# GERMLINE HOMOLOGOUS RECOMBINATION DEFICIENCY INFLUENCES THE FITNESS IMPACT OF ONCOLOGIC THERAPY ON DNA DAMAGE RESPONSE GENE CLONAL HEMATOPOIESIS

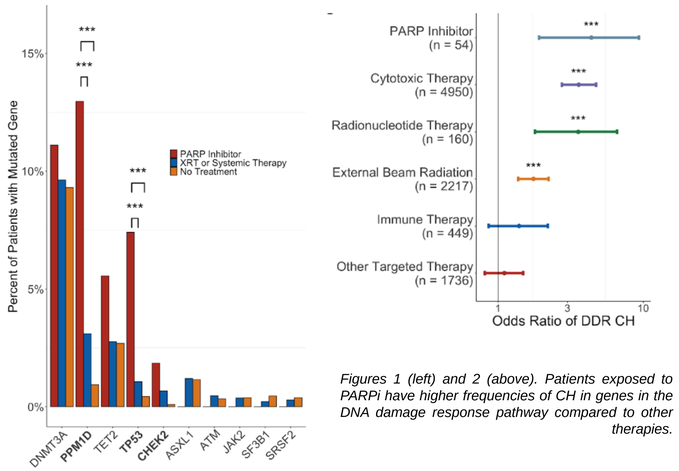
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## INTRODUCTION

Poly (ADP-ribose) polymerase inhibitors (PARPi) are a promising new class of targeted therapy used in a variety of solid tumors with homologous recombination deficiencies (HRD). Data from clinical trials and observational studies have linked PARPi and platinum-based agents to therapy-related myeloid neoplasia (tMN). However, interpretation of these findings is limited by confounding variables including germline mutation status and prior therapeutic exposure. We and others have shown that the oncologic therapies known to confer an elevated risk of leukemia also promote the expansion of clonal hematopoiesis (CH) due to mutations of genes in the DNA damage response (DDR) pathway, including TP53, PPM1D, and CHEK2, and we previously showed in retrospective studies that incidence of DDR mutations is increased in patients that received PARPi therapy. However, the extent to which CH is directly associated with PARPi is not clear.

**Are PARP inhibitor and platinum-based therapies driving expansion of DDR CH, increasing risk of transformation to tMN?**




*Figures 1 (left) and 2 (above). Patients exposed to PARPi have higher frequencies of CH in genes in the DNA damage response pathway compared to other therapies.*

## HYPOTHESES

1. **Exposure to PARPi confers a competitive advantage to HSPCs containing mutations in DDR pathway genes.**
2. **Germline mutations in HRD genes, such as BRCA1/BRCA2, increase genotoxic stress induced by PARPi or cytotoxic agents, further enhancing the fitness of HSPCs carrying DDR gene mutations.**

## RESULTS

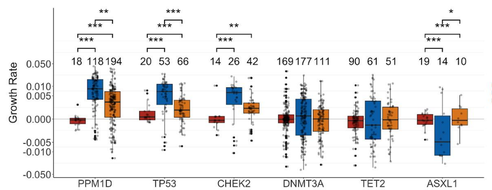



*Figure 3 (left). Change in growth of most common CH gene mutations after Carboplatin or PARPi. Significance determined by linear regression.*

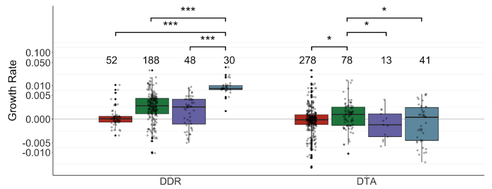



*Figure 4 (left). Comparison of different PARP inhibitors. Change in growth of CH mutations after PARPi in DDR genes or DTA (DNMT3A, TET2, and ASXL1). Significance determined by linear regression.*

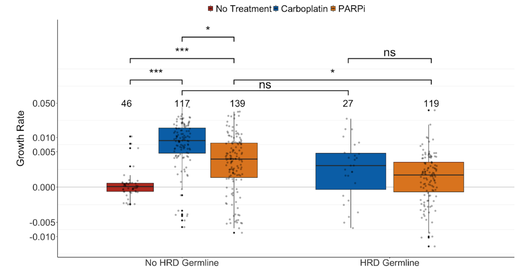
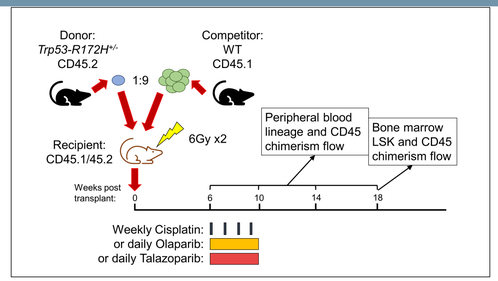



*Figure 5 (left). Change in growth of CH mutations after Carboplatin or PARPi for patients with HRD germline mutations, compared to no germline mutations. Significance determined by linear regression.*



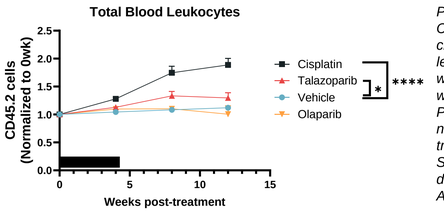




*Figure 6 (left). Peripheral blood CD45.2(Trp53$^{R172H/+}$) chimerism in total leukocytes after 4 weeks of treatment with platinum or PARPi. Fold change normalized to pre-treatment, Significance determined by 2-way ANOVA. n=9-21*

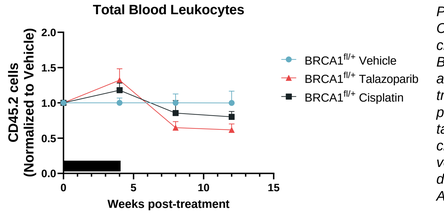



*Figure 7 (left). Peripheral blood CD45.2 (Trp53$^{R172H/+}$) chimerism on mutant BRCA1 background, after 4 weeks of treatment with platinum or talazoparib. Fold change normalized to vehicle, Significance determined by 2-way ANOVA. n=23-25*

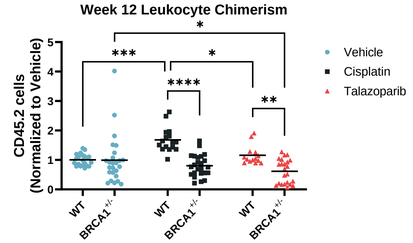



*Figure 8. Comparison of peripheral blood CD45.2 (Trp53$^{R172H/+}$) chimerism in WT BRCA1 or mutant BRCA1 background, 12 weeks post-treatment with platinum or talazoparib. Fold change normalized to vehicle, Significance determined by 2-way ANOVA. n=14-25*

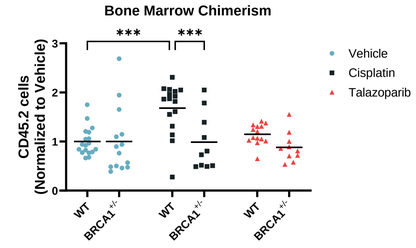



*Figure 9. Comparison of total bone marrow CD45.2 (Trp53$^{R172H/+}$) chimerism in WT BRCA1 or mutant BRCA1 background, 12 weeks post-treatment with platinum or talazoparib. Fold change normalized to vehicle, Significance determined by 2-way ANOVA. n=14-25*

## METHODS

Sequencing and analysis: Sequencing of 942 patients' pre-treatment and post-treatment blood samples (112 PARPi, 177 Carboplatin, 182 Control) was performed with ArcherDX VariantPlex UMI-based panel allowing us to calculate the rate of growth of nine of the most commonly mutated CH genes.

Clonal hematopoiesis mouse model: Bone marrow from *Trp53-R172H* mutant and WT mice carrying different CD45 isoforms are mixed 1:9 and 5 million cells transplanted retro-orbitally into lethally irradiated recipients expressing both CD45 isoforms. After 6 weeks to allow complete engraftment and reconstitution, mice are treated with drug or vehicle.

Lineage chimerism flow cytometry: Peripheral blood: Gr1, CD115, CD3e, B220, CD45.1, and CD45.2. Bone marrow: lineage panel, Sca1, ckit, CD45.1, and CD45.2.

## CONCLUSIONS

- Both platinum and PARP inhibitor treatment significantly increased the growth rate all CH mutations, driven almost entirely by mutations in DDR genes.
  - Platinum treatment had significantly higher rates of mutational growth compared to PARPi therapy.
- The strongest PARP-trapping member, Talazoparib, had the largest effect on the growth rate of mutations in DDR genes.
  - Only Talazoparib, not Olaparib, expanded Trp53-mutant cells in CH mouse model
- Germline HRD variants do **NOT** enhance this fitness advantage.
  - Patients with HRD variants have no increase in DDR CH growth rate with either therapy.
  - The fitness advantage of Trp53-mutated mouse HSPCs is lost in cells with a *Brca1*$^{+/-}$ mutations.
- PARPi therapy may have less of an impact on leukemia risk compared to carboplatin and in fact may show synergistic effects with HRD in blocking the competitive advantage of DDR CH during genotoxic stress.