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Abstract: 3510

IDH1-R132H tumor cells are not robustly sensitive to PARP inhibition in a 2-HG–dependent manner

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BACKGROUND

- Mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) 1 or 2 arise in a variety of malignancies and lead to the production of the oncometabolite (D)-2-hydroxyglutarate (2-HG).[1,2]
- FDA approval of the mutant IDH (mIDH) 1 and 2 inhibitors ivosidenib (IVO; AG-120) and enasidenib (AG-221), for the treatment of adults with mIDH1 or mIDH2 relapsed or refractory acute myeloid leukemia (AML), underscores the clinical benefit of blocking the production of 2-HG.
- Parallel investigations have suggested that IDH1/2 mutation leads to a "BRCAness" phenotype and sensitivity to poly(ADP-ribose) polymerase (PARP) inhibition via a reduced capability for DNA damage repair owing to high levels of 2-HG.[3,4]
- PARP inhibition has been proposed as an alternative strategy for the treatment of mIDH1/2 tumors, with the associated hypothesis that mIDH1/2 inhibition may lead to a loss of sensitivity to PARP inhibition.[3,4]

OBJECTIVES

- To investigate the relationship between 2-HG and DNA damage and repair in mIDH1 tumor cells.
- To assess the sensitivity of mIDH1 cells to PARP inhibition *in vitro* and *in vivo*.
- To investigate the potential for antagonism between IVO and PARP inhibitors in mIDH1 cells and mouse xenografts.

METHODS

- Mutant and corresponding parental control cell lines were purchased from Horizon Discovery:
  - HCT-116 human colon carcinoma cells heterozygous for a knock-in IDH1-R132H mutation
  - DLD-1 human colorectal adenocarcinoma cells with BRCA2–/– knock-out.
- THP-1 (acute monocytic leukemia) and U87MG (glioblastoma) cells were stably transduced using lentiviral constructs encoding for IDH1-R132H or an empty vector (EV) control.
- 2-HG levels were measured by liquid chromatography-mass spectrometry in all cell lines and are summarized in **Table 1**.
- Levels of DNA damage were measured by immunofluorescence of γH2Ax foci:
  - γH2Ax immunofluorescence staining was performed using anti-phospho-histone H2A.X (Ser139) antibody, clone JBW301 (Millipore, Ref: 05-636). Cells were classified as positive for DNA damage when ≥10 foci per nucleus were counted.
  - Foci quantification was performed using the FindFoci plugin for ImageJ.[5]
- Total and phosphorylated ATM and H2Ax protein levels were estimated by western blot. Acute bleomycin treatment (10 μM for 1 hr) was used as a positive control for DNA damage signaling induction.
- Baseline homologous recombination (HR) activity was estimated by GFP reporter assays (TopoGEN).

Table 1. 2-HG levels across cell lines

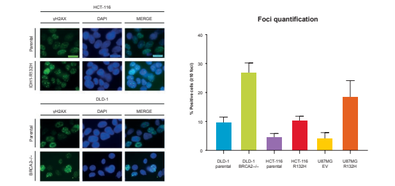
	2-HG level, mean (SD), ng/1 × 10⁴ cells
DLD-1 parental	169.25 (3.03)
DLD-1 BRCA2–/–	47.55 (2.75)
HCT-116 parental	5.38 (0.07)
HCT-116 IDH1-R132H	968 (98.36)
U87MG EV	48.9 (3.37)
U87MG IDH1-R132H	2090 (75.87)
THP-1 EV	6.8 (0.23)
THP-1 IDH1-R132H	400 (6.06)

RESULTS

mIDH1 cells show increased baseline DNA damage

- Solid tumor mIDH1 cell lines showed an increased percentage of foci-positive cells compared with WT cells, reproducing previously published data (**Figure 1**).[3]

Figure 1. mIDH1 protein expression leads to increased baseline DNA damage

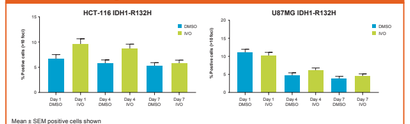


Mean + SEM positive cells shown

Treatment of mIDH1 cells with IVO fails to reduce DNA damage

- mIDH1 HCT-116 and U87MG cells were treated with 1 μM IVO for 1, 4, and 7 days. 2-HG levels were reduced by >90% after treatment for 24 hr.
- In contrast to previous publications,[3] IVO treatment had little to no effect on DNA damage levels (**Figure 2**).

Figure 2. Reduction of 2-HG by mIDH1 inhibition has little to no effect on the DNA damage observed

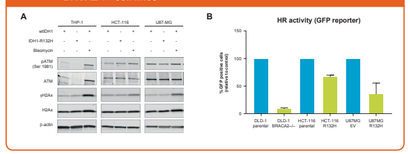


Mean ± SEM positive cells shown
DMSO = dimethyl sulfoxide

mIDH1 cells have reduced baseline HR activity

- As previously reported,[6] total ATM levels were reduced in THP-1 cells expressing mIDH1. No effect on ATM was observed in HCT-116 and U87MG cells (**Figure 3A**).
- mIDH1 cells demonstrated decreased efficiency of HR repair compared with IDH WT cells (**Figure 3B**). HR activity was reduced by 90% in DLD-1 BRCA2–/– cells compared with the isogenic BRCA2+/+ parental cells (**Figure 3B**).

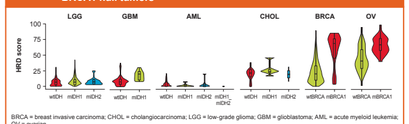
Figure 3. Estimate of DNA repair protein levels and HR activity in mIDH1 or BRCA2–/– cell lines



Exome sequencing of tumor samples does not reveal greater HR deficiency in mIDH1 tumors

- Analysis of exome sequencing data can identify genomic 'scars' indicative of HR deficiency (HRD). The TCGA PanCancer Atlas study reported an 'HRD score' based on the sum of:[7]
  - HRD loss of heterozygosity[8]
  - Large-scale state transitions[9]
  - NtAI (number of telomeric allelic imbalances).[10]
- HRD scores for IDH mutant tumor samples are not in the same range as those for BRCA1 null tumors (**Figure 4**).

Figure 4. Mutant IDH tumors fail to recapitulate the HRD score observed in BRCA1 null tumors



BRCA = breast invasive carcinoma; CHOL = cholangiocarcinoma; LGG = low-grade glioma; GBM = glioblastoma; AML = acute myeloid leukemia; OV = ovarian

mIDH1 cells show discordant sensitivity to PARP inhibitors *in vitro*

- Little to no sensitivity to talazoparib (TALA), olaparib (OLA), and niraparib (NIRA) was observed in mIDH1 cells based on 7-day CellTiter Glo (CTG) assay (OLA data shown in **Figure 5**). IC_{50} values for all compounds and cell lines are summarized in **Table 2**.
- In contrast to CTG assays, mIDH1 cells showed enhanced sensitivity to PARP inhibition in clonogenic survival assays (**Figure 6**), as previously reported.[3]

Figure 5. Mutant IDH1 cells were insensitive to PARP inhibition as determined by cell viability following treatment for 7 days

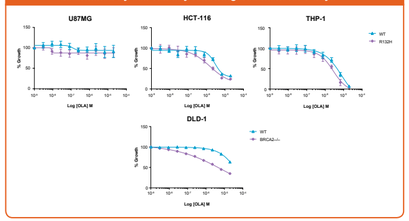
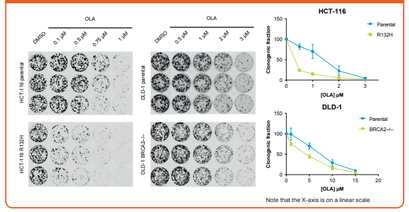


Table 2. IC_{50} values in IDH1 WT and mutant cell lines treated with PARP inhibitors

Cell line	TALA GI_{50}, μM		OLA GI_{50}, μM		NIRA GI_{50}, μM	
	Parental	IDH1-R132H	Parental	IDH1-R132H	Parental	IDH1-R132H
HCT-116	<0.001	<0.001	0.62	0.56	2.85	1.55
U87MG	>20	>20	>20	>20	>20	>20
THP-1	0.22	0.16	1.4	0.85	8.08	2.7
	Parental	BRCA2–/–	Parental	BRCA2–/–	Parental	BRCA2–/–
DLD-1	0.93	~0.01[a]	8.07	0.85	>20	8.82

[a]Ambiguous owing to the slope of the curve
GI_{50} = drug concentration causing 50% reduction in cell proliferation

Figure 6. Mutant IDH1 cells are sensitive to PARP inhibition in clonogenic growth assays



Note that the X-axis is on a linear scale

Table 3. 50% Clonogenic survival values in IDH1 WT and mutant cell lines treated with PARP inhibitors

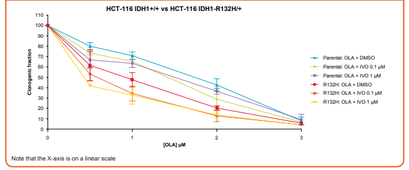
Cell line	TALA SF_{50}, μM		OLA SF_{50}, μM		NIRA SF_{50}, μM	
	Parental	IDH1-R132H	Parental	IDH1-R132H	Parental	IDH1-R132H
HCT-116	0.0042	0.0017	0.68	0.17	1.2	0.33
	Parental	BRCA2–/–	Parental	BRCA2–/–	Parental	BRCA2–/–
DLD-1	0.0217	0.0091	2.4	0.48	6.92	3.39

SF_{50} = 50% survival fraction (concentration that inhibits cell survival to 50%)

IVO does not reverse sensitivity to PARP inhibition in HCT-116 IDH1-R132H cells

- 14-day clonogenic assays were performed in HCT-116 IDH1 WT and mutant cells combining increasing concentrations of PARP inhibitors with 0.1 or 1 μM IVO (**Table 4**).
- IVO treatment of mIDH1 cells did not reverse sensitivity to PARP inhibition in clonogenic growth assays (**Figure 7**), in contrast to previously published work.[3]

Figure 7. mIDH1 inhibition does not reverse sensitivity to PARP inhibition in clonogenic growth assays



Note that the X-axis is on a linear scale

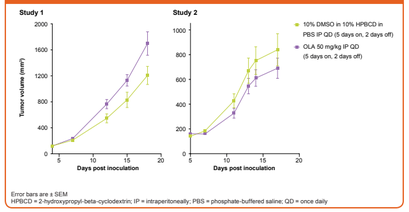
Table 4. 50% Clonogenic survival values in IDH1 WT and mutant cell lines treated with PARP inhibitors in combination with IVO

Cell line	TALA SF_{50}, nM	NIRA SF_{50}, μM	OLA SF_{50}, μM
HCT-116 WT			
+ DMSO	4.20	0.83	1.60
+ 0.1 μM IVO	3.65	0.65	1.25
+ 1 μM IVO	3.84	0.72	1.19
HCT-116 R132H			
+ DMSO	2.66	0.57	0.79
+ 0.1 μM IVO	1.75	0.29	0.58
+ 1 μM IVO	1.71	0.28	0.42

HT-1080 IDH1-R132C/+ subcutaneous mouse xenografts are insensitive to PARP inhibition alone

- Tumor growth inhibition upon treatment with a PARP inhibitor was not observed in two independent studies in HT-1080 IDH1-R132C/+ subcutaneous mouse xenografts (**Figure 8**).
  - Study 1 was conducted as a prophylactic treatment model consistent with previous reports describing OLA sensitivity in HT-1080 mouse xenografts.
  - Study 2 was conducted as an established tumor model assessment of OLA sensitivity in HT-1080 mouse xenografts.
- OLA plasma exposures were analyzed and found to be within the expected concentration range (**Table 5**).

Figure 8. HT-1080 IDH1-R132C/+ subcutaneous mouse xenografts are insensitive to PARP inhibition alone



Error bars are ± SEM
HPBCD = 2-hydroxypropyl-beta-cyclodextrin; IP = intraperitoneally; PBS = phosphate-buffered saline; QD = once daily

Table 5. Summary of plasma OLA exposure and tumor 2-HG production across HT-1080 studies

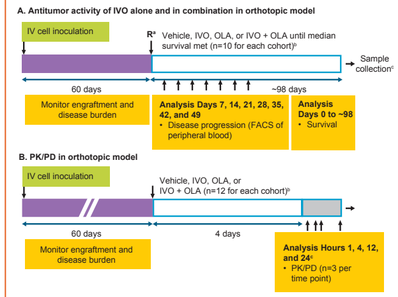
Study	Analyte	Sample	Treatment	AUC_{0-24hr} mean (SD)
Study 1	2-HG	Tumor	Vehicle	1.92 (0.11) × 10^7
			OLA	1.87 (0.13) × 10^7
	OLA	Plasma	OLA	1.21 (0.29) × 10^4
Study 2	2-HG	Tumor	Vehicle	1.75 (0.03) × 10^7
			OLA	1.41 (0.09) × 10^7
	OLA	Plasma	OLA	2.28 (0.26) × 10^4

AUC = area under the curve for plasma (hr•ng/mL) or tumor (hr•ng/g)

IVO/PARP inhibitor combination shows superior activity over single agents in an IDH1-R132H+ AML patient-derived xenograft (PDX)

- Reduction of 2-HG (>90%) by IVO led to survival benefit compared with vehicle (**Figure 10**).
- OLA treatment alone led to survival benefit compared with vehicle treatment alone.
- Combination of IVO and OLA provided added survival benefit compared with either treatment alone.
- Survival correlated with the onset of increased disease burden in peripheral blood (hCD45+ AML PDX cells).

Figure 9. *In vivo* study designs for assessing efficacy and PK



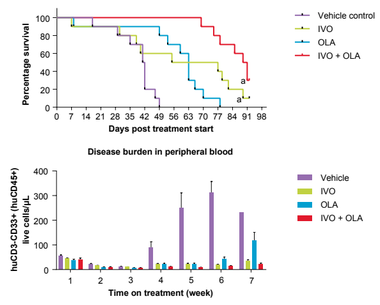
[a]Animals were randomized on the basis of AML PDX cells/μL in peripheral blood
[b]Doses were IVO 450 mg/kg QD orally, OLA 50 mg/kg QD IP 5 days on and 2 days off
[c]Upon termination, bone marrow, spleen, and plasma were collected
FACS = fluorescence-activated cell sorting; IV = intravenous; PD = pharmacodynamics; PK = pharmacokinetics; R = randomized

Table 6. Summary of PK/PD analysis in plasma, spleen, and bone marrow following 4 days of treatment

Dose group	Analyte	Dose, mg/kg	Plasma	Spleen		Bone marrow	
			AUC_{0-24hr} mean (SD)	AUC_{0-24hr} mean (SD)	% 2-HG inhibition*	AUC_{0-24hr} mean (SD)	% 2-HG inhibition*
IVO	2-HG	–	–	9.36 (0.39) × 10^5	96	1.62 (0.32) × 10^5	94
	IVO	450	7.67 (1.77) × 10^6	8.84 (1.74) × 10^4	–	4.21 (0.85) × 10^4	–
IVO + OLA	2-HG	–	–	6.76 × 10^5	97	2.73 (0.05) × 10^5	90
	IVO	450	2.00 (0.86) × 10^6	3.36 × 10^5	–	1.44 (0.22) × 10^5	–
	OLA	50	8.8 (6.1) × 10^5	2.19 × 10^4	–	1.03 (0.10) × 10^4	–
OLA	2-HG	–	–	1.78 (0.18) × 10^7	23	2.48 (0.38) × 10^6	8
	OLA	50	1.52 (0.51) × 10^6	3.89 (3.54) × 10^5	–	4.1 (0.90) × 10^3	–
Vehicle	2-HG	–	–	2.32 (0.18) × 10^7	–	2.63 (0.75) × 10^6	–

*Compared with vehicle

Figure 10. Ivosidenib/PARP inhibitor combination shows superior activity to single agents alone



AML PDX model genetics: IDH1-R132H; NPM1 W288fs*12; DNMT3A A576fs; FLT3-ITD+
Error bars are + SEM
Disease burden in peripheral blood monitored by weekly survival bleeds
[a]Median survival met

Table 7. Median survival

Treatment	Median survival (days post treatment start)
Vehicle	41.5
IVO	66
OLA	63
IVO + OLA	90
Survival comparison (Mantel-Cox test)	**p-value**
Vehicle vs IVO	0.035
Vehicle vs OLA	0.0002
Vehicle vs combination	<0.0001
IVO vs combination	0.0179
OLA vs combination	<0.0001
IVO vs OLA	Not significant

CONCLUSIONS

- In contrast to published reports, reduction of 2-HG by IVO has little to no effect on baseline DNA damage.
- mIDH1 cells show reduced HR activity *in vitro* compared with IDH1 WT cells. However, these findings were not comparable to a BRCA-deficient phenotype.
- Exome sequencing analysis did not identify the presence of HRD marks in mIDH1 tumors across different indications.
- *In vivo* experiments in HT-1080 xenografts demonstrated no sensitivity to single-agent PARP inhibitor treatment, in contrast to previously published data.
- Combined treatment of an mIDH1 AML PDX with IVO and a PARP inhibitor led to a significant survival benefit compared with either treatment alone.

Disclosures
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