Antiangiogenics are standard of care for clear cell carcinomas but outcomes in mPCR are not known. We present the clinical results of treatment antiangiogenics on patients (pts) with mPCR.
Material and Methods: An exhaustive retrospective monocentric review of the medical records of patients with mPCR treated with antiangiogenics in first or second line was performed. Papillary cell carcinoma diagnosis was pathologically established after surgery. Evaluation of the tumor response was done according to RECIST criteria, survival data were estimated using Kaplan-Meier method.
Results: A total of 30 pts (23 men, 77%), with a median age of 58 years [range: 29−77] has been analyzed. All underwent radical nephrectomy. At the diagnosis of mPCR, 20 pts (67%) and 5 pts (17%) had an ECOG performance status (PS) of 0 and 1 respectively. Twenty one pts (70%) had a single metastatic site. Metastatic sites were lung (n = 16, 53%), lymph node (n = 11, 37%), bone (n = 3, 10%), liver (n = 3, 10%) and other (n = 8, 27%). Thirteen pts (43%) were classified as intermediate and one as poor risk group according to Motzer criteria at the onset of first line therapy. After a median follow-up of 55.7 months, 13 pts (43%) were still alive and their median survival time was 38.2 months. Clinical outcomes for the first line are summarized in table 1. Five-teen pts received antiangiogenics as a second line. Partial response and SD were noted for 3 pts (20%) and for 12 pts (80%) respectively. Progression-free survival was 12.7 months.
Conclusions: Clinical benefit and survival of mPCR treated with antiangiogenics seem to be promising, but pathological classification of those tumors remains controversial. Reproductive criteria for diagnosis are necessary for valuable therapeutic evaluation.

Clinical outcomes of first-line for mPRC

	Partial response, n (%)	Stable disease, n (%)	Progression-free survival (months)
Cytokines (n = 15)	0	11 (73%)	6.8
Antiangiogenics (n = 6)	1 (16%)	4 (66%)	6.9
Chemotherapy (n = 9)	0	3 (33%)	2.6

Animal models

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Neurobehavioral properties of penclomedine (PEN) and derivatives

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Introduction: 4-Demethyl-4-cholesteryloxycarbonylpenclomedine (DM-CHOC-PEN) is a non-neurotoxic cholesteryl carbonate derivative of neurotoxic PEN developed by DEKK-TEC. DM-CHOC-PEN has begun clinical trials because of superior intracranial (IC) anticancer activity in gliomas (*vs*. other DM-PEN carbonates, carbamates and BCNU [AACR 48, Abst. 5614, 2007]), with no apparent neurotoxicity noted in animal studies. The current study was initiated to screen DM-CHOC-PEN in rats in a water maze to quantitate influences on memory and cognitive behavior.
Methods: The Morris water maze was modified to screen agents for memory and cognitive behavior. Adult female rats (5−10) were dosed IP once with therapeutic amounts of PEN and analogs and pyrimidines and compared vs. controls for learning/memory abilities after 1, 2, 3 & 24 h of dosing. The testing tank was 85×50 cm with 12 cm of water and a 15×15 cm wire pedestal rising 3 cm above the water. A monolayer of peanuts covered the water and pedestal. The time required for each rat to swim 29 cm & find the pedestal was compared. Each rat was tested six consecutive swims and the avg. and SD of the 1st & 6th trials were compared.
Results: PEN and 5-FU had the greatest impact on memory and learning with 65 & 50% impairment, resp., after 3 hr., with >50% for PEN at 24 h PEN – which continued for >5 days, despite normal appearances. 5-FU impairment reversed after 24 h. DM-CHOC-PEN demonstrated a *35% improvement* in memory and learning with no impairment of cognitive abilities noted. Gemicitabine demonstrated no impairment in memory/learning.
Conclusion: Any drug that is a pyrrole, pyridine or could form a pseudo-N-containing ring configuration with an available −N− could interact with or antagonize the hippocampal complex -NMDA receptor. The latter is a memory transmitter pathway that has a variety of neurotransmitter substrates – serine, glycine, N-methyl-D-aspartate, glutamate all of which can exist as pseudo-pyridine or pyrroles at the NMDA receptor. The interactions of PEN and 5-FU with the NMDA and phencyclidine (PCP) receptors and learning/memory will be discussed. DM-CHOC-PEN is a high energy carbonate, that in addition to being an effective anticancer agent for IC tumors (that localizes in IC tumor tissue) also improved memory and cognitive behavior in the rat model and serves as a springboard for new anticancer structures – carbonated aryl anticancer agents. The present described screen is easy to use and should be useful in screening new agents for behavioral modification properties.
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Hypoxic conditions increase hypoxia response element and vascular endothelial growth factor promoter reporter activity within the hollow fibre assay in vivo

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Background: The hollow fibre assay has been adopted by the NCI to facilitate short-term assessment of cancer drug efficacy in vivo. However, the current technique requires complete cell recovery and ex vivo outgrowth to determine cell numbers in conditions not reflective of the in vivo environment. We therefore aimed to develop a real-time imaging hollow fibre system in which cell viability and assessment of hypoxia can be quantified in vivo.
Material and Methods: The Flp-In system (Invitrogen) was used for the establishment of stable transfected cell lines – a Flp Recombination Target (FRT) site was randomly inserted into the host cell genome, and reporters were then inserted into the FRT site by recombination. Reporters were constructed by cloning a Far Red Fluorescent Protein gene (HcRed) into pcDNA5/FRT to track transfected cells either alone or followed by (i) three tandem hypoxia response elements (HRE) preceding a SV40 minimal promoter linked to a firefly luciferase gene, or (ii) a vascular endothelial growth factor (VEGF) promoter linked to firefly luciferase. Transfected cells were implanted into hollow fibres, cultured for 24 h, then subcutaneously embedded into MF1 nude mice to mimic tumour conditions. Fluorescence and luminescence (by intraperitoneal injection of 60 mg/kg D-Luciferin) were monitored over 14 days and imaged using an IVIS® 100 (Caliper Life Sciences).
Results: Cell lines transfected with HcRed alone (4e5 cells) displayed fluorescence detectable by the IVIS 100, whereas no fluorescence could be detected from cells transfected with a red fluorescent protein gene (DsRed). Cell lines stably transfected with the HRE-SV40 and VEGF promoter reporters displayed luciferase activity when exposed to hypoxic (1% oxygen) conditions in vitro in cell culture conditions and when loaded into hollow fibres. Upon implantation into mice within hollow fibres, HcRed fluorescence was detected in real-time and provided a means of monitoring cell viability. Using the HRE-SV40 reporter construct, luminescence intensity increased over time with increasing tumour size. Similarly, the VEGF promoter reporter also showed an increase in luminescence over time in response to the hypoxic environment.
Conclusions: The data validate the use of a Far Red Fluorescent Protein (HcRed) for quantifying cell viability, and HRE-SV40 and VEGF promoter reporters for monitoring hypoxic changes within hollow fibres in vivo. This model provides the basis for further investigations to assess short-term cellular responses to cancer drugs, and will define the need for second stage xenograft studies.

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Synergistic anti tumor effect of histone deacetylase inhibitor MS-275 in combination with interleukin 2 in a murine model of malignant melanoma

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Background: A high dosage of interleukin-2 (IL-2) is a standard treatment option at the National Cancer Institute in the USA for metastatic malignant melanoma patients. However, the toxicity and limited clinical benefits associated with IL-2 has limited its use. Histone deacetylase (HDAC) inhibitors have demonstrated antitumor activity in different tumor models including malignant melanoma, and also possess immunomodulatory properties.
In our study we tested the efficacy of a combination of IL-2 and HDAC inhibitor, MS-275, in a murine melanoma model.

Materials and Methods: B16 F10 cells were implanted subcutaneously on the back of C57BL/6 mice. Animals were randomly divided into four groups and treated with 50,000 IU IL-2 daily by subcutaneous injection, 5 mg/kg MS-275 daily by oral gavage (5 days/week for two weeks), or a combination thereof. Treatment was started 3 days after tumor cell injection.
Results: Weekly measurement of tumor size and tumor weight after 3 weeks of treatment showed significant tumor inhibition (>60%) in the combination therapy-group compared to the IL-2 (no significant inhibition) or MS-275 (no significant inhibition) groups. Kaplan-Meier analyses showed a statistically significant increase in the survival rate of the combination group, compared with control and single agent-groups. The percentage of CD4+ and Fox-p3+ T cells decreased in the lymph nodes of tumor-bearing animals treated with the combination of MS-275 and IL-2, whereas in the control and single agent-group the percentage had increased. Similarly, the percentage of CD8+ and CD69+ (activated CD8) cells had increased in the lymph nodes of the tumor-bearing animals treated with the combination of MS-275 and IL-2 in comparison with the control and MS-275-treated groups. These results suggest that a combination of IL-2 and MS-275 has a synergistic antitumor effect in vivo in an immunocompetent murine model of malignant melanoma. The antitumor effect was associated with a decreased number of regulatory T cells and increased activated CD8 cells.
Conclusion: These data provide the rationale for clinical testing of the combination of IL-2 and HDAC inhibitors in the treatment of malignant melanoma patients.

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A relevant panel of human uveal melanoma xenografts directly established from primary and/or metastatic patient's tumor for pharmacological preclinical assays

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Background: Uveal melanomas, which are the most common intraocular malignancy, have a pejorative outcome with about 50% of the patients who die of metastases. No efficient chemotherapy was still available and new therapeutic approaches should be evaluated to improve the prognosis. Human cancer xenografts transplanted into immunodeficient mice, that mimic the patients' tumor genomic heterogeneity, constitute a useful preclinical tool for testing new agents and protocols and for further exploration of the biological basis of drug responses. The aim of this study was then to develop, establish, and characterize an in vivo panel of xenografts directly obtained from uveal melanoma patients.
Materials and Methods: Samples obtained from primary tumors after enucleation or from liver metastases, after patients' consent, were subcutaneously xenografted into immunocompromised mice. A characterization of the xenografts growing into mice was then performed and compared to originated tumors, including histopathological, genetic (karyotype or FISH, and CGH-array), and molecular assays, as well as in vivo response to conventional chemotherapy.
Results: Thirty-seven xenografts have been obtained among 95 patient's tumor sample transplantations in which 10 that have still grown after at least three transplantations in mice and that have been characterized. Pathological analyses of these ten xenografts confirmed the diagnosis of uveal melanoma and showed, for the five models derived from primary tumors, similar chromosome 3 status, namely 2 monosomies and 3 disomies in which one probable isodisomy. Bcl-2 protein was overexpressed in all but 2 xenografts. NA17 and Melan-A antigen expressions were positive in all tested samples, tyrosinase antigen expression was positive in all but 2 xenografts, and MAGE-(1/2/3/4/6/10), LAGE-1, and MAGE-C2 antigens expression were negative in all studied cases. Finally, In vivo therapeutic assessments are currently performed and will be further presented.
Conclusion: Our in vivo human uveal melanoma xenografts present the same histopathological and genomic characteristics of the patient's originated tumors. This observation supports the use of our panel for pharmacological preclinical evaluations that could serve as a bridge linking pre-clinical and clinical research, and drug development.

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Sensitivity of a disseminated in vivo model of L363 plasma cell leukaemia against antitumoral compounds sorafenib, bortezomib, and dexamethasone

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For the better understanding of multiple myeloma (MM), the establishment of reproducible in vivo models is pursued worldwide. We have established a cell line-based, disseminated MM model in NOD/SCID-IL2-receptor-gamma-chain$^{-/-}$ (IL2$^{-/-}$) mice. In the current study, this model was validated in various treatment groups, using 1. bortezomib (B: 0.7 mg/kg/day (d); d: 0, 4, 11), 2. sorafenib (S: 200 mg/kg/d; d: 0−11), 3. dexamethasone (D: 3 mg/kg/d, d: 0−4 + 7−11), in comparison with 4. a control group. L363 cells were injected intratibialy (it) into IL2$^{-/-}$ mice and respective therapies were started 7 days after L363-implantation (d0). Tumor growth was monitored with (a) flow-cytometry (FACS; detection of human HLA-A, B, C + CD138), (b) daily monitoring of MM symptoms, (c) fluorescence-based in vivo imaging (FI) and (d) serum osteocalcin analyses. Tumor inhibition was calculated as the median percentage of MM cells at respective compartments of the test- vs. control-group multiplied by 100 (optimal test/control (T/C) in %). L363 engrafted reliably (take rate=100%) at the injection site and in distant organs, such as bone marrow (BM; 100%), spleen (38%) and rarely liver (8%). Control mice developed MM symptoms, such as hind limb pareses, weight loss and osteolyses. L363 cells were detected by FACS and FI, not only at injection sites, but also in the BM, hollow bones and spleen. Primary tumor development was markedly reduced by S (optimal T/C of 23% on d14), as well as with D or B, albeit to a much lesser extend (optimal T/C: 81% + 62% on d14, respectively). BM metastases were also significantly reduced by S with an optimal T/C value of 67% on d28. D and B, possibly due to subclinical doses (determined after titration and toxicity experiments), had no relevant influence on BM metastases (97% + 100% optimal T/C on d28, respectively). Thus, L363 engraftment into IL2$^{-/-}$ is a valuable in vivo model for MM which exhibits high reproducibility, take- and metastases-rates and closely mimics the clinical situation. Collection of whole-body FI data proved to be a time- and animal-saving analysis that allows to closely monitor MM growth. Further investigations will validate the very promising antitumor activity of S and evaluate the potentially synergistic effect of B and S. The evaluation of new therapeutic approaches in comparison to standard agents was thus successfully conducted, suggesting that this model serves as a valuable tool in the development of new anticancer strategies.

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Evaluating the duel kinase inhibitor lapatinib: Bioanalytical method development and pharmacokinetic analysis in mouse, rat, and human and determination of in vivo efficacy in a panel of EGFR wildtype and mutant human tumor xenograft models

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The EGFR/Erb-2 (Her2/neu) duel tyrosine kinase inhibitor lapatinib is currently approved as part of a combination therapy in some types of metastatic breast cancer. Because of its mechanism of action and positive preclinical results, clinical trials are underway examining the possibility of expanding the use of this agent in other indications both alone and in combination with approved chemotherapeutics or novel agents in later stages of development. Several early-stage compounds have shown promising single agent activity in preclinical studies and may be useful when combined with lapatinib in treatment of certain cancer types.
Our goal was to benchmark lapatinib in various preclinical systems for subsequent studies evaluating effects of early-stage anticancer agents on factors including pharmacokinetic (PK) parameters, tumor and tissue deposition, and antitumor activity as well as a rodent to human PK comparison. To accomplish this, we developed a bioanalytical method for quantification of lapatinib in mouse, rat, and human plasma and rodent tissue; experimental determinations included half-life with single and repeated dosing, oral versus intravenous bioavailability, and determination of plasma: tumor and tissue ratios. In addition, we screened a panel of human tumor xenograft models including non-small cell lung based on EGFR mutation status, practical tissue types, and published results. Activity of lapatinib was also compared to standard agents in several of these models.