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

C.C. Cazaux[1], V.B. Bergoglio[1], F.L. Lemee[1], A.V.F. Vidal-Fernandez[1], A.M. Machado[1], A.L.M. Martin[2], H.R. Roche[3], R.D.W. Wood[4], J.C.B. Bourdon[5], J.S.H. Hoffmann[1]. [1]*Institute of Pharmacology and Structural Biology, Genetic Instability and Cancer, Toulouse, France,* [2]*French Federation of Cancer Centers, Cancer Centers, Paris, France,* [3]*Institute Claudius Regaud, EA3035, Toulouse, France,* [4]*University of Texas, MD Anderson Cancer Center, Smithville, USA,* [5]*University of Dundee, INSERM European Associated Laboratory, Dundee, United Kingdom*

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

Y. Pignochino[1], S. Inghilleri[2], M. Zorzetto[2], M. Luisetti[2], P. Morbini[3], M. Aglietta[1], E. Pozzi[2], G. Stella[2]. [1]*IRCC, Oncological Sciences, Candiolo-Torino, Italy,* [2]*University of Pavia and Fondazione IRCCS Policlinico San Matteo, Hematological Pneumological and Cardiovascular Sciences, Pavia, Italy,* [3]*University of Pavia and Fondazione IRCCS Policlinico San Matteo, Pathology, Pavia, Italy*

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

Y. Pignochino[1], G. Grignani[1], M. Basirico[1], M. Motta[2], S. Bruno[3], G. Cavalloni[1], S. Aliberti[1], P. Picci[4], M. Alberghini[4], M. Aglietta[1]. [1]*IRCC, Department of Oncological Sciences, Candiolo-Torino, Italy,* [2]*A.O Mauriziano, Unit of Pathology, Torino, Italy,* [3]*Center for Molecular Biotechnology and Center of Experimental Research and Medical Sciences, 3 Department of Internal Medicine, Torino, Italy,* [4]*Istituti Ortopedici Rizzoli, Department of Musculoskeletal Oncology, Bologna, Italy*

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

C. Festuccia[1], G.L. Gravina[1], D. Dondi[2], M. Piccolella[2], M. Ruscica[2], V. Tombolini[1], M. Motta[2]. [1]*University of L'Aquila, Experimental Medicine, L'Aquila, Italy,* [2]*University of Milan, Endocrinology Department Center of Endocrinological Oncology, Milan, Italy*

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

(PTEN positive) and LnCaP (PTEN negative) cells in absence of androgen or in presence of the antiandrogen compound, bicalutamide to derive bicalutamide-resistant (BCLR) clones. Similarly, 22rv1 cells were grown subcutaneously in castrated and intact male nude mice receiving or not 50 mg/Kg/day bicalutamide.
We demonstrated that Akt is activated after treatment with androgen deprivation therapies or bicalutamide. In addition the Akt inhibition as well as Akt gene knock down slowed down the development of androgen independent or BCLTR cell strains. We observed also an increment in DNMT3a and DNMT3b expression as well as in HDAC-2, HDAC-4 and HDAC-6. In vitro treatment with DNMT inhibitor, 5-azacytidine, or a pan histone deacethylase inhibitor, PXD101, upmodulated PTEN levels in PTEN positive and bicalutamide resistant 22rv1 cells and reduced Akt activity down-modulating Akt protein expression both in PTEN positive or negative BCLT treated cells. Azacitidine treatment was able to slow-down the development of a BCLTR phenotype and to restore the effectiveness to bicalutamide. Our study suggests that, after exposure to androgen deprivation therapies, prostate cancer cells undergo a series of coordinated changes which eventually result in the development of androgen independence. A major factor in this process is the induction of DNMT activity by increased expression of DNMT3a and DNMT3b, responsible to de novo/gene specific DNA methylation, reducing the expression of tumour suppressor genes. Similarly the induction of HDAC activities are responsible to stabilization of several oncogenetic molecules (growth factor or intracellular key regulator such as Akt) which may contribute to the development of androgen independence through: (i) maintaining cell proliferation; (ii) inhibiting apoptosis; and/or (iii) inducing AR activation in a ligand-independent fashion. These effects may be mediated, at least in part, through activation of the PI3K/Akt pathway.

136 Validation study of the prognostic significance of β-microseminoprotein and cysteine-rich secretory protein-3 after radical prostatectomy using automated image analysis

A. Dahlman[1], E. Rexhepaj[2], D. Brennan[3], W. Gallagher[2], T. Schlomm[4], K. Jirström[5], A. Bjartell[1]. [1]*University Hospital Malmö, Division of Urological Cancers, Malmö, Sweden,* [2]*UCD Conway Institue University College Dublin, UCD School of Biomolecular and Biomedical Science, Dublin, Ireland,* [3]*UCD Conway Institue University College Dublin, UCD School of Medicine and Medical Science, Dublin, Ireland,* [4]*University Medical Center, Martini-Clinic Prostate Cancer Center, Hamburg, Germany,* [5]*University Hospital Malmö, Center of Molecular Pathology, Malmö, Sweden*

Background: Despite prostate cancer being the most frequent cancer in males in the Western world, there are still no clinically reliable tissue biomarkers for predicting disease recurrence after surgery. We have previously identified β-microseminoprotein (MSMB) and cysteine-rich secretory protein-3 (CRISP3) as independent outcome predictors of biochemical recurrence after radical prostatectomy. In the healthy male, MSMB is second only to prostate specific antigen (PSA) as the most predominant protein expressed, but levels are known to decrease, or even disappear in prostate cancer. In seminal plasma, MSMB can be found in a complex with CRISP3. In this study, we wanted to validate our previous findings in a larger cohort, and to use automated image analysis enabling quantitative determination of MSMB and CRISP3 expression.
Material and Methods: Tissue cores from 3 261 patients undergoing radical prostatectomy at the Department of Urology, University Medical Center Hamburg-Eppendorf between 1992 and 2005 were organised in tissue microarray blocks, and immunohistochemically stained for MSMB and CRISP3. Whole-slide digital images were captured using a 20x objective and the Aperio ScanScope CS Slide Scanner (Aperio Technologies). A positive pixel count algorithm (Aperio Technologies) was used to develop a qualitative scoring model for cytoplasmic staining.
Results: Low expression of MSMB (<20% of tumour cells staining positive) correlated with biochemical recurrence after radical prostatectomy ($P = 0.001$), and with overall survival ($P = 0.001$). High expression of CRISP3 (>80% of tumour cells staining positive) was not associated with biochemical recurrence ($P = 0.085$), but with overall survival ($P = 0.03$). Multivariate analysis revealed that MSMB expression was an independent predictor of decreased risk of recurrence (hazard ratio, 0.68; 95% confidence interval, 0.57–0.81; $P < 0.001$).
Conclusion: In the current study, we were able to validate the prognostic significance of the suggested biomarkers MSMB and CRISP3, using a large independent cohort, and novel image analysis technology. Prostate cancer tumours expressing low MSMB and high CRISP3 levels are associated with higher risk of recurrence and adverse outcome after radical prostatectomy. MSMB in particular, is a strong independent biomarker for prostate cancer recurrence.

137 A chemical genetics screen identifies novel steroid inhibitor drugs that inhibit the growth of glioma stem cells

D. Kamnasaran[1], D. Poirier[2], M. Rana[1], N. Ajewung[1]. [1]*Université Laval, Paediatrics, Québec, Canada,* [2]*Université Laval, Laboratory of Medicinal Chemistry Oncology and Molecular Endocrinology, Québec, Canada*

Background: Glioma stem cells represent a fraction of cells within a tumour mass which are postulated to be responsible for tumour re-growth. Moreover, recent studies have associated glioma stem cells with impeccable chemo-resistance mechanisms, leading to an overall poor survival and failure among patients treated by conventional adjuvant chemotherapy. Since a wide range of steroid receptors are expressed in gliomas, our objective was to investigate whether novel classes of steroid inhibitor drugs can be used efficiently to inhibit glioma growth. To achieve this, we studied the effect of these drugs on the growth of glioma stem cells.
Methods and Results: We screened using a candidate chemical structure approach, a library of 400 steroid inhibitor drugs on 5 human glioma stem cells established from surgeries ($n = 2$) and cell lines ($n = 3$), and a normal human neuroprogenitor cell line. We discovered 5 potent new steroid inhibitor drugs belonging to the methyl-piperazine family, can induce significant death of glioma stem cells ($n = 5/5$) within a 24 hour period, and with some death of normal human neuro-progenitor cells. These drugs induced significant apoptosis resulting in an overall decreased viability and proliferation of the cells in a dose dependent manner (5 μM and 10 μM). Furthermore, significant inhibition of transformation was noted.
Conclusions: We have discovered a novel chemically distinct class of drugs that can significantly inhibit the growth of glioma stem cells. Current efforts are undertaken to study more of the mechanistic function of these drugs.

138 Metastatic breast cancer survival according to triple receptor status

N. Todorovic-Rakovic[1], Z. Neskovic-Konstantinovic[2], D. Nikolic-Vukosavljevic[1]. [1]*Institute for Oncology and Radiology of Serbia, Department of Experimental Oncology, Belgrade, Serbia,* [2]*Institute for Oncology and Radiology of Serbia, Department of Clinical Oncology, Belgrade, Serbia*

Background: Although the prognosis of metastatic breast cancer (MBC) patients is poor, better knowing of useful prognostic markers could make a difference. The value of known prognostic factors is not well established, mainly because there is a lack of studiesin MBC. The aim of this study was to identify the influence of combined so called “triple receptor status” i.e. estrogen and progesteron receptor (ER, PR) and human epidermal growth factor receptor-2 (HER2) status on prognosis in MBC patients, beside other known clinicopathoogical parmeters.
Materials and Methods: The study included 109 MBC patients with known clinicopathological characteristics. ER/PR status was determined by ligand-binding assay i.e. in cytosol fraction of primary breast cancer tissue using dextran-coated method. HER2 amplification was determined by chromogenic in situ hybridization (CISH) on the same paraffin embedded primary tumour samples.
Results: According to survival analysis, among available clinicopathological parameters as relevant for follow up of MBC patients are years, DFI (disease free interval) and ER/PR status. Combined ER/PR status showed that patients with ER−PR− phenotype have poorer prognosis and that this negative effect is more pronounced with addition of the effect of HER2 amplification. (ER−PR−HER2+ phenotype). Furthermore, survival analysis of extreme receptor combinations (ER−PR−HER2+ and ER+PR+HER2−) in different age subgroups (⩽50 and >50) showed that negative impact of ER-PR-HER2+ phenotype is age related. Patients older than 50 years, with ER−PR−HER2+ phenotype, had the mortality rate 100% and median survival time of 14 months.
Conclusion: These findings confirm that biology of breast cancer could be significantly affected by patient’s age. There is a strong indication for use of combined triple receptor status for follow-up of MBC patients. Finding that ER-PR-HER2+ phenotype in a restricted subgroup of patients (>50 years) means extremely poor prognosis and a highest mortality rate, indicates further consideration regarding therapy efficiency.

139 Annexin A10 (ANXA10) is a marker for metastasis and disease progression in bladder cancer

F. Mansilla[1], P. Pinholt Munksgaard[1], A. Brems Eskildsen[1], K. Birkenkamp-Demtroder[1], N. Fristrup[1], B. Parm Ulhøj[2], M. Agerbæk[3], T.F. Ørntoft[1], L. Dyrskjøt[1]. [1]*Skejby Hospital, Molecular Medicine, Aarhus N, Denmark,* [2]*Skejby Hospital, Institute of Pathology, Aarhus N, Denmark,* [3]*Aarhus University Hospital, Department of Oncology, Aarhus N, Denmark*

Background: Bladder cancer is among the most common type of cancers worldwide. Bladder cancer is clinically divided into two distinct groups; non-muscle-invasive (stages Ta and T1) treated with a local, organ-sparing approach, and muscle-invasive cancer (stages T2-T4) where radical cystectomy with lymphadenectomy is applied. Presently, no molecular biomarkers are