Pharmacological effects of GSK2656157, a novel PERK kinase inhibitor, on tumor growth and angiogenesis using DCE-MRI in pancreatic tumor model

Hasan Alsaid[1], Charity Atkins[2], Sarah Lee[3], Tinamarie Skedzielewski[1], Mary V Rambo[1], Shu-Yun Zhang[2], David J Figueroa[2], Brandon Whitcher[3], Rakesh Kumar[2], and Beat M Jucker[1]

[1]Preclinical & Translational Imaging, LAS, PTS, GlaxoSmithKline, King of Prussia, PA, United States, [2]Oncology R&D, GlaxoSmithKline, Collegeville, PA, United States, [3]Mango Solutions, London, United Kingdom

Introduction: The eukaryotic initiation factor 2-alpha kinase 3 (EIF2AK3) or PERK is one of three mediators of the unfolded protein response signal transduction pathway. Activation of PERK has been shown to regulate angiogenesis (1). GSK2656157 is the first-in-class, small molecule inhibitor of PERK enzyme activity in cells with an IC_{50} in the range of 10-30 nM as reflected by inhibition of stress-induced PERK autophosphorylation. In this study, we investigated the pharmacological effect of GSK2656157 on tumor growth and angiogenesis using dynamic contrast enhanced MRI (DCE-MRI) in the BxPc3 xenograft tumor model.

Method: All procedures were approved by the Animal Care and Use Committee of GlaxoSmithKline and were specifically designed to minimize animal discomfort. Twelve week old nude female mice (n=18) were used in the study. Mice were treated with the PERKi, GSK2656157 (150 mg/kg, PO, BID) for 14 days. The groups were vehicle (n=6) and PERKi (n=12). MRI was performed pre-treatment at baseline (D0), and post-treatment at day 7 (D7), and day 14 (D14). All experiments were performed on a 9.4T/30 cm Bruker system. The mice were anesthetized with continuously inhaled isoflurane (1.5–2%), respiration was monitored and temperature maintained at 37°C. Following a scout image, axial T2-weighted images were acquired using a fast spin-echo sequence with TR/TE=5000/12 ms, Rare factor = 8, FOV=3.2X3.2 cm, Matrix =128X128, slice thickness =2 mm, and AVR =4. A saturation recovery sequence with nine repetition times (TR=0.2, 0.4, 0.8, 1.2, 2, 4, 6, 8, 12 sec) was used to acquire a T1 map before contrast administration to estimate the intrinsic T1 relaxation rate in tumor. Dynamic contrast enhanced (DCE) images were acquired using a 2D-FLASH sequence before (17 frames) and after (33 frames) Gd-DTPA injection (0.3 mmol/kg) with the following parameters: TR/TE=64.85/2.62 ms, Flip Angle =58 deg, and a temporal resolution of 7 sec/frame. The arterial input function (AIF) was obtained by analyzing blood samplesover time to determine the concentration of Gd-DTPA in the blood of the mice. Image processing and data analysis were performed on a voxel-by-voxel basis. Regions of interest (ROIs) that cover the tumor were defined and the T1 relaxation rate was calculated from the T1 map. The signal intensities from the dynamic acquisition (DCE) were converted to gadolinium contrast-agent concentration before estimating the K^{trans} value for each voxel. The median K^{trans} values (min^{-1}) in the ROI from each imaging session, across all subjects, were determined (2), and mean tumor size was calculated at D0, D7, and D14. Vascular density was analyzed using immunohistochemistry (IHC) with rat anti-mouse pan-endothelial cell antigen (MECA-32) and anti-von Willerbrand factor.

Results: No mortality was observed in the vehicle group. Six PERKi treated mice died between D7 and D14 post treatment. One PERKi treated mouse was excluded due to a failure of Gd-DTPA injection at baseline. DCE-MRI images (K^{trans} maps) showed differences in the PERKi treated groups at D7 and D14 compared to D0 (Fig 1a). The K^{trans} values (Fig 1d) were significantly decreased (one-way ANOVA) in the PERKi treated group from (mean±SEM, 0.98±0.23) at baseline to 0.23±0.09 (-72% from baseline, $p<0.01$) at D7, 0.30±0.07 (-57%, $p<0.05$) at D14. In comparison no significant differences were observed in the vehicle group: 0.90±0.30 at baseline, 0.74±0.22 (+6.22%) at D7, and 0.48±0.12 (-4.47%) at D14. In addition, quantitative IHC analysis showed a significant decreas in vascular density (Fig 1b) in response to PERKi treatment (↓ 66%) compared to vehicle. Tumor size was significantly increased in the vehicle group at D14 compared to D0; however, tumor growth was much reduced in the PERKi treated group (Fig 1c).

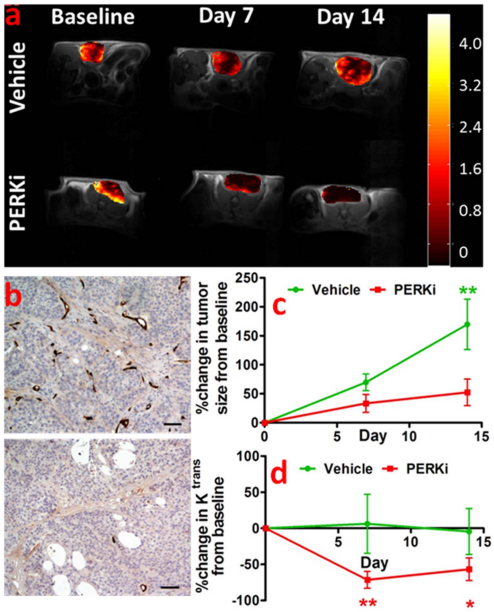

Figure 1 illustrates K^{trans} maps (a), IHC of vascular density (b) changes in tumor size (c) and change in K^{trans} values from baseline (d). PERKi inhibited tumor growth compared to the vehicle group. Median K^{trans} in PERKi treated group (red) was significantly decreased from baseline compared to vehicle (green) which was associated with a decrease in vascular density as shown by IHC.

Conclusion: DCE-MRI was used to assess therapeutic efficacy of PERK inhibition in pancreatic xenografts tumor. These results suggest DCE-MRI can be used as a PD marker to monitor the antiangiogenic effect of a PERK inhibition in human subjects.

References: (1) Blais et al Mol. Cell. Biol. 2006; 26: 9517–32. (2) Yankeelov et al MRI. (2005) 519–529.