Errata

Volume 27 (2007), No 6B, pages 4152, 4153: Figures 1 and 2 should be printed in color:

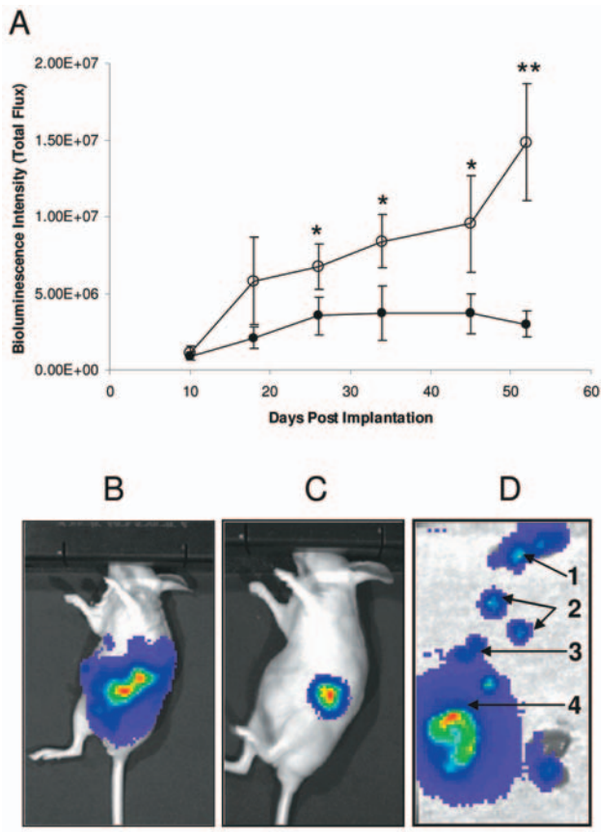

Figure 1. *SD-208 reduces growth kinetics of the primary tumor and inhibits formation of metastatic lesions: A) tumor growth kinetics of the primary tumor monitored by measuring bioluminescence intensity in vehicle and SD-208 at 60 mg/kg/po/bid treated mice with pancreatic cancer. Data was collected in a longitudinal mode from each animal repeatedly through the study period. SD-208 time-dependently reduced bioluminescence intensity in the primary tumor and the data is statistically significant during the last two-thirds of the study (*$p<0.05$ and **$p<0.005$); B) representative whole-body bioluminescence imaging for vehicle-treated; C) SD-208-treated animal on day 56; and D) representative ex vivo imaging in tissues from vehicle-treated animal to confirm metastatic lesions where: 1, metastatic lesions in spleen; 2, metastatic lesions in intestinal lymph nodes; 3, metastatic lesions in the liver; and 4, primary tumor. Values are reported as the mean±SD, n=12.*

the treatment period (day 10-56 of the 56 days study), with p value <0.05 on day 26, 34, and day 45, and $p<0.005$ on day 52. Inhibition of bioluminescence intensity in the primary tumor on day 56 is consistent with inhibition of the tumor weights reported on day 56 in our earlier communication (18). As shown in Figure 1B and D, both local and distal metastatic lesions (spleen, lymph nodes and liver) are evident in the vehicle treated group and SD-208 markedly reduced those lesions (Figure 1C). This finding also agrees with the reported reduced incidence of metastases, particularly to distal sites (18).

SD-208 affects both primary tumor and tumor microenvironment. SD-208 reduces tumor size, proliferation, angiogenesis and increases apoptosis. Gene array findings from our earlier study (18) raised the hypothesis that blockage of TGF-β signaling could affect tumor cell proliferation directly as well as indirectly through effects on its microenvironment. In the present study, we test this hypothesis *in vivo* by analyzing tumors histologically. As shown in the Figure 2A, hematoxylin-eosin staining revealed that animals from the SD-208 treatment group had smaller tumors with intact fully functional beta cells assoicated pancrei when compared to the vehicle treated group ($p<0.001$; Figure 2A) and it is in agreement with our earlier findings on tumor weight (18) In addition, tumors from the vehicle group were highly mitotically active as indicated by Ki-67 IHC staining, whereas tumors from SD-208 treated animals had a significantly lower percentage of Ki-67 positive tumor cells ($p<0.01$: Figure 2B). Caspase 3 IHC staining as a marker for apoptosis revealed that tumors from SD-208-treated animals had a significantly higher level of apoptosis compared to the vehicle-treated tumors ($p<0.05$; Figure 2C). Intriguingly, these effects are not seen in cell-based assays (18), underscoring the role of the tumor microenvironment with respect to TGF-β signaling and the importance of tesing *in vitro* generated hypotheses with *in vivo* experimentation. Together these results show that inhibition of TGF-β signaling reduced cell proliferation and cell survival in the native pancreatic tumor environment. To address whether TGF-β inhibition may have affected tumor growth by decreasing angiogenesis, we quantified microvessel density (MVD) and number in non-necrotic areas of tumors from both vehicle and SD-208-treated animals using IHC for the endothelial cell marker CD34. We found no difference in the number of vessels between SD-208 treated and untreated animals (data not shown). Interestingly, staining in the SD-208 treatment group appeared to have lower intensity than in the vehicle group ($p<0.07$; Figure 2D). Although the difference was not statistically significant,

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Figure 2. *SD-208 affects primary tumor and microenvironment as assessed by histology: Photomicrograph of pancreatic tumors stained with A) hematoxylin-eosin for tumor size, B) Ki-67 (marker for proliferation), C) caspase-3 (marker for apoptosis), D) CD34 (marker for angiogenesis), E) Masson's trichrome for fibrosis, and F) PAX-5 (marker for B-cell) from vehicle group (left panel) and 60 mg/kg/po/bid SD-208-treated group (middle panel). Sections were scored quantiatively for each marker and presented as bar graphs. As shown in the respective graphs (right panel), SD-208 significantly reduced tumor size, proliferation, and fibrosis and significantly increased apoptosis when compared to the vehicle-treated group (**$p<0.05$; ***$p<0.01$; ****$p<0.001$). It also reduced angiogenesis and increased B-cell infiltration into the tumor when compared to the vehicle group (*$p<0.07$). Values are reported as the mean±SD; n=12.*