

## FA Pathway Deficient Cells are Hypersensitive to CHK1 Inhibition

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**Objective:** During carcinogenesis, cancer cells develop genomic instability. This often involves the loss of DNA damage response pathways and an increase in the amount of template DNA damage encountered by DNA polymerases during S phase. To prevent a failure in DNA replication the cell constantly needs to respond to damaged DNA in S phase and can be said to exist in a state of “S phase stress.” Under normal circumstances the cancer cell can deal with this S phase stress through the activation of ATR and CHK1 and the subsequent use of other S phase specific DNA damage response pathways. These particular cancer cells may therefore be susceptible to therapeutic inhibition of CHK1.

**Methods:** In order to identify DNA damage response defects that cause a hyper reliance on CHK1 function we used a high throughput siRNA screening approach. We individually knocked down 230 genes known to be required for DNA repair and measured the sensitivity to a CHK1 inhibitor, Go6976.

**Results:** Disruption of pathways involved in DNA synthesis, chromatin remodeling, and S phase specific repair resulted in specific sensitivity to CHK1 inhibition. Amongst the top targets were several Fanconi anemia genes, components of a DNA repair pathway known to be disrupted in cancer. Human fibroblast lines mutant for FA genes were selectively more sensitive to Go6976 than paired corrected cell lines as measured by viability and chromosomal breakage. In addition whole zebrafish embryos depleted for FANCD2 by a morpholino approach were selectively sensitive to Go6976. The human ovarian cancer cell line 2008, which has epigenetic silencing of the FANCF gene, was also more sensitive to Go6976 than a paired FANCF corrected cell line.

**Conclusions and Translational Applicability:** These data suggest that FA pathway deficient cells are hyperdependent on CHK1 function. Cancers that are deficient in FA pathway function may be sensitive to monotherapy with CHK1 inhibitors, a class of compounds that are currently entering phase I clinical studies. Moreover, these data suggest that FA patients may have a systemic requirement for CHK1 function and therefore CHK1 inhibitors may not be suitable for the management of malignancy in these individuals. Alternative therapies which augment the activity of CHK1 or CHK1-activated DNA repair pathways may improve the growth and survival of FA cells.