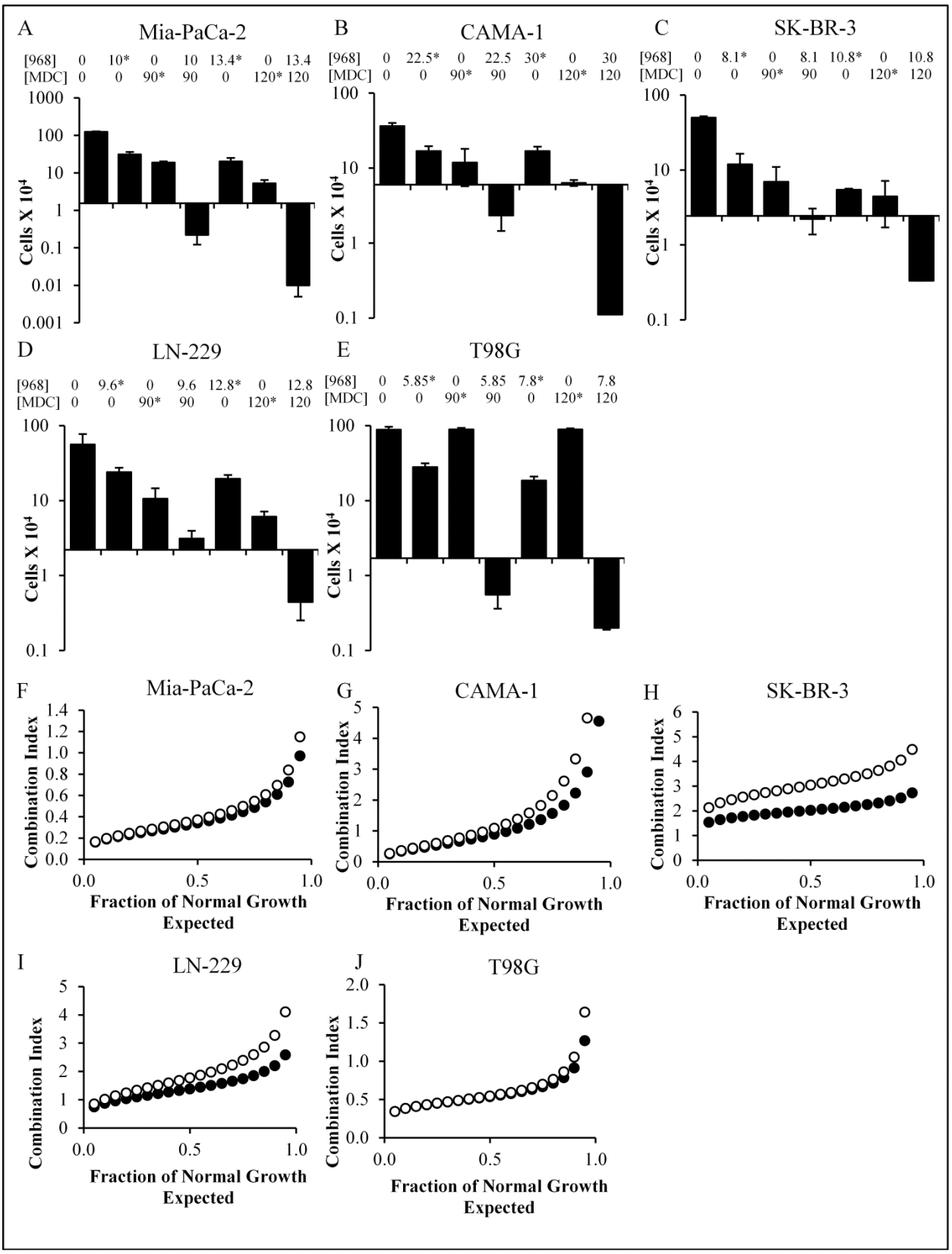
A
Mia-PaCa-2
[968] 0 10* 0 10 13.4* 0 13.4
[MDC] 0 0 90* 90 0 120* 120
Cells X 10^4
B
CAMA-1
[968] 0 22.5* 0 22.5 30* 0 30
[MDC] 0 0 90* 90 0 120* 120
C
SK-BR-3
[968] 0 8.1* 0 8.1 10.8* 0 10.8
[MDC] 0 0 90* 90 0 120* 120
D
LN-229
[968] 0 9.6* 0 9.6 12.8* 0 12.8
[MDC] 0 0 90* 90 0 120* 120
E
T98G
[968] 0 5.85* 0 5.85 7.8* 0 7.8
[MDC] 0 0 90* 90 0 120* 120
F
Mia-PaCa-2
G
CAMA-1
H
SK-BR-3
Combination Index
Fraction of Normal Growth Expected
I
LN-229
J
T98G

Figure SI1: Combination treatment of numerous cancer cell lines using 968 and MDC. A-E) Histograms depicting specific data points collected from dose curves for 968, MDC, or 968 and MDC in the indicated cell lines. The Y-axes represent the number of cells in culture after 6 days of drug treatment, while the X-axes are positioned at the starting number of cells. Values indicated with * were calculated from dose curves, and their error bars represent the standard deviation from the nearest experimental measurement. Drug concentrations are reported in μM. F-J) Combination Index (CI) calculated for 968 and MDC when used to treat the indicated cell lines, used at a ratio of IC_{50}(968) μM 968 to 60 μM MDC in any given case, where IC_{50}(968) was the IC_{50} for 968 for that particular cell line. The CI was calculated at regular intervals that represent a specific fraction (5%) of normal cell growth. Plots were determined considering the two drugs as either mutually exclusive (black circles) or mutually nonexclusive (white circles). Error bars in A-E represent the standard deviation of three separate experiments.

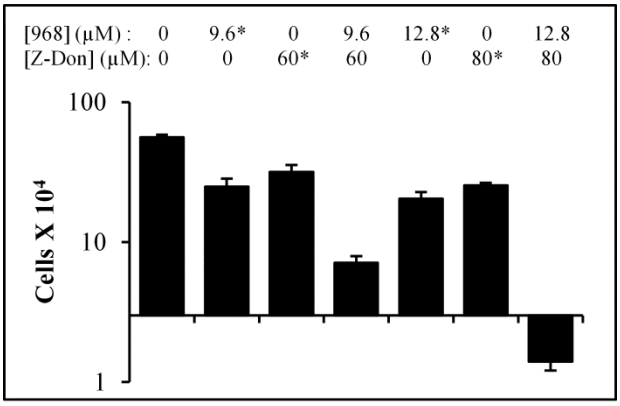

Figure SI2: LN-229 treated with 968 and Z-Don. Cells were cultured in the presence of 968, Z-Don, or a combination of 968 and Z-Don (at a ratio of 6.4 μM 968: 40 μM Z-Don) for 6 days and then counted. The Y-axis represents the number of cells in culture after 6 days of drug treatment, while the X-axis is positioned at the starting number of cells. Values marked with * were calculated from dose curves, and error bars represent the standard deviation from the nearest experimental measurement.

Supplemental Methods:

Method of Chou and Talalay:

The method of Chou and Talalay was used to determine drug synergism.[38] The results from the growth assays (performed as described in materials and methods) were used to determine the fraction of normal cell growth (Fg) for each drug treatment (the drugs were used individually, or in combination, over a range of concentrations), with the 'no drug' treatment being considered the maximum Fg (i.e. 100% cell growth). The data were then plotted as linearized dose curves: log[(1/ Fg)-1] was plotted against the log of the drug dose. The resulting plots were fitted using Excel, and the slope and IC_{50} (determined from the X intercept) of each line was determined. These values allowed estimations of the concentration of each drug, or combination of drugs, required to obtain a certain fraction of the maximum growth of a cell line ($Dose_{Fg}$) by the formula:

$$Dose_{Fg} = Dose_{IC_{50}} \left[\frac{1 - Fg}{Fg}\right]^{\frac{1}{m}}$$

where $Dose_{IC_{50}}$ is the IC_{50} of a drug or combination of drugs as calculated from the linearized dose curve, and m is the slope of that line.

$Dose_{Fg}$ was calculated for values of Fg between 0.95 and 0.05 in 0.05 increments. Because drugs in combinations were administered at a constant ratio $Fraction(A):Fraction(B)$ (in this study, generally IC_{50}(A):IC_{50}(B)), the relative concentration of each individual drug ($Dose_A$ or $Dose_B$) in any given value of $Dose_{Fg}$ for the drug combination is determined by the equation:

$$Dose_A = Dose_{Fg}\left[\frac{Fraction(A)}{Fraction(A) + Fraction(B)}\right]$$

Synergistic, additive, or antagonistic effects of various drug combinations on cells were then determined via calculation of the combination index (CI):

$$CI = \frac{Dose_A}{\left(Dose_{Fg}\right)_A} + \frac{Dose_B}{\left(Dose_{Fg}\right)_B} + \alpha * \frac{Dose_A Dose_B}{\left(Dose_{Fg}\right)_A \left(Dose_{Fg}\right)_B}$$

where $\left(Dose_{Fg}\right)_A$ is the concentration of drug A needed to obtain a given Fg value, and $Dose_A$ is the concentration of that drug required to obtain the same value of Fg when drug A and drug B are used simultaneously. $\left(Dose_{Fg}\right)_B$ and $Dose_B$ are analogous values for the second drug. The value α is equal to 1 if the two drugs are mutually nonexclusive, and 0 if the drugs are mutually exclusive. CI values equal to 1 indicate drug combinations that produce additive effects, those greater than 1 indicate antagonistic effects, and those less than 1 indicate synergistic effects. The CI value was determined for each value of Fg and plotted for either value of α.

Sample CI Calculation:

MDA-MB-231 cells were cultured with varying concentrations of 968, Z-Don, or a combination of 968 and Z-Don, as indicated. Linearized dose curves (plotted as log[(1/ Fg)-1] vs. log [total drug]) for the conditions are shown in Figure SI3. Data obviously outside the linear measurement range are not included on the plots.

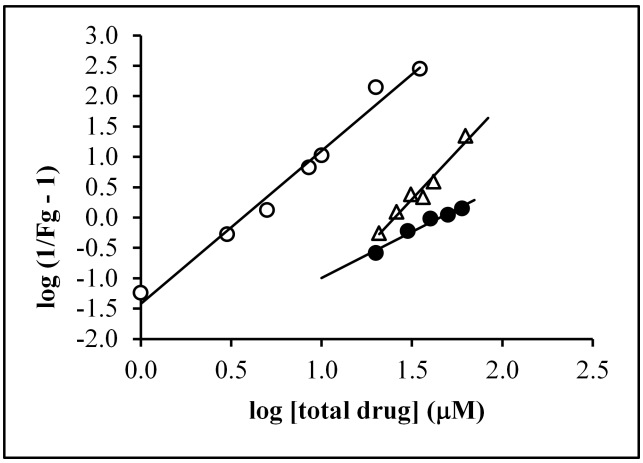

Figure SI3: Dose curves for 968 (white circles), Z-Don (black circles), and the combination of 968 and Z-Don (white triangles) when applied to MDA-MB-231 cells.

The IC_{50} for each drug or drug combination was calculated from the lines on the graph (Table SI1).

	Z-Don	968	Co-dose
y-intercept	- 2.52	- 1.42	- 4.46
slope	1.52	2.52	3.18
x-intercept = -1 * y-intercept / slope	1.65	0.56	1.40
IC_{50} = 10^x-intercept	45.07	3.66	25.37
IC_{50} determined in Sigmaplot	37.5	4.2	n.a.

Table SI1: IC_{50} values for assorted drug treatments. IC_{50} values (micromolar) were determined from best-fit linearized dose curves in Excel, and from sigmoidal dose curves in Sigmaplot.

Below is a step-by-step calculation of CI for the combination of Z-Don and 968 in MDA-MB-231 cells, at an arbitrarily selected Fg value of 0.9. Of note is that two IC_{50} values were

determined for each drug/cell dose curve. We report IC_{50} values derived from sigmoidal curves in Sigmaplot throughout the work, and we similarly use these values as the arbitrary ratio of drug A to drug B in combination dose curves. Thus, the ratio of Z-Don to 968 ($Fraction(A):Fraction(B)$) in this example was 37.5:4.2 - these values allow the calculation of $Dose_A$ and $Dose_B$. However, the calculation of $Dose_{Fg}$, requires the use of the slope of the linearized dose data. Therefore, the IC_{50} values derived from those lines are used to calculate $Dose_{Fg}$. Also note that Figure SI3 shows that the plots for 968 (white circles) and Z-Don (black circles) are not parallel. As such, it cannot be conclusively determined if 968 and Z-Don are mutually exclusive or mutually nonexclusive. Thus, CI calculations were conducted for both possibilities.

Step-by-step calculation (units removed for clarity):

$\left(Dose_{Fg}\right)_A$ (for Z-Don) = $IC_{50} * \left(\frac{1-Fg}{Fg}\right)^{\frac{1}{m}} = 45.07 * \left(\frac{1-0.9}{0.9}\right)^{\frac{1}{1.52}} = 10.64$

$\left(Dose_{Fg}\right)_B$ (for 968) = $IC_{50} * \left(\frac{1-Fg}{Fg}\right)^{\frac{1}{m}} = 3.65 * \left(\frac{1-0.9}{0.9}\right)^{\frac{1}{2.52}} = 1.53$

$\left(Dose_{Fg}\right)_C$(for the co-dose) = $IC_{50} * \left(\frac{1-Fg}{Fg}\right)^{\frac{1}{m}} = 25.37 * \left(\frac{1-0.9}{0.9}\right)^{\frac{1}{3.18}} = 12.70$

$Dose_A$ (for Z-Don) = $\left(Dose_{Fg}\right)_C \left[\frac{Fraction(A)}{Fraction(A)+Fraction(B)}\right] = 12.70 * \frac{37.5}{37.5 + 4.2} = 11.42$

$Dose_B$ (for 968) = $\left(Dose_{Fg}\right)_C \left[\frac{Fraction(B)}{Fraction(A)+Fraction(B)}\right] = 12.70 * \frac{4.2}{37.5 + 4.2} = 1.28$

and finally

$$CI = \frac{Dose_A}{\left(Dose_{Fg}\right)_A} + \frac{Dose_B}{\left(Dose_{Fg}\right)_B} + \alpha * \frac{Dose_A Dose_B}{\left(Dose_{Fg}\right)_A \left(Dose_{Fg}\right)_B}$$

$$= \frac{11.42}{10.64} + \frac{1.28}{1.53} + (1\ or\ 0) * \frac{11.42 * 1.28}{10.64 * 1.53}$$

giving CI = 1.91 for mutually exclusive drugs (α = 0) or CI = 2.81 for mutually nonexclusive drugs (α = 1).

Table SI2 shows these calculations for a full range of Fractional growth (Fg) values, and Figure SI4 shows the resulting CI plot.

Fg	$\left(Dose_{Fg}\right)_A$	$\left(Dose_{Fg}\right)_B$	$\left(Dose_{Fg}\right)_C$	$Dose_A$	$Dose_B$	CI α=0	CI α=1
0.95	6.52	1.14	10.04	9.03	1.01	2.27	3.51
0.90	10.64	1.53	12.70	11.42	1.28	1.91	2.81
0.85	14.42	1.84	14.69	13.21	1.48	1.72	2.46
0.80	18.13	2.11	16.39	14.74	1.65	1.60	2.23
0.75	21.90	2.36	17.95	16.14	1.81	1.50	2.06
0.70	25.83	2.61	19.43	17.47	1.96	1.43	1.93
0.65	30.01	2.86	20.88	18.77	2.10	1.36	1.82
0.60	34.53	3.11	22.33	20.08	2.25	1.30	1.72
0.55	39.51	3.38	23.82	21.42	2.40	1.25	1.64
0.50	45.07	3.66	25.37	22.81	2.56	1.20	1.56
0.45	51.42	3.96	27.02	24.30	2.72	1.16	1.48
0.40	58.83	4.30	28.82	25.92	2.90	1.12	1.41
0.35	67.69	4.68	30.83	27.72	3.11	1.07	1.35
0.30	78.63	5.12	33.13	29.79	3.34	1.03	1.28
0.25	92.75	5.66	35.86	32.25	3.61	0.99	1.21
0.20	112.04	6.34	39.26	35.30	3.95	0.94	1.14
0.15	140.84	7.28	43.81	39.40	4.41	0.89	1.06
0.10	190.85	8.75	50.68	45.57	5.10	0.82	0.96
0.05	311.79	11.77	64.12	57.67	6.46	0.73	0.84

Table SI2: Calculations of CI values for different values of Fg for MDA-MB-231 cells treated with 968 and Z-Don. $\left(Dose_{Fg}\right)_A$ and $Dose_A$ are calculated for Z-Don, while $\left(Dose_{Fg}\right)_B$ and $Dose_B$ are calculated for 968, and $\left(Dose_{Fg}\right)_C$ is calculated for the combination dose.

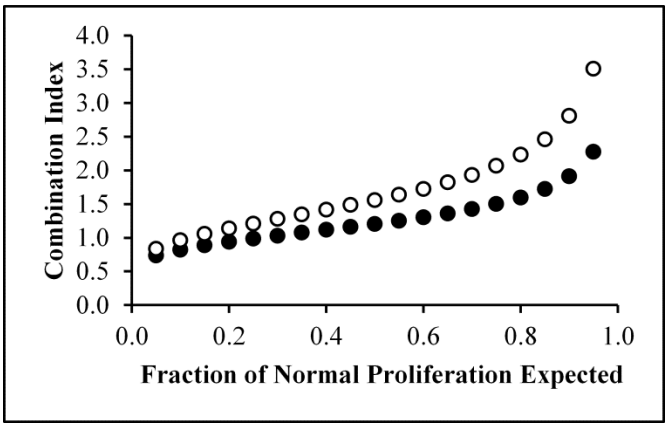

Figure SI4: Combination Index (CI) plot for MDA-MB-231 cells treated with 968 and Z-Don. CI values were determined assuming that the drugs were mutually exclusive (black circles) or mutually nonexclusive (white circles).