survival. The 1q gain was also related to a profile of cell cycle deregulation and to overexpression of *DTL* in an independent **38-tumour set**. DTL proved to be a potent regulator of the cell cycle by abrogating p53, p21 and p27 and to be a major contributor to the 1q gain-expression profile. Moreover, tumours with this alteration showed higher proliferation rates as assessed by Ki-67 immunohistochemistry.
Other relevant findings from the CNA study were: the percentage of genome affected by CNA per sample proved a tight and progressive correlation with overall survival. The gain of chromosome 8 was associated with metastasis location in lungs. Several smallest regions of overlap were defined, containing relevant candidate genes.
Conclusions: CNA have a marked impact on ES outcome. The gain of 1q molecularly defines a substantial subset of ES patients with worse outcome who could benefit from new prognostic biomarkers (1q gain/*DTL* overexpression) and from specific targeted therapies.

132 POLQ up-regulation is associated with poor survival in breast cancer, perturbs DNA replication and promotes genetic instability

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Background: "Replicative stress", one of the main factors underlying neoplasia from its early stages, can arise from a deficiency in DNA replication. Genes involved in DNA synthesis may therefore represent an under-explored source of prognostic markers with therapeutic potential in cancer.
Material and Methods: Gene expression profiles were generated here from two independent cohorts (France and the UK; n = 206 and n = 117, respectively) of previously untreated primary breast cancers. We also generated human cells that mimicked the observed genotype.
Results: We report here that among the 13 human nuclear DNA polymerase genes, the Polq gene (or *POLQ*) is the only one significantly up-regulated in breast cancers compared with normal breast tissues. Importantly, *POLQ* up-regulation significantly correlates with poor clinical outcome, with a 3.1-fold increased risk of death. *POLQ* expressionwas independent of Cyclin E expression, which is also correlated with a poor prognosis in breast cancer. In addition, we show that POLQ expression can discriminate the survival outcome of patients with a high number of positive lymph nodes, considered to date as a negative marker for breast cancer.
POLQ is a specialized DNA polymerase believed to function primarily in the replicative bypass of endogenous DNA damage. Aiming to decipher the molecular consequences of *POLQ* up-regulation, we generated human cells stably over-expressing this polymerase. Our data shows that a high level of *POLQ* gene expression was directly associated with defective DNA replication fork progression and double strand break induction, resulting in cancer-associated chromosomal aberrations.
Conclusions: We propose that *POLQ* over-expression may facilitate tumour selection and progression through DNA replicative stress. In addition, it is a new promising prognostic marker with therapeutic potential.

133 mTOR is a druggable molecule in Malignant Pleural Mesothelioma targeted therapy: antiproliferative effect of sorafenib and everolimus in preclinical models

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Background and Rationale: Malignant Pleural Mesothelioma (MPM) is an aggressive tumour, with no effective therapies and an increasing incidence as a result of widespread exposure to asbestos. The identification of molecular targets for novel therapeutical strategies is mandatory. With this work, we aim at (i) indagate activated oncogenic pathways in MPM focusing on PI3K–AKT–mTOR and the BRAF/KRAS/MAPK pathways and (ii) explore the preclinical efficacy of the multikinase inhibitor sorafenib (SOR) and the mTOR specific inhibitor, everolimus (EV).
Methods: FFPE sections were obtained from patients diagnosed and followed at IRCCs San Matteo of Pavia. Phospho-mTOR expression was checked by immunohistochemistry. Genomic DNA was then extracted from MPM and corresponding surrounding non tumoural tissues. The mutational status of oncogenes in PI3K and MAPK pathways was assessed by PCR and sequencing. 3 human MPM cell lines established from the pleural effusion were treated with scalar doses of SOR, EV and their combination for 72 hours. The IC50 values and the combinatorial index (CI) were calculated with Calcusyn.
Results: The mTOR kinase was activated in all the MPM samples and in the adjacent hyperplastic mesothelium but not in normal not-transformed mesothelium. No mutations were found in "hot spot" coding regions in EGFR (exons 18−21), KRAS (exon 2), BRAF (exon 15) PIK3CA (exons 9−20) genes. In vitro assays demonstrated that SOR and EV have synergistic effect in the inhibition of MPM cell line proliferation: SOR showed a dose dependent inhibition effect (IC50 = 1.72 μM). EV alone is able to affect no more than 35% cell line proliferation. In combination they displayed synergistic effects (the IC50 of SOR was reduced to 0.91 μM and the IC50 of EV was calculated (45.54 nM) in combinatorial schedule.
Conclusions: Our results suggest that mTOR kinase is highly activated in MPM onset. This status is not consequent to the occurrence of activating mutations affecting the PI3K and MAPK signaling. SOR is a promising therapeutic molecule in MPM mostly in combinatorial schedule with EV. Molecular studies will further elucidate the cross-talk between pathways.

134 Dissecting the mTOR pathway in osteosarcoma

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Background: Osteosarcoma (OS) is the most common bone tumour of the paediatric age. Despite improved prognosis, recurrent or metastatic forms are still fatal. A better understanding of molecular mechanisms involved in OS onset, progression and metastatization is a clear priority both in the assessment of targeted agents and to identify and select patients that are likely to achieve a clinical benefit. Our work is focused on the identification of OS activated oncogenic pathways and we specifically are studying the PI3K/AKT/mTOR pathway. To this goal we planned to use a triple approach (i) analysis of immunophenotype and mutational profile on 30 OS samples (ii) *in vitro* studies with molecular targeted drugs commercially available such as the multikinase inhibitor sorafenib (SOR) and the mTOR specific inhibitor, everolimus (EV) (iii) *in vivo* experiments on OS xenografts.
Material and Methods: The activation of mTOR pathway was assessed by immunohistochemistry in 30 samples from patients diagnosed at Istituti Ortopedici Rizzoli of Bologna. Primary antibodies against phospho-mTOR (Abnova) and PTEN (C-term, Millipore) were used. Mutational analysis by PCR and Sanger sequencing was performed starting from DNA extracted from OS and surrounding non tumoural tissues when present. Effects of EV (from 500 to 15.125 nM) and SOR (from 10 to 0.3125 μM) were tested on cell proliferation (Cell Titre GLO assay), cell cycle (flow cytometry) and apoptosis (Annexin V/PI). Synergism (SOR+EV) was calculated through normalized isobologram and combination index (CI). 10^6 OS cells were injected in SCID mice. After tumour establishment, mice were orally treated for 6 wks with SOR (5 and 1 mg/kg/day) or EV (1 and 0.1 mg/kg/day) and their combination.
Results: Phospho-mTOR is overexpressed in only 5/30 samples. PTEN expression is lost in the majority of samples (28 out of 30). No "hotspot" mutations were found in KRAS (exons 1−2), PIK3CA (exons 9−20) and Akt (exon 1). *In vitro* assays demonstrated that EV alone is able to inhibit no more than 40% of cell proliferation but it synergistically potentiates the antiproliferative effect of SOR after 72 hours of treatment. The activity of the 2 drugs as single agent or in combination orally administered to OS-bearing NOD/SCID mice will be presented.
Conclusions: This work shows the in vitro and in vivo antiproliferative effect of SOR, EV and their combination. This pharmacological approach warrants to be tested in OS clinical setting.

135 Epigenetic control of pi3k/akt activity in prostate cancer during hormone manipulation

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In advanced stages of prostate cancer, the phosphatidylinositol-3′ kinase (PI3K)/Akt signaling cascade, one of the major survival pathways in the cell, is frequently and constitutively activated due to increased loss of the tumour suppressor protein phosphatase and tensin homolog deleted on chromosome 10 (PTEN) and/or increased expression/activity of growth factor receptors.
Here we asked whether the increase in Akt phosphorylation may contribute to the development of androgen independence.
To mimic the clinical situation and to test the role of androgen manipulation in prostate cancer progression we cultured androgen receptor positive 22rv1