p53-Y220C-BET bifunctionals (tPRIMEs) drive p53-Y220C-mutant cancer cells into apoptosis

Charlotte Kelley, Jae Young Ahn, Scott Rusin, Joshua Murtie, Florence Fevrier-Wagner, Alexandra Joseph, Moses Moustakim, Alexandra Lantermann

Photys Therapeutics, Waltham MA, ajoseph@photys.com (Alexandra Joseph, CSO) & alantermann@photys.com (Alexandra Lantermann, Director of Biology)

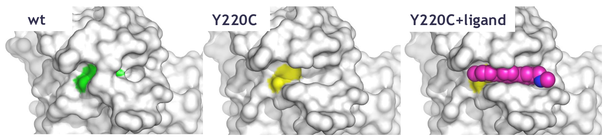
Abstract

The tumor suppressor p53 is one of the most frequently mutated genes in cancer and has been difficult to target therapeutically due to its intrinsically disordered regions. The hotspot mutation p53-Y220C, a mutation thermodynamically destabilizing p53, creates a unique extended crevice on the surface of the protein for which chemical matter has recently been identified. Advanced p53-Y220C binders stabilize p53-Y220C in its wildtype conformation, thereby restoring its role in target gene expression and inhibiting the growth of p53-Y220C mutant cancer cell lines. We rationalized that direct recruitment of the transcriptional elongation machinery to p53-Y220C and its target genes may potentiate effects beyond protein stabilization alone. We leveraged induced proximity to discover bifunctional molecules, p53-Y220C-targeted **PR**oximity **I**nduced **M**odulators of **E**xpression (tPRIMEs), that specifically recognize the BET bromodomain proteins and induce stable ternary complexes with p53-Y220C. P53-Y220C-tPRIMEs potently inhibit proliferation and induce apoptosis of p53-Y220C mutant cancer cell lines to a greater extent than the parental ligands alone or in combination. Gene expression analyses revealed that p53-Y220C-tPRIMEs induce an increase in p53 target gene expression compared to parental binders. The superior antiproliferative activity, enhanced apoptosis, and increased p53 target gene expression are dependent on ternary complex formation. These data strongly suggest that a p53-Y220C-tPRIME-mediated induced proximity approach between transcriptional regulators and p53-Y220C can change the fate of p53-Y220C mutant cells from cell cycle inhibition to an apoptotic response, providing a compelling therapeutic modality for p53 mutant cancers.

p53-Y220C is a druggable p53 mutation that can be exploited to drive tumor-suppressive activity

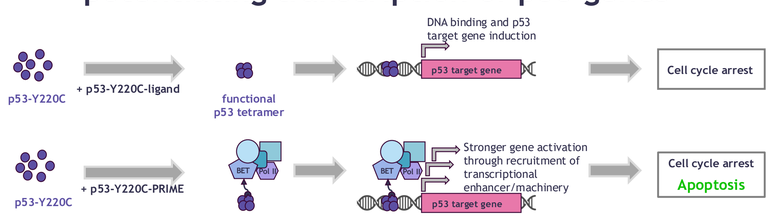
Fischer M. Census and evaluation of p53 target genes. Oncogene. 2017

- p53 is a tumor suppressor, mutated in ~50% of human cancers[1]
- p53-Y220C is the 9th most prevalent p53 mutation and present in ~1% of human cancers[2,3]
- The p53-Y220C mutation induces a surface crevice that destabilizes the protein, lowering its melting temperature and making it susceptible to aggregation[2]
- Ligands which bind to p53-Y220C mutation-induced surface crevice have been identified, resulting in stabilization and activation of the p53-Y220C protein[4,5]
- p53-Y220C-ligands have entered the clinic but demonstrate dose limiting toxicity and limited efficacy[5]



1 Vogelstein, Lane, and Levine, Nature 2000; **2** Petitjean et al, Hum Mutat. 2007; **3** Bouaoun et al, Hum Mutat. 2016; **4** Bullock, Henckel and Fersht, Oncogene 2000; **5** Vu et al ASC Med Chem Lett 2025

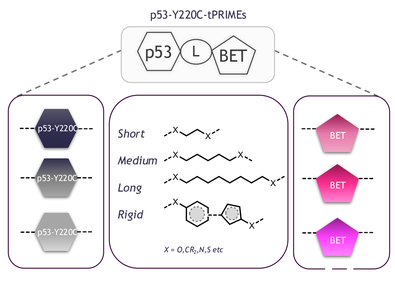
p53-Y220C-tPRIME Hypothesis: Photys' p53-Y220C-tPRIME molecules go beyond stabilization by further potentiating transcription of p53 genes



targeted **PR**oximity **I**nduced **M**odulator of **E**xpression

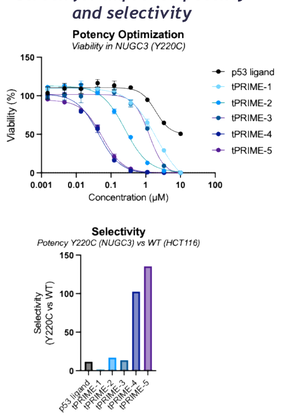
Gourisankar S et al, Nature 2023; Sarott RC et al, Science. 2024; Zhu X et al, bioRxiv. 2024

Approach: Design a diverse set of p53-Y220C-tPRIMEs & look for selective viability reduction

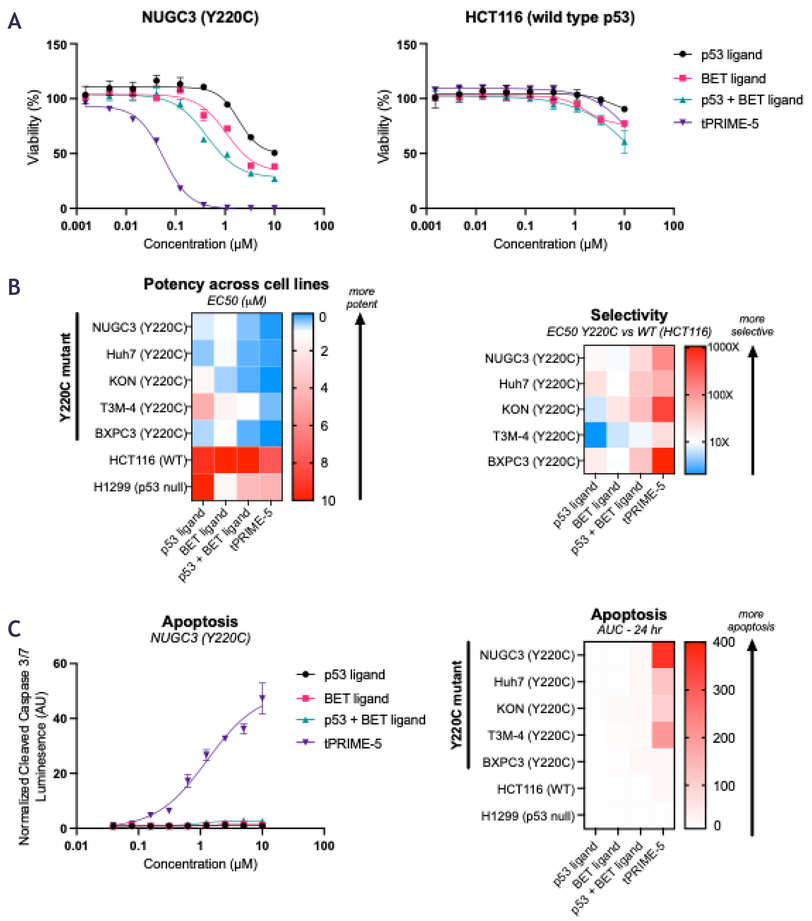


Multiple ligands • Multiple exit vectors • Multiple linkers

Screen for improved potency and selectivity

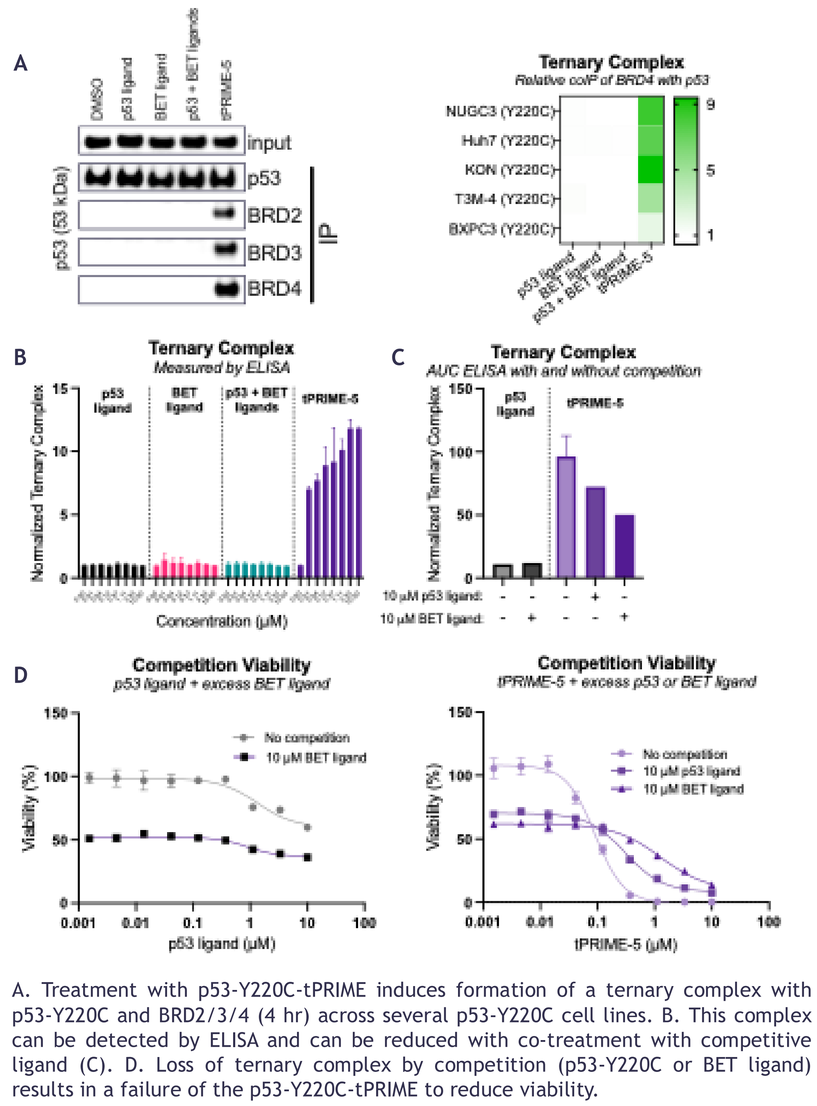


p53-Y220C-tPRIME treatment potently reduces cell viability & induces apoptosis of p53-Y220C-mutants



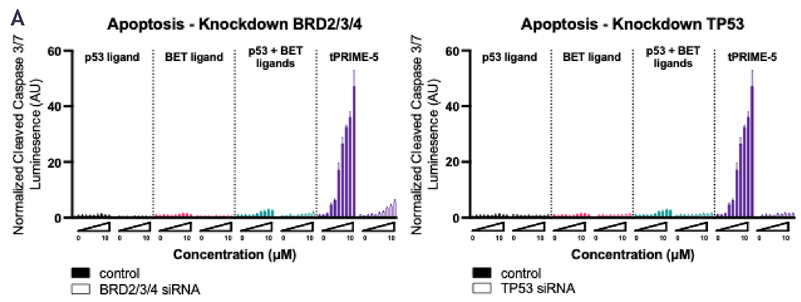
A. p53-Y220C-tPRIME selectively reduces viability of NUGC3 p53-Y220C-mutant cells compared to p53-Y220C or BET ligands alone or in combination. B. p53-Y220C-tPRIME selectively inhibits viability of a panel of p53-Y220C mutant cell lines. C. p53-Y220C-tPRIME selectively induces apoptosis in p53-Y220C-mutant cell lines, measured by cleaved-caspase 3/7 activation.

p53-Y220C-tPRIME-mediated ternary complex is required for the induction of apoptosis



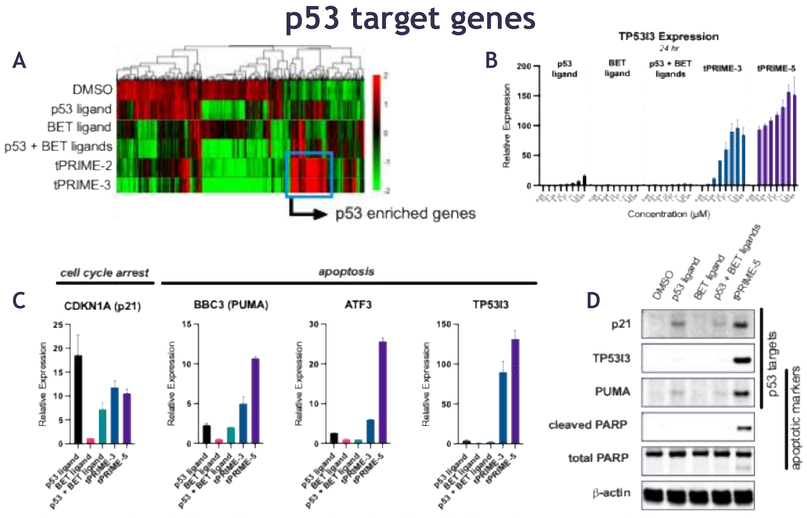
A. Treatment with p53-Y220C-tPRIME induces formation of a ternary complex with p53-Y220C and BRD2/3/4 (4 hr) across several p53-Y220C cell lines. B. This complex can be detected by ELISA and can be reduced with co-treatment with competitive ligand (C). D. Loss of ternary complex by competition (p53-Y220C or BET ligand) results in a failure of the p53-Y220C-tPRIME to reduce viability.

p53-Y220C-tPRIME treatment requires expression of p53-Y220C & BRD2/3/4 to induce apoptosis



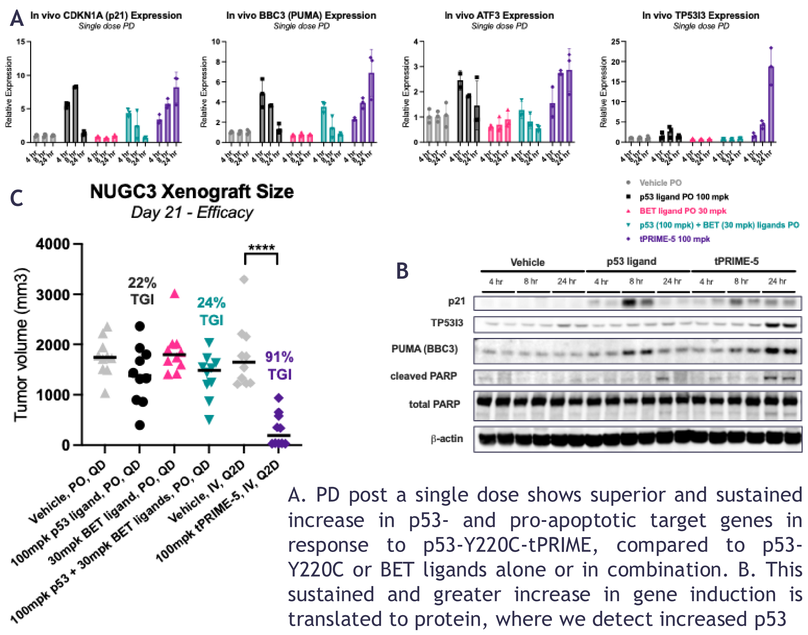
A. Knockdown of the combination of BRD2, BRD3, and BRD4, or knockdown of TP53 using siRNA in NUGC3 p53-Y220C cells reduces the tPRIME-5 induced apoptosis, detected by cleaved caspase 3/7 (24 hr).

p53-Y220C-tPRIMEs lead to strong induction of p53 target genes



A. RNAseq of NUGC3 cells treated with p53-Y220C-tPRIMEs revealed strong induction of p53 target genes. B. Example of one of these canonical p53-target genes shows p53-Y220C-tPRIME treatment induces a greater Emax and more potent gene expression induction of TP53I3. C. Greater induction of gene expression of several p53-target and pro-apoptotic genes in response to 1μM p53-Y220C-tPRIMEs compared to ligands alone or combined. D. p53-gene induction translates to protein, where 1μM p53-Y220C-tPRIME treatment results in increased apoptosis at 24 hr in NUGC3 compared to control ligands.

p53-Y220C-tPRIME treatment induces p53 target gene expression & reduces tumor growth in vivo



A. PD post a single dose shows superior and sustained increase in p53- and pro-apoptotic target genes in response to p53-Y220C-tPRIME, compared to p53-Y220C or BET ligands alone or in combination. B. This sustained and greater increase in gene induction is translated to protein, where we detect increased p53 target proteins and apoptosis (cleaved PARP). C. In a 21-day efficacy study, p53-Y220C-tPRIME, dosed Q2D 100 mpk IV, significantly shrunk NUGC3 xenograft tumors compared to vehicle, p53, and BET ligands alone or in combination.

Key Takeaways & Next Steps

1. p53-Y220C-tPRIMEs, through induction of a ternary complex between p53-Y220C and BRD2/3/4, more strongly and selectively induce apoptosis than p53-Y220C ligands, which act through stabilization alone.
2. p53-Y220C-tPRIMEs achieve this apoptotic response through strong induction of the p53-dependent apoptotic pathway (BBC3, TP53I3).
3. These findings translate to in vivo models, where p53-Y220C-tPRIMEs induce similar gene pathways as in vitro, leading to a meaningful reduction in xenograft tumor growth.

Come to our talk on Feb 19 at 3pm session to hear more on tPRIMEs

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