**Figure S11**: 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 IC₅₀(968) µM 968 to 60 µM MDC in any given case, where IC₅₀(968) was the IC₅₀ 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 S12: 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 ($F_g$) 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 $F_g$ (i.e. 100% cell growth). The data were then plotted as linearized dose curves: $\log[(1/F_g) - 1]$ 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 ($Dose_{F_g}$) by the formula:

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

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.

$Dose_{F_g}$ was calculated for values of $F_g$ 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 $Dose_{F_g}$ for the drug combination is determined by the equation:
Synergistic, additive, or antagonistic effects of various drug combinations on cells were then determined via calculation of the combination index (CI):

\[
CI = \frac{Dose_A}{(Dose_{fg})_A} + \frac{Dose_B}{(Dose_{fg})_B} + \alpha \cdot \frac{Dose_A Dose_B}{(Dose_{fg})_A (Dose_{fg})_B}
\]

where \((Dose_{fg})_A\) 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. \((Dose_{fg})_B\) and \(Dose_B\) are analogous values for the second drug. The value \(\alpha\) 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 \(\alpha\).

**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 S13: 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<sub>50</sub> for each drug or drug combination was calculated from the lines on the graph (Table S11).

|                   | 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<sub>50</sub> = 10<sup>x</sup>x-intercept | 45.07  | 3.66  | 25.37   |
| IC<sub>50</sub> determined in Sigmaplot | 37.5   | 4.2   | n.a.    |

Table S11: IC<sub>50</sub> values for assorted drug treatments. IC<sub>50</sub> 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<sub>50</sub> 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):

$$(Dose_{Fg})_A \text{ (for Z-Don)} = IC_{50} \times \left( \frac{1-F_g}{F_g} \right)^{1/\mu} = 45.07 \times \left( \frac{1-0.9}{0.9} \right)^{1/1.52} = 10.64$$

$$(Dose_{Fg})_B \text{ (for 968)} = IC_{50} \times \left( \frac{1-F_g}{F_g} \right)^{1/\mu} = 3.65 \times \left( \frac{1-0.9}{0.9} \right)^{1/1.52} = 1.53$$

$$(Dose_{Fg})_C \text{ (for the co-dose)} = IC_{50} \times \left( \frac{1-F_g}{F_g} \right)^{1/\mu} = 25.37 \times \left( \frac{1-0.9}{0.9} \right)^{1/3.18} = 12.70$$

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

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

and finally
\[ CI = \frac{Dose_A}{(Dose_{Fg})_A} + \frac{Dose_B}{(Dose_{Fg})_B} + \alpha \cdot \frac{Dose_ADose_B}{(Dose_{Fg})_A(Dose_{Fg})_B} \]

\[ = \frac{11.42}{10.64} + \frac{1.28}{1.53} + (1 \text{ or } 0) \cdot \frac{11.42 \cdot 1.28}{10.64 \cdot 1.53} \]

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

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

| \( Fg \) | \( (Dose_{Fg})_A \) | \( (Dose_{Fg})_B \) | \( (Dose_{Fg})_C \) | \( Dose_A \) | \( Dose_B \) | \( CI \alpha=0 \) | \( CI \alpha=1 \) |
|---------|-----------------|-----------------|-----------------|---------|---------|--------------|--------------|
| 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. \( (Dose_{Fg})_A \) and \( Dose_A \) are calculated for Z-Don, while \( (Dose_{Fg})_B \) and \( Dose_B \) are calculated for 968, and \( (Dose_{Fg})_C \) is calculated for the combination dose.
**Figure S14:** 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).