

SUPPLEMENTARY MATERIALS

Targeted therapy of pyrrolo[2,3-*d*]pyrimidine antifolates in a syngeneic mouse model of high grade serous ovarian cancer and the impact on the tumor microenvironment

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Supplementary Table S1: Epithelial ovarian cancer (EOC) tissue cDNA array for real-time RT-PCR The cDNA array (Hort502) (Origene, Inc) contained 48 samples, including 8 normal, 8 stage I, 9 stage II, 17 stage III, and 6 stage IV EOC. Age, pathology, diagnosis, tumor grade and stage for each patient are summarized below.

Specimen	Age	Pathology	Diagnosis	Tumor grade	Stage
1	46	Normal	Leiomyoma of myometrium	Not Applicable	0
2	49	Normal	Leiomyoma of myometrium	Not Applicable	0
3	46	Normal	Leiomyoma of myometrium	Not Applicable	0
4	42	Normal	Endometrium, secretory	Not Applicable	0
5	33	Normal	No residual malignancy	Not Applicable	0
6	40	Normal	Cyst of ovary, follicular	Not Applicable	0
7	45	Normal	Endometriosis	Not Applicable	0
8	35	Normal	Endometriosis	Not Applicable	0
9	70	Tumor	Carcinoma of ovary, papillary serous	FIGO G2: Poorly differentiated	IA
10	68	Tumor	Adenocarcinoma of ovary, papillary cell	FIGO G2: Moderately differentiated	IA
11	48	Tumor	Adenocarcinoma of ovary, clear cell	Not Reported	IA
12	58	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G1: Well differentiated	IB
13	55	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IB
14	74	Tumor	Adenocarcinoma of ovary, endometrioid, papillary serous	FIGO G3: Poorly differentiated	IB
15	65	Tumor	Carcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IC
16	58	Tumor	Carcinoma of ovary, endometrioid	FIGO G2: Moderately differentiated	IC
17	78	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIA
18	44	Tumor	Carcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIA
19	73	Tumor	Adenocarcinoma of ovary	FIGO G3: Poorly differentiated	IIA
20	67	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIB
21	67	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIB
22	58	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IIB
23	58	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIB
24	59	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIC
25	63	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIC
26	51	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	III
27	73	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	III
28	91	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	III

29	75	Tumor	Adenocarcinoma of ovary, endometrioid	FIGO G3: Poorly differentiated	IIIA
30	65	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IIIA
31	55	Tumor	Adenocarcinoma of ovary, mucinous	FIGO G2: Moderately differentiated	IIIA
32	66	Tumor	Adenocarcinoma of ovary, serous	FIGO G2: Moderately differentiated	IIIA
33	80	Tumor	Adenocarcinoma of ovary, serous	Not Reported	IIIB
34	64	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIB
35	66	Tumor	Carcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIB
36	52	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIB
37	71	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIC
38	48	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIIC
39	50	Tumor	Carcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IIIC
40	58	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G2: Moderately differentiated	IIIC
41	53	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IIIC
42	66	Tumor	Adenocarcinoma of ovary, endometrioid, papillary serous	FIGO G3: Poorly differentiated	IIIC
43	45	Tumor	Adenocarcinoma of ovary, serous, metastatic	Not Reported	IV
44	80	Tumor	Adenocarcinoma of ovary, endometrioid, papillary serous	FIGO G3: Poorly differentiated	IV
45	68	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IV
46	61	Tumor	Adenocarcinoma of ovary, serous	FIGO G3: Poorly differentiated	IV
47	60	Tumor	Adenocarcinoma of ovary, papillary serous, metastatic	Not Reported	IV
48	63	Tumor	Adenocarcinoma of ovary, papillary serous	FIGO G3: Poorly differentiated	IV

Supplementary Table S2. Primer sequences for C1 genes and housekeeping genes for murine (“m”) and human cells.

Gene	Forward Sequence	Reverse Sequence
Mouse		
mAICARFTase	cccaaacttcccatcacagt	cgttcaaagcgtcacacaag
mGARFTase	acattggcttccgtgctg	tccggagtccttgaagtcaa
mFR	ctcctggtgtgtcacaggatt	ggcttcctttgctttctca
mRFC	gggtctccaagtccgtgat	ccacgtggcagtacctta
mPCFT	taccgggccactctgaact	tccgggtgtggattaacct
m β -actin	ctaaggccaaccgtgaaaag	accagaggcatacagggaca
Human		
β -actin	caaccgcgagaagatgac	gtccatcacgatgccagt
GARFTase	ccctgagaaaacttggggtaga	ctgctgcaacctgagaaaa
AICARFTase	gagggactgcaaaagctctc	ggaaatcccgtcaactcaga

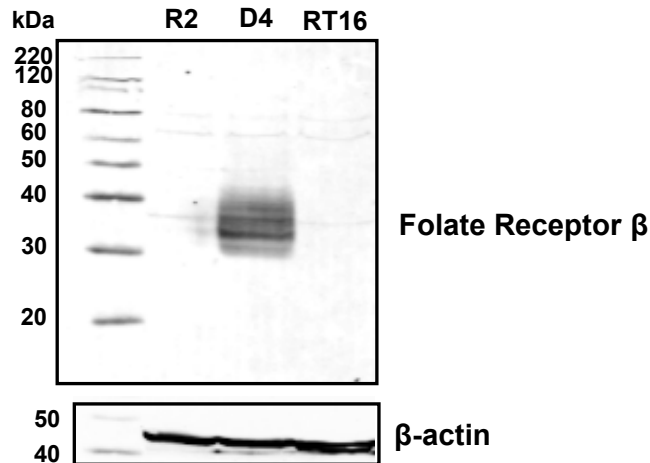


Figure S1. Folate Receptor (FR) β specificity by western blot of FR β -expressing Chinese hamster ovary cells. Chinese hamster ovary cells null for FRs (R2) or expressing FR β (D4) or FR α (RT16) were measured from a particulate membrane fraction by SDS polyacrylamide gel electrophoresis and Western blotting probed with FR β -specific antibody (Genetex, Catalog: GTX105822); detection was with IRDye700CW-conjugated goat anti-rabbit IgG secondary antibody (LI-COR Biosciences, Lincoln, NE). Membranes were scanned with an Odyssey® infrared imaging system (LI-COR Biosciences, Omaha, NE). Protein loading was normalized to levels of β -actin using anti- β -actin mouse antibody (Sigma-Aldrich).

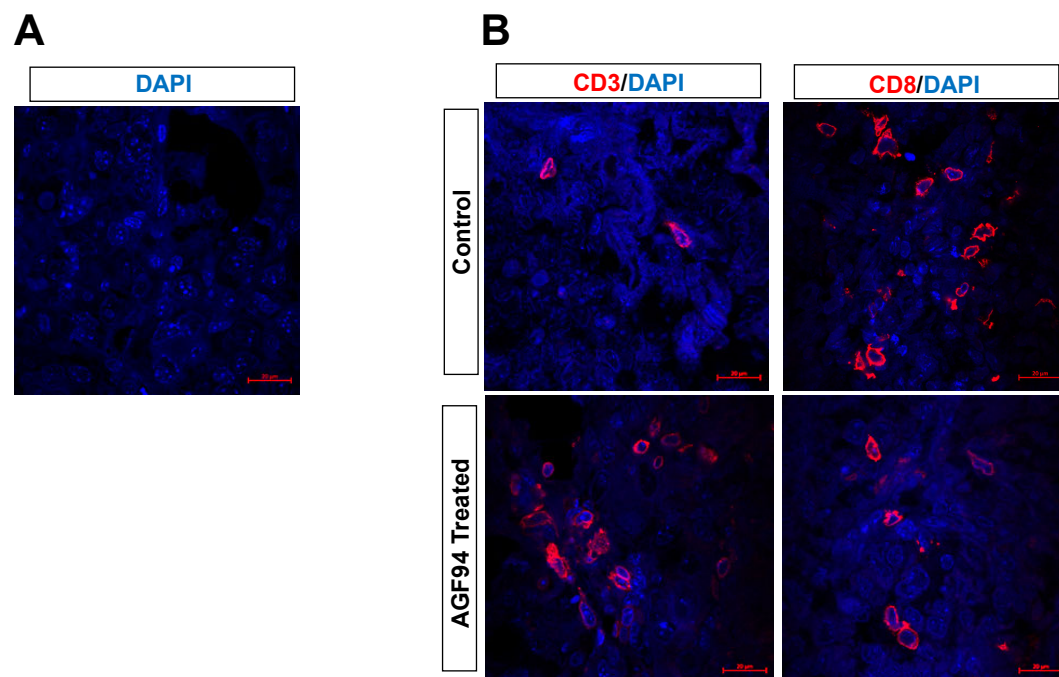


Figure S2. Negative control for immunofluorescence and immunofluorescence staining for CD3 and CD8. Panel A. Control immunofluorescence staining with secondary antibody (anti-Rabbit IgG (H+L) Alexa Fluor 647 (1:200, Thermo Fisher, Catalog# A-21245). Panel B. Immunofluorescence staining for CD3 (Cell Signaling) and CD8 (Cell Signaling) with goat anti-rabbit IgG (H+L) Alexa Fluor 647 as described in the Materials and Methods. Scale bar set to 20 μm.