The Role of Poly ADP Ribose Polymerase (PARP) Inhibitors in the treatment of Acute Myelogenous Leukemia

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Background

Acute Myeloid Leukemia (AML) is a rare but deadly cancer that occurs in both adults and children. While the overall cure rates of pediatric cancer have reached nearly 80%, the overall survival of children diagnosed with AML is approximately 60%. In the last twenty years very few changes have been made to the standard of care treatment regimen of pediatric AML¹. This is true despite a much stronger scientific understanding of the disease process and underlying genetics. In the era of precision medicine and epigenetic targeted therapy, the need to drive AML therapy in that direction is high. Poly ADP ribose polymerase (PARP) inhibitors have shown good efficacy in clinical trials of adult solid tumors and promising pre-clinical data in AML^{2,4}.

This project focuses on further investigation of PARP inhibitors and their potential for use in combination therapy targeting AML. Initial in vitro single agent and combination drugging experiments demonstrate that AML human cell lines with high risk mutations and/or rearrangements are sensitive to PARP inhibitors as single agents and in synergy with conventional chemotherapy drugs. This sensitivity is being evaluated in vivo through drugging experiments in murine models of leukemia and further investigation into the mechanisms of sensitivity is underway.

Materials & Methods

Cell Line	Mutation
CHRF-288-11	M7; NUP98-KDMA5A fusion [BRCA1 mutated per RNASeq]
CTS	M1; MLL-AF6
CMS	M7; CBAF2T3-GLIS2
MOLM-13	M5; MLL-AF9 with FLT3 ITD
AML-193	M5; NRA5
ME-1	M4; CBFB-MYH11 [inv (16)]
Kasumi1	M2, AML1-ETO [t(8;21)]

Table 1. Cell lines used for in vitro drugging experiments representative of a wide range of pediatric AML. Cell lines in red have high risk lesions while those in blue have lower risk lesions. Active compounds from single agent drugging experiments were chosen and screened in combination with talazoparib, the PARP inhibitor identified to be the most efficacious. Delta synergy drug screening was performed and analyzed using the BRAID method.

Figure 1. Primary transplants using mouse bone marrow that had been transduced with a vector containing luciferase, a GFP reporter, and one of four driving leukemic mutations/rearrangements (MLL-AF6, AML1-ETO9a, NUP98-KDMA5A, and CBAF2T3-GLIS-2)

Results

Figure 2. Dose response curves for single agent talazoparib in seven cell lines with EC50. Talazoparib, an orally available potent inhibitor of PARP was shown to be efficacious in four of the seven cell lines tested including CHRF288-11, CTS, CMS, and MOLM-13. These cell lines contain fusions that when found in patients have been associated with refractory and difficult to treat disease. EC50 can be compared to the positive control ovarian cell lines UWB1.289 that contains a BRCA mutation and is known to be sensitive to PARP inhibitors (EC50 19.96 nM) and negative control UWB1.289R that has restored BRCA function and PARP inhibitor resistance (EC50 317.6 nM).

Figure 3. a. Heat map of delta synergy experiments evaluating talazoparib in combination with one of 16 compounds. Color represents kappa (i.e. synergy) while dot size is a measure of IAE90 or feasibility of combination. Large white dots are additive while pink/red dots indicate synergy. PF477736 is a checkpoint inhibitor. As predicted based on mechanism of action, topoisoemase inhibitors topotecan and SN-38 (Irinotecan) were found to be the most synergistic. b. BRAID modeling showing potentiation of both topotecan by talazoparib and vice versa is indicative of synergy between these two compounds. c. BRAID modeling showing potentiation of gemcitabine by talazoparib but no potentiation of talazoparib by gemcitabine is indicative of additivity but not synergy. Based on these results and a previous phase I clinical trial performed by Kolb et al. the combination of talazoparib, topotecan, and gemcitabine was chosen for in vivo efficacy experiments.

Figure 4. Combination screening with Talazoparib and AZD0156, an ATM inhibitor demonstrates extreme synergy between the two compounds in cell lines sensitive to PARP inhibition. Cell lines that were resistant to PARP inhibition do not demonstrate the same synergy and were more resistant to ATM inhibition as a single agent. Since ATM plays a significant role in homologous recombination, this indicates that there is an intrinsic homologous recombination defect in cell lines sensitive to PARP inhibition.

Discussion

PARP inhibitors show promising results in AML cell lines with historically difficult to treat cytogenetic aberrations in the single agent drug testing. Several other compounds that are known to inhibit double stranded DNA damage repair were also shown to be effective. Combination in vitro screening predicts that the combination of talazoparib, topotecan, and gemcitabine in combination will be efficacious in treating in vivo murine leukemias that are currently in development. This combination has also been shown to be tolerated in non-leukemia bearing mice at dose levels that are clinically relevant. High degree of synergy between PARP and ATM inhibitors in high risk cell lines indicate a baseline intrinsic deficiency in double stranded DNA repair through homologous recombination in AML cell lines with high risk mutations.

Future Directions

- In vivo efficacy studies to test drug combination.

	Day							
Treatment	-14	1	2	3	4	5	6-15	15-19
BMN (Talazoparib)	200 rads							
GEM TPT	200 rads							
GEM TPT BMN	200 rads							
DOX AraC	200 rads							
DOX AraC BMN	200 rads							

- Further functional assays to evaluate for intrinsic defects in homologous recombination.

References

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