Poster Board Number: 584
Abstract Number: 584

COMBINATION TREATMENT OF TEMOZOLOMIDE + PARP INHIBITOR SENSITIZE OVARIAN CANCER CELLS FOR GAMMA-DELTA T CELL KILLING THROUGH NKG2DL EXPRESSION

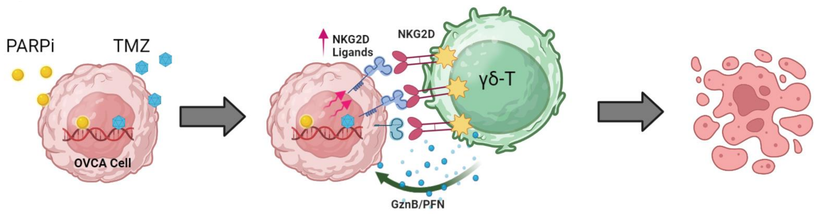
Lei Ding, PhD[1], Gabrielle Siegers, PhD[1], Yanjie Li, PhD[1], Mariska ter Haak[1], Amber Jones[2], Trishna Goswami, MD, MBA[1], Anita Hjelmeland[2], PhD and Lawrence Lamb Jr., PhD[1]
[1]IN8bio, Inc., New York, NY 10118, USA, web: www.IN8bio.com, e-mail: info@IN8bio.com;
[2]Department of Cell, Developmental, and Integrative Biology, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA

UAB MEDICINE
The University of Alabama at Birmingham

Introduction

With a five-year survival rate of less than 50%, ovarian cancer (OVCA) is the deadliest cancer impacting women. The most common and devastating histological subtype is high-grade serous ovarian cancer (HGSOC), comprising ~70-80% of deaths. While first-line surgery and platinum chemotherapy are initially effective, most cases recur. Over the last decade, poly ADP-ribose polymerase inhibitors (PARPi) have revolutionized HGSOC therapy and improved patient outcomes; however, recurrence remains a significant obstacle, with the emergence of cross-resistance to both platinum and PARPi. As such, new approaches are needed to tackle this unmet need. Alkylating agents such as temozolomide (TMZ) increase NKG2D-ligand (NKG2DL) expression on cancer cells through the DNA damage response (DDR) pathway, enhancing gamma-delta (γδ) T cell cytotoxicity. We hypothesized that the combination of PARPi + TMZ would synergize to further potentiate γδ T cell targeting in the context of HGSOC.

1



Materials and Methods

Cell Viability Assay: OVCA lines (OVCAR-3, OVCAR-4, OVCAR-8, OVSAHO, and Kuramochi) were plated in 96-well flat-bottom microplates at density of 4,000-60,000 cells/well. After 24 hour recovery, 10 µl cell culture medium (six control wells/plate) or of culture medium with test compound was added. The test compounds were applied at 9 concentrations in duplicates in half-log increments up to a top concentration of 300µM, 100 µM or 30 µM and treatment continued for 24 hours or five days. Viability of cells was quantified by the CellTiter-Glo® One Solution Assay. Experiment and data analysis services provided by Charles River Laboratories, Germany.

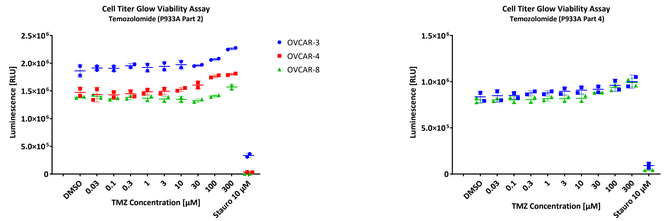
NKG2DL Expression Analysis: OVCA lines (OVCAR-3, OVSAHO, and Kuramochi) were treated for 24 hrs with DMSO, TMZ (200 µM), Niraparib(5 µM), or TMZ(200 µM) and Niraparib(5 µM) combination. Cells were labeled with isotype controls, anti MIC-A, anti MIC-B, anti ULBP-1, and anti ULBP-2,5,6 monoclonal antibody for flow cytometry and the data was further analyzed and visualized using FACSDiva and Flowjo. Median fluorescent intensity (MFI) was established for each treatment group. Relative expression data for each treatment group was normalized with MFI of medium only control for each respective cell line.

γδ T Cytotoxicity Assay: Untreated or drug treated OVCA cells (OVCAR-3, OVSAHO, and Kuramochi) were labeled with **CFSE** and seeded on 48-well plates overnight before they were co-cultured with expanded and activated Vδ2+ γδ T cells (EAGDT) at the indicated effector to target (E:T) ratios for 24 or 36 hours in vitro, stained with 7-AAD and acquired by flow cytometry. CFSE+/7AAD+ population indicate dead cells, cytotoxicity data are normalized with spontaneous cell death (E:T=0).

Results

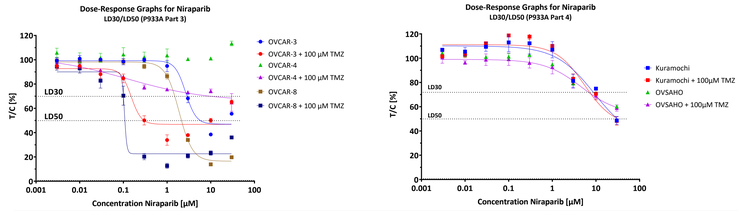
Effect of TMZ monotherapy or in combination with the PARPi niraparib on the growth and viability of various OVCA cell lines.

2A



No significant cytotoxicity from TMZ single-agent treatment up to 300µM

2B



2C

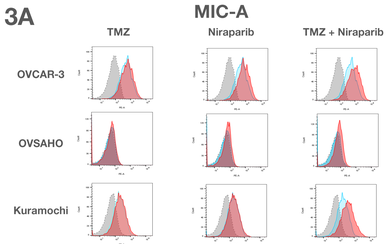
Cell Line	Treatment	Concentration	LD30 [µM]	LD50 [µM]
OVCAR-3	Niraparib	(30 µM-0.003 µM)	2.85	5.77
	Niraparib//TMZ	(30 µM-0.003 µM) //100 µM	0.15	0.3
OVCAR-4	Niraparib	(30 µM-0.003 µM)	nr	nr
	Niraparib//TMZ	(30 µM-0.003 µM) //100 µM	9.69	nr
OVCAR-8	Niraparib	(30 µM-0.003 µM)	1.5	2.14
	Niraparib//TMZ	(30 µM-0.003 µM) //100 µM	0.1	0.11
Kuramochi	Niraparib	(30 µM-0.003 µM)	9.63	30.84
	Niraparib//TMZ	(30 µM-0.003 µM) //100 µM	7.83	32.94
OVSAHO	Niraparib	(30 µM-0.003 µM)	7.9	n.r.
	Niraparib//TMZ	(30 µM-0.003 µM) //100 µM	8.03	n.r

n.r.: not reached

OVCA cells are more sensitive to niraparib treatment. In addition, combination treatment with Niraparib + low dose TMZ (100 µM) showed enhanced cytotoxicity over single-agent treatment in some OVCA cells.

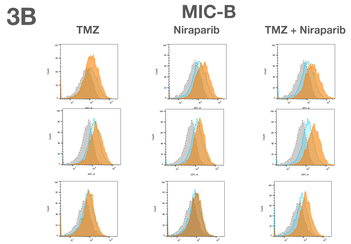
Upregulation of NKG2DL expression in 3 OVCA cell lines after 24hr. single treatment of TMZ (200 µM), Niraparib (5 µM), or combination treatment of TMZ (200 µM) + Niraparib (5 µM).

3A



Grey dotted: Isotype; Cyan filled: DMSO; Red filled: Drug treatment

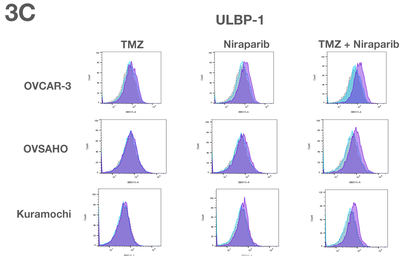
3B



Grey dotted: Isotype; Cyan filled: DMSO; Orange filled: Drug treatment

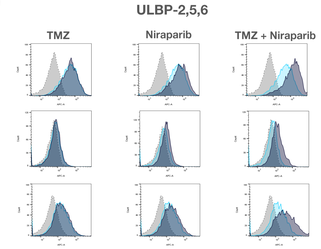
Results

3C



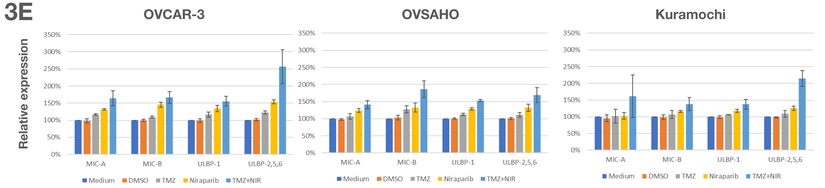
Grey dotted: Isotype; Cyan filled: DMSO; Purple filled: Drug treatment

3D



Grey dotted: Isotype; Cyan filled: DMSO; Black filled: Drug treatment

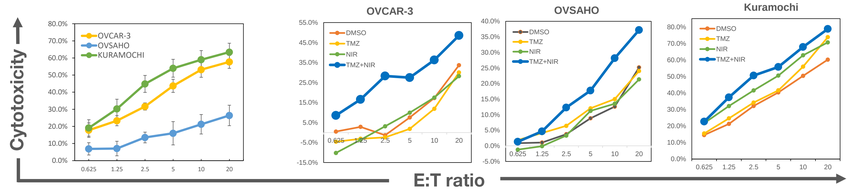
3E



EAGDT demonstrated varied baseline cytotoxicity against OVCA cell lines in a 36hr. co-culture assay. Following treatment with TMZ, niraparib, or combination for 24hr, EAGDT cells showed enhanced cytotoxicity against OVCA cells treated with combination of TMZ + niraparib.

4A EAGDT vs. OVCA w/o treatment: 36h coculture

4B EAGDT vs. OVCA with drug treatment: 24h coculture



Conclusions

- TMZ alone has little effect on the viability of OVCA cells up to 300µM; Combination of PARPi + low dose TMZ reduces viability of OVCA cells significantly more compared to Niraparib treatment alone in 3/5 OVCA lines tested.
- TMZ and PARPi combination treatment significantly upregulates NKG2DL expression in OVCA lines with synergistic effect. OVCA cells with combination treatment are more sensitive to killing by expanded and activated γδ T cells.
- We believe OVCA is a potential candidate extracranial tumor indication for treatment with concurrent infusion of chemo-protected γδ T cells in combination with TMZ + PARPi.