2X-121/ stenoparib – a novel, dual inhibitor of PARP and tankyrase in phase 2 clinical trials in advanced ovarian cancer- blocks the WNT signaling pathway and inhibits growth of human colorectal cancer cell lines at clinically relevant drug concentrations

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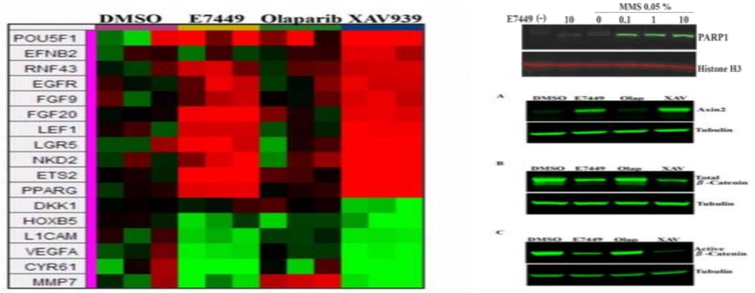
Abstract

2X-121 (stenoparib/ E7499) is a novel inhibitor of PARP1/2 (1nM ~IC50) and Tankyrase 1/2 (IC50 ~50nm). As such, 2X-121 impairs DNA repair while simultaneously inhibiting the WNT/ß-catenin oncogenic signaling pathway. 2X-121 has shown durable clinical benefit in a phase 2 study in patients with advanced, platinum resistant and refractory ovarian cancer as a single agent dosed twice daily, regardless of BRCA status. A new protocol is currently enrolling platinum resistant or ineligible ovarian cancer patients to further deepen the clinical understanding of 2X-121 mediated clinical benefit.

The clinical experience in ovarian cancer patients has shown benefit in BRCA^{wt} patients- patients who typically do not show durable clinical benefit from first generation PARP inhibitors. These data may suggest that, in addition to PARP 1/2 inhibition, the added activity of 2X-121 inhibiting tankyrase and the WNT pathway may contribute to the therapeutic mechanism of action for 2X-121. Accordingly, 2X-121 may be therapeutically useful as an inhibitor of the WNT pathway for cancers not typically sensitive to PARP 1/2 inhibition. Colorectal cancers are not typically sensitive to PARP1/2 inhibition. However, approximately 80% of colorectal cancers (CRCs) do show mutational activation of the canonical WNT pathway, which may impart resistance to standard chemotherapy. Moreover, WNT pathway activation may also enable a cancer initiating cell/ cancer stem cell-like phenotype enabling the cellular plasticity that often characterizes advanced malignancies.

We therefore sought to explore the therapeutic activity of 2X-121 in a panel of CRC cell lines chosen for a spectrum of WNT pathway activating mutations. We show that 2X-121 inhibits growth of multiple colorectal cancer cell lines in monolayer and 3D culture conditions. 2X-121 also inhibits the WNT pathway, stabilizing Axin, reducing activated beta-catenin, and generally blocking WNT pathway activation in CRC cell lines harboring TCF-LEF reporters. The reduction in cell number following 2X-121 treatment may reflect both cytostasis and direct cell killing. Importantly, these effects are evident at clinically relevant drug concentrations for 2X-121. Collectively, these data provide the foundation to explore the clinical potential of 2X-121 in colorectal cancers as well as other cancers where WNT pathway activation is prevalent.

2X-121: a Dual PARP and WNT Pathway Inhibitor



Left Panel shows gene expression profiles for 2X-121/ E7449 compared to DMSO control, the PARP inhibitor Olaparib and the Tankyrase inhibitor XAV939. The Right Panel Top shows PARP trapping by 2X-121/ E7499 from DT40 cells at the indicated concentrations. The remaining three panels on the right show western blots for WNT pathway components - Axin and Beta catenin- in SW480 cells. All were taken from treatment with 10uM drug as indicated. Data from McGonicle et al. 2015. E7449: A dual inhibitor of PARP1/2 and tankyrase1/2 inhibits growth of DNA repair deficient tumors and antagonizes Wnt signaling. Oncotarget. 6(38):41307-23.

2X-121 Plasma Concentrations Phase 1 Solid Tumor Pts

C_{max} (50 mg)	265 ng/mL	0.835 µM
C_{max} (100 mg)	284 ng/mL	0.895 µM
C_{max} (200 mg)	996 ng/mL	3.140 µM
C_{max} (400 mg)	999 ng/mL	3.149 µM
C_{max} (600 mg)	2250 ng/mL	7.092 µM
C_{max} (800 mg)	4430 ng/mL	13.964 µM
$AUC_{(0-24)}$ (50 mg)	768 ng*h/mL	2.421 µmol*h/L
$AUC_{(0-24)}$ (100 mg)	879 ng*h/mL	2.771 µmol*h/L
$AUC_{(0-24)}$ (200 mg)	3670 ng*h/mL	11.569 µmol*h/L
$AUC_{(0-24)}$ (400 mg)	4690 ng*h/mL	14.784 µmol*h/L
$AUC_{(0-24)}$ (600 mg)	4690 ng*h/mL	14.784 µmol*h/L
$AUC_{(0-24)}$ (800 mg)	7930 ng*h/mL	24.997 µmol*h/L

Plasma concentrations from a Phase 1 clinical study in patients with Pancreatic, Breast, lung, colorectal and other solid cancers. Data from a single dose at the indicated concentrations. Data derived from Plummer et al., 2020. British J Cancer 123: 525-533.

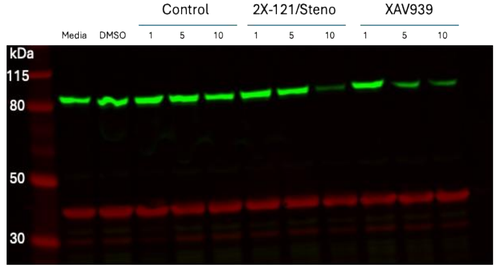
2X-121 Inhibits Proliferation of Colorectal Cancer Cells

Cell Line	Doubling Time (h)	MSI/MSS	WNT path	PIK3CA	TP53	KRAS	BRAF	Stenoparib - IBRI	XAV939	JPI-547	Olaparib	Niraparib
								IC50 (uM)				
Colo205	28	MSS	APC; bCat	wt	fs		V600E	6.3	>10	>10	9	1.1
Colo320	30	MSS	APC	wt	ms			4.3	>10	>10	>10	4.1
DLD-1	36	MSI	APC	E545K	S241F	G13D		7.5	>10	4.8	>10	7.6
HCT15	31	MSI	APC	E545K	R273H		G596R	5.7	>10	>10	>10	3.6
HCT116	24	MSI	bCat	H1047R		G13D		4.7	>10	ND	6.5	ND
HT29	22	MSS	APC	P449T	R273H		V600E	3.4	>10	ND	>10	ND
LoVo	28	MSI	APC	wt		G13D		>10	>10	>10	>10	>10
LS174T	28	MSI	bCat	H1047R		G12D		2.7	>10	ND	3.7	ND
RKO	27	MSI	n/a	H1047R			V600E	6.2	>10	ND	>10	ND
SW480	91	MSS	APC	wt	R273H	G12V		4.0	>10	5.5	>10	4.7
SW620	74	MSS	APC	wt	R273H	G12V		2.3	>10	8.4	6.0	0.7

Colorectal Cancer Cell Line Panel. Data from cell treatment for 8 days with the indicated PARP inhibitors Olaparib and Niraparib as well as the Tankyrase inhibitors XAV939 and JPI-547. Proliferation was assessed by Cell Titre Glo assays (Promega).

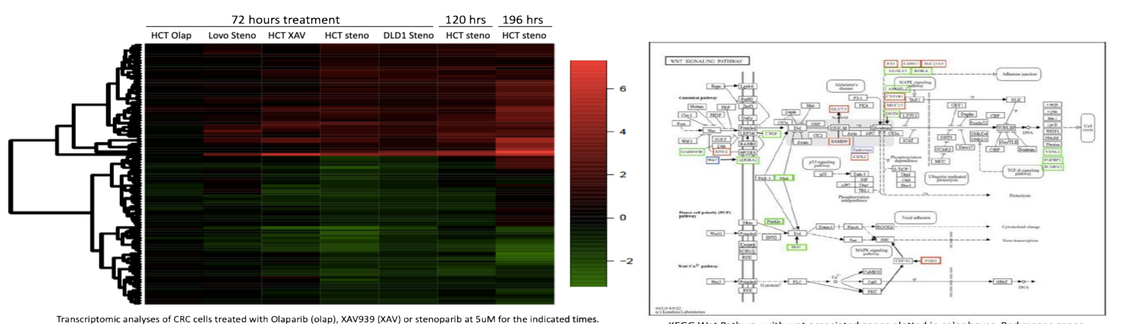
2X-121/ Stenoparib Inhibits the WNT/ Beta Catenin Pathway in CRC Cells

Active (non-phosphorylated) Beta Catenin



Western Blot Analyses. SW620 Colorectal Cancer Cells treated 24 hours at the indicated concentrations (in uM) with controls (media, DMSO or inactive control), 2X-121/ stenoparib or the Tankyrase specific inhibitor control (XAV939). Active Beta-Catenin (green) and GAPDH (red).

Transcriptional Analyses Reveal WNT Pathway Modulation



Transcriptomic analyses of CRC cells treated with Olaparib (olap), XAV939 (XAV) or stenoparib at 5uM for the indicated times. Heatmap of log2 fold changes of genes under different conditions. Fold change is calculated relative to DMSO control. A color bar indicates the scale of log2 fold change. A dendrogram groups genes according to similarity of fold change.

KEGG Wnt Pathway with wnt-associated genes plotted in color boxes. Red means genes upregulated by stenoparib, green means genes downregulated by stenoparib.

TCF-LEF Promoter Reporter Activity (Colo320 72 hrs)

Conclusions

- 2X-121 inhibits CRC proliferation at clinically achievable drug concentrations
- 2X-121 inhibits PARP1/2 as well as PARP5/ Tankyrase
- Tankyrase is a key regulator of the WNT pathway
- 2X-121 inhibits WNT pathway activity at clinically achievable drug concentrations