The Magic Bullet approach to cancer drug screening has been replaced by the Personalized Healthcare approach, which seeks to exploit differences between cancer types and subtypes as therapeutic targets. Thus, potential drugs need to be evaluated not only for selective toxicity, but also their ability to promote cytostasis or apoptosis (programmed cell death), to reduce chemoresistance to other drugs and/or enhance their effectiveness, and to suppress metastasis. This is particularly true of pancreatic cancer (PC), with a 5-year survival rate of only 1-3% that has not improved for decades, and is characterized by (1) suppression of pro-apoptotic signaling, (2) rapid development of chemoresistance to gemcitabine (the current standard of care), and (3) ability to metastasize through epithelial-mesenchymal transition (EMT), a hallmark of PC. Here we demonstrate how Confluence Discovery Technologies, as part of an early stage testing funnel for PC drug discovery, evaluates compounds in a variety of cancer cell lines for (a) impact on proliferation, cytostasis, cytotoxicity, or apoptosis, (b) ability to enhance sensitivity to drugs like gemcitabine, and (3) ability to suppress EMT. Mechanism of action is confirmed through the correlation of potency in cellular assays with target modulation. For the readouts described above, we utilize inhibitors of transforming growth factor TGF-beta-activated kinase 1 (TAK1), an enzyme that has been shown to sit at a nexus of pathways that control functions such as apoptosis and EMT, with implications for chemoresistance. Interestingly, by employing CellTiter-Glo and Methylthiazolyltetrazolium bromide (MTT) to assess viability, CellTox Green to assess onset of cell death, and Caspase-Glo 3/7 to assess induction of apoptosis, and by analyzing EMT, we demonstrate that TAK1 inhibitors can have differential effects on various pancreatic cancer cell lines. Thus, investigation of the mechanism of death and metastasis, rather than relying on cell viability IC$_{50}$ alone, results in a more complete understanding of drug candidate function and more informed decisions on compound advancement and drug candidate selection. For more information on these approaches to characterize potential anti-cancer drugs please visit the Confluence Discovery Technologies Booth #1510.

### OVERVIEW
- **The Magic Bullet approach:** Replaced by the Personalized Healthcare approach.
- **Key challenges:** Suppression of pro-apoptotic signaling, rapid development of chemoresistance, and metastasization through EMT.
- **Confluence Discovery Technologies' approach:** Evaluates compounds in various cell lines for proliferation, cytostasis, cytotoxicity, and apoptosis.
- **Key findings:** TAK1 inhibitors show differential effects on pancreatic cancer cell lines.

### RESULTS
- **Figure 1:** Target modulation by TAK1 inhibitor (5Z)-7-Oxozeaenol (5Z)-7-Oxozeaenol sensitized KrasG12D cells to gemcitabine.
- **Figure 2:** Cell-type specific effects of TAK1 inhibitors on cell viability. (A) PATU8902 (Pancreatic), Colo205 (Colorectal), and BxPC3 (Pancreatic) were treated with (5Z)-7-Oxozeaenol (Oxozeaenol) and cell viability was assessed using CellTiter-Glo (Promega). IC$_{50}$s were 1368, 54, and 5581 nM respectively. (B) MTT was used to measure cell viability in primary normal and SKCO1 cancerous colon cells using Oxozeaenol (left panel) or Inhibitor B (right panel). Oxozeaenol is more potent in SKCO1 cancerous colon cells in comparison to primary normal colon cells while no differential was observed using Inhibitor B. Results are consistent with those in the literature indicating SKCO1 cells to be especially sensitive to Oxozeaenol (Singh et al. Cell. 2012).
- **Figure 3:** Differing effects of TAK1 inhibitors on various pancreatic cancer cell lines. MiaPaCa2 and BxPC3, and PATU8902 PC cell lines were treated with Oxozeaenol for 72 h and cell viability was assessed using Caspase-Glo 3/7.
- **Figure 4:** Cell-type specific effects of TAK1 inhibitor A upon cytostasis, cytotoxicity, and apoptosis. (A) Cytotoxic time course for Inhibitor A demonstrates cytotoxic effects in MiaPaCa2 (left panel) and BxPC3 (middle panel) but cytostatic effects in PATU8902 (right panel) using CellTox Green (Promega). Death onset apparent between 24 h to 48 h. (B) TAK1 Inhibitor A exhibits greater apoptotic effect upon the BxPC3 pancreatic cancer cell line than upon MiaPaCa2, with minimal effect upon PATU8902 by Caspase-Glo 3/7 (Promega). Staurosporine was used as a positive control (right panel).
- **Figure 5:** TAK1 inhibitors did not increase sensitivity to gemcitabine in pancreatic cancer cell lines. Cell lines were treated with or without Gemcitabine (Gem) and either TAK1 Inhibitor A or Oxozeaenol. (A and B) PATU8902 cells with Inhibitor A and Oxozeaenol, respectively. (C and D) MiaPaCa2 cells with Inhibitor A and BxPC3 cells with Oxozeaenol, respectively.
- **Figure 6:** Effects of TAK1 inhibitors on EMT. (A) TGF-beta induced EMT was demonstrated in epithelial PATU8902 cells (right panel). The addition of Oxozeaenol blocked EMT (middle panel). (B) EMT was confirmed by immunoblotting using biomarkers of epithelial cells (E-cadherin) and mesenchymal cells (Zeb1). GAPDH was used as a loading control.

### CONCLUSIONS
Confluence Discovery Technologies has demonstrated the ability to develop an early stage cell testing funnel to test drug candidates. First, target modulation is confirmed to provide confidence in mechanism. Next, compound mechanism of action and selective toxicity are assessed. A variety of assays such as CellTiter-Glo, MTT, CellTox Green, and Caspase-Glo 3/7 are used to distinguish between cytostatic, cytotoxic, and apoptotic effects of the compounds on several cell lines. Finally, potential impact on disease-relevant processes such as sensitivity to other drugs or metastasis is assessed. In the example shown, it was found that (1) a TAK1 inhibitor can be selectively toxic in a cancer cell line as compared to a normal primary cell line, (2) different TAK1 inhibitors can have either cytostatic or cytotoxic effects on PC in a cell-line dependent manner, (3) TAK1 inhibitors do not increase sensitivity of PC cell lines to gemcitabine, and (4) a TAK1 inhibitor suppresses TGF-beta-induced EMT, suggesting a potential anti-metastatic application.