the only independent risk predictor for recurrence (HR=4.326, p=0.014) and death (HR=4.915, p=0.012) from RCCs when tumor size, tumor grade and stage were included. No significant correlation with clinical or pathological parameters and outcome was observed for 8-OHdG. p27^{kip1} total protein levels showed a variable behaviour at WB analysis with a modest trend toward a global reduction but an elevation in some cases.

Conclusions: loss of p27^{kip1} is frequent in human RCCs and is a strong predictor of poor outcome. p27^{kip1} alteration are not related to endogenous oxidative DNA damage. The behaviour of p27^{kip1} at WB analysis is probably related to the elevation of the cytoplasmatic (and inactive) fraction.

C10 FUNCTIONAL CHARACTERIZATION OF TRAP1 PATHWAY IN MULTIDRUG RESISTANCE IN HUMAN COLORECTAL CARCINOMA

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Background: TRAP1 has been recently characterized by our group as a mitochondrial chaperone up-regulated in human colorectal carcinoma (CRC) and involved in favoring a phenotype resistant to apoptosis and chemotherapeutic agents in tumor cells. Interestingly, this finding correlates with the observation that TRAP1 is a component of a mitochondrial pathway which antagonizes the proapoptotic activity of cyclophilin D and is responsible for maintaining mitochondria integrity and favoring cell survival.

Materials and Methods: Preliminary GST-pulldown experiments and mass spectrometry assays led us to the identification of several TRAP1 ligands and, among others, sorcin, a Ca^{2+}-binding protein involved in the emergence of the MDR phenotype in leukemia cells. Thus, we further characterized the role of TRAP1 and sorcin in inducing multi-drug resistance in human CRC.

Results: TRAP1 and sorcin co-immunoprecipitation was observed in CRC cells and preliminary experiments suggest a concomitant enrichment of TRAP1 and sorcin, which was known to be a cytosolic protein, in the mitochondrial fraction of CRC cells resistant to 5-fluoruracil, irinotecan and oxaliplatin and in human CRC specimens. Indeed TRAP1 and sorcin are up-regulated in 60-70% of human CRCs where a significant correlation between the two proteins is observed (Pearson Correlation test r=0,60; p=0,001). These findings are in agreement with the observation that HT-29 CRC cells transfected with TRAP1 exhibit a phenotype resistant to 5-fluorouracil-, oxaliplatin- and irinotecan-induced apoptosis and that the inhibition of TRAP1 activity by the TRAP1 ATPase antagonist, shepherdin, or the transfection of a dominant negative TRAP1mutant increase the sensitivity to apoptosis induced by chemotherapeutic agents in wild type CRC cells or CRC cells resistant to the single agents.

Conclusions: It is likely that sorcin and TRAP1 cooperate in a pro-survival pathway responsible for resistance to chemotherapy and that such a pathway may represent a novel molecular target to overcome drug resistance in human CRCs.

C11 IN VITRO SCHEDULE-DEPENDENT INTERACTION BETWEEN DOCETAXEL AND GEMCITABINE IN HUMAN ERCC1+ NSCLC CELLS LINES

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Purpose: we aimed to assess the activity of Docetaxel (DOC) and Gemcitabine (GEM) and to define the most effective treatment scheme in human ERCC1+ NSCLC cells lines.

Experimental Design: DOC and GEM were tested using different combination and sequences. DOC and GEM were tested at all three concentrations (0.01, 0.1 and 1 ng ml^{-1}) in combination schemes and when they were used as the first drug in the sequential schemes. The lowest concentration (0.01 ng ml^{-1}) was used when DOC or GEM was administered as the second drug in the sequential schemes. Cytotoxic activity was evaluated by MTT colorimetric assay, potential clinical activity was estimated by relative antitumor activity (RAA). To quantify deviations from additive effects induced by the sequential administration of two drugs, a statistical Student's t-test was employed. For a given drug dose, we determined a surviving

fraction (Sf) of cells. The performance index (PI) statistic model was used to evaluate type of interaction. Cell cycle perturbations and apoptosis were evaluated by flow cytometry.

Results: The sequential scheme of a 24-h treatment with GEM followed by DOC produced a weak synergistic effect (PI = 1.15). The opposite sequence of drugs administration (DOC followed by GEM) induced an antagonistic interaction. Cell cycle perturbations were analysed by flow cytometric analysis in an attempt to explain the mechanism underlying the synergistic interaction. DOC induced a characteristic cell block in the G2/M phase after a 24-h treatment, while GEM induced the block in the S-phase. On the basis of cell cycle perturbations, it can be hypothesized that cells recovering after phase S block induced by GEM receive as they progress in M phase a cytotoxic effect by DOC, producing a powerful synergistic effect.

Conclusions: Our preliminary findings suggest that the interaction of DOC and GEM in ERCC1+ NSCLC cell lines is highly schedule dependent and has been used recently to plan a Phase II clinical protocol.

C13 PREDICTIVE VALUE AND BIOLOGIC SIGNIFICANCE OF CIRCULATING TUMOR CELLS (CTC) IN VON HIPPEL LINDAU (VHL) RENAL CANCER

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Background: Haematogenous spreading of tumor cells is a key step toward metastasis; the automated analysis of CTC by Cellsearch platform represents an improvement to serially investigate and quantify these rare cells. At present, CTC count is indicated in the follow-up of some metastatic cancers. Von Hippel-Lindau disease (VHL) is a rare, autosomal dominant genetic conditionin in which hemangioblastomas can be associated with several pathologies including renal cysts, renal cell carcinoma and phaeochromocytoma. VHL results from a mutation in the tumour-suppressor gene on chromosome 3p25.3 Preliminary reports indicate that CTC are present in patients with various metastatic carcinomas but lack at presents At the moment, no extensively analysis of renal cell carcinoma (RCC) in patients with VHL disease have been done.

Materials and Methods: To investigate if VHL patients present CTC, we have designed a pilot study enrolling VHL patients, at diagnosis and naive for treatment. The first clinical objective of the study was to correlate CTC count with major prognostic factors determined at diagnosis. CTC and M30+ were measured in 25 consecutive patients affected by VHL disease. In 6 of them a RCC was found, while in 3 renal cysts. CTC were measured in a group of healthy donors too. The study is ongoing

Results and conclusions: Preliminary data obtained indicate that:
70% of RCC samples VHL presented CTC
2 patients with benign renal cysts presented CTC
No CTC were detected in healthy donors
66% of VHL patients with RCC presented 100% of apoptotic CTC

C14 PRECLINICAL EVIDENCES ON THE ACTIVITY OF EGFR1 INHIBITORS IN POORLY-DIFFERENTIATED THYROID CARCINOMA CELLS

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Background: Poorly-differentiated thyroid tumors are characterized by poor prognosis and lack of sensitivity to standard therapies, thus new therapeutic strategies are needed. The expression of EGFR1 is up-regulated in human poorly-differentiated thyroid carcinomas, but its role as a therapeutic target is still unclear. We recently reported novel evidences in favor of a role for EGF signaling in driving the transition toward a less differentiated and invasive phenotype in human thyroid carcinoma cells.

Material and Methods: The antiproliferative activity of the EGFR1 TK inhibitor erlotinib, and panitumumab and cetuximab, two monoclonal antibodies which target the extracellular domain of EGFR1 was evaluated in poorly-differentiated FRO, follicular differentiated WRO and papillary differentiated TPC-1 thyroid carcinoma cells. The potential synergism of action between erlotinib and anti-EGFR1 antibodies and erlotinib and chemotherapeutic agents was also tested in FRO cells.

Results: Erlotinib exhibited a dose-dependent antiproliferative activity which was higher in FRO cells (70% inhibition at 10 uM erlotinib) than in WRO and TPC-1 cells (40% inhibition at 25 uM erlotinib). By contrast, cetuximab and panitumumab were completely inactive in WRO and TPC-1 cells and exhibited a minimal activity in FRO cells. The combination of erlotinib with anti-EGFR monoclonals did not result in any further down-regulation of cell proliferation. Based on the hypothesis that EGF signaling is responsible for the induction of antiapoptotic mechanism we tested the ability of either erlotinib or cetuximab to enhance the proapoptotic activity of doxorubicin and paclitaxel. Interestingly, erlotinib-pretreated FRO cells exhibited a significant up-regulation of doxorubicin and paclitaxel-induced cell death, whereas cetuximab did not enhance the cytotoxic activity of either antiblastic agents.

Conclusions: Poorly-differentiated thyroid tumor cells are more sensitive to EGFR1 inhibitors than differentiated thyroid tumor cell lines and the TK inhibitor erlotinib seems to be more active compared to agents which target the extracellular domain of EGFR1. Furthermore, combination therapy with erlotinib and chemotherapeutic drugs deserves to be evaluated as a novel treatment in undifferentiated thyroid carcinomas.

C15 SERUM LIPID ABNORMALITIES AND HYPOTIROIDISM IN PATIENTS TREATED WITH SUNITINIB

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Hypothyroidism is a known adverse event of Sunitinib. The overall prevalence of elevated TSH levels after Sunitinib has been recently reported in 40-70% of patient. The basis of thyroid impairment in those patients is not established yet. Others common metabolic abnormalities in patients on Sunitinib are hypercholesterolemia, hypertriglyceridemia.

Between July 2007 and February 2008 20 patients affected by metastatic renal cell carcinoma (mRCC) referred to our institution. Median age was 61 (range 50-75 year-old). All those patients received a first line Sunitinib, with a daily administration according to the following 6 weeks schedule: 4-week daily administration (ON) and 2-week withdrawal (OFF). Blood samples (complete of thyroid function tests, lipid asset and pancreatic function biomarkers) were collected on the first and last day of both ON and OFF periods. Antithyroid antibodies were collected, before the beginning of the treatment and every three months. They have been found in a normal range.

At enrolment, TSH levels, pancreatic function test, serum triglycerides and cholesterol were in the normal range. 12 patients (58%) developed subclinical hypothyroidism. TSH levels were increased in all these patients and ranged from 5,97 mU/L to 27,2 mU/L (nv: 0,25-3,5 mU/l). Free T3 and free T4 always resulted within a normal range in all patients. Thyroid test function abnormalities were detected after a median of 3,3 cycle (range 1-7 cycles).

15/20 patients (75%) developed hypertriglyceridemia and hypercholesterolemia, within 2 cycles of treatment. 10/20 patients (50%) developed both thyroid and lipid abnormalities.

This is a significant issue because we don't know whether hypertriglyceridemia and hypercholesterolemia might be due to Sunitinib-induced hypothyroidism or are a direct consequence of the drug. In the first case these metabolic alterations might be reversible only with thyroid hormone replacement. In our experience levothyroxine was administered to patients with subclinical hypothyroidism without a significant result on lipid asset suggesting that serum lipid changes could be related to the drug rather than to Sunitinib-induced hypothyroidism.

C16 DUAL-TARGETING OF AMPK AND PI3K/MTOR IN HER2 OVEREXPRESSING BREAST CANCER

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Background: Metformin is a widely used diabetes drug known to reduce cancer risk and to improve cancer prognosis. The aim of this study was to explore the anti-tumor effect of metformin alone or in combination with PI3K/mTOR inhibitors in breast cancer cell lines.

Methods: MCF-7 and MCF-7/HER2 breast cancer cells reaching a confluence of 70% were exposed to escalating dose of metformin with and without a fixed concentration of PI3K/mTOR inhibitor. Levels of phosphorylated AMPK, MAPK, Akt, and S6 were evaluated by western blot analysis. Cell proliferation assays were performed in triplicates using the MTT and cristal violet colorimetric assays.

Results: Metformin induces dose-dependent growth inhibition of MCF-7/-HER2 breast cancer cell lines. At the IC50 of 20 mM, metformin is able to increase the levels of AMPK, which decrease the activity of the PI3K/Akt downstream target S6K, suggesting the mechanism for its anti-proliferative effect. Metformin in combination with the PI3K/mTOR inhibitor results in a more profound inhibition of the levels of p-Akt and

p-MAPK in parallel with a strong up-regulation of the levels of p-AMPK, which finally translates in a superior anti-proliferative effect over each single agent.

Conclusions: Metformin significantly inhibits the growth of breast cancer cell lines overexpressing HER2. Combined targeting of AMPK with metformin and PI3K/mTOR results in a superior anti-tumor effect over each single strategy. Further preclinical studies are being conducted to determine the applicability.

C17 EXPRESSION AND PROGNOSTIC ROLE OF EXCISION REPAIR CROSS COMPLEMENTING GROUP 1 (ERCC1) IN EARLY STAGE BREAST CANCER (BC) PATIENTS (PTS)

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Triple-negative (TN) BCs are defined by negativity for ER, PgR, and HER2 and most of them possess a basal phenotype (BLBC) showing varying degrees of basal markers expression. These tumours are associated with a poor outcome and chemotherapy is the standard treatment but no specific regimen is recommended. Heritable BRCA1 BCs are often TN and in the majority of cases express basal keratins (CK) and EGFR but BRCA1 dysfunction seems to play an important role in sporadic TNBCs explaining the sensitivity to DNA-damaging agents. Cisplatin-DNA adducts are repaired by nucleotide excision repair and ERCC1 is a critical protein within this pathway. ERCC1 mRNA, but also the levels measured by IHC, seem to predict the survival benefits in NSCLC treated with platinum-based therapy.

Methods: The expression of ERCC1 by IHC was retrospectively evaluated in 135 early stage BC pts of whom 85 were TN and 49 represented the control group and correlated with pts 'characteristics and prognosis. Tumour sections were immunostained using MoAb (ERCC1, clone 8F1) and slides were evaluated by a semiquantitative scoring system with a cut-off of ≥1. CKs 5/6 and 14 (MoAb) were considered positive if ≥10% of tumour cells stained.

Results: Stage I tumours were less frequent in the TN group while high grade tumours were more common. Twenty-eight (32.9%) TN pts and 26 (54.2%) control group pts were ERCC1-positive with a statistically significant difference (p=0.017). At a median follow-up of 4.2 years, there were no significant differences in DFS and OS according to ERCC1 expression in both groups. In the group of TNBC 48% of pts had at least one CK positive but no correlation with the clinical outcome was observed.

Conclusion: In this limited series of pts a significantly higher percentage of ERCC1 positivity was observed in the control group but ERCC1 had no prognostic role. The role of ERCC1 expression as a predictive factor of response to cisplatin should be evaluated in a prospective trial.

C18 PERI-OPERATIVE CIRCULATING TUMOR CELLS DETECTION IN PATIENTS WITH PRIMARY BREAST CANCER UNDERGOING SURGERY

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Background: Circulating Tumor Cells (CTCs) in blood have been demonstrated to represent an adverse prognostic factor in patients with metastatic breast cancer, while little data exist in operable patients. If the presence of CTCs in early breast cancer would predict an increased risk for relapse, this method might be used as an early marker for treatment efficacy and could help in deciding treatment continuation.

Methods: We analyzed 30 ml of peripheral blood from 69 T1-T3, any N, M0 breast cancer pts, before surgery and after 5 days. In case of positivity of one of the perioperative samples, a further sample was taken after 30 days. The presence of CTCs was assessed with the CellSearch System (Veridex, USA). A sample was considered positive when 1 or more cells were detected.

Results: Data are available for 56 pts. We found ≥1 CTC in 29% of the pts (16/56) before surgery, in 30% of the pts (n= 14/47) at 5 days and at day 30 (8/27). The median number of CTC was 1 (range 1-3). No association was pathological data was found, apart a borderline significant association between presence of CTC at baseline and presence of vascular invasion (p=0.07). In 47 patients data of CTCs at baseline and on day 5 were available: 28 patients showed concordant results. Among the 19 patients with discordant results, 10 were negative at baseline and positive at day 5: 8 out of these 10 pts had positive nodes.

Conclusions: In a considerable number of patients CTCs can be detected in peripheral blood before (29%) and after (30%) surgery. No association with the pathological variables was found, except for vascular invasion which was associated with the presence of pre-operative CTC. Of note 8 of the 10 patients with negative CTCs at baseline and positive CTCs at day 5 showed nodal involvement. Long-term follow-up will be required to understand the significance of CTCs in patients with operable breast cancer.

C19 THE PROTEASE INHIBITOR GABEXATE MESYLATE HAS ANTI-TUMOR EFFECTS AND ENHANCES GEMCITABINE ACTION ON PANCREATIC CANCER CELL LINES

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Background: Gemcitabine is standard treatment of advanced pancreatic adenocarcinoma. Cancer cells, as their normal counterparts, produce various proteases involved in invasion and metastatic spread. Gabexate mesylate (GM), a serine protease inhibitor approved for acute pancreatitis, may therefore reduce invasive and metastatic capabilities and also enhance chemotherapy effects. This work aims to test this hypothesis.

Materials and Methods: Studies have been performed on the poorly differentiated PANC-1 cell line. After preliminary sensitivity tests of gemcitabine and GM, various sequential protocols (GM pretreatment followed by gemcitabine) have been evaluated for efficacy. Aspects studied included cell viability (MTT colorimetric assay), cell invasiveness and migration (Boyden chamber assay), matrix metalloproteinase 2 (MMP-2) activity (gelatin zymography) and angiogenesis (endothelial tube formation assay on EA.hy926 cells). All assays have been performed in triplicate.

Results: Continuous exposition to GM 100 μM has time-dependent effects on PANC-1 cell viability, decreasing it by 40% after 4 days ($p < 0.001$); gemcitabine alone 250 μM has negligible effects (-15%, p ns). GM is therefore active against PANC-1 cell line and we chose 100 μM as the effective concentration. Further tests on various sequential protocols showed maximal activity in the case of GM pretreatment followed by 24-hour rest and eventually by gemcitabine 250 μM. Effects on cell viability almost doubles with respect to gemcitabine alone (-28%), while cell invasiveness and MMP-2 activity decrease by 50% ($p<0.001$). Endothelial tube formation by EA.hy926 endothelial cells exposed to the conditioned medium of PANC-1 cells decreases by >95% after treatment ($p<0.001$). GM concentration escalation does not significantly alter these parameters.

Conclusion: These preliminary data suggest that GM has strong action in pancreatic cancer, especially on angiogenesis, and can enhance gemcitabine effects. Results need confirmation in other cell lines before definitive conclusions be made.

C20 MOLECULAR ALTERATIONS AS CANDIDATE BIOMARKERS FOR PREDICTING RESPONSE TO THERAPY AND PROGNOSIS IN STAGE IIIB BREAST CANCER PATIENTS

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Background: Patients with T4 breast carcinomas of any type, with or without lymph node involvement, and without metastases are classified with the highest stage of non-metastatic disease (Stage IIIB). Characterization of molecular mechanisms associated with such a disease could help with patients' stratification and management. In present study, we examined alterations in survivin-p53-pERK1-2 expressions and *CyclinD1-h-prune* gene copy number among stage IIIB breast carcinomas, in order to determine their association with clinico-pathological parameters and patients' prognosis.

Methods: Paraffin-embedded samples from 53 consecutive stage IIIB patients underwent immunohistochemistry and FISH analysis. Chi-square and Fisher's exact tests were used to evaluate correlation with treatment responses [complete clinical response (cCR), partial clinical response (cPR), pathological complete response (pCR), major pathological response (MpR; corresponding to pT0-pT1 classification after primary chemotherapy)] and survivals.

Results: Overexpression of survivin, p53, and pERK1-2 as well as amplification of *h-prune* and *cyclinD1* were evaluated for association with several histological tumour characteristics: estrogen and progesterone status, HER2 amplification, Ki67 proliferation index. No statistically significant correlation was observed, with the exception of an inverse distribution of positive pERK1-2 and Ki67 expressions [absence of pERK1-2 staining in 16/42.(38%) Ki67+ cases *vs.* 4.(15%) pERK1-2+ tumours in 26/42.(62%) Ki67- cases]. The Ki67 and HER2 parameters were significantly associated with better clinical response rates [5/7.(71%) cCR *vs.* 11/35.(31%) cPR and 8/8.(100%) cCR *vs.* 29/45.(64%) cPR, respectively], whereas pERK1-2 expression was significantly associated with worse clinical response rates [0/8 cCR *vs.* 5/45.(11%) cPR]. Univariate analysis showed a significant association to better survivals in breast cancer cases with absence of *h-prune* amplification, pERK1-2 immunostaining, and survivin expression. After multivariate analysis, pathological response to primary chemotherapy and survivin expression remained the only parameters closely correlated to prognosis.

Conclusions: Although our study was retrospective and based on relatively small numbers of patients, our findings provided some important indications about either the prediction of the response to therapy or the role on prognosis in stage IIIB breast cancer patients.

C21 THE IMPORTANCE OF MONITORING CIRCULATING MITOTANE LEVELS IN THE MANAGEMENT OF ADRENOCORTICAL CANCER PATIENTS

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Adrenocortical carcinoma (ACC) is a rare disease with a reported incidence of 1-2 per million population per year. Mitotane (o,p'-DDD) is the reference agent for treatment of ACC. This drug has notoriously a long half-life in serum concentration; numerous side effects (gastrointestinal, neurological, haematological) limited its long-term clinical tolerance. Experience with monitoring blood mitotane concentrations is still limited but appears promising to improve drug efficacy and avoid severe toxicity. It's generally accepted that antitumor efficacy is observed with dose reaching plasma o,p'-DDD concentrations >14mg/liter while severe toxicities are usually associated with concentrations >20 mg/l (Van Slooten et al.,1984). However, it is actually unclear how many patients are able to attain and maintain over time effective drug levels. In this study we report the application of a validated methodology (De Francia et al.,2006) for serum mitotane assessment in management of ACC patients admitted to S.Luigi Hospital of Orbassano (Turin). Mitotane levels were measured, after specific drug extraction, by HPLC-UV (High Pressure Liquid Chromatography). From 2006 to 2009 we collected every 3 month blood samples of 66 patients (35 women, 31 men) treated with mitotane (2-3 grams daily) to correlate continuously drug levels to each patient case-history. Median drug level achieved was 13.5μg/ml (range:0.15-49.3) in overall cases without difference according to sex; specifically 13.3μg/ml (0.26-38.7) for women and 13.7μg/ml (0.15-49.3) for men. As a whole mitotane levels correlated with the dose assumed although wide differences in concentrations were reached in some patients receiving the same dose, probably due to a wide inter-patients variability of mitotane kinetics, mainly at its biotransformation. Furthermore, in our experience, mitotane levels within therapeutic range correlated with clinical response. Drug dosage was reduced in patients with adverse reactions, while it was increased in patients with levels below the therapeutic range. As consequence no severe side effects were reported. In conclusion monitoring circulating mitotane levels may be useful in preventing severe side effects and in increasing potentially drug efficacy.

C22 EVALUATING THE PROGNOSTIC ROLE OF SERUM EXTRACELLULAR DOMAIN (ECD) OF HER-2/neu (S-HER2) IN PATIENTS (PTS) WITH EARLY (E) BREAST CANCER (BC)

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Since the last decades, many efforts have been made to identify different subsets of BCs on the basis of molecular markers that might help in defining the prognosis. The overexpression of HER2/neu (20%-30% of BCs) is associated with a poor prognosis. The ECD of HER2 protein can be cleaved from the cell-surface and thus detected and measured in the serum. It has been reported that elevated s-HER-2 levels are observed in 9-19% of EBC pts and have a potential prognostic and predictive role.

We prospectively evaluated the s-HER2 levels of women with EBC (ELISA immunometric test: cut-off 13ng/ml), to investigate its prognostic role and the possible correlation with various clinical features. The assessment was done (after a written informed consent) at the first visit (bs-HER2) and then every 3 months.

From January 2005 to December 2008, 110 consecutive EBC pts have been included. The median follow-up is 37 months (1-59). The main characteristics were: median age 60 years (28-84); 29 pts (26.3%) stage I, 67 (61%) stage II, 14 (12.7 %) stage III; ER+ 72 pts (75%); PgR+ 66 pts (69.5%); IHC evaluation of HER2 was available in 85 pts [27 pts (32%) positive (IHC3+ or IHC2+ FISH+), 58 pts (68%) negative].

A total of 386 samples have been tested (mean=3.5/pt). Elevated bs-HER2 were found in 24(22%) of pts, of which 5 HER2+ and 15 HER2-: no correlation was seen between bs-HER2 and HER2 status. Similarly, no correlation was found between bs-HER2 and T/N/HR status/G (Fisher's exact test).

At time of writing 7 pts have relapsed. Six had bs-HER2 < 13 and 1 (HER2-) > 13; 2 pts out of these 6 had elevated s-HER2 levels after disease progression. No significant correlation have been found between bs-HER2 and PFS regardless of HER2 status.

In this series no significant association have been found between basal levels, trend of s-HER2 and different clinical parameters. The study is ongoing.

C23 ANALYSIS OF SERUM PROTEOMA IDENTIFIES CLUSTERIN AND ALPHA-1-MICROGLOBULIN AS POTENTIAL PREDICTIVE BIO-MARKERS OF CLINICAL RESPONSE/RESISTANCE IN ADVANCED PANCREATIC CANCER PATIENTS UNDERGOING GEMCITABINE-OXALIPLATIN CHEMOTHERAPY

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Studies based on novel technologies for proteomic profiling of body fluids can now provide new biomarkers predictive of response/resistance to treatment in human cancer. The aim of our research program is the identification, through proteomic analysis, of plasma proteins with potential value of response to chemotherapy by highlighting differential protein profiles between responsive and non-responsive patients. In an exploratory analysis, we enrolled 6 patients with diagnosis of locally advanced or metastatic pancreatic adenocarcinoma who receveid bi-weekly gemcitabine 1000 mg / sqm on day 1 and oxaliplatin 85 mg / sqm on day 2 (Louvet et al. J Clin Oncol 2005) as first-line chemotherapy. The plasma samples were collected before chemotherapy (day 1 of the first course) and 48 hours after the end of the same treatment, according to standards of the Human Proteome Organization (HUPO). The proteomics analysis was performed by the use of two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) coupled to liquid chromatography tandem mass spectrometry (LC-MS/MS). We specifically identified in the post-treatment plasma samples the shedding of two proteins, clusterin and alpha-1-microglobulin, which in the light of their biological function might indeed correlate with treatment resistance: the former in fact might inhibit cancer cells apoptosis induced by drugs (Zhang et al. World J Gastroenterol. 2005), while the latter might increase the metabolism of radical oxygen species (ROS), that are associated with the antiproliferative activity of the drug (Olsson et al. Free Radic Biol Med 2007). We then analyzed clusterin and alpha-1-microglobulin on human pancreatic adenocarcinoma cell lines BXPC-3 and PSN-1 *in vitro* by Western blot and we found that tumor cell exposure to gemcitabine and oxaliplatin actually resulted in overexpression of the two proteins. We are now enlarging the prospective series of pancreatic cancer patients for assessing the biomarker value of clusterin and alpha-1-microglobulin on the bases of the here reported proof of principle findings. (supported by the Ministry of Welfare).

C24 PATHOLOGICAL AND MOLECULAR FINGERPRINTS TO DISTINGUISH CONTRALATERAL SECOND PRIMARY BREAST CANCER FROM DISEASE RECURRENCE

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Background: Patients with history of breast cancer have an increased risk of developing a second primary breast cancer with cumulative lifetime risk of 2%-15%. Contralateral breast cancer is either a metastatic or a second primary cancer. From biological and therapeutic viewpoints, it's important to differentiate metastatic from second primary. Because there are no estabilished criteria to distinguish these different entities we investigated if the pattern of chromosome X-inactivation in the two tumors is able to determine if the tumors derive from different progenitor cells.

Patients and Methods: The clonality of bilateral breast cancer was evaluated through the clonal X-inactivation analysis of human tumors using the human androgen receptor gene (HUMARA) polymorphism. The results of molecular analysis were compared to those obtained by histopathologic determination. A different pattern of X-inactivation was considered as indicator of a second primary cancer. An identical pattern was considered not informative. We considered morphological indicators of a new primary cancer a different histologic type or grading.

Results: 10 patients with synchronous or metachrounos bilateral breast cancer diagnosed between 1993 and 2006 were evaluated. Among these 10 patients, morphologic criteria indicated that eight(80%) were second primary. Molecular analysis confirmed that the majority of contralateral tumors are second primary. However 2 cases(25%) classified as recurrence accorging to morphlogic criteria were classified as second tumor by molecular analysis.

	Histology	ER(%)	Grade	CLINICAL DIGNOSIS	HUMARA
1	D/D	81/0	2/2	recurrence	2nd tumor
2	D/D	78/6	3/2	2nd tumor	2nd tumor
3	L/D	43/47	2/3	2nd tumor	2nd tumor
4	D/D	0/94	1/2	2nd tumor	n.i.
5	L/D	17/47	2/2	2nd tumor	2nd tumor
6	A/D	1/97	2/2	2nd tumor	2nd tumor
7	L/L	97/99	2/2	recurrence	2nd tumor
8	L/D	93/96	2/2	2nd tumor	2nd tumor
9	D/P	96/0	1/1	2nd tumor	n.i.
10	D/D	7/0	2/3	2nd tumor	2nd tumor

Abbreviations: D, ductal; L, lobular; A, apocrin; n.i., not-informative.

Conclusion: Our results show that the HUMARA clonality assay can improve the histological parameters in differentiating metastatic cancer from second primary cancer.

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C25 TRYPTASE EXPRESSION PARALLELED WITH ANGIOGENESIS IN EARLY BREAST CANCER PATIENTS

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Literature data indicate that mast cells (MCs) are involved in tumor angiogenesis due to the release of several pro-angiogenetic factors among which tryptase, a serine protease stored in MCs granules, is one of the most active. In this study, we have evaluated the correlations between the number of MCs positive to tryptase (MCDPT), the area occupied by MCs positive to tryptase (MCAPT) and microvascular density (MVD) and endothelial area (EA) in a series of 88 primary T1-3, N0-2 M0 female breast cancer, by means of immunohistochemistry and image analysis methods. For this aim a three layer biotin-avidin-peroxidase system was utilized. Briefly, six-micrometers thick serial sections of formalin-fixed and paraffin-embedded bioptic tumor samples were deparaffinized. Then, for antigen retrieval, sections were microwaved at 500 W for 10 min., after which endogenous peroxidase activity was blocked with 3% hydrogen peroxide solution. Next, slides were incubated with human-specific monoclonal antibodies anti-CD34 (QB-END 10; Bio-Optica Milan, Italy) diluted 1:50 for 1h at room temperature and anti-tryptase (clone AA1; Dako, Glostrup, Denmark) diluted 1:100 for 1h at room temperature. The bound antibody was visualised using biotinylated secondary antibody, avidin-biotin peroxidase complex, and and 3-amino-9-ethylcarbazole or 3,3 diaminobenzidine. The five most vascularized areas (''hot spot'') were selected at low magnification and both individual vessels and MCs were counted at x 400 magnification. Single brown stained endothelial cells and red MCs positive to tryptase were also evaluated in terms of immunostained area at x 400 magnification. Data demonstrated a significantly (r= ranging from 0.78 to 0.89; p: ranging from 0.001 to 0.002 by Pearson's analysis respectively) correlation between MCDPT, MCAPT, MVD, EA to each other. No correlation concerning MCDPT, MCAPT, MVD, EA and the main clinical pathological features was found. Our results suggest that tryptase-positive MCs play a role in breast cancer angiogenesis. In this context several tryptase inhibitors such as gabexate mesilate and nafamostat mesilate might be evaluated in clinical trials as a new antiangiogenetic approach.

C26 SOMATOSTATIN RECEPTOR SUBTYPE 2 EXPRESSION IN GASTRIC CANCER: AN IMMUNOHYSTOCHEMICAL STUDY

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Objective: Somatostatin analogues induce cell proliferation inhibition mediated by somatostatin receptors (SSTR). Although SSTR subtypes are extensively studied in gastroenteropancreatic endocrine tumor, scanty data are available about their distribution in gastric cancer. This study aimed to assess the presence of SST2A in gastric cancer and to correlate its expression with histological type, grade and clinical outcome.

Patients and Methods: 51 patients affected with gastric cancer, consecutively observed in our institution, were included in the study. Gastric cancer specimens were collected after surgical resection in 48 cases and at upper endoscopy in the remainder. The evaluation of SST-2A was optimised employing slides of neuroendocrine pancreatic tumor as positive control. The immunoreactivity for SST-2A was graded on a semiquantitative scale considering the extent (score: 0-4) and the intensity (score: 0-3) of staining. The product was used to obtain an immunostaining score : low: 1-4, middle: 4-8, high: 8-12.

Results: 36 cases were intestinal and 15 diffuse type. 24 tumors were classified as G1-2 while 27 as G3. SST-2A were expressed in 38 (74.5 %) patients, including a case of pleural metastases. Low score was present in 20 cases (53%), middle in 12 (31%) and high in 6 (16%). 35 out 36 (97.2%) intestinal cancer stained positively for SST-2A (17 with a middle-high score) in respect to 3 out of 15 (20%) of diffuse tumors ($P<0.05$). All 24 moderately to well differentiated (G1-2) gastric cancers were SST-2A positive versus 13 out of 27 (48%) undifferentiated tumors (G3) ($P<0.05$). A reverse correlation between grade and staining score was also observed ($P<0.05$). At a median follow-up time of 23 months (range 9-106), 8 patients experienced a progressive disease : 6 were SST-2A positive and 4 have a middle-high score.

Conclusions: Somatostatin receptor subtype 2A staining (middle-high score) was present in 35% of specimen observed. SST-2A expression was more frequent in intestinal and in moderately to well differentiated tumors.

C27 THE 70-GENE MAMMAPRINT SIGNATURE FOR RISK STRATIFICATION IN BREAST CANCER: A COMPARISON WITH TRADITIONAL PATHOLOGIC AND CLINICAL TOOLS

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Background: The identification of early stage breast cancer patients at risk for relapse is a crucial point to avoid overtreatment; the introduction of gene signature in clinical practice as a complement to clinico-pathologic parameters may lead to a better identification of population at risk of developing metastases.

Methods: In our single institution since Sept 2008 patients diagnosed with invasive breast cancer and submitted to surgical procedure, selected on the basis of the availability of sufficient amount of fresh frozen tissue, have been stratified for clinical risk with different tools: NPI (Nottingham Prognostic Index), Adjuvantonline, St Gallen guidelines and Mammaprint. The results with each method were compared.

Results: As to March 31st 2009 data have been obtained from 62 patients, aged 38-87, mean 65; 18 women underwent mastectomy and 44 breast conserving surgery. NPI was undetermined in 19 cases with lymphnode micrometastases not submitted to subsequent axillary node dissection; in the remaining 43 cases, 18 were low risk, 20 intermediate and 5 high risk.

According to Adjuvantonline 20 and 42 patients were respectively low and high risk; applying St Gallen criteria only 5 cases were at low risk, 17 at high risk and the great majority (40 patients) were at intermediate risk. Mammaprint identified a number of low risk cases superior to Adjuvantonline (28/62).

The relatively low number of patients actually classified does not allow head-to-head comparison between different tools, as well as identification of clinical characteristics of cases with discordant risk evaluation.

Conclusion: As in literature also in our small but omogeneous casistic there are discrepancies between results obtained with different clinico-pathological tools; this can contribute to uncertainty in decision making in a significative percentage of early breast cancer patients as of use of chemotherapy: Mammaprint could help to reduce such uncertainty, also because it seems to be predictive for chemotherapy benefit.

C28 DRUG-DRUG INTERACTION BETWEEN ANTINEOPLASTICS DRUG-BASED PROTOCOLS AND GENERAL MEDICATIONS: A NOVEL METHODOLOGICAL APPROACH IN THE CLINICAL PRACTICE

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Background: Patients with cancer are usually treated with drugs for comorbilities while they have anti-neoplastic and biological agents. The probability of drug-drug interactions (DDI) is associated with the number of such drugs. Despite this, little is known about DDI between general medications and anti-neoplastic drugs in particular with antineoplastic protocols. Currently, no instruments are available to predict DDI between general medications and antineoplastic protocols in the clinical practice.

Methods: The data were extracted from Drugs.com powered by Micromedex™, Cerner Multum™, Wolters Kluwer™ and others, PubMed National Library of Medicine. Data elaboration were performed by using Excell electronic datasheet. We focused our observation on antifungal, antiviral and antibiotic drugs.

Results: The visual support emerging from our research, consists of a table reporting possible interactions ordered in three grade of significance, from absolutely prohibited (red colour), allowed with relevant limitations (orange colour) to allowed associations (yellow colour). The contra-indications to certain types of association for the risk of DDI is overcome by changes in dose or route of administration for such drugs. From this analysis emerges that beta-lactam antibacterials are among the safest when associated with antiblastic regimens; a moderate-severe interaction is with methotrexate due to the synergistic renal toxicity of the two classes of drugs. There are contraindications to the co-administration of platinum derivatives, temsiroliums, and methotrexate regiments with the antiviral cidofovir, or of irinotecan-based regimens with the antiviral atazanavir and antifungal drugs.

Conclusions: For the first time it has been addressed the problem of interactions between most common cancer protocols used in solid tumours and antimicrobial drugs. Our table is rationally created because of the use of different colours in order to provide rapid and effective means of consulting the data. Data will be available in electronic format, implemented and updated annually with other classes of drugs such as NSAIDs, antihypertensives, hypoglycaemic and other drugs used in combination with antiblastics.

The authors gratefully acknowledge EISAI for supporting the DDI program.

C29 EVALUATION OF DNA DAMAGES BY ALKALINE COMET ASSAY IN EARLY BREAST CANCER PATIENTS

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Background: Breast cancer comprises an extraordinarily diverse group of diseases in terms of presentation, morphology, molecular profile and treatment response. Gene expression analysis identifies four main breast cancer subgroups that are biologically and clinically distinct. The Triple-negative (TN) sub-group shows an unfavourable prognosis with aggressive behaviour and few effective treatment strategies. Alkaline comet assay is broadly accepted as a standard method for assessing DNA damage and DNA repair ability in individual cells, it provides valuable information about intrinsic DNA characteristic and responses to external factors, including radiation, chemicals and drugs. This information may prove particularly relevant in the diagnosis, prognosis and treatment of cancer. Our aim was to determine if a correlation exists between breast cancer molecular sub-groups and DNA damage in order to identify patients with a cancer defective in DNA repair machinery.

Materials and Methods: Scraping from 44 primary breast cancers and respective healthy tissues were collected for alkaline comet assays (Trevigen). Percentage of DNA in comet tail, linearly related to DNA break frequency, and tail moment (TM), which combines both tail length and tail intensity, were measured using COMET IV software (Perspective). Samples were classified as Luminal A, Luminal B, Her 2+ and TN by IHC markers (ER, PGR, HER2, Ki67) and grade. Moreover, other histopathological parameters were collected. Differences between samples were analysed by Kruskal-Wallis and ANOVA tests.

Results: Our data highlights the presence of different degrees of DNA damage between healthy and cancer tissues and between breast cancer sub-groups. Particularly, TN samples show the highest TM and percentage of DNA in tail. An analysis is ongoing to assess the level of heterogeneity in test results within the TN cohort.

Conclusion: Comet assay is an easy and cheap tool able to identify patients with a defective capacity to repair DNA damage and may be used as a complementary instrument to identify patients potentially sensitive/resistant to DNA damage cytotoxics.

C30 CT FINDINGS AND SERUM MESOTHELIN IN WORKERS EXPOSED TO ASBESTOS

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Background: Serum levels of mesothelin and its derivatives (soluble mesothelin-related proteins - SMRP) proved promising, albeit in a limited number of patients, as predictive biomarkers of disease in asbestos exposed populations. This study is part of a survey on a large cohort of previously asbestos-exposed workers aimed at evaluating the usefulness of SMRP in screening subjects at risk of MM. Here we correlated SMRP values with thoracic CT findings in a subgroup of exposed subjects.

Subjects and Methods: One-hundred and seventy-seven subjects with SMRP dosage and thoracic CT (median age 62.7 years) have been studied. One-hundred and fourteen subjects had abnormal TC (Pleural fibrosis or thickening). SMRP mean value was 0.76±0.4 nM/l and 1.09±2.0 nM/l in subjects with normal and abnormal CT, respectively (p=0.07). Assuming 1.5 nM/l as cut-off value, 19 subjects had higher SMRP values. Abnormal CT was found in 89.5% of subjects with higher SMRP levels and in 61.4% of subjects with lower SMRP (p=0.02).

Conclusions: SMRP mean value is slightly higher in patients with abnormal CT. CT abnormalities are more frequent in subjects with high SMRP. Correlation between high SMRP levels and CT abnormalities should be deepened with a larger series.

rxtc * SMRP1.5 Crosstabulation

			SMRP1.5		Total
			,00	1,00	,00
rxtc	7-9	Count	61	2	63
		% within rxtc	96,8%	3,2%	100,0%
		% within SMRP1.5	38,6%	10,5%	35,6%
	pos x thickening or fibrosis	Count	97	17	114
		% within rxtc	85,1%	14,9%	100,0%
		% within SMRP1.5	61,4%	89,5%	64,4%
Total		Count	158	19	177
		% within rxtc	89,3%	10,7%	100,0%
		% within SMRP1.5	100,0%	100,0%	100,0%

C31 FUNCTIONAL AND METABOLIC MYOCARDIAL EVALUATION WITH MR IN PATIENTS TREATED WITH CARDIOTOXIC CHEMOTHERAPY: A PILOT STUDY

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Background: Cardiotoxicity from antiblastic therapy is becoming a clinical relevant problem for the wider use of chemotherapeutic regimens, the rise of long-survivor population, and the use of new agents intrinsically cardiotoxic. Echocardiography (ECO) is the gold-standard to evaluate the cardiac function; recently Cardiac Magnetic Resonance (CMR), particularly with ^{31}P spettroscopy, has proved the ability to evaluate early metabolic disfunction potentially useful in detecting subclinical myocardiocyte damage.

Patients and Methods: We prospectively enrolled consecutive patients scheduled to receive a potentially cardiotoxic chemotherapeutic regimen. Before starting chemotherapy (baseline) all patients underwent cardiological evaluation, Troponin T serum level dosage, electrocardiography (ECG), left ventricular volumes (VV) and ejection fraction (EF) was obtained from ECO and CMR. Fosfocreatin/βATP ratio (PCr/βATP) was measured with a CMR ECG-triggered chemical shift imaging ^{31}P spettroscopy sequence. Before each chemotherapeutic cycle ECG and Troponin T dosage were repeated, whereas instrumental evaluations were repeated every three months during therapy and every six months during a two years follow-up.

Results: From January 2008, we enrolled 12 patients (1 male; mean age 58±11 years); 11 underwent baseline and at least two follow-up evaluations. Troponin T serum level remained <0.1 ng/ml in each measurement. ECO and CMR shown a high grade of reproducibility for VV and EF, while statistically significant difference was found between mean diastolic volume at baseline (p 0.009) and at six month (p 0.012). Five spectra were not assessable due to patient movement or suboptimal acquisition, but initial data analysis suggest that the oscillating trend of PCr/βATP during the treatment may detect subclinical myocardial damage.

Conclusions: Preliminary results showed no worsening of functional nor metabolic parameters. This could be explained by the cumulative dose of the drugs used, lower than cumulative risk dose for cardiotoxicity. Long term results, further studies and larger samples with more heavily treated patients are needed to evaluate the real ability of CMR to assess early metabolic alterations.

C32 EGFR AND TOPOISOMERASI II ALFA IN A SUBSET OF TRIPLE NEGATIVE BREAST CANCER NOT METAPLASTICS (PRELIMINARY)

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Introduction and Objectives: The triple negative breast cancer (TNB) (estrogen and progesterone receptors, Her2 negative) are aggressive carcinomas(ca), with high pathological response to chemotherapy but worse survival. Aim of this work was to investigate the role of egfr and Topoisomerasi II α for the characterization of a group of meta-neoplasticsTNB that could respond to targeted therapies

Materials and Methods: 311 breast ca were processed by immunohistochemistry (IHC) for the assessment of estrogen receptors, progesterone, the expression of the protein Her2 and proliferative marker (Ki67). In 23 result TNB is evaluating the expression of EGFR by IHC and the status of egfr and topoisomerasi II α method by fluorescent in situ hybridisation (Fish)

Results: The histological diagnosis was: 22 ca ductal, lobular ca 1. In 74% cases the grade were G3 and Ki67 average = 34%, median = 30%.

In 82% of TNB was present sporadic, focal or diffuse overexpression of EGFR with positivity membrane complete and / or incomplete (2/3+ intensity).

In 65% TNB, the number of egfr copies was increased by amplification and/or polisomia of chromosome 7.

Fish test showed significant correlation between:

number of egfr copies amplified and EGFR over-expression p=0,1 Risk Relative (RR) 2,1

number of copies of polisomia egfr and EGFR over-expression p=0,022 RR 2,7

number of major egfr, for amplified or for polisomia, egfr and EGFR over-expression p=0,008 RR 4,7

No TNB had amplification of topoisomerase II α and in 39% TNB there was deletion of topoisomerase II α in a variable number of cells.

Conclusions: The correlation between number of copies of egfr and EGFR protein over-expression and lack of amplification topoisomerasi II α is a characteristic of a TNB not metaplastic group. If this result will be confirmed by a larger study, this characterization could help for a choice of a ''gold target therapy''.

C33 CARDIOPROTECTIVE EFFECT OF TELMISARTAN IN CANCER PATIENTS TREATED WITH EPIRUBICIN

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Background: We had previously shown in 31 cancer patients (pts) that early cardiac abnormalities occurred at epirubicin (EPI) doses of 200 mg/m^2 and persisted throughout subsequent EPI doses and even up to 18 months. Early contractility impairment, i.e. Strain rate (SR) reduction was detected by tissue doppler imaging (TDI) associated with high levels of inflammatory/oxidative stress markers. Renin-angiotensin system activation has been suggested to play an important role in the pathogenesis of Anthracycline-induced cardiotoxicity.

Methods: A phase II placebo-controlled study was designed to investigate the possible role of Telmisartan (an antagonist of angiotensine II type I receptor) in preventing both early preclinical and late myocardial damage induced by EPI. The correlation with changes of biochemical/inflammatory markers was also assessed. Planned sample size was 100 pts (2:1 randomization, telmisartan vs placebo). Inclusion criteria: 18–70 y, histologically confirmed cancer, previously untreated and candidates for an EPI-based regimen; LVEF ≥55%; ECOG PS 0-2, no history of cardiac disease and previous mediastinal irradiation. Eligible pts were randomized to receive Telmisartan 40 mg (1 tablet)/day or placebo starting 1 week before EPI up to 6 months after the end of EPI administration. TDI as well as inflammatory/oxidative stress markers were assessed at baseline, 24 hours and 7 days at EPI doses of 100, 200, 300, and 400 mg/m^2.

Results: At April 2009 we enrolled 33 pts (M/F: 7/26, mean±SD age 58±14 years): 20 in the Telmisartan arm and 13 in the placebo arm. Twenty-six pts completed EPI treatment (16 Telmisartan and 10 placebo). A significant reduction of SR peak was observed at 200mg/m^2 and 300 mg/m^2 of EPI both in the placebo arm ($p<0.001$ and $p<0.01$, respectively) and the Telmisartan arm ($p=0.03$ and $p=0.04$, respectively). *Viceversa* the diastolic function as assessed by Em/Am was significantly reduced in the placebo arm both at 200 and 300 mg/m^2 of EPI ($p<0.05$ for both) whilst it was not reduced significantly in the telmisartan arm. Proinflammatory cytokines and oxidative stress markers did not change in the telmisartan arm whilst reactive oxygen species and IL-6 increased significantly in the placebo arm from 200 mg/m^2 of EPI.

Conclusions: The study is in progress.

C34 ELABORATION OF A BIOKINETIC MODEL FOR THE RADIOPHARMACEUTICAL [^{153}SM]SM-EDTMP AND EVALUATION OF INCIDENCE OF ZOLEDRONIC ACID ADMINISTRATION ON ITS UPTAKE

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Since the radiopharmaceutical [^{153}Sm]Sm-EDTMP is administered with a standard activity (37 MBq/kg), in bone metastases palliation cares, we studied a suitable patient-specific biokinetic model. Moreover we investigated how a previous administration of bone-target Zoledronic Acid affects radiopharmaceutical uptake.

In five over ten treatments Zoledronic Acid administration was suspended at least 4 weeks before [^{153}Sm]Sm-EDTMP injection (Group A). In three cases the bisphosphonate was suspended later (Group B) and in two it was not administered (Group C).

We measured the radioactivity of serial blood samples and urine within 24 h after injection, we evaluated [^{153}Sm]Sm-EDTMP blood clearance and excretion and we compared data with body imaging information.

We elaborated a biokinetic model with a central, blood, and two peripheral compartments, soft tissues and bone.

For all the patients, uptake and k_{12}/k_{13} ratio are summarised in Table 1, where k_{12} is blood to soft tissue rate transfer constant, while k_{13} is blood to bone rate transfer constant (in brackets median value is indicated).

	Group A	Group B	Group C
Uptake (%)	28 – 96 (90)	43 – 90 (54)	40 – 44 (42)
k_{13}/k_{12}	0 – 1.2 (0.3)	0.2 – 0.9 (0.3)	0.1 – 0.8 (0.2)

Considering the primary tumour, the parameters are showed in Table 2:

	Group A		Group B		Group C	
Primary tumour	Prostate (2 cases)	Breast (3 cases)	Prostate (2 cases)	Breast (1 case)	Prostate (1 case)	Breast (1 case)
Uptake (%)	90 – 96 (93)	28 – 90 (57)	43 – 90 (67)	53 – 55 (54)	40 – 42 (41)	42 – 44 (43)
k_{13}/k_{12}	0 – 1.8 (0.7)	0 – 12 (0.3)	0.1 – 0.9 (0.3)	0.2 – 0.5 (0.3)	0.1 – 0.8 (0.5)	0.1 – 0.4 (0.2)

An early Zoledronic Acid suspension seems more effective in [^{153}Sm]Sm-EDTMP uptake, while Group C lower values can be due to patient better clinical conditions. Particularly in prostate cancer primary tumour it seems to increase also the bone to soft tissue transfer ratio, but presently our statistics is rather low and we need to analyse more cases.

C35 IMPROVING CHEMOTHERAPY CAPACITY BY SWITCHING FROM INTRAVENOUS (NVBIV) TO ORAL VINORELBINE (NVBO): THE ITALIAN EXPERIENCE WITH TAMINO (TIME AND MOTION INTERNATIONAL STUDY WITH NAVELBINE® ORAL)

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Background: Efficiency, safety and patient-centred care are key factors for a chemotherapy service of quality. NVB with its two formulations is a good model to perform capacity studies to assess whether the use of an oral chemotherapy *vs* intravenous brings a benefit to patients (pts) and chemotherapy service organization. A time and motion audit was thus performed in eight centers of four European Countries. 123 pts were enrolled and treated with NVBo (74) or NVBiv (51) monochemotherapy. Although some heterogeneity was observed, results showed 36% reduction of time spent in hospital for pts treated with NVBo (2h31' *vs* 3h56'). Time for preparation and dispensing was 33' *vs* 1h8' respectively. Here we specifically report the experience of the 3 Italian Centres who conducted TAMINO: two public hospitals (a medium size and a major) and one private cancer centre.

Material and Methods: 50 pts (40% of the international sample) were treated with NVBo (32) or NVBiv (18). Treatment pathways were identified in each centre and for each process the average and range was calculated.

Results: Two steps of the whole process mostly benefited of the use of NVBo: total time pts spent in hospital and time elapsed between end of consultation and start of administration. Pts treated with NVBo spent on average 3h19' [1h25'-5h45'] in hospital *vs* 4h18' [2h15'-6h59'] with NVBiv (25% reduction). Time elapsed between end of consultation and start of administration was on average 50' [5'-1h59'] *vs* 1h42' [8'-4h10'] (37% reduction). The width of ranges shows heterogeneous patient pathways among the three Italian centres as it was among European centres. Two of the three centres clearly favoured NVBo with average time spent by patients reduced by 3h47' and 1h16', whilst in another this time was slightly increased (16') pointing out the necessity of modifing the organizational model of nurses staff.

Conclusion: On average NVBo reduces the time spent by patients and pharmacists in chemotherapy service delivery *vs* its iv form. These audits are useful to quantify and highlight any critical step in the organizational model. The introduction of recent oral drugs urges the answer as to which is the optimal model to provide effective and safe cancer care while optimising capacity, competency, time and expenses, including the possibility of home delivery.

C36 SAFE DRUG ADMINISTRATION IN ONCOLOGY: PREVENTING ERRORS USING FAILURE MODE AND EFFECTS ANALYSIS (FMEA)

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Background: Pharmacological treatment is a key factor in hospital care and is subject to potentially serious errors. With the aim of improving safety in the processes involved in non-chemotherapy drug ordering and administration, we thoroughly examined all the work-flow using FMEA (according with standards of the USA Joint Commission III ed. QPS 10 and Accreditation Canada, ROP 2008).

Methods: A team consisting of oncologists, nurses and a team advisor identified a flow chart of the process under review, and the subprocesses involved. A hazard analysis was conducted identifying the failure modes of each subprocess, determining the potential effects and ranking severity, frequency and detectability of each failure mode. A critical index score (CIS) was calculated by multiplying the values of each failure. Finally corrective actions and outcome measures were planned.

Results: 104 potential effects of failure were isolated. The team decided to address all potential failure modes with CIS greater than the mean. The 12 steps with high priority (CIS=48) were related to the prescription form and the communication between physicians and nurses. The overall index score was 1941. A new form was prepared in

which parameters such as signatures, date of treatment prescription, allergy, treatment as needed were added. An operative procedure describing rules and legends was filled. A plan of periodic controls was made and the incident reporting of errors or near-errors was encouraged. A daily meeting between doctors and nurses was introduced in clinical practice. After implementing the new system the overall index score was found to be 1431, with a reduction of 26% compared with the base-line index.

Conclusions: Our analysis suggests that the majority of medication errors are made in the ordering phase. The prospective adoption of a prevention system is critical. The methodology of FMEA is feasible and was useful in planning a safer process. This method should be applied in other critical processes in oncology in order to enhance patients safety.

C37 USE OF HUMAN SERUM ALBUMIN MICROAGGREGATES LABELLED IN THE BREST OCCULT RADIOGUIDED LOCALITATION (ROOL)

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In last years has been propagated many pre-operating localization techniques of brest non palpable lesions. Nuclear medicine propose ROOL method use (radiogiuded occult lesion localization); this method allows lesion localization precisely, aside tracer inoculation place and allowed to surgeon to localize lesion during operating all phases.
Aim of Study: Our experience of breast cancer radioguided surgery is of 216 cases from 2000 until now. Concerning suspect non palpable lesions localization and treatment we valued the use of human serum albumin microaggregates labeled.ed.

Materials and Methods: We analysed 50 patients averages value 53 (range 35-75) with suspect breast lesion detected mammographically or ultrasonically or citologically. 36 cases showed unifocal microcalcification areas evocative DCIS (with variable extension by 5 to 25mm);14 cases remaining showed suspect lesions US detected with diameter always inferior to 1 cm, citological analysis was C4 or C5.Female patients have been subjected to operation whit variable distance of 4- 6 hours from 20MBq Tc99 labelled particles of human serum albumin injection of size lower to 80 nm into volume of 2 ml. Concerning occult lesions detected mammographically, mammographic equipment with a computerised stereotactic system has been used to guide the injection. A 22 G spinal needle mounted in the stereotassic frame has been introduced into lesions and tracer has been injected mostly into lesions and littleness on the outside followed by 1 ml of radiopaque contrast and effectuating the five minutes from injection of a mammographic particolar to prove contrast exact localization into lesion. Concerning occult lesions detected ultrasonically radiotracer has been injected under US guidance. Needle has been plugged manually into lesion and radiotracer has been injected mostly into lesion and littleness into immediate neighbourhood. Scintigraphic images have been acquired in anterior and lateral proiections to 10 minutes in the distance to 4 or 6 hours from radiotracer injection. The images a 10' has been useful for localization cutaneous projection area of lesion tattooed with dermo-graphic pencil. The late images to 4 or 6 hours have been useful to value presence of radiotracer spred perilesional and, for effectuated cases (42/50), have been useful for sentinel node scintigraphic search. Excisional surgery procedure has been carried out with aid of hand-held gamma-detecting probe, allowing always control resection adequacy, trough hot-spot individuation during all phases of operating and residual activity in resection cavity.

Results and Conclusions: The management allowed in a lot of cases 49/50 an adequate excision of suspect lesion, it has been furnished through radiological control and rarely ecographic in the surgical specimen. In one only case has been needed surgery radicalisation of the resection margins resultes Ca intraductal istologically involved. Our esperienze founded on case limited but meaningful shows validity of human serum albumin microaggregates use breast occult lesions search.

C38 CYTOTOXIC DRUGS MANAGEMENT, PRESCRIPTION, PREPARATION AND ADMINISTRATION: CRITICAL ANALYSIS OF AN HIGH RISK PROCESS USING THE HFMEA (HEALTHCARE FAILURE MODE AND EFFECTS ANALYSIS)

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Introduction: The management of cytotoxic drugs is an high risk process for adverse events (AE) and medical errors (ME). The aim of this study is to investigate the potential risks and the critical steps in the chemotherapy practices, using the Healthcare Failure Mode and Effects Analysis (HFMEA) a proactive method designed specifically for healthcare, in order to develop and implement improvement actions to prevent potential AE.

Methods: The HFMEA process includes five steps. The first one is to define the topic and the process to be studied. The second step is to create a multidisciplinary team to conduct the analysis. The third step is to describe the process of the analysis and consecutively to number each process step identified with the relative sub-processes in order to create a flow diagram. The Hazard Analysis is the fourth step: the team lists all possible/potential failure modes (FM) under each activity composing sub-processes; then determines the severity and the probability of the potential FM and records these on a worksheet. After this, the team looks up the Hazard Score on the Safety Assessment Code (SAC) Matrix and record this numbers and, using the Decision Tree, determines if the FM warrants further action. The final step is to determine the Actions and Outcome Measures that allow to eliminate or control the FM founded.

Results: The project has started on March 2009 and it's still ongoing. The multidisciplinary team includes the Oncological staff (Oncologists and Nurses) and some advisors (Anesthetist, Pharmacologist, Nurses, Engineer, Safety Coordinator, Quality Coordinator and Hospital Managers). We create a flow diagram of the process including 10 blocks and 225 activities. We decide to analyze only 4 blocks in order to concentrate the team efforts only on the highest risk activities which probably present the gravest FM. The final results of the analysis with the actions and outcome measures used will be presented at the meeting.

Conclusion: The systematic approach of HFMEA conducted by a multidisciplinary team is a valid method to investigate the potential risks and the critical steps in the chemotherapy practices, in order to develop and implement improvement actions for preventing AE and ME.

C39 PHARMACOLOGICAL INTERACTIONS AMONG BREAST CANCER PATIENTS IN A OUTPATIENTS CLINIC

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Background: Older oncology patients with multiple comorbidities are at risk of adverse drug events associated with polypharmacy and drug-drug interactions due to patients' altered pharmacokinetic/pharmacodynamic status.

Objective: To evaluate and describe characteristics of preventable adverse drug events (pADEs) in the outpatient after diagnosis of breast cancer.

Materials and Methods: We performed a retrospective cohort study in patients with multiple comorbidities. The medical records of 100 consecutive patients attending oncological ambulatory care at the Parodi Delfino Hospital were selected and reviewed.

The pADEs were classified as moderate or relevant based on evaluations shared within the working group, according to the texts: S. Garattini, A. Nobili. ''Interazioni tra farmaci'' Selecta medica. 2001, and ''Guida all'uso dei farmaci 5'', 2008 edition.

Results: 38 patients (male 2, female 36, age 69.05±12.42) out of 100 were in follow-up after a diagnosis of breast cancer: 3 patients not taking medications, 8 patients a single drug, 7 patients two drugs, 20 patients three or more drugs (up to 10 different drugs). Cardiovascular medications (34,2%), followed by hormonal therapy (20%) (anastrozole 47%, tamoxifene 23.5, letrozole 17.8%, exemestane 11.7%), anticoagulants (6,7%) and hypoglycemics (3,3%), were the most common medication categories prescribed. The associations of drugs prescribed to 12 patients (44.5%) did not expose them to pADEs. In 8 patients (29.6%), pADEs were considered moderate. In 7 patients (25.9%), pADEs were relevant.

Most pADEs were classified as moderate. Cardiovascular events were the most common types of pADEs, followed by electrolyte/renal events.

Older patients, those with comorbidities conditions and those taking many medications were at greater risk of drug interactions

Conclusions: The data suggest that hormonal therapy is not associated with pADEs, in this group of patients.

Our results suggest some practical targets for intervention, including improved information concerning the administration of medication both in the clinic and at home, and the monitoring of prescribed medications.