Supplementary information for

Therapeutic advantage of targeting PRMT5 in combination with chemotherapies or EGFR/HER2 inhibitors in triple-negative breast cancers

Rayan Dakroub [1,2], Solène Huard [1], Yara Hajj-Younes [1], Samyuktha Suresh[1], Bassam Badran [2], Hussein Fayyad-Kazan [2], and Thierry Dubois [1]

[1] Breast Cancer Biology Group, Translational Research Department, Institute Curie-PSL Research University, 75005 Paris, France

[2] Laboratory of Cancer Biology and Molecular Immunology, Faculty of Sciences-I, Lebanese University, Hadath 1003, Lebanon

Correspondence: Thierry Dubois

Breast Cancer Biology Group, Translational Research Department, Institute Curie-PSL Research University, 75005 Paris, France

Te+33 156246250

Email: thierry.dubois@curie.fr

This PDF file includes:

Supplementary Tables S1 to S3

Supplementary Figures S1 to S5

Table S1: Doubling time of the breast cell lines, and the assays used to measure proliferation

Cell line	Doubling Time (hours)	Treatment duration (days)	Proliferation assay
MCF10A	16	3	MTT
T47D	43	7	MTT
MCF7	45	7	MTT
HCC1954	40	7	MTT
BT474	40	7	MTT
HCC38	60	7	WST-1
MDA-MB-453	55	7	MTT
MDA-MB-468	45	7	MTT
BT20	50	7	MTT
HCC70	50	7	MTT
MDA-MB-231	25	4	CTG

Abbreviations: CTG, CellTiter-Glo luminescent cell viability assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-based assay; WST-1, water-soluble tetrazolium salt based assay.

Table S2: Sensitivity of breast cell lines to EPZ015938 and EPZ015666

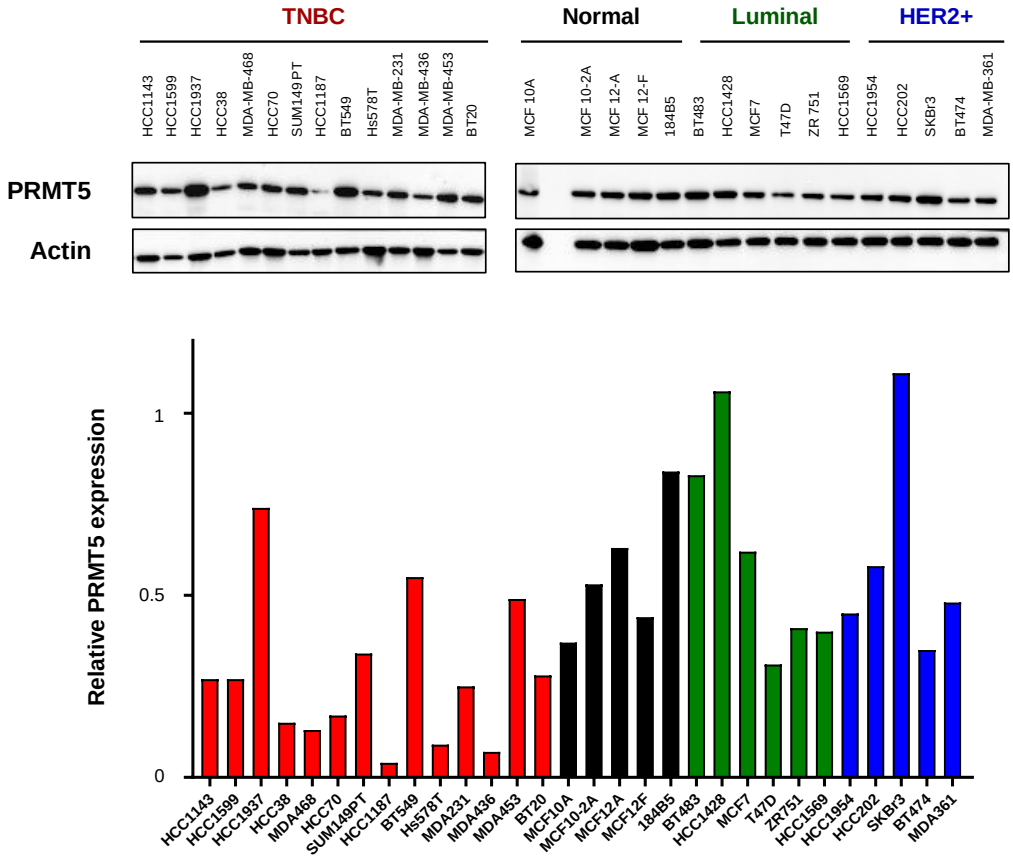
Cell line	Classification	EPZ015938 IC_{50} (nM ± SD) This study	EPZ015666 IC_{50} (nM ± SD) From Ref. 23
MCF10A	Normal epithelial	722.8 ± 122	42684 ± 14200
MCF12A	Normal epithelial	ND	47078 ± 11200
T47D	Luminal	304 ± 244.8	ND
MCF7	Luminal	191.5 ± 47	2642 ± 1010
HCC1954	HER2-positive	54.2 ± 19.4	812 ± 140
SKBr3	HER2-positive	ND	3939 ± 1930
BT474	HER2-positive	625.5 ± 217.6	ND
HCC38	TNBC (BL1)	21.9 ± 8.7	2770 ± 1430
MDA-MB-453	TNBC (LAR)	109.4 ± 13.4	985 ± 256
MDA-MB-468	TNBC (BL1)	319.3 ± 226.2	2224 ± 909
BT20	TNBC (unclassified)	>1000	12316 ± 7800
HCC70	TNBC (BL2)	>1000	29852 ± 5650
MDA-MB-231	TNBC (M)	>1000	ND
MDA-MB-157	TNBC (M)	ND	33365 ± 1729
Hs578T	TNBC (M)	ND	67847 ± 17600

Abbreviations: BL1, basal-like 1; BL2, basal-like 2; IC_{50}, half-maximal inhibitory concentration; LAR, luminal androgen receptor; M, mesenchymal; ND, not determined; SD, Standard deviation; TNBC, Triple-negative breast cancer.

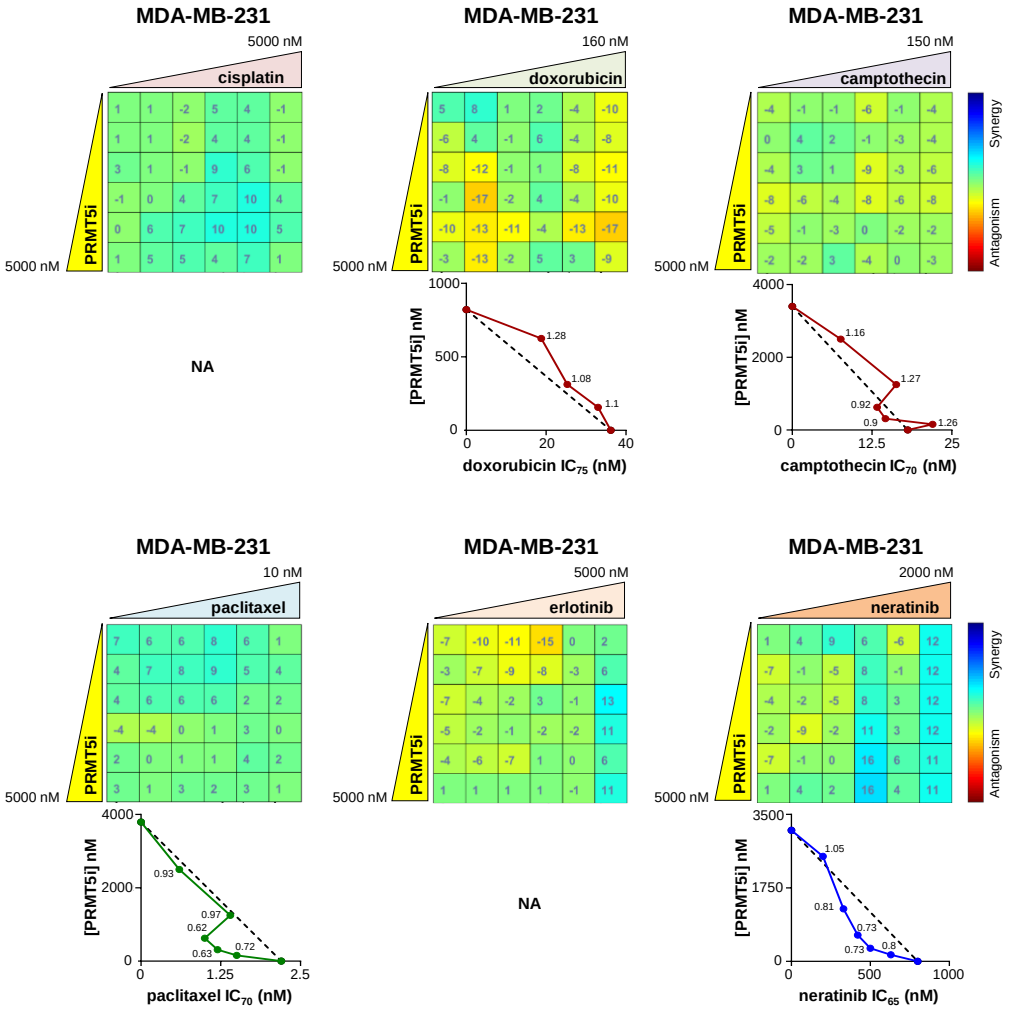
Table S3: IC_{50} of the inhibitors used in the study

	IC_{50} (nM ± SD)				
	BT20	MDA-MB-231	MDA-MB-468	MDA-MB-453	HCC1954
Neratinib	1502 ± 519	918.3 ± 478	30.9 ± 14	22.6 ± 10	14.2 ± 1.8
Tucatinib	>5000	ND	>5000	431.2 ± 162	508.6 ± 61
Erlotinib	2379 ± 911	>5000	1950 ± 490	>5000	ND
Cisplatin	155.7 ± 56.5	>5000	133.8 ± 49.5	949.1 ± 340	ND
Camptothecin	6.2 ± 2.1	53.8 ± 15	7.7 ± 2.9	6.9 ± 0.4	ND
Paclitaxel	1.39 ± 0.5	5.8 ± 3	5.4 ± 0.4	1.05 ± 0.2	ND
Doxorubicin	7.08 ± 1.2	0.07 ± 0.03	19.9 ± 6.7	0.04 ± 0.01	ND

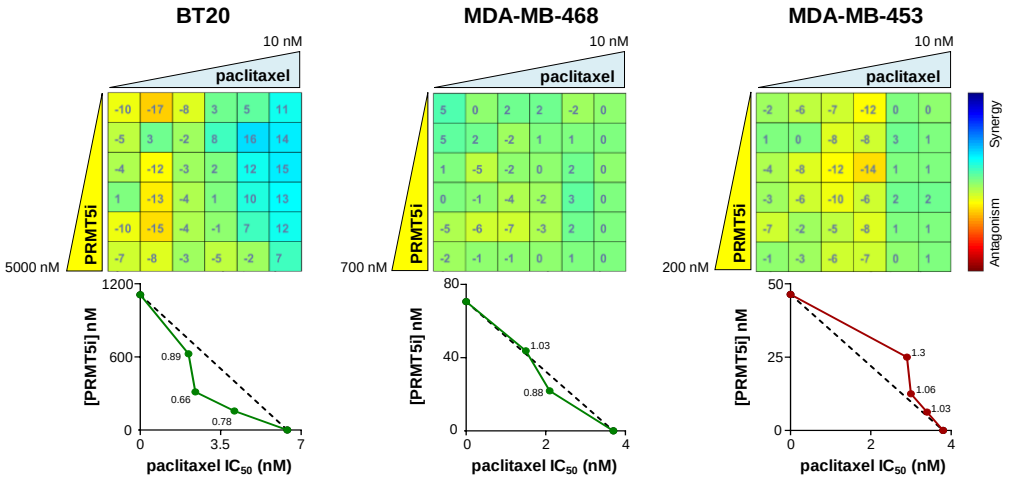
Abbreviations: IC_{50}, half-maximal inhibitory concentration; ND, not determined; SD, Standard deviation.



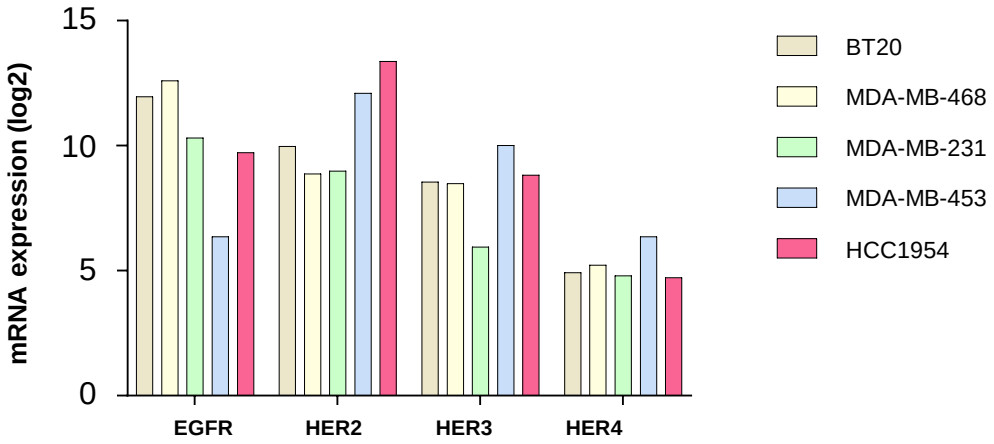
Supplementary figure 1. PRMT5 expression in breast cell lines. PRMT5 expression was assessed in breast cancer cells of the different subtypes (TNBC, luminal, HER2-positive) and normal breast cell lines by western blotting. Actin was used as a loading control. The graphs represent the relative amount of PRMT5 normalized to actin. Noteworthy, the levels of actin may vary across the different cell lines.



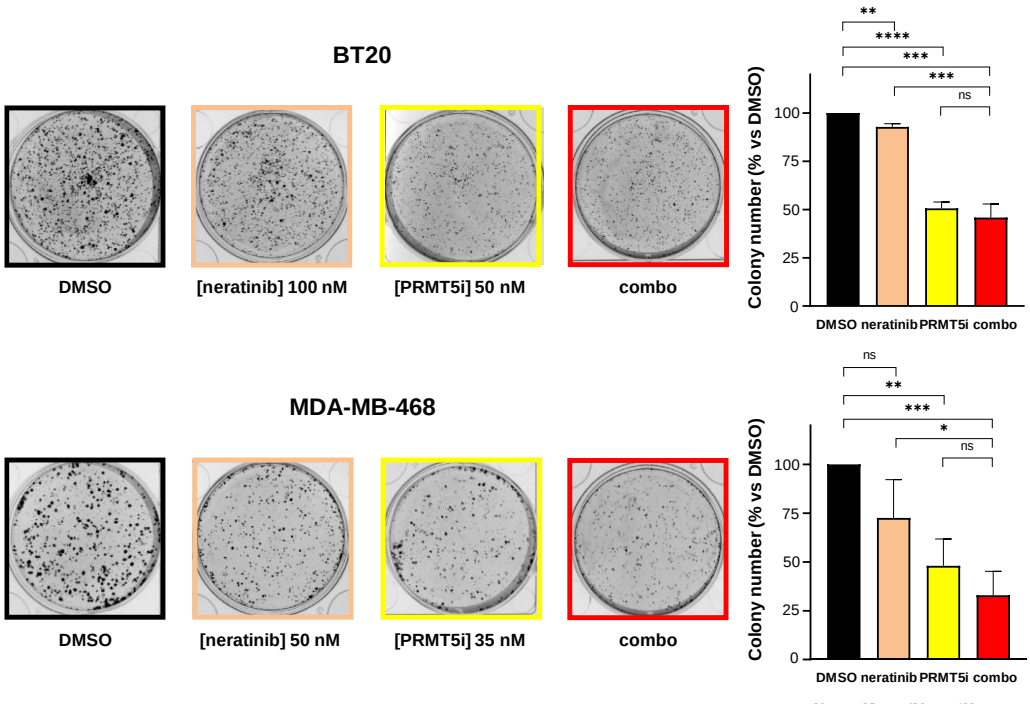
Supplementary figure 2. Effect of the inhibition of PRMT5 in combination with chemotherapies, erlotinib and neratinib on MDA-MB-231 cell proliferation. MDA-MB-231 TNBC cells were seeded in 96-well plates and treated with varying concentrations of EPZ015938 (PRMT5i) and/or cisplatin, doxorubicin, camptothecin, paclitaxel, erlotinib, or neratinib, then cell proliferation was measured after four mitotic cycles (4 days). Percentage of viable cells was normalized to DMSO or DMSO + H2O (when cisplatin was used)-treated cells. Each drug was used at a maximal concentration of 2xIC_{50} or 5 µM maximum when MDA-MB-231 are resistant to the inhibitor, followed by two-fold serial dilutions. The nature of drug interaction between EPZ015938 and the different inhibitors was assessed using the Loewe model on the Combenefit software. The synergy matrix (upper panel) and isobologram (bottom panel) for each combination are shown. The isobolograms represent the IC_{60} of neratinib, IC_{70} of paclitaxel and camptothecin or IC_{75} of doxorubicin (X-axis) obtained at various EPZ015938 concentrations (Y-axis). CI were calculated at the different EPZ015938 concentrations used and are shown on the isobolograms. Isobolograms of the combination between EPZ015938 and cisplatin or erlotinib could not be plotted as the treatment with cisplatin or erlotinib alone did not impair cell viability more than 20% and are indicated as NA (not applicable). Data are representative of at least three independent experiments.



Supplementary figure 3. Effect of the inhibition of PRMT5 in combination with paclitaxel on TNBC cell proliferation. BT20, MDA-MB-468, and MDA-MB-453 TNBC cells were seeded in 96-well plates and treated with varying concentrations of EPZ015938 (PRMT5i) and/or paclitaxel, then cell proliferation was measured after four mitotic cycles (7 days). Percentage of viable cells was normalized to DMSO-treated cells. Each drug was used at a maximal concentration of 2xIC_{50} for sensitive cell lines (5 µM maximum for resistant cells), followed by two-fold serial dilutions. The nature of drug interaction between EPZ015938 and paclitaxel was assessed using the Loewe model on the Combenefit software. The synergy matrix (upper panel) and isobologram (bottom panel) for each cell line are shown. The isobolograms represent the IC_{50} of paclitaxel (X-axis) obtained at various EPZ015938 concentrations (Y-axis). CI calculated at the different EPZ015938 concentrations used are shown on the isobolograms. CI were calculated at the different EPZ015938 concentrations used and are shown on the isobolograms. Data are representative of at least three independent experiments.



Supplementary figure 4. mRNA expression of the four HER family members in breast cancer cell lines. mRNA expression (log2 transformed) of EGFR, HER2, HER3 and HER4 in BT20, MDA-MB-468, MDA-MB-231, MDA-MB-453, and HCC1954 cells.



Supplementary figure 5. Effect of the inhibition of PRMT5 in combination with neratinib on TNBC cell colony formation. BT20 and MDA-MB-468 cells were seeded at low densities then treated with DMSO, EPZ015938 (PRMT5i), neratinib, or a combination (combo) of the two inhibitors. Colonies were quantified using ImageJ software. An image for each condition is shown and is representative of three independent experiments (left panel). Quantification of colony number is expressed as a percentage relative to DMSO-treated cells and represented as a mean ± SD of three independent experiments (right panel). P values were calculated using student t-test and presented as: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns = non-significant.