Methods: Retrospective analysis of 17 patients with SCC due to metastatic breast cancer treated from 2005 to 2009. The patients have been submitted to 4 different RT schedule: 2000 cGy (400 cGy × 5), 3000 cGy (300 cGy × 10) and a split-course regimen of 5 Gy × 3, 4 days rest, and then 3 Gy × 5, and a short-course regimen of 8 Gy, 7 days rest, and then 8 Gy.
Results: At presentation 6 patients were ambulant with mild neurological deficit, 8 patients were paraparetic and 3 patients were paraplegic. Diagnosis was established by CT-scan or MRI of the spine, 15 patients presented dorsal or lumbar pain requiring opioid treatment on average 25 days before onset of neurological symptoms (range 10–230 days). All patients underwent steroid treatment; the 14 patients underwent radiotherapy alone and 3 radiotherapy and laminectomy. Overall 10/17 patients were ambulant after treatment. 2 out of 3 patients treated by laminectomy and radiotherapy were ambulant after treatment versus 8 out of 14 patients treated by radiotherapy alone. 14 patients died during follow-up with a median survival of 3.7 months (2 weeks to 41 months), while 3 patients were alive at the last control. No patient complained of spinal cord morbidity.
Conclusions: The patients' prognosis with spinal cord compression from metastatic breast cancer is poor. Cord compression should be treated promptly, late cases with loss of ambulation and sphincter function is associated with poor prognosis and poor outcome. The golas of treatment are (4Ps): a) preservation or recovery of neurological function, b) palliation of pain, c) prevention of recurrence, d) preservation of spinal stability. Besides if treatment is started within 24 to 48 hours of onset of symptoms neurological damage may be reversible. Heightened awareness of the significance of back pain is the most important factor in successful treatment of cord compression. Efforts must be concentrated on early diagnosis and on prevention of spinal cord compression: Moreover patients with spinal cord compression from metastatic breast cancer who develop persistent back pain should undergo imaging studies (bone scan, spine CT-scan or MR) to the purpose to identify precocious lesions and to begin the radiant treatment.

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HIGH ACTIVITY OF SEQUENTIAL COMBINATION OF LOW DOSE CHEMO-MODULATING TEMOZOLOMIDE (TMZ) + FOTEMUSTINE (FM) IN METASTATIC MELANOMA (MM). A FEASIBILITY STUDY

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Background: MM is an incurable and chemoresistant cancer with poor prognosis. Preclinical and clinical experiences, support the concept that continuous exposure to alkilating agent, can effectively deplete cells of the DNA repair enzyme O^6-methylguanine DNA methyltransferare which is the primary mechanism of tumor resistance to alkylating agents like nitrosurea analogs. Our study was finalized to verify this hypothesis using a sequential combination of low dose chemo-modulating TMZ with FM. Primary endpoints were safety and tumor response evaluation.
Methods: 14 consecutive MM pts were enrolled into two well balanced cohorts of 7 pts each using 2 schedules of TMZ+FM (Cohort A: TMZ *os* 100 mg/m^2 d1,2; 7,8; FM *iv* 100 mg/m^2 d2, 8, 4 h after TMZ, every 4 weeks for 2 cycles; then every 3 weeks for further 6 cycles. Cohort B: TMZ+FM at the same dose but every 3 weeks for a total of 9 cycles).
Results: Main results are reported in the table.

Cohort	Schedule	Toxicity profile G3-G4			Response	
		Neutrop.	Thrombop.	Anemia	Number	Site
Cohort A	1,8,28	1/7 pts	4/7 pts	1/7 pts	1 CR, 2 PR, 2 SD	LN, soft tissue, bowel
Cohort B	1,21	1/7	1/7	0/7	2 PR, 3 SD	LN, soft tissue, adrenal gland, liver

Conclusions: sequential combination of low dose TMZ and FM demonstrated a high activity in our pts population. d1–21 schedule showed a more acceptable toxicity with respect to d1–8-28 schedule maintaining his antitumoral activity. Thus schedule d1–21 has been used in our phase II ongoing study.

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PAIN MANAGEMENT AND QUALITY LIFE IN BONE METASTASIS FROM BREAST CANCER: ROLE OF RADIOTHERAPY

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Background: In this study we have evaluated the impact on the control of the pain and on the quality of life of different schemes of dose fractions and total dose of radiotherapy in patients affected by bone metastasis.
Methods: Between 2003 and 2008, 78 patients, with bone metastasis from breast cancer were treated at "Hospital Pugliese-Ciaccio", Catanzaro. At December 2008, 78 patients with a median age at diagnosis of 64 years (range 35–74 years) were analyzed, of these, 57 (73%) were males and 21 (27%) females. Forty-five patients introduced multiple metastasis while in the remaining patients the bone metastasis was unique. The intensity of pain was assessed by WHO Criteria. The average follow-up time was 7 months (range 2–45 months).
Results: All patients manifested moderate (23/78) or severe (55/78) pain and were in treatment with transdermic fentanyl (25, 50, 75 mg/h) in association or less to FANS. In sixty-seven patients we have used a scheme of dose fractions of 300 cGy × 10 fractions (total dose, TD 3000 cGy), in 9 patients a schemes of dose fractions of 400 cGy × 5 fractions (TD, 2000 cGy) and in two patients a schemes of dose fractions of 600 cGy ×2 fractions (TD, 1200 cGy), in all patients was associated treatment with Zoledronic acid. Fifty five patients, at the end of the treatment, have obtained an improvement in the intensity of the pain, in ten the total disappearance of the same. For fifteen patients has been necessary to perform a new treatment after a median of 6.5 months (range 2–9 months).
Conclusions: In summary, our data confirm the results of literature on the control of the pain and on the improvement of the quality of life of the patients with bone metastases treated with radiotherapy and Zoledronic acid. Moreover, with regard the different schemes of radiotherapy dose fractions and total dose, the short course regimen (600 cGy × 2) can become the treatment of choice for the majority of patients with bone metastasis.

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EFFECT OF miR-21, miR-182 AND let-7i ON TSP-1 EXPRESSION IN COLON CANCER CELL LINE

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Background: MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of different genes, including genes involved in cancer progression, angiogenesis and metastasis. Thrombospondin-1 (TSP-1) has been shown to contrast angiogenesis in vivo. TSP-1 expression levels are inversely correlated with tumor vascularity and metastasis in colon cancer. Bio-informatic statistical analysis indicated that TSP-1 is hypothetical target of miR-21, miR-182, overexpressed in CRC, and let-7i which expression is down-regulated in this tumor. In this work we investigated whether TSP-1 expression could be regulated by miR-21, mir-182 and let-7i in HT29 colon cancer cell line.
Methods: To investigated whether miR-21, mir-182 and let-7i directly modulates TSP-1 expression, we transfected HT29 cell line with pre-mir21, pre-mir182 and pre-let7i by using siPortNeo FX tranfection agent and after 48h we evaluated TSP-1 mRNA, using Quantitative Real Time-PCR, and intracellular and secreted protein level performed by Western blotting and ELISA. To confirm the modulation of TSP-1 by miRNAs we transfected HT29 cell line with anti-mir to target the mature form of miR-21, miR182 and let-7i.
Results: Using Real-Time PCR we did not find any variation of TSP-1 mRNA expression levels after transfection with pre-mir21 in HT29 cell line, but we observed a down-regulation of cytosolic and secreted protein by Western blot and ELISA. In cells transfected with pre-mir182 we did not observe any down-regulation both

TSP-1 mRNA and cytosolic and secreted protein. Finally, we did not find any variation of TSP-1 level in cells transfected with let-7i. Results were confirmed by transfection with anti-mir21, anti-mir182 and anti-let7i and, using the same method, we evaluated TSP-1 expression.
Conclusions: Data suggest that mir-182 induces degradation of TSP-1 mRNA in HT29 cell line, whereas mir-21 affects probably by blockage of TSP-1 translation. Let-7i does not seem involved in regulation of TSP-1 expression in HT29 cells. Understanding the molecular mechanism by which miRNAs regulate TSP-1 expression could be used to restore TSP-1 expression to contrast angiogenic events in colon cancer.

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AZD1152 PLUS GEMCITABINE FOR PANCREAS CANCER TREATMENT: *IN VITRO* AND *IN VIVO* STUDY

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Background: AZD1152 is a prodrug that, after activation in AZD1152-HQPA, impairs cytokinesis by inhibition of the activity of its specific target Aurora B kinase. Aurora B kinase is known to be involved to determining the correct chromosome alignment, kinetochore-microtubule biorientation, and activation of the spindle assembly checkpoint. In this report, we verify the possibility of combine this novel drug with gemcitabine widely used in chemotherapy for pancreas cancer patients.
Methods: Pancreatic (MiaPaCa-2) cancer cells were used and the capability of the drug to enhance gemcitabine effectiveness has been evaluated as cell growth inhibition, apoptosis induction and cell cycle perturbation.
Results: Our results showed that AZD1152-HQPA strongly modifies cell structure and activity, with an increase in cell size, in polyploidia and chromosome numbers. Its activity was through the inhibition of Histone 3 phosphorylation even if it also seemed to modulate other signal transduction pathways, such as survival one with the implication of p53.
Kinetic experiments evidenced that AZD1152-HQPA was an enhancer of gemcitabine effectiveness in MiaPaCA-2 cells and the best schedule was that in which our aurora kinase B inhibitor was given before the chemotherapeutic drug, with a gain of about 20–30% of efficacy.
Then, the promising *in vitro* combination of AZD1152 with gemcitabine has been tested *in vivo* with MiaPaCa-2 xenografts in CD *nu/nu* male mice. At the appearance of a measurable subcutaneous tumor (~100 mm^3), mice were grouped randomly and treated as follows: *i*) control (vehicle alone), *ii*) AZD1152 alone (25 mg/kg daily for four days), *iii*) gemcitabine alone (120 mg/kg four times at 3-day intervals) and *iv*) the sequential combination of AZD1152 and gemcitabine. AZD1152 and gemcitabine alone significantly inhibit tumour growth in absence of toxicity. When mice were treated sequentially with the two compounds, the tumor growth was delayed and the inhibition of both tumor volumes and weights was markedly enhanced.
Conclusions: In conclusion, our results suggest that AZD1152, a novel selective inhibitor of Aurora kinase B, could be a promising therapeutic approach in combination with gemcitabine in pancreas cancer treatment.
AZD1152 and AZD1152-HQPA are trademarks of the AstraZeneca group of companies.

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DNA DOUBLE STRANDS BREAK REPAIR GENES EXPRESSION ANALYSIS REVEAL RAD51 AS A NEW POTENTIAL BIOMARKER IN BREAST CANCER.

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Background: We determined expression for genes that play key roles as sensors, modulators or effectors in this pathway. We analyzed Mrna expression of 15 DSB related genes from 20 breast cancers in order to classify them into homogeneous clusters. For genes *ATR*, *G22P1/ku70* and *RAD51* was developed a mRNA relative quantification method that was used to analyze additional 55 cases.
Methods: *RAD51* protein expression was determined by immunohistochemestry on 58 tumours represented on a commercial available tissue microarray. Hierarchical clustering analysis of the DSB repair genes analyzed identified *ATR*, *G22P1/ku70* and *RAD51* as differentially expressed among the breast cancer cases.
Results: The analysis of the additional 55 tumours for these three genes indicate an association between *RAD51* increased mRNA levels and ER-positive/PR-negative breast cancers ($P = 0.09$). This result was confirmed at protein expression level when a tissue microarray including 58 breast cancers was analyzed by immunohistochemestry ($P = 0.003$).
Conclusions: Our results indicate that the *RAD51* gene is differentially expressed in breast cancer characterized by different steroid hormone receptor status and may represent a novel potential breast cancer biomarker.

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DETECTION OF KRAS MUTATIONS IN COLORECTAL CARCINOMA PATIENTS WITH AN INTEGRATED PCR/SEQUENCING AND REAL TIME PCR APPROACH

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Background: Patients with metastatic colorectal carcinoma (mCRC) carrying activating mutations of the *KRAS* gene do not benefit of treatment with anti-epidermal growth factor receptor (EGFR) monoclonal antibodies. Therefore, *KRAS* mutation testing of mCRC patients is mandatory in the clinical setting for the choice of appropriate therapy.
Methods: We developed a cost/effective approach for the determination of *KRAS* mutations in codons 12 and 13 in clinical practice based on a sensitive PCR/sequencing technique and the commercially available Real-Time PCR-based Therascreen kit (DxS).
Results: The PCR/Sequencing test was able to detect 10% mutant DNA in a background of wild-type DNA. By using this assay, we determined the mutational status of *KRAS* in 527/540 (97.6%) formalin-fixed paraffin-embedded (FFPE) tissues from mCRC patients. PCR/sequencing was not conclusive in 13 cases in which low-intensity peaks suggestive of potential mutations were identified. DxS, which showed a sensitivity of 1%, identified mutations in 11/13 inconclusive cases. Interestingly, 5 of these 11 cases showed high levels of DNA fragmentation. No significant difference was found in the ability of PCR/sequencing and DxS to identify *KRAS* mutations within 160 cases with >30% tumor cells. However, in 24 samples with ≤30% tumor cells DxS showed an higher sensitivity.
Conclusion In conclusion, our findings suggest that PCR/sequencing can be used for mutational analysis of the majority of tumor samples that have >30% tumor cell content, whereas more sensitive and expensive tests should be reserved for inconclusive cases and for samples with a low amount of tumor cells.

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HYPOXIA INDUCES DECREASED EXPRESSION OF *BRCA2* IN BREAST CANCER CELL LINES

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Background: The hypoxic tumor microenvironment is a key factor that induces genetic instability. Several studies have demonstrated