Supplementary Table 1

Breast cell lines with Combination Index (CI) and p53 mutation status

Cell Line	CI	p53 status[a]
BT20	0.44	K132Q
BT549	0.53	R249S
CAL120	0.61	c.672+2T>G
CAL51	0.73	wild-type
CAMA1	0.76	R280T
HCC1569	0.29	E294X
HCC70	0.52	R248Q
HMT3552	0.64	H179N
JIMT-1	1.01	R248W
MCF10A	0.77	wild-type
MCF12A	0.81	wild-type
MCF7	1.04	wild-type
MDA-MB-134	1.16	E285K
MDA-MB-361	1.05	E56X
MDA-MB-436	0.74	E204fsX45
MDA-MB-453	0.59	homozygous deletion exons 10/11
MFM223	1.27	K132R
S68	0.47	mutant; established by V. Catros, Rennes, France
SKBR3	0.59	R175H
SUM149	1.14	M237I
SUM225	0.79	L265P
SUM44	1.11	E28fsX42
SUM52	0.98	R213X
T47D	0.84	L194F
ZR75.1	0.99	wild-type

[a] Cell lines were assigned to *TP53* wild-type and mutant groups according to the IARC TP53 database (www-p53.iarc.fr), or previous publications (1, 2). MCF10A and MCF12A are breast epithelial cell lines.

1. Rennstam K, Jonsson G, Tanner M, et al. Cytogenetic characterization and gene expression profiling of the trastuzumab-resistant breast cancer cell line JIMT-1. Cancer Genet Cytogenet 2007; 172: 95-106.

2. Wasielewski M, Elstrodt F, Klijn JG, Berns EM, Schutte M. Thirteen new p53 gene mutants identified among 41 human breast cancer cell lines. Breast Cancer Res Treat 2006; 99: 97-101.

Supplementary Table 2

Cell cycle distribution of pH3/PI FACS analysis in Figure 3A

Time	HU Release			HU+MK Release		
	2N DNA G1/early S	S-phase	4N DNA G2/M	2N DNA G1/early S	S-phase	4N DNA G2/M
0h	66.9	16.4	15.6	64.4	16.7	14.3
8h	10.4	39.6	47.3	59.5	18.9	12.9
16h	28.2	8.5	62.2	51.6	20.9	19.9
24h	49.4	14.0	33.7	53.0	20.3	18.6
Asynch	53.2	14.9	28.9			

Supplementary Figure Legends

Supplementary Figure 1 Inhibition or silencing of WEE1 triggers premature mitosis

A. Mitotic index of CAL120 and MCF7 cells treated with gemcitabine for 24 hours followed by different WEE1 inhibitors for 8 hours: 5 μM PHCD (681637, Calbiochem), 10 μM WEE1 inhibitor II (681641, Calbiochem), or PD0166285 (Pfizer) at the indicated doses.

B. CAL120 and MCF7 cells were transfected with siCON or WEE1 siRNA SMARTpool (siWEE1) for 48 hours prior to gemcitabine (Gem) treatment for 48 hours. Mitotic cells were quantified by pH3/PI FACS analysis. Western blot analysis confirmed WEE1 silencing in CAL120 cells at 72 hours after transfection. β-Tubulin (β-Tub) was used as loading control.

Supplementary Figure 2 Gemcitabine and MK-1775 combination in various breast cancer cell lines

A. Mitotic index in various breast cancer cell lines either untreated, or treated with gemcitabine or MK-1775 alone. Mitotic cells with <4N DNA content (grey bars) and 4N DNA content (black bars) were quantified by pH3/PI FACS.

B. Representative PI cell cycle profiles and pH3 dot plots of the indicated cell lines after treatment with gemcitabine (Gem), MK-1775 or the combination. 2N DNA content indicates cells in G1 phase. 4N DNA content indicates cells in either G2 or M phase.

Supplementary Figure 3 Time course and dose analysis of gemcitabine and MK-1775

A. Time course analysis of mitotic index as determined by pH3/PI FACS analysis. MK-1775 (1 μM) was added to gemcitabine-treated cells for the indicated durations. MK-1775 only (9 hours), gemcitabine only (33 hours) and untreated (ctr) cells were included as controls.

B. Cells were treated as described in part A but treated with 300 nM MK-1775. ND, not determined.

Supplementary Figure 4 HU and MK-1775 combination treatment induces apoptosis

A. Annexin V positive cells were detected by flow cytometry using the Annexin V-FITC Apoptosis Detection Kit I (BD Pharmingen) according to the manufacturers' instructions in two independent experiments. Representative Annexin V-FITC/PI dot plots are shown for CAL120 cells treated with HU for 24 hours followed by MK-1775 for the indicated durations.

B. Quantification of early apoptotic cells (Annexin V +ve, PI -ve) as described in part A.

C. Increased caspase 3/7 activation in CAL120 cells treated with HU (24 hours) and MK-1775. Caspase 3/7 activity was assessed in 96-well format using the Caspase-Glo 3/7 Assay (Promega) using triplicate measurements per condition in three independent experiments. Caspase activities were corrected for differences in cell number (assessed by Cell Titer-Glo Luminescent Cell Viability assay, Promega) and normalized to the level of apoptosis in untreated control cells.

Supplementary Figure 5 Combined inhibition of CHK1 and WEE1

A. CAL120 and MCF10A cells were exposed to various doses of MK-1775 in the presence of 100 nM AZD7762 or without (DMSO). Survival was assessed after 3 days exposure with Cell Titer-Glo cell viability assay, and expressed relative to no treatment.

B. CAL120 cells were either untreated or pre-treated with gemcitabine for 24 hours followed by treatment with AZD7762 and/or MK-1775 for an additional 2 hours (top panels) or 8 hours (bottom panels), prior to lysis. Western blot analysis of WEE1 (phospho-S642 and total), CHK1 (phospho-S317), CDC25C (phospho-S216 and total) expression, and β-tubulin as loading control. During interphase CDC25C is constitutively phosphorylated at S216, while in mitosis this residue is not phosphorylated. Loss of CDC25C-Ser216 phosphorylation may therefore be the consequence of mitotic entry, as well as a primary effect of drug treatment.

Supplementary Figure 6 Premature mitosis is prevented by p53, $p21^{Waf1/Cip1}$ and CDK1 activity

A. CAL120 cells were transfected with siCON or two selected siRNA duplexes targeting CDK2 for 24 hours prior to gemcitabine and MK-1775 combination treatment (+Gem/MK). Mitotic cells were quantified by pH3/PI FACS analysis. At 48 hours post-transfection, CDK1 and CDK2 expression were assessed by Western blot analysis; β-Tubulin was used as loading control.

B. MCF7 cells were transfected with siCON or various siRNA duplexes targeting $p21^{Waf1/Cip1}$ (sip21) or p53 (siTP53) for 48 hours prior to gemcitabine and MK-1775 combination treatment. At 72 hours after siRNA transfection, mitotic index was determined by pH3/PI FACS and $p21^{Waf1/Cip1}$ expression was assessed by Western blot analysis. β-Tubulin was used as loading control.

Supplementary Figure 7 CDK1 and cyclin B1 expression levels in breast cancer cell lines

A. Western blots of lysates from CAL120 cells synchronized by HU for 24 hours (early S-phase), released for 4 hours (S-phase) or 10 hours (late S/G2 phase) after which nocodazole was added for 16 hours (Noc, mitotic cells) and then released for 6

hours (partial G1). Western blots probed for WEE1, Cyclin B1, phospho-Y15 and total CDK1, and β-tubulin as loading control. Asynchronous cells (Async) were included as controls. Corresponding PI FACS profiles and percentage of mitotic cells (pH3+ve) are shown.

B. Indicated *TP53* mutant cells were cultured in the presence or absence of gemcitabine for 24 hours, harvested and subjected to western blotting with antibodies against WEE1, Cyclin B1, CDK1 (phospho-Y15 and total), CHK1 (phospho-S317), and β-tubulin as loading control.

Supplementary Figure 8 Molecular determinants of unscheduled mitotic entry

A. CAL120 cells were transfected with siCON, two different siRNA duplexes or SMARTpool targeting EZH2 for 72 hours. EZH2 expression was assessed by Western blot analysis using β-Tubulin as loading control.

B. SUM44 cells were transfected with siCON, individual $p21^{Waf1/Cip1}$ siRNA duplexes or SMARTpool and treated with gemcitabine/MK-1775 48 hours post transfection. Mitotic index was determined by pH3/PI FACS analysis (* denotes P=0.02, Student's t-test compared to siCON transfected cells, n.s. not significant).

Supplementary Figure 9 Gemcitabine and MK-1775 combination in WiDr cells

A. WiDr cells were treated with gemcitabine (Gem), MK-1775 or the combination and subjected to pH3/PI FACS analysis. Top panels PI alone, and bottom pH3/PI. 2N DNA content indicates cells in G1 phase. 4N DNA content indicates cells in either G2 or M phase.

B. PI cell cycle profiles of single cell suspensions derived from WiDr xenograft tumours after treatment with gemcitabine (Gem, 100 mg/kg, I.P.) and MK-1775 (MK, 30 mg/kg, P.O.) 24 hours later. Tumours were taken 8 hours post MK-1775 administration. The peak representing mouse stromal G1 phase cells (corresponding

to ~1N DNA content in the PI profile relative to aneuploid WiDr cells) was excluded from the analysis and is therefore not displayed.