

### ***Cell lines and in vitro culture conditions***

The cell lines ASPC1, MiaPaca2, HS766T, L3.6PL were obtained from the American Tissue Culture Collection (Manassas, VA), and are derived from pancreatic carcinomas (passages +5 to +10 were used). The cell lines Panc1, Panc203, Panc430, and Panc1005 are low-passage pancreatic cell lines and were a kind gift from Dr. Elizabeth Jaffee (passages +5 to +10 were used). The cell lines XPA3 and XPA4 are low-passage pancreatic cell lines and were a kind gift from Dr. Anirban Maitra (passages +3 to +5 were used). The cell lines E3.LZ10 and E3.JD13 are low-passage pancreatic cell lines obtained from a rapid autopsy program and were a kind gift from Dr. Christine Iacobuzio-Donahue (passages +3 to +5 were used). In vitro drug sensitivity to ON 01910.Na and gemcitabine concentrations ranging from 0 to 10  $\mu$ M was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma, St Louis, MO). For cyclin B1 protein and mRNA expression analysis the cell lines were grown in 6-well plates. When the cells reached a 60-70% confluence they were washed twice with chilled PBS, lysis buffer or RLT (Qiagen, Valencia, CA) were added to the plates and the cells were scraped for protein or mRNA extraction.

### ***Western Blot analysis***

Equal amounts of protein were resolved on 10% polyacrylamide gels. Gels were transferred onto nitrocellulose membranes that were incubated overnight at 4°C with antibodies against cyclin B1 (eBioscience, San Diego, CA), MPM-2 (Upstate, Charlottesville, VA), Plk1 (Santa Cruz Biotechnology, Santa Cruz, CA), phospho-CDC25C, phospho-H3 and actin (Cell Signaling Technology, Beverly, MA). The

immunoreactive proteins were detected using the enhanced chemiluminescence method (ECL; Amersham, Piscataway, NJ).

### ***Small interference RNA (siRNA) analysis***

Cyclin B1 and Plk1 siRNA and transfection reagent (Dharmacon RNA technologies, Lafayette, CO) were optimized in 24-well cell culture plates where 30,000 cells were added to each well in 500  $\mu$ L growth media and grown overnight at 37°C. Each plate contained wells for antibiotic-free media alone, wells for 0.2%, 0.4% and 0.6% of transfection reagent to final volume with antibiotic-free media to check toxicity and wells for the varying transfection reagent amount plus 100 nM and 200 nM siRNA concentrations in antibiotic-free media. Conditions were tested for 24 and 48 hours. Cells were harvested with RLT buffer (Qiagen Valencia, CA). After optimization of siRNA conditions for MiaPaca2 and HS766T, the optimal concentration of transfection reagent, siRNA and time were 0.4%, 200 nM and 48 hours, respectively. For the efficacy studies by MTT, cells were seeded in 96-well plates, transfected during 48 hours with the above conditions, and then exposed to either vehicle, gemcitabine, or ON 01910.Na for 72 hours.

### ***Quantitative Real-Time RT-PCR Analysis***

Total RNA was extracted from cell pellets and tumors using the RNeasy™ Mini Kit (Qiagen, Valencia, CA). cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) following the manufacturer's instructions. Relative quantification of cyclin B1, cyclin D1, *c-myc*, *c-fos* and ubiquitin C (UBC, used as

housekeeper gene) mRNA was achieved using an iCycler iQ real-time PCR detection system (Bio-Rad) using ABI Taqman probes (Foster City, CA). The experiments were repeated twice and samples were run in quadruplicate.

### ***Statistical analysis***

The comparisons between means and proportions and the correlations obtained from the biologic studies were done using Student's t-test, chi-square method, and Spearman's test respectively, using  $P = 0.05$  as the cut-off for significance, and two-tailed analysis. Type I error was set as .10, Type II error was set at .05 by default. Relative *in vivo* tumor growth inhibition was calculated by relative tumor growth of treated mice divided by relative tumor growth of control mice since the initiation of therapy (T/C). A T/C of less than 50% was considered significant. For statistical analyses of IHC endpoints, an index of intensity x percentage of cells staining positive was calculated and compared by means analysis.