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Isogenic CRISPR anchor screens identified actionable nodes to CHK1/2 inhibitor Prexasertib in TP53 mutant cancer

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TP53 KO sensitizes A549 cells to CHK1/2 inhibitor Prexasertib

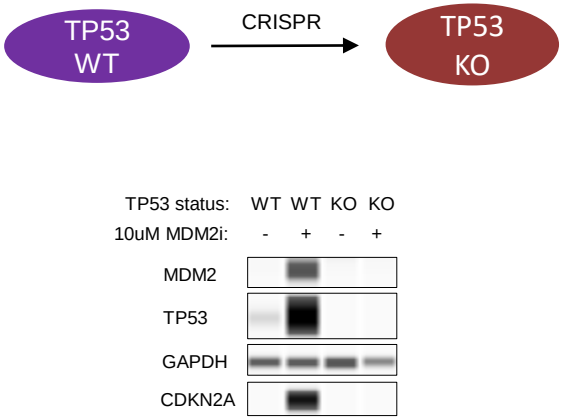

Figure 1. CRISPR mediated TP53 knockout (KO) created isogenic pairs for high resolution screening in A549 cell line. The functional status of TP53 in the isogenic pair cell lines were confirmed by the expression of TP53 and its downstream effector molecules MDM2 and CDKN2A with and without MDM2 inhibitor treatment. Representative western blot for A549-Cas9 isogenic matched-pair cell lines shown.

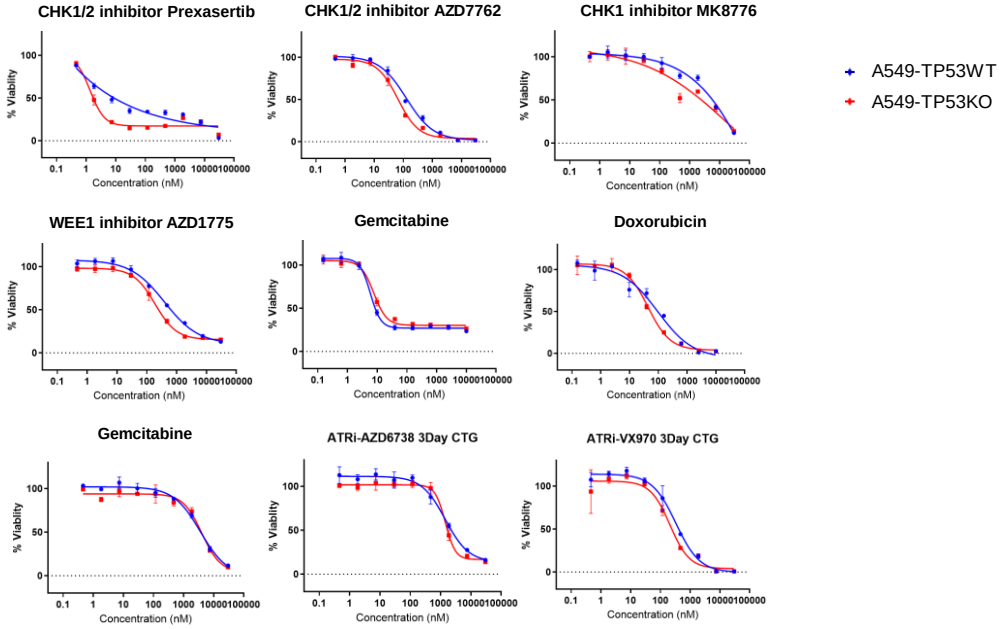

Figure 2. TP53 Knockout (KO) in A549 cells leads to sensitization to CHK1/2 inhibitor Prexasertib among a panel of DDR pathway inhibitors. Representative 3 Day viability results shown (CellTiter-Glo assay). Data presented as mean ± SEM, n=3.

Isogenic CRISPR anchor screens for genes that further sensitize TP53 KO to Prexasertib

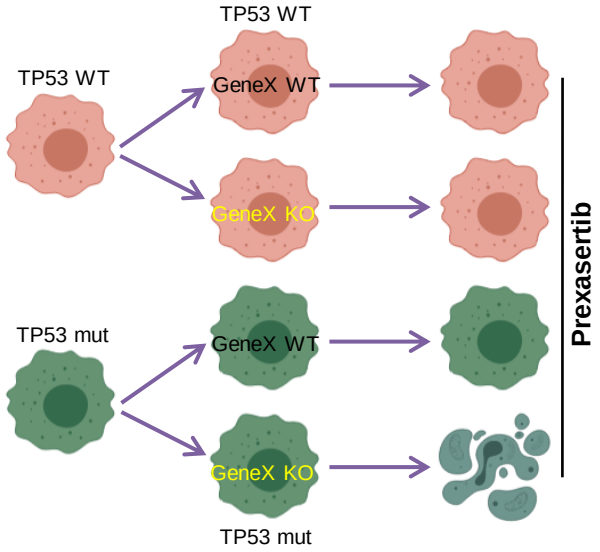

Figure 3. Therapeutic rationale for treating TP53 mutant cancer with Prexasertib. GeneX defines a combination target or a genetic context that sensitizes TP53 mutants to Prexasertib.

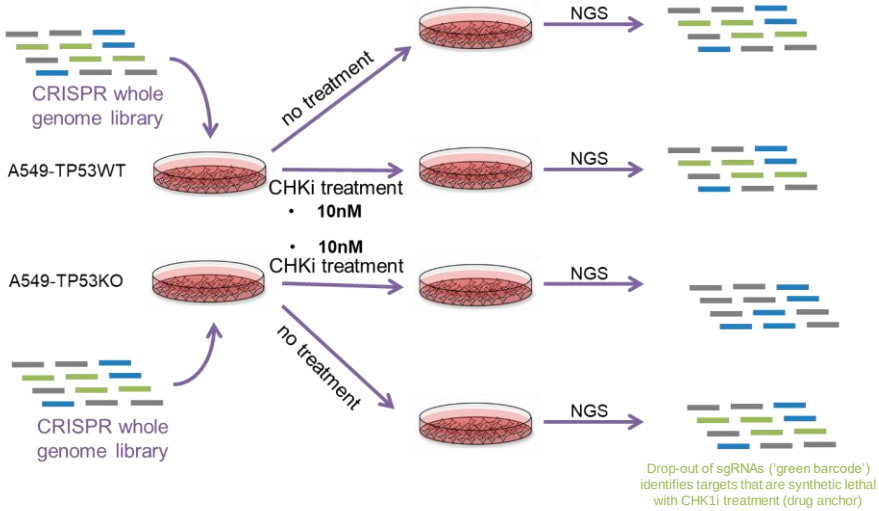

Figure 4. Isogenic CRISPR anchor screen scheme. Screens were carried out in the same manner using CRISPRn (gene knock-out), CRISPRi (gene knock-down) and CRISPRa (gene 'activation') platforms

Common sensitizer and rescuer hits confirm the mechanism of action

CRISPR screen

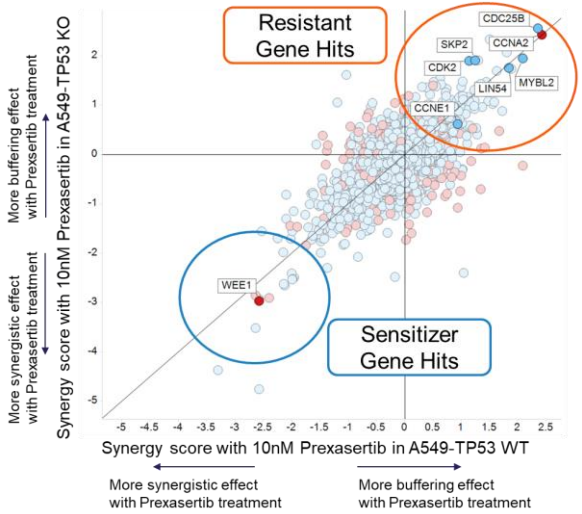

Figure 5. CRISPR screen identified top Prexasertib sensitizer and rescuers regardless of TP53 status. Similar hits were also identified in CRISPRi screen.

CRISPRa screen

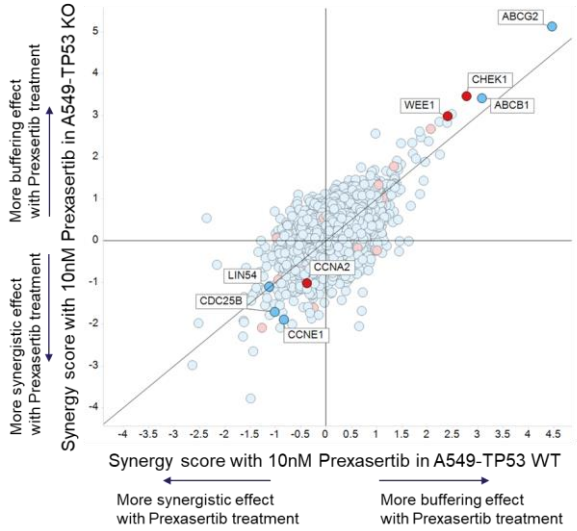

Figure 6. Similar top Prexasertib sensitizer and rescuers as shown in Figure 5 were also identified in CRISPRa screen in reversed direction. CHK1, the target of Prexasertib; and ABCB1 and ABCG2, two drug transporters were also among the top rescuers in CRISPRa screen, confirming the robust performance of CRISPRa platform and the on-target activity of the small molecule kinase inhibitor Prexasertib

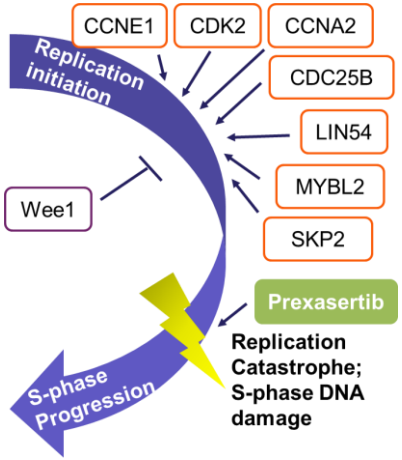

Figure 7. Top hits from Figure 5 and 6 all converge on DNA replication stress and reflect Prexasertib's mechanism of action

Folate metabolism pathway genes preferentially sensitize TP53KO mutant to Prexasertib

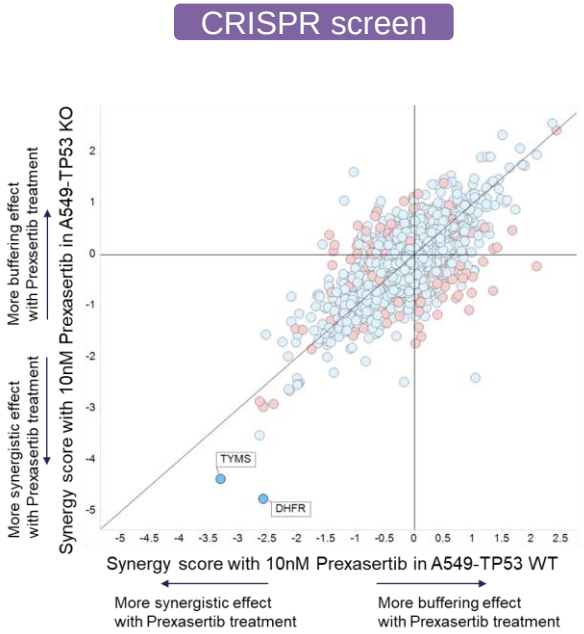

Figure 8. TYMS and DHFR knockouts make TP53KO cells more sensitive to Prexasertib than TP53 wildtype.

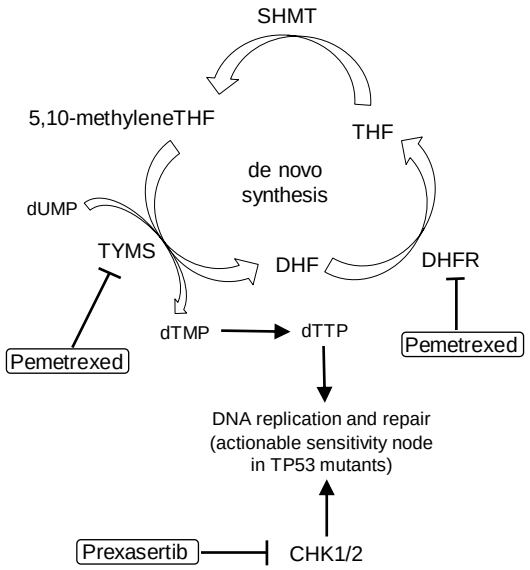

Figure 9. Working model demonstrating that TP53 mutants are more sensitive to DNA replication stress caused by combo therapy targeting CHK1/2 and TYMS/DHFR. TYMS and DHFR are key enzymes regulating de novo dTMP synthesis which contributes to DNA replication and repair. Loss of TP53 activity renders cancer cells more sensitive to DNA replication stress caused by CHK1/2 inhibition and can be further enhanced with TYMS/DHFR targeting agents such as Pemetrexed.

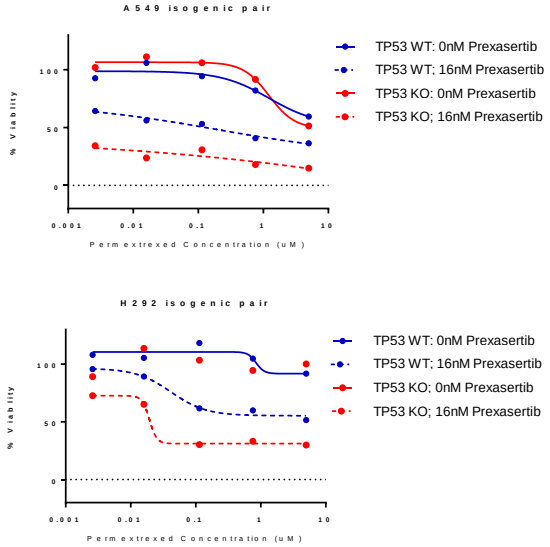

Figure 10. Low doses of Prexasertib sensitize TP53 KO to Pemetrexed in A549 and H292 cells. Data presented as mean ± SEM, n=3.

Additional novel synthetic lethal targets and contexts identified through Tango CRISPR platform

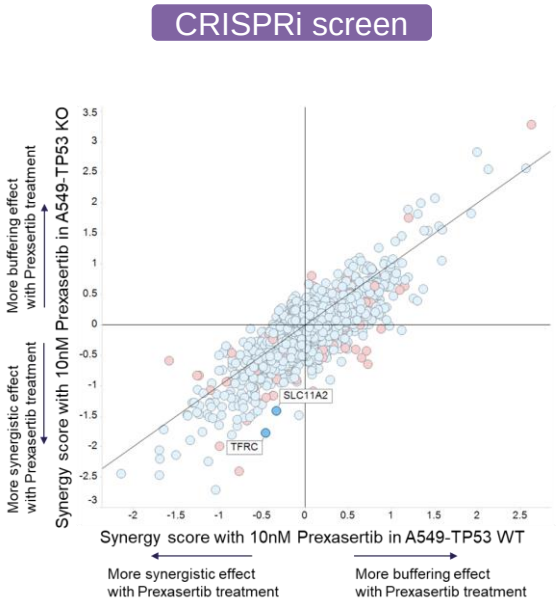

Figure 11. Knockdown of iron transporters TFRC and SLC11A2 make TP53KO cells more sensitive to Prexasertib than TP53 wildtype.

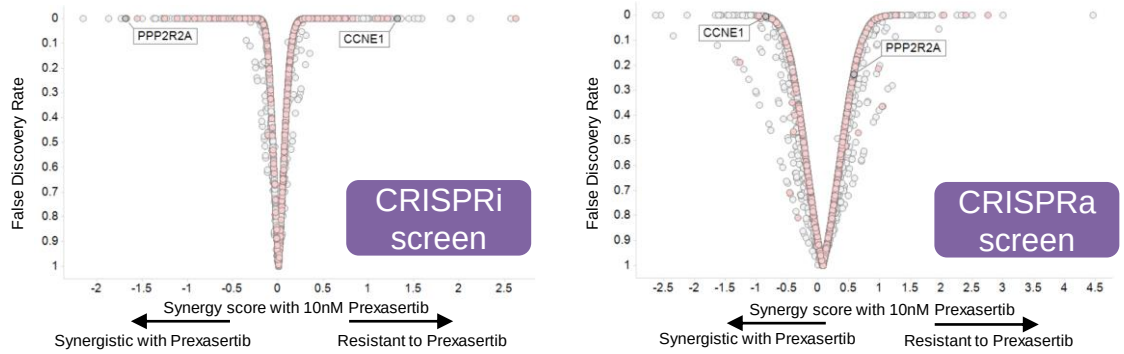

Figure 12. CCNE1 and PPP2R2A showed up as top hits in different screening platforms

Figure 13. CCNE1 amplification or PPP2R1A mutations are frequent in Bladder, Ovarian and Uterine cancer, representing potential patient stratification strategies for CHK1/2 inhibitors such as Prexasertib. Analysis shown prevalence of alterations in selected genes in TCGA dataset using cBioPortal tool.

Conclusion

- At Tango Therapeutics, we use functional CRISPR-based genomics screens to identify novel, synthetic lethal drug targets
- Our genome-wide CRISPR, CRISPRi and CRISPRa platforms are routinely employed in all phases of the drug discovery and development cycle
- Drug anchor screens informs on lead molecules and clinical development:
  - ✓ biomarkers of drug sensitivity and resistance and informs patient selection
  - ✓ rational drug combinations and translational medicine opportunities
  - ✓ compound MOA and selectivity profile (on/off-targets)

Thank You!

- Tango Therapeutics:

Contact information

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