gender and treatment (79 of the patients were treated with standard stupp protocol).
Conclusions: This is the first study assessing RKIP expression levels in GBMs. We conclude that, in contrast to other solid tumours, the percentage of RKIP negative GBM cases is low and that the absence of RKIP expression seems not to be associated with poor survival in GBMs patients.

79 Association of JAK-STAT pathway related genes with lymphoma risk

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Background: Non-Hodgkin Lymphoma (NHL) belong to the seventh most common cancer in Europe and constitute the tenth most commonly diagnosed cancer worldwide. Apart from risk factors such as certain infectious agents and immunodeficiency syndromes, genetic variants related to immunity have been associated with lymphomagenesis. Previous studies suggested an important role of the JAK-STAT signalling pathway in tumour development. Therefore, we explored genetic variants in the JAK-STAT pathway associated with lymphoma risk.
Material and Methods: In total, 1481 lymphoma cases and 1491 age, sex and study centre matched controls of the EpiLymph study, a multi-centre case-control study on the aetiology of lymphomas among adults in Europe, were genotyped for 1536 single nucleotide polymorphisms (SNPs) using GoldenGate BeadArray™ Technology (Illumina, San Diego, CA).
Association between selected SNPs and haplotypes of the JAK-STAT pathway and risk of Hodgkin lymphoma (HL), NHL and most frequent NHL subtypes were estimated by calculating Odds Ratios (OR), the corresponding 95% confidence intervals (CI) and p-values using unconditional logistic regression using SAS (version 9.2).
Results: Among 220 relevant JAK-STAT pathway SNPs, polymorphisms in several genes (*STAT3, STAT6, IFNG, BMF, STAT5A*) were significantly associated with lymphoma risk. Reduced risk for NHL overall and diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) were seen in association with seven *STAT3* SNPs in high linkage disequilibrium and respective haplotypes. Variant rs4103200 conferred an about 20% reduced NHL risk (OR_{CG} 0.79, 95% CI 0.66–0.94, OR_{GG} of 0.78, 95% CI 0.66–0.91, ptrend = 0.002). Reduced risk in association with this variant was also evident for DLBCL and FL. A putatively functional variant in STAT6 previously associated with IgE levels (rs324011) was inversely associated with HL risk ($OR_{TC/CC}$: 0.61, 95% CI 0.45–0.82, p = 0.001).
Conclusion: Our results implicate a relevant role of the JAK-STAT signalling in the development of lymphoma. Furthermore, our data support previously found associations between genetic variants of STAT genes and immune phenotypes.

80 NAT2 gene polymorphisms and risk susceptibility to childhood acute leukemia

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Background: Maternal exposures to a variety of carcinogens, such as those found in cigarette smoke, diet, drugs and environment during pregnancy play a role in the etiology of childhood leukemia. These compounds are acetylated by NAT2 resulting in activation or detoxification of a variety of heterocyclic amine drugs and carcinogens. Individuals may be classified as rapid or slow acetylators according to the rates at which drugs are acetylated by NAT2. Epidemiological studies suggest that the *NAT2* acetylation polymorphisms may modify the risk of developing childhood acute leukemia. To identify the distribution of *NAT2* polymorphisms in Brazilian children and the effects of the polymorphisms on the development of childhood acute leukemia, we performed a case-control study.
Material and Methods: DNA samples from a total of 194 childhood acute leukemia cases and 285 age-matched controls were analyzed. The genotypes of polymorphisms were assessed by PCR-RFLP and the phenotypes of subjects were defined as fast- or slow-acetylators based on their genotypes. Unconditional logistic regression methods were used.
Results: Point mutations at positions 191 and 341 were more frequent in children with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) than in control group (7.3% and 9.7% vs. 3.7%, respectively of 191 position; and 46.5% and 48.6% vs. 34.3%, respectively of 341 position). We found an association of *NAT2* slow-acetylation alleles and increased risk of ALL and AML (odds ratio [OR] = 2.29; 95% confidence interval [CI], 1.69–3.11; and OR = 2.80; 95% CI, 1.55–5.07; respectively), due to a high frequency of *NAT2*5A* allele within the leukemia group. On the other hand, because of the underrepresentation of *NAT2*4* and **12A* alleles in leukemia group, *NAT2* rapid-acetylation alleles were associated with a protection role of ALL and AML (OR = 0.44; 95% CI, 0.32–0.59; and OR = 0.36; 95% CI, 0.20–0.65; respectively).
Conclusions: In conclusion, ours findings suggest that *NAT2* slow-acetylation phenotype modifies the risk of ALL and AML development in Brazilian children.

81 Catumaxomab: a causal therapy for malignant ascites

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Background: Malignant ascites is a typical complication of several epithelial tumours due to the spread of malignant cells into the peritoneal cavity and is associated with a poor prognosis. Catumaxomab (Removab®, anti EpCAM x anti-CD3) is the first approved causal therapy for malignant ascites. The safety and efficacy data reported here are from the international pivotal trial (NCT00836654).
Material and Methods: The trial was a two-arm, randomized (2:1) open-label, phase II/III study. Patients (pts) received either paracentesis plus catumaxomab or paracentesis alone (control). Following randomisation, pts were stratified into groups with ovarian and non-ovarian cancer. Catumaxomab treatment consisted of 4 i.p. infusions of 10, 20, 50, and 150 μg on day 0, 3, 7, and 10. The primary endpoint was puncture-free survival, defined as time to first therapeutic puncture or death, whichever occurred first. Main secondary endpoints were time to next therapeutic puncture, and overall survival (OS).
Results: Overall, 258 pts (129 ovarian and 129 non-ovarian cancer pts) were randomized. Statistical significant improvement was demonstrated for the catumaxomab group for both median puncture-free survival ($p < 0.0001$, 46 days versus 11 days for control), as well as time to first need of therapeutic puncture with 77 versus 11 days in the control group, ($p < 0.0001$). The study was neither designed nor powered for overall survival, however the pooled analysis showed a positive trend for catumaxomab, and statistical significant OS was shown in the gastric cancer subgroup. The benefit of catumaxomab was confirmed independent of the primary tumour or prognostic factors like number of previous chemotherapies or presence of distant metastases. Catumaxomab was well tolerated with more than 80% of the pts receiving all four infusions. The observed safety profile was expected due to the mode of action and consisted of cytokine release related symptoms like pyrexia, nausea or vomiting. The side effects were generally mild to moderate and fully reversible.
Conclusions: Catumaxomab treatment resulted in a clear clinical benefit in patients with symptomatic malignant ascites. Based on these results catumaxomab (Removab®) was granted approval in the European Union April 2009 for the treatment of malignant ascites in patients with EpCAM-positive cancer.

82 PET and MRI determination of the effects of Sunitinib on hypoxia and vasculature on a rat brain tumour model

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Background: The use of anti-angiogenic treatments has proven highly efficient for solid tumour including brain tumours [1,2]. However, it has also been shown that these treatments led, paradoxically and at least transiently, to a normalization of the vasculature instead of its disappearance [3]. Although the normalization process should result in more functional vasculature associated with a decrease in tumour hypoxia, until now, no direct proof has been brought in vivo for a correlation between hypoxia and vasculature following an anti-angiogenic treatment. Consequently, the aim of the present study was to analyse, using MRI and PET imaging, the effects of an anti-angiogenic treatment (Sunitinib) on tumour growth, vasculature and hypoxia.
Methods: A rat brain tumour model has been used after inoculation of C6 glioma cells in Wistar rats (5.10^4 cells/3μl). Rat received Sunitinib orally from Day 17 to Day 24 daily (20 mg/Kg) and underwent MRI and PET imaging on Day 17 and 24. MRI was performed on a 7 teslas magnet (Bruker) using (i) T2w RARE imaging; (ii) T2 maps; (iii) T2* maps; (iv) ADC maps and (v) T1w imaging. T2 and T2* maps were performed prior and after an intravenous injection of Sinerem (200 μmol/kg; Guerbet SA) to compute CBV and VSI maps [4]. Hypoxia detection was performed using a microPET imaging (Inveon, Siemens) 120–150min after injection of ^{18}F-FMISO (600 μCi/rat).

Voxels were defined hypoxic when the signal was up to 1.2 fold the contralateral value. Image analysis was performed with Image J.
Results: Our results show the efficiency of the anti-angiogenic treatment despite a delayed administration (i.e. 17 Days) with a decrease in tumour volume by 51% in the Sunitinib group as compared to the Control group ($p < 0.01$). Along with this anti-tumour effect, we observe an increase in CBV (Control: 4.6±0.7%; Sunitinib: 5.9±1.03%; $p < 0.05$) and VSI (DR2*/DR2; Control: 1.13±0.13; Sunitinib: 1.22±0.14; $p < 0.05$) but also a reduction of hypoxia (Mean = Control: 1787±348 nCi/cc, Sunitinib: 1512±134 nCi/cc; Max = Control: 3134±1099 nCi/cc, Sunitinib: 2181±414 nCi/cc; $p < 0.05$) detected following the Sunitinib treatment.
Conclusions: Using both MRI and PET imaging, we present data demonstrating a vascular normalization following an anti-angiogenic treatment in a rat glioma model. We are currently trying to elucidate mechanisms associated with these vascular effects which may reflect a better vascular supply (high CBV, low hypoxia) paradoxically to a slowdown of tumour growth.
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83 Colorectal cancer susceptibility loci on chr 8q23.3 and 11q23.1 as modifiers for disease expression in Lynch syndrome

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Background: Recently, six colorectal cancer (CRC) susceptibility loci have been identified and two SNPs; rs16892766 (8q23.3) and rs3802842 (11q23.1) have been found to be significantly associated with an increased CRC risk in Lynch syndrome patients. In the current study we have genotyped 9 SNPs within these six loci to confirm previous finding and investigate whether they act as modifiers of disease risk in Lynch syndrome patients.
Methods: The patient cohort consisted of 684 mutation positive Lynch syndrome patients from 298 Australian and Polish families. A total of 9 SNPs were genotyped: rs16892766 (8q23.3), rs7014346 and rs6983267 (8q24.21), rs10795668 (10p14), rs3802842 (11q23.1), rs10318 and rs4779584 (15q13.3) and rs4939827 and rs4464148 (18q21.1). The data was analysed to investigate possible associations between the presence of variant alleles and risk of developing disease.
Results: An association between SNPs rs3802842 on chromosome 11q23.1 and rs16892766 on chromosome 8q23.3 and the risk of developing CRC and age of diagnosis was found in MLH1 mutation carriers. Female MLH1 mutation carriers harbouring the homozygous variant genotype for SNP rs3802842 have the highest risk of developing CRC. When analysing the number of risk alleles for the two SNPs combined, a difference of 24 years can be detected between individuals carrying 3 risk alleles compared to 0 risk alleles.
Conclusion: In conclusion, we were able to replicate the association between the CRC susceptibility loci on chromosome 8q23.3 and 11q23 and the risk of developing CRC in Lynch syndrome patients but the association could only be detected in MLH1 mutation carriers in the current study.

84 Genomic rearrangements in BRCA1/2 and CHEK2 genes in Czech high-risk breast/ovarian cancer patients

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Background: *BRCA1* and *BRCA2* are major breast cancer predisposing genes. Large genomic rearrangements (LGRs) represent substantial proportion of pathogenic mutations in the *BRCA1* gene. On the contrary, the frequency of rearrangements in the *BRCA2* gene is low in many populations. The deletion of 5395 bp in *CHEK2* gene has been described in the Czech breast cancer patients.
Material and Methods: LGRs in the *BRCA1/2* genes 5395 bp del in *CHEK2* and were examined in 558 unrelated patients, previously tested negative for *BRCA1/2* point mutations and small deletions or insertions, selected from 700 Czech high-risk breast and/or ovarian cancer patients. For mutation screening and characterization multiplex ligation-dependent probe amplification (MLPA), long range PCR, and genomic sequencing were used. Location of several deletions was disclosed using chromosome 17-specific oligonucleotide-based array comparative genomic hybridization (aCGH).
Results: We identified 15 patients with 8 different LGRs in the *BRCA1* gene that accounted 12.2% (15/123) of all pathogenic *BRCA1* mutations. Among 268 patients from hereditary cancer cases, we found 12 large deletions (4.5%), whereas in 290 non-familial cancer cases 3 deletions were revealed (1.0%). Deletions of exons 1−2, 5−14, and 21−22 were already described in the Czech Republic or in other populations. Five LGRs were novel, namely, deletions of exons 1−17, 5−10, 13−19, 18−22, and 21−24. Deletions of exons 1−17 and 5−14 were both identified in four families, and represented two Czech-specific founder mutations. LGRs at the *BRCA1* locus explained 2.14% (15/700) of all cancer cases in the study group. No LGRs were found in the *BRCA2* gene, but BRCA2-specific MLPA revealed 5 carriers of 5395bp deletion in *CHEK2* gene.
Conclusions: Our results indicate that screening for genomic rearrangements in *BRCA1* gene should include patients from breast/ovarian cancer families as well as patients with non-familial cancer, in particular cases with early-onset breast or ovarian cancer. On the contrary, our analyses do not support the need to screen for rearrangements in the *BRCA2* gene. Chromosome-specific oligonucleotide-based aCGH accurately located deleted regions, which markedly facilitated the design of primers for amplification and sequence analysis of junction fragments.
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85 Risk of non-Hodgkin lymphoma development in patients carrying mutations in CHEK2 gene or polymorphism R72P in P53 gene

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Background: The risk of non-Hodgkin lymphoma (NHL) development is modified by genetic background. Checkpoint kinase 2 protein (CHK2) participates in regulation of DNA double-strand break repair and among others phosphorylates P53 protein. Functions of these proteins could be negatively influenced by gene alterations. Not only *P53* gene, but also *CHEK2* gene has been reported as a cancer susceptibility gene in different types of cancer (e.g. breast, colorectal or prostate), but the relevance to the NHL remains unclear. The aim of our study was to determine the frequencies of *CHEK2* alterations and *P53* R72P polymorphism in NHL patients in order to evaluate their impact on the risk of NHL development.
Material and Methods: Mutation analysis of the whole coding sequence of *CHEK2* gene and of *P53* gene exon 4 was performed in 340 NHL patients. Genomic DNA was isolated from peripheral blood of patients that signed approved informed consent prior genetic testing. Individual exons and intron-exonic boundaries were PCR-amplified and analyzed by denaturing high-performance liquid chromatography (DHPLC; WAVE3500; Transgenomic). Samples with aberrant elution profiles were sequenced from independent amplification (ABI 3100; Applied Biosystems). The population frequencies of alterations were estimated in group of non cancer controls.
Results: The *CHEK2* region (exons 2 and 3) coding for highly conservative forkhead-associated (FHA) domain was shown to contain the majority of gene alterations [e.g. c.470T>C (I157T), c.542G>A (R181H), IVS1−5T>A, IVS2+1G>T]. Frequency of alterations in FHA region was significantly higher in NHL patients (5.6%; 19/340) compared to controls (2.8%; 19/683) with OR = 2.1 (95% CI 1.1−3.9; $p = 0.03$). The frequencies of polymorphisms in exon 1 [c.122C>T (S41F), c.252A>G (E84E), IVS1+39dupA] and in exon 4 (IVS4−78_-100dup23) were the same in NHL cases and controls. Alterations in other exons of *CHEK2* gene were rare (with minor allele frequency <1%). Several *CHEK2* alterations found in NHL patients has not been described previously [e.g. c.1067C>T (p.S356L), c.1201A>G (p.T401A), IVS10+1G>C, IVS10+28A>G, c.1336A>G (p.N446D), c.1421G>A (p.R474H)]. The frequency of *P53* R72P polymorphism was similar in NHL cases as in controls (21.8% and 22.4% of alleles, respectively).
Conclusions: We conclude that inherited alterations of *CHEK2* gene, but not R72P in *P53* gene, could modify the risk of NHL development.
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