597 POSTER

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.

598 POSTER

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.

599 POSTER

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.

600 POSTER

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,