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## ABSTRACT

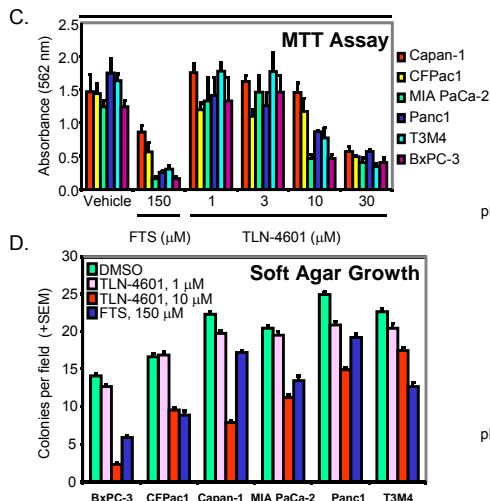
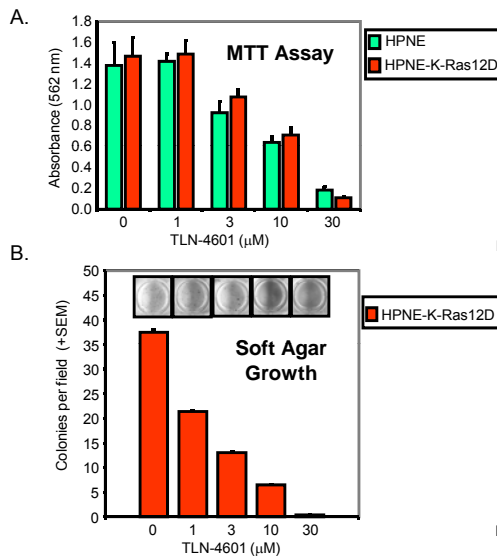
TLN-4601 (formerly ECO-4601) is a structurally novel farnesylated dibenzodiazepinone discovered through Thalion's DECIPHER® technology platform. The compound has demonstrated broad anti-tumor activity in vitro and in vivo against various tumor models. The proposed mechanism of action of TLN-4601 involves its ability to disrupt the activity of Ras signaling by interacting at the level of Ras and Raf-1. **MATERIAL AND METHODS:** Since mutational activation of KRAS is associated with 90% of pancreatic cancer, we have assessed the activity of TLN-4601 in two cell models for KRAS-driven pancreatic cells by MTT viability and soft agar colony formation assays. To determine the ability of TLN-4601 to modulate Ras function, western blot analysis was used to evaluate the steady-state levels of total K-Ras and the Raf-1, MEK1 and MEK2 protein kinases, which are activators of the ERK MAPKs. **RESULTS:** We determined that TLN-4601 potently inhibited the anchorage-dependent and -independent growth of KRAS-transformed human nestin-positive (HPNE) pancreatic duct-derived cells. We also found that the growth of KRAS mutation-positive pancreatic carcinoma cell lines (PDAC) was inhibited by TLN-4601. We then assessed the ability of TLN-4601 to antagonize Ras signal transduction. Consistent with the ability to directly antagonize Ras, we found that TLN-4601 treatment caused cell context-dependent reduction in Ras and Raf-1 protein expression and in MEK1/2 phosphorylation.

## BACKGROUND

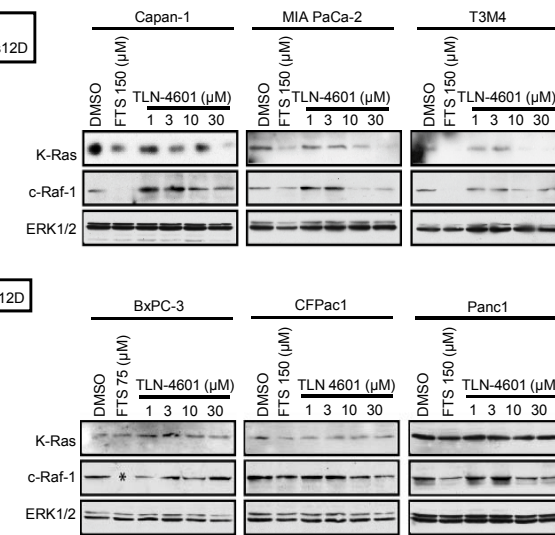
The therapeutic options for pancreatic cancer (PDAC) are poor and essentially all patients will die of the disease. K-Ras is mutationally activated in >90% of human pancreatic tumors, and therefore an important therapeutic target for PDAC. However, there are currently no effective anti-Ras therapies approved for clinical application. Therefore, there is an acute need for effective anti-Ras therapeutic agents. Our recent data indicate that the anti-tumor activity of TLN-4601 may involve inhibiting Ras and Ras-dependent signaling. Here we investigate the efficacy of TLN-4601 in PDAC model systems.

## RESULTS

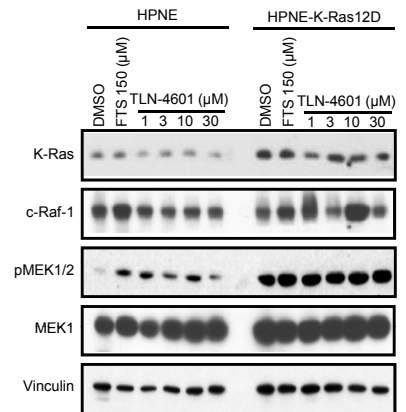
### TLN-4601 reduces anchorage-dependent (A,C) and -independent (B,D) proliferation of HPNE-K-Ras and PDAC cells



### TLN-4601 decreases K-Ras and Raf-1 abundance and downstream signaling in PDAC cells



### TLN-4601 decreases K-Ras and Raf-1 abundance and downstream signaling in HPNE-K-Ras cells



## CONCLUSIONS

While TLN-4601 shows dose-dependent efficacy against cell proliferation and contact-independent soft agar colony formation in both Ras-transformed pancreatic duct-derived cells and PDAC cells, the most striking reductions of signaling proteins are observed in the cancer lines. Our ongoing research will further define the mechanisms of action of TLN-4601 in pancreatic and other tumor types. Our results support the use of TLN-4601 for PDAC treatment as well as other Ras-mediated tumors and are consistent with a model where the anti-tumor activity of TLN-4601 is mediated, in part, through antagonism of Ras signaling. The mechanism of action of TLN-4601 is cell context-dependent and is associated with antagonism of multiple facets of Ras signal transduction.