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Merkel cell polyomavirus (MCPyV)-negative Merkel cell carcinomas harbor frequent TP53 mutations, express p53, and are associated with unfavorable prognosis

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Background: Merkel cell polyomavirus (MCPyV) DNA is present in approximately 80% of Merkel cell carcinomas (MCCs). The viral genome encodes a large T antigen, which binds some tumor suppressor proteins including the retinoblastoma protein (Rb) and p53. Little is known about the associations between presence of MCPyV and expression of key cell cycle proteins and cell regulatory proteins.
Material and Methods: Expression of Ki-67, cyclin D1, cyclin E, p16, Rb, phospho-Rb, p53, and MDM2 proteins were analyzed using immunohistochemistry from a tissue microarray generated from 114 MCCs. MCPyV DNA was detected using quantitative PCR. All p53-positive tumors in the series with tissue available (n = 12) and 30 p53-negative tumors were sequenced for presence of mutations in *TP53* exons 4 to 9.
Results: Rb was expressed more often in MCCs of female than male patients [54 (79.4%) out of 68 vs. 15 (50.0%) out of 30, respectively; p = 0.003], whereas p53 expression showed a reverse association [9 (13.8%) out of 65 vs. 9 (34.6%) out of 26; p = 0.025]. Phospho-Rb expression was associated with a small tumor diameter (median, 13.5 vs. 25 mm; p < 0.0001). Rb expression was much more frequent in MCPyV DNA-positive cancers than in MCPyV-negative cancers [65 (84.4%) out of 77 vs. 4 (19.0%) out of 21; p < 0.0001], whereas tumors that contained mutated *TP53* and those that expressed p53 rarely contained MCPyV DNA compared to *TP53* mutation-negative and p53-negative MCCs [3 (25.0%) out of 12 vs. 20 (66.6%) out of 30, p = 0.014; 9 (50.0%) out 18 vs. 62 (84.9%) out of 73, p = 0.001, respectively]. No significant associations were detected between presence of MCPyV DNA and Ki-67, cyclin D1, cyclin E, p16 or MDM2 expression. Patients who had Rb-positive MCC had more favorable 5-year MCC-specific survival compared to those with Rb-negative MCC (78.6% vs. 46.3%, log-rank p < 0.0001) and more favorable overall survival (50.5% vs. 7.7%, p < 0.0001, respectively), whereas tumor p53 expression was associated with unfavorable 5-year survival (16.7% vs. 39.7%, p = 0.013).
Conclusions: MCCs likely have either a viral or a non-viral origin. Presence of MCPyV DNA is associated with Rb-expression, and absence of MCPyV DNA with *TP53* mutations, p53 expression and unfavorable survival.

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Association of Merkel cell polyomavirus infection with p53, KIT, PDGFR-alpha, stem cell factor and survival in Merkel cell carcinoma

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Background: Most Merkel cell carcinomas (MCCs) contain Merkel cell polyomavirus (MCPyV), and the virus likely has a role in tumor pathogenesis. p53 and the KIT receptor tyrosine kinase have also been implicated in MCC pathogenesis, but their association with MCPyV infection and clinical significance are unknown.
Methods: We identified 87 patients diagnosed with MCC in Finland in 1979 to 2004 using the files of the Finnish Cancer Registry, and with adequate clinical information, tumor tissue and DNA available. Presence of MCPyV DNA was assessed by quantitative PCR; p53, KIT, phospho-KIT, stem cell factor (SCF), and PDGFRα expression using immunohistochemistry; and presence of mutations in KIT exons 9, 11, 13 and 17 and PDGFRA exons 10, 12, 14 and 18 by DNA sequencing.
Results: Majority (77.0%) of the 87 tumors contained MCPyV DNA and 37 (42.5%) expressed KIT, whereas PDGFRα, p53, SCF and pKIT expression was less common (31.9%, 22.8%, 8.6% and 4.8%, respectively). No *KIT* or *PFGFRA* mutations were detected. Tumor p53 and KIT expression were associated with absence of MCPyV DNA ($p = 0.01$ and 0.009, respectively). Tumor p53 expression was associated with poor MCC-specific survival ($p = 0.021$) and overall survival ($p = 0.046$), but tumor KIT expression only when stratified by presence of MCPyV DNA.
Conclusions: The results suggest that p53 and KIT expression are associated with lack of MCPyV DNA in MCC, and that the molecular pathogenesis of MCC is multifactorial.

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Antiangiogenic effects of linifanib (ABT-869) in xenograft models and patients with solid tumors

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Background: Linifanib is a novel VEGF/PDGF RTK inhibitor that has exhibited single-agent anti-tumor activity in preclinical solid tumor models and in early clinical trials. The current study was undertaken to evaluate in preclinical and clinical settings the antiangiogenic mode of action of linifanib on tumor vasculature, which is characterized by reduced microvessels and vessel leakiness, elevated circulating endothelial cells (CEC) and improved integrity of vascular wall. Antiangiogenic agents such as linifanib are expected to normalize the aberrant tumor vasculature.
Methods: Preclinically, tumor vessel density/diameter and maturation markers were assessed by immunohistochemistry in the human ectopic SCID mouse HT1080 fibrosarcoma and Fischer 344 rat orthotopic 9L glioma tumors. CEC were assessed using 4 color flow cytometry on whole blood. Microvessel permeability (K^{trans}) was assessed longitudinally using DCE-MRI in the rat glioma model. Clinical data were obtained from an open-label, dose-escalation study of linifanib in pts with advanced solid tumors. Pts (n = 33) received linifanib 10 mg or a weight-based dose of 0.1, 0.25 or 0.3 mg/kg daily. CEC were assessed at baseline, day (d) 8, 15 and 42. DCE-MRI scans were obtained at baseline and on d 15 after the start of treatment.
Results: Linifanib normalized tumor vessels (reduced vessel density, diameter and leakiness, and increased pericyte coverage), and reduced CEC by 70−80% when compared to controls in the preclinical tumor models. In solid tumor pts, CEC were elevated at baseline compared to healthy subjects (16.2 *vs*. 5.2 CEC/ml; p < 0.0001). CEC returned to the normal range (11.5 CEC/ml) in 83% pts after 15 d on drug. Elevated CEC during treatment was associated with worse outcome (mTTP 52 *vs.* 135 d, p = .026; mean best tumor size change +12.7% *vs.* −13.2%, p = .0032). Changes in tumor vasculature were also seen in preclinical and clinical DCE-MRI studies. In the rat glioma model, linifanib-treatment reduced K^{trans} from baseline at 2−24 hours after dosing, lasting 4−7 d. In pts, reduced K^{trans} was seen across all dose groups. Mean reductions for the 0.1 and 0.25 mg/kg groups were 31% and 29%. A >10% reduction in K^{trans} was associated with increased mTTP (220 d vs 95 d, $P < 0.012$).
Conclusions: Changes in CEC levels and K^{trans} DCE-MRI, potential consequences of vasculature normalization, were observed in preclinical and clinical settings. These biomarkers provide insight into the antiangiogenic dose of linifanib required to normalize tumor vascular pathways and provide clinical benefit.

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Preclinical pharmacokinetics–pharmacodynamics (PK−PD) modeling of TAK-733, an investigational MEK inhibitor

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Background: TAK-733 is an investigational potent and selective MEK inhibitor that is currently being evaluated in a Phase 1 clinical trial. PD assessment (inhibition of phosphorylated ERK [pERK]) in blood is under evaluation as a marker of biological activity of TAK-733. pERK is a suitable PD marker of MEK inhibition because it is proximal to the target, can be measured in both blood and tumor, and relevant assays have been developed in multiple species. To address biological relevance of the blood PD assay, we assessed the kinetic and dynamic properties of pERK modulation in blood and tumor at efficacious exposures in mice.
Materials and Methods: The relationship between plasma PK and tumor PD was examined in the A375 xenograft mouse model, based on a single ascending dose study design with mice (N = 3) dosed at 0.1, 0.3, 1, 3, 10, 30 mg/kg. Tumor efficacy was determined in separate studies with the A375 xenograft mouse model, with oral QD dosing for 14d at 1, 3,

10 and 30 mg/kg doses. For pERK assessments in blood, BalbC mice were dosed at 1, 10 and 30 mg/kg and blood samples were taken at the same time points for PK and PD measurements. pERK was measured via flow cytometry in CD3+ lymphocytes after whole blood ex-vivo stimulation with phorbol myristate acetate. We applied a linear compartmental PK framework to describe plasma PK. The time course of tumor PD in-vivo was described by an indirect response model. A sigmoidal E_{max} model was used to describe the dose response relationship of blood PD. Fitted PK−PD models were then used to simulate the PD time course in plasma and tumor at efficacious doses in the A375 model. The models were further extended to simulate human PK−PD profiles.
Results: Simulations of blood and tumor PD profiles upon repeat dosing suggest that continuous and substantial inhibition of both tumor and blood PD is associated with drug response. The EC50s of tumor and blood PD were in broad agreement indicating biological relevance of measuring pERK inhibition in the blood. Simulations of the PD profile demonstrated that the trough PD response has better dynamic range than peak PD response, suggesting sampling strategies of blood PD should focus on trough levels.
Conclusions: A continuous and substantial inhibition of blood p-ERK level is expected to associate with TAK-733 response. Sampling time for PD response at trough levels offers an advantage to peak levels because of higher dynamic range.

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Comparative tissue distribution of the HDAC inhibitor JNJ-26481585

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Background: The histone deacetylase inhibitor JNJ-26481585 has been shown in preclinical testing to have significant efficacy against a number of solid tumour xenografts and improved potency when compared to other HDAC inhibitors such as Vorinostat (Arts et al, Clin Can Res 15, 6841, 2009). We hypothesised that this might be due to a combination of intrinsic potency against target HDACs and also improved tissue distribution. In this study we determined the comparative tissue distribution of JNJ-26481585 and compared it to that of other hydroxamic acid HDAC inhibitors.
Material and Methods: Male nude mice were dosed once per day by the oral route for up to 7 days with 40 mg/kg of Vorinostat, Panobinostat and JNJ-26481585. Dosed animals were sacrificed at 0, 0.5, 1, 2, 4, 7 and 24 hours (3 animals per timepoint), either for single or repeat dose, and plasma and tissues were prepared for compound analysis by LC-MS/MS. Tissues sampled included bone marrow, brain, heart, kidney, large intestine, liver, lung, muscle, prostate, skin and fat. In addition, tissue samples from skin, liver, lung and bone marrow were selected for immunohistological examination for markers of HDAC inhibition.
Results: Comparative exposures (AUC) showed highest levels of all compounds in the large intestine. After this, exposures were highest in the kidney, lung, prostate skin and heart. In all cases JNJ-26481585 showed superior tissue distribution to that of Vorinostat and Panobinostat, reaching levels up to 6 times those of Vorinostat in lung and up to 3 times in prostate, skin and kidney. JNJ26481585 showed better tissue penetration than Panobinostat particularly in brain, liver, muscle and skin. No tissue accumulation was noted after multiple dosing. Tissue levels of JNJ-26481585 exceeded those of plasma levels, with T/P ratios being in excess of 100 in large intestine, kidney and lung and over 50 in heart, prostate and skin.
Preliminary analysis of pharmacodynamic changes in tissues in response to the HDAC inhibitors showed a significant increase in histone acetylation concurrent with a significant reduction in the Ki67 marker of proliferation. Further comparative analysis will be presented.
Conclusions: JNJ-26481585 shows excellent tissue penetration in nude mice, superior to that of Vorinostat and Panobinostat. This property may make JNJ-26481585 an attractive candidate for clinical trials in solid tumours.

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Identification of HSP105 as a novel non-Hodgkin lymphoma restricted antigen

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Background: We reported that vaccination of relapsed indolent non-Hodgkin lymphoma (NHL) patients using dendritic cells loaded with killed autologous tumor achieved clinical benefits associated with humoral immunity. To identify novel NHL-restricted antigens (ags), we exploited the antibody (Ab) repertoire of responder patients (R) compared to that of non-R (NR), using both pre- and post-vaccine serum samples.
Methods: Purified pre- and post-vaccine Abs from R and NR were biotin-conjugated and tested by immunohistochemistry (IHC), flow cytometry (FC) and western blot (WB) both on autologous and allogeneic NHL specimens and cell lines. Ag discovery was performed applying a modified serological proteomic-based approach (SERPA) followed Mass Spectrometry (MS) analysis. MS-identified cancer-related proteins were further investigated for their role in lymphomagenesis.
Results: By IHC and FC, we found that post-vaccine Abs from R reacted not only on autologous but also on allogenic NHL biopses and cell lines at significantly higher levels than matched pre-vaccine R samples or NR pre- and post-vaccine Abs, respectively. Furthermore, Abs from post-vaccine R serum significantly impaired NHL cell line growth when added for 72 hours in culture as compared to Abs from normal human serum ($p = 0.001$). Towards the identification of novel potential targets for NHL, WB analyses of the follicular lymphoma (FL) cell line DOHH2, tested either as total cell lysate or acidic protein fractions, revealed one differential band migrating at about 100 kDa only when post-vaccine samples from R was used. MS analysis identified the heat shock protein (HSP) 105 as possible ag candidate. By FC, we observed that HSP105 was expressed both on the tumor cell surface and in the cytoplasm of a panel of B-NHL cell lines and, at lower levels, in normal B cells. On the other hand, no reactivity was found following FC analysis of normal T cells or T- lymphoma cell lines. In addition, by IHC on 50 lymphoma specimens, we determined that HSP105 expression levels increased at the increasing of tumor aggressiveness. Accordingly, in vitro blocking assays using a commercial anti-HSP105 rabbit serum revealed a higher anti-tumor activity directed to Burkitt's lymphoma than diffuse large B cell lymphoma or FL cell lines, respectively.
Conclusions: Our preliminary results suggest that HSP105 may represent a novel B-NHL-restricted ag that could be exploited as potential immunotherapeutic target. In vivo studies are ongoing to corroborate our working hypothesis to target HSP105 for the treatment of B-cell lymphoma.

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Baseline circulating tumor cell (CTC) counts enhance the performance of the Royal Marsden Hospital (RMH) Prognostic Score and improve patient selection for phase 1 clinical trials

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Background: CTCs are prevalent in many patients with advanced cancer with higher CTC counts portending a worse prognosis. The use of the RMH Prognostic Score for patient selection for phase 1 clinical trials has been previously validated in prospective analyses. (Arkenau et al, JCO 2009). We evaluated the incorporation of baseline CTC counts to further improve the utility of this prognostic score and enhance patient selection in phase I trials at the RMH.
Methods: We performed a retrospective analysis on the patients who had CTC enumeration as part of their phase 1 trial between January 2006 and December 2009. Blood samples were collected at baseline, during and post therapy for CTC counts and analysed using the CellSearch system (Veridex). Patient characteristics and baseline CTC counts were correlated with the RMH Phase 1 Prognostic Score, which is based on 3 objective markers (albumin <35 g/dL, lactate dehydrogenase [LDH] > upper limit of normal [ULN], and >2 sites of metastases).
Results: Data from 128 patients, male:female ratio (1.1:1), median age 60.5 years (range, 17.5–79.1 years) were collected. The most frequent tumor sites were genitourinary ($n = 31$), gastrointestinal ($n = 30$) and breast ($n = 18$). Median CTC count was 1 (range 0–134). Multivariate analysis indicated that both higher baseline CTC counts and RMH Prognostic Score were independent prognostic factors (HR 1.014, $p = 0.006$). The addition of baseline CTC count enhanced the performance of the RMH Prognostic Score and classified patients eligible to participate in Phase 1 clinical