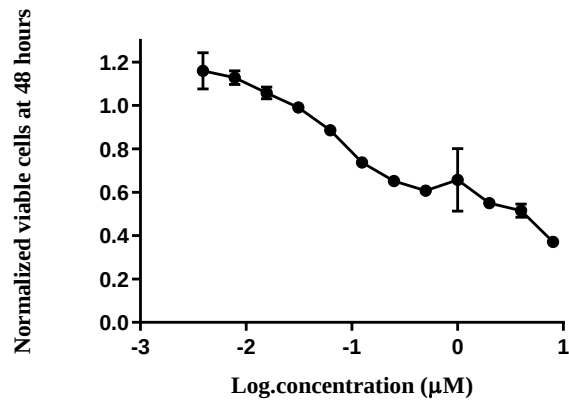
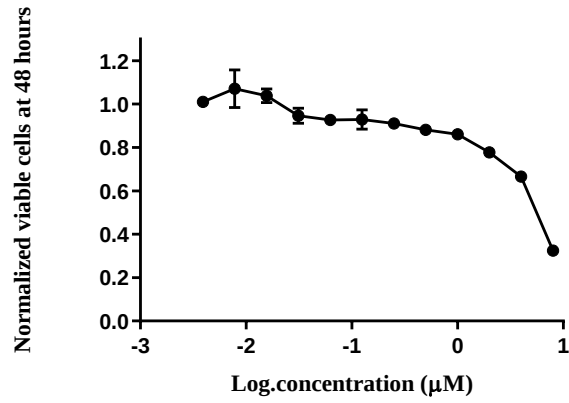


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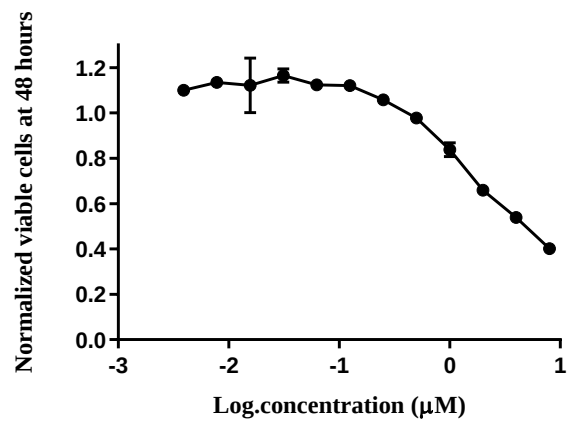
May 5, 2023



((a)) Normalized cell viability of MOLM-13 after 48 hours in various concentrations of palbociclib

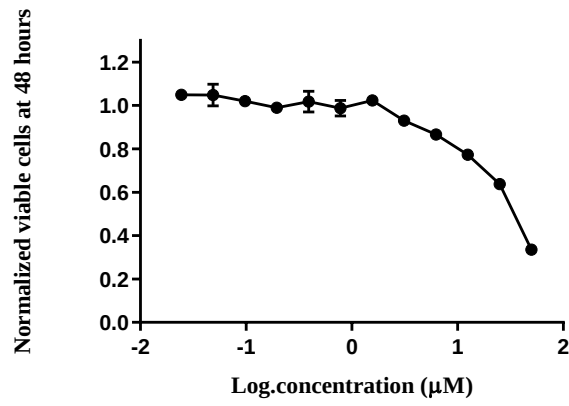


((b)) Normalized cell viability of MOLM-14 after 48 hours in various concentrations of palbociclib

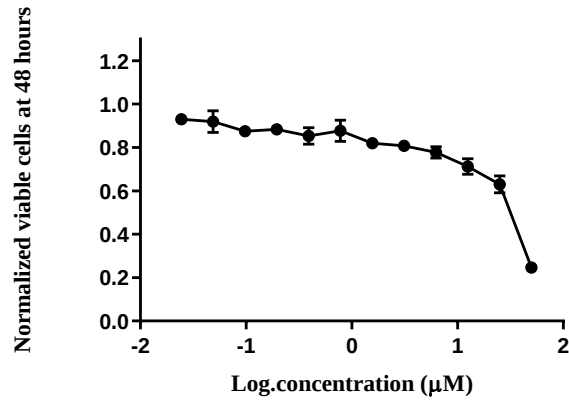


((c)) Normalized cell viability of MV4-11 after 48 hours in various concentrations of palbociclib

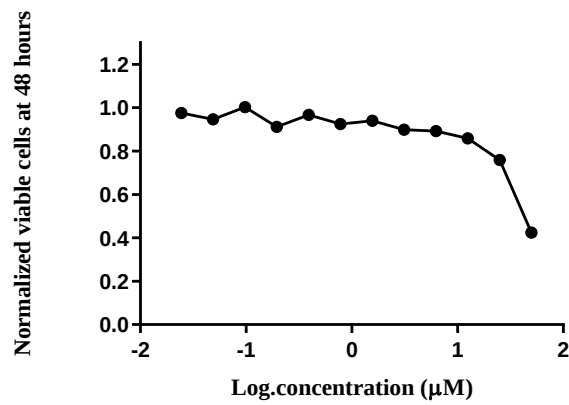
Figure S1: Normalized cell viability of AML cell lines after 48 hours in various concentrations of palbociclib



((a)) Normalized cell viability of MOLM-13 after 48 hours in various concentrations of idelalisib

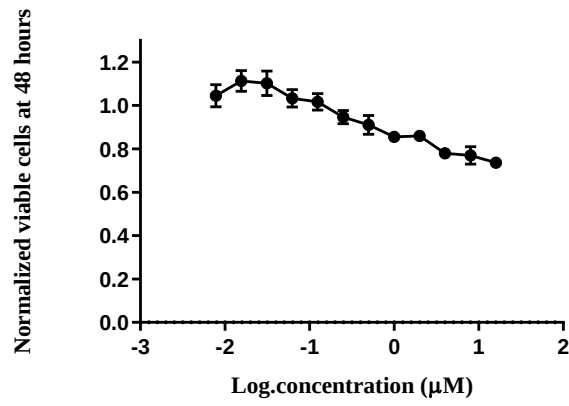


((b)) Normalized cell viability of MOLM-14 after 48 hours in various concentrations of idelalisib

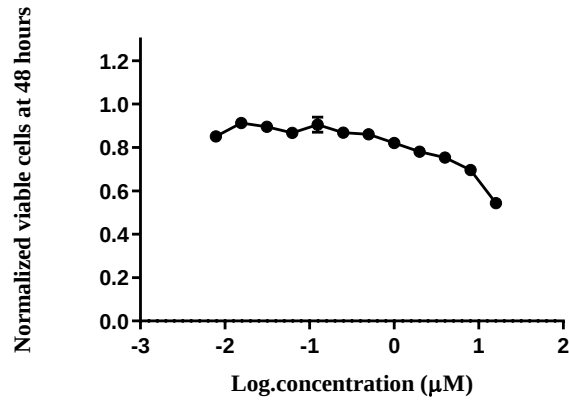


((c)) Normalized cell viability of MV4-11 after 48 hours in various concentrations of idelalisib

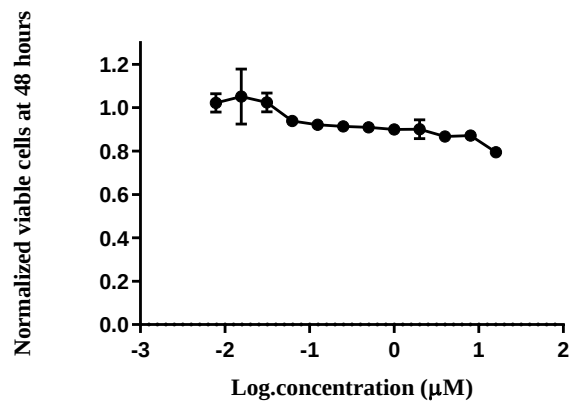
Figure S2: Normalized cell viability of AML cell lines after 48 hours in various concentrations of idelalisib



((a)) Normalized cell viability of MOLM-13 after 48 hours in various concentrations of duvelisib

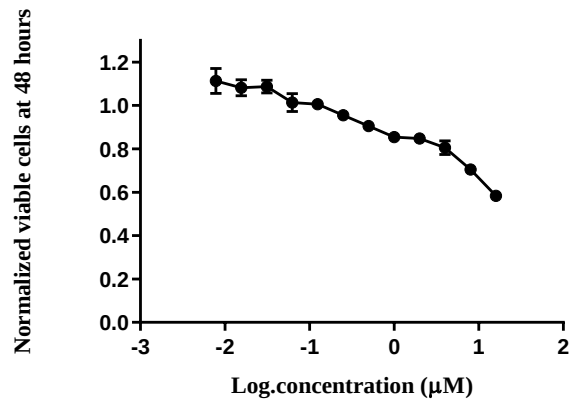


((b)) Normalized cell viability of MOLM-14 after 48 hours in various concentrations of duvelisib

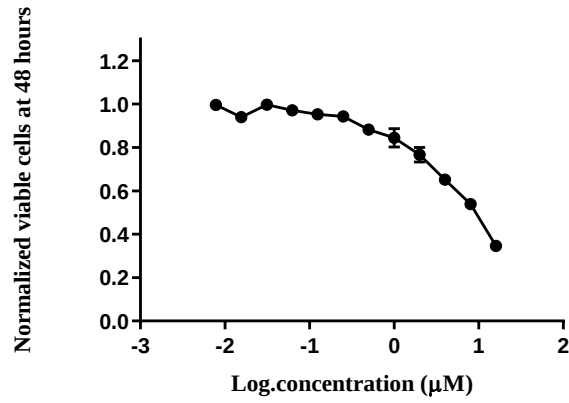


((c)) Normalized cell viability of MV4-11 after 48 hours in various concentrations of duvelisib

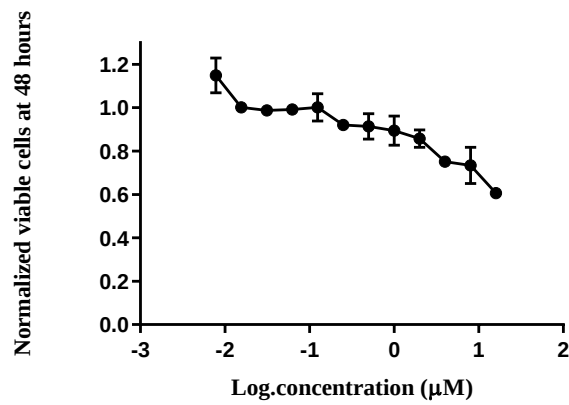
Figure S3: Normalized cell viability of AML cell lines after 48 hours in various concentrations of duvelisib



((a)) Normalized cell viability of MOLM-13 after 48 hours in various concentrations of alpelisib

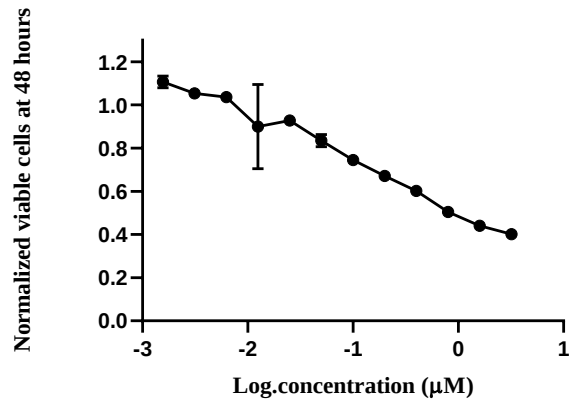


((b)) Normalized cell viability of MOLM-14 after 48 hours in various concentrations of alpelisib

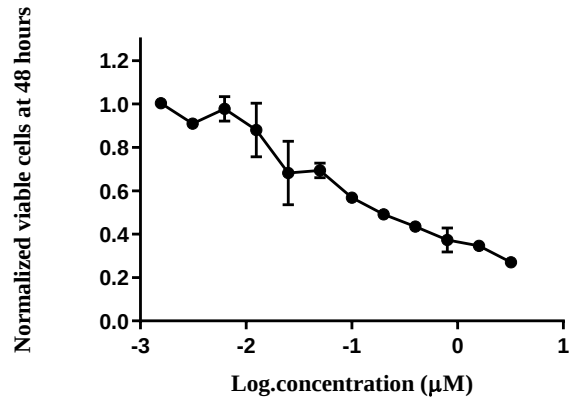


((c)) Normalized cell viability of MV4-11 after 48 hours in various concentrations of alpelisib

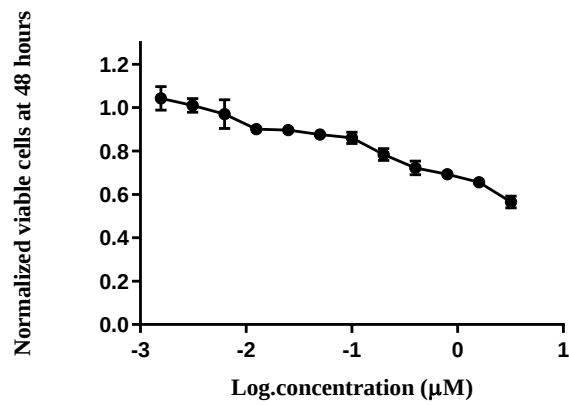
Figure S4: Normalized cell viability of AML cell lines after 48 hours in various concentrations of alpelisib



((a)) Normalized cell viability of MOLM-13 after 48 hours in various concentrations of copanlisib

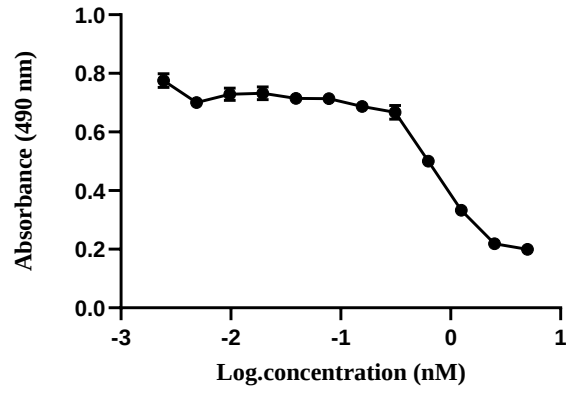


((b)) Normalized cell viability of MOLM-14 after 48 hours in various concentrations of copanlisib

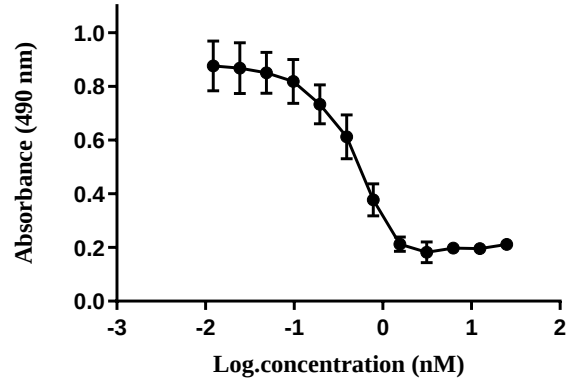


((c)) Normalized cell viability of MV4-11 after 48 hours in various concentrations of copanlisib

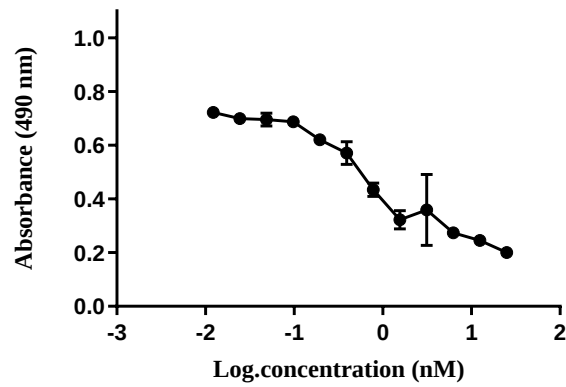
Figure S5: Normalized cell viability of AML cell lines after 48 hours in various concentrations of copanlisib



((a)) Normalized cell viability of MOLM-13 after 48 hours in various concentrations of quizartinib

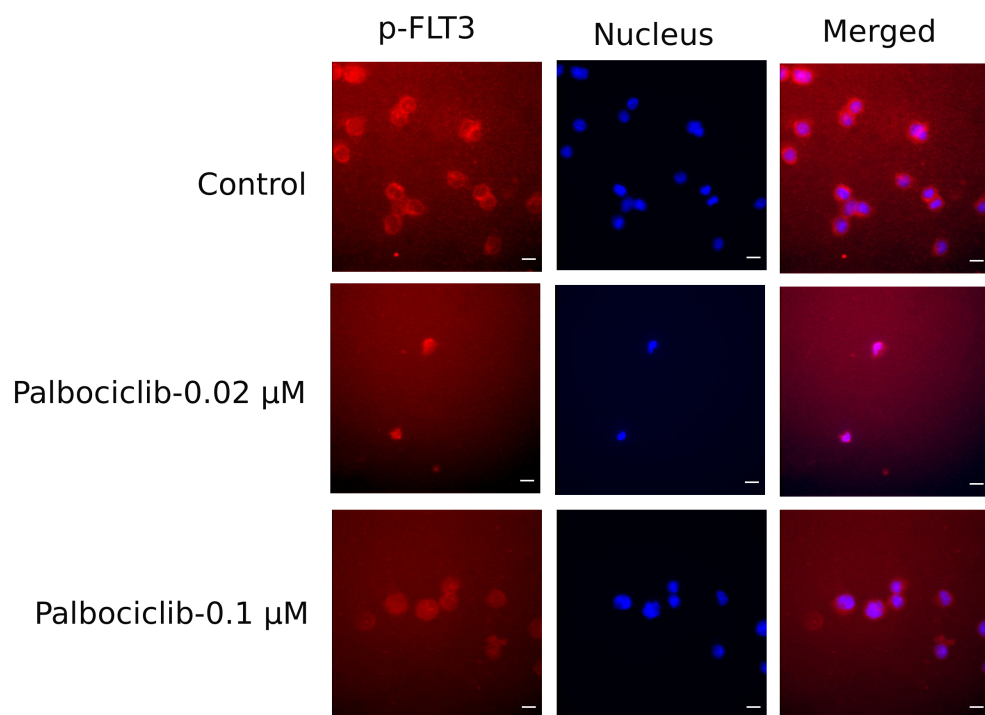


((b)) Normalized cell viability of MOLM-14 after 48 hours in various concentrations of quizartinib

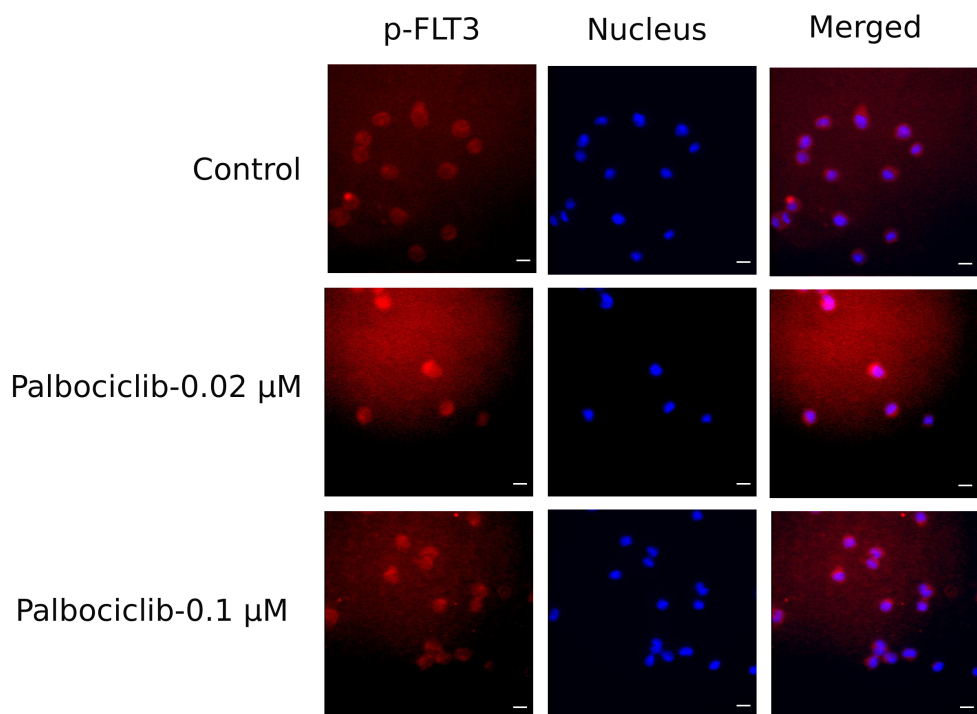


((c)) Normalized cell viability of MV4-11 after 48 hours in various concentrations of quizartinib

Figure S6: Dose-response curve with FLT3 inhibitor quizartinib alone in the AML cell lines. Three independent experiments were carried out.



((a)) Staining of phosphorylated FLT3 in MOLM-14 cell line with different concentration of palbociclib treatment after 24 hours



((b)) Staining of phosphorylated FLT3 in MV4-11 cell line with different concentration of palbociclib treatment after 24 hours

Figure S7: Confocal imaging to assess⁸ the expression of phosphorylated FLT3 in MOLM-14 (a) and MV4-11 (b) following treatment with palbociclib. Immunofluorescence analysis was performed using an antibody against phosphorylated FLT3. Left panel: phosphorylated FLT3 antibody staining (red signal); Middle panel: DAPI nucleus staining (blue signal); Right panel: merged image. Scale bar 200 μ m

Table S 1: Growth rate of MOLM-13 at day 2 under different treatments

Treatment-generation	Growth rate
Control-G1	0.6108
Control-G2	0.6879
Control-G3	0.7058
Control-G4	0.7327
Palbociclib-G1	0.7316
Palbociclib-G2	0.7017
Palbociclib-G3	0.6450
Palbociclib-G4	0.4529
Idelalisib-G1	0.4566
Idelalisib-G2	0.6740
Idelalisib-G3	0.8718
Idelalisib-G4	0.7294
Copanlisib-G1	0.5241
Copanlisib-G2	0.5894
Copanlisib-G3	0.6723
Copanlisib-G4	0.4876
Alpelisib-G1	0.4016
Alpelisib-G2	0.5163
Alpelisib-G3	0.6443
Alpelisib-G4	0.7250
Duvelisib-G1	0.4729
Duvelisib-G2	0.6849
Duvelisib-G3	0.6748
Duvelisib-G4	0.6029
Quizartinib-G1	0.6264
Quizartinib-G2	0.6498
Quizartinib-G3	0.6476
Quizartinib-G4	0.7589
Idelalisib+Palbociclib-G1	0.4653
Idelalisib+Palbociclib-G2	0.2663
Idelalisib+Palbociclib-G3	0.2891
Idelalisib+Palbociclib-G4	0.3298
Copanlisib+Palbociclib-G1	0.2394
Copanlisib+Palbociclib-G2	0.7215
Copanlisib+Palbociclib-G3	0.5032
Copanlisib+Palbociclib-G4	0.1806
Alpelisib+Palbociclib-G1	0.4491
Alpelisib+Palbociclib-G2	0.2989
Alpelisib+Palbociclib-G3	0.2646
Alpelisib+Palbociclib-G4	0.4423
Duvelisib+Palbociclib-G1	0.2924
Duvelisib+Palbociclib-G2	0.5407
Duvelisib+Palbociclib-G3	0.3913
Duvelisib+Palbociclib-G4	0.4804
Quizartinib+Palbociclib-G1	-
Quizartinib+Palbociclib-G2	-
Quizartinib+Palbociclib-G3	-
Quizartinib+Palbociclib-G4	0.1018

Table S 2: Growth rate of MOLM-14 at day 2 under different treatments

Treatment-generation	Growth rate
Control-G1	0.7921
Control-G2	0.9372
Control-G3	1.0049
Control-G4	0.6368
Palbociclib-G1	0.2443
Palbociclib-G2	0.2430
Palbociclib-G3	0.3464
Palbociclib-G4	0.3091
Idelalisib-G1	0.5824
Idelalisib-G2	0.9121
Idelalisib-G3	0.6483
Idelalisib-G4	0.8395
Copanlisib-G1	0.5399
Copanlisib-G2	0.7521
Copanlisib-G3	0.6730
Copanlisib-G4	0.5098
Alpelisib-G1	0.4766
Alpelisib-G2	0.6316
Alpelisib-G3	0.6997
Alpelisib-G4	0.5783
Duvelisib-G1	0.5681
Duvelisib-G2	0.6613
Duvelisib-G3	0.8026
Duvelisib-G4	0.7726
Quizartinib-G1	0.6830
Quizartinib-G2	0.9791
Quizartinib-G3	0.8501
Quizartinib-G4	0.7653
Idelalisib+Palbociclib-G1	0.2435
Idelalisib+Palbociclib-G2	0.0905
Idelalisib+Palbociclib-G3	0.1820
Idelalisib+Palbociclib-G4	0.2883
Copanlisib+Palbociclib-G1	0.2300
Copanlisib+Palbociclib-G2	0.2354
Copanlisib+Palbociclib-G3	0.1225
Copanlisib+Palbociclib-G4	0.2112
Alpelisib+Palbociclib-G1	0.0632
Alpelisib+Palbociclib-G2	0.1958
Alpelisib+Palbociclib-G3	0.2621
Alpelisib+Palbociclib-G4	0.2951
Duvelisib+Palbociclib-G1	0.2090
Duvelisib+Palbociclib-G2	0.2294
Duvelisib+Palbociclib-G3	0.1715
Duvelisib+Palbociclib-G4	0.2480
Quizartinib+Palbociclib-G1	0.1768
Quizartinib+Palbociclib-G2	-
Quizartinib+Palbociclib-G3	0.1941
Quizartinib+Palbociclib-G4	-

Table S 3: Growth rate of MV4-11 at day 2 under different treatments

Treatment-generation	Growth rate
Control-G1	0.6513
Control-G2	0.7778
Control-G3	1.0971
Control-G4	0.6761
Palbociclib-G1	0.3541
Palbociclib-G2	0.4108
Palbociclib-G3	0.7060
Palbociclib-G4	0.4992
Idelalisib-G1	0.6409
Idelalisib-G2	0.8090
Idelalisib-G3	0.6802
Idelalisib-G4	0.5328
Copanlisib-G1	0.6917
Copanlisib-G2	0.8024
Copanlisib-G3	0.7784
Copanlisib-G4	0.6936
Alpelisib-G1	0.9883
Alpelisib-G2	1.0517
Alpelisib-G3	0.5690
Alpelisib-G4	0.1978
Duvelisib-G1	0.5358
Duvelisib-G2	0.2303
Duvelisib-G3	0.1895
Duvelisib-G4	0.1919
Quizartinib-G1	0.6226
Quizartinib-G2	0.8228
Quizartinib-G3	0.7207
Quizartinib-G4	0.5078
Idelalisib+Palbociclib-G1	0.2285
Idelalisib+Palbociclib-G2	0.2603
Idelalisib+Palbociclib-G3	0.3798
Idelalisib+Palbociclib-G4	0.1973
Copanlisib+Palbociclib-G1	0.1764
Copanlisib+Palbociclib-G2	0.2920
Copanlisib+Palbociclib-G3	0.3486
Copanlisib+Palbociclib-G4	0.2269
Alpelisib+Palbociclib-G1	0.2312
Alpelisib+Palbociclib-G2	0.2095
Alpelisib+Palbociclib-G3	0.2604
Alpelisib+Palbociclib-G4	0.1906
Duvelisib+Palbociclib-G1	0.2415
Duvelisib+Palbociclib-G2	0.1460
Duvelisib+Palbociclib-G3	0.2377
Duvelisib+Palbociclib-G4	0.2658
Quizartinib+Palbociclib-G1	0.1976
Quizartinib+Palbociclib-G2	-
Quizartinib+Palbociclib-G3	0.1684
Quizartinib+Palbociclib-G4	-

Table S 4: Growth rate of MOLM-13 at day 2 under different treatments

Treatment-generation	Growth rate
Control-G1	0.1290
Control-G2	0.1674
Control-G3	0.1694
Control-G4	0.2125
Gilteritinib-G1	0.0186
Gilteritinib-G2	0.0210
Gilteritinib-G3	0.0577
Gilteritinib-G4	0.0176
Abemaciclib-G1	0.0222
Abemaciclib-G2	0.0254
Abemaciclib-G3	0.0230
Abemaciclib-G4	0.0174
Gilteritinib+Palbociclib-G1	0.0226
Gilteritinib+Palbociclib-G2	0.0216
Gilteritinib+Palbociclib-G3	0.0215
Gilteritinib+Palbociclib-G4	0.0212
Gilteritinib+Abemaciclib-G1	0.0224
Gilteritinib+Abemaciclib-G2	0.0269
Gilteritinib+Abemaciclib-G3	0.0251
Gilteritinib+Abemaciclib-G4	0.0177
Abemaciclib+Copanlisib-G1	0.0242
Abemaciclib+Copanlisib-G2	0.0264
Abemaciclib+Copanlisib-G3	0.0291
Abemaciclib+Copanlisib-G4	0.0184
Abemaciclib+Quizartinib-G1	0.0215
Abemaciclib+Quizartinib-G2	0.0209
Abemaciclib+Quizartinib-G3	0.0171
Abemaciclib+Quizartinib-G4	0.0244

Table S 5: Growth rate of MOLM-14 at day 2 under different treatments

Treatment-generation	Growth rate
Control-G1	0.3373
Control-G2	0.2852
Control-G3	0.4583
Control-G4	0.4265
Gilteritinib-G1	0.0895
Gilteritinib-G2	0.0209
Gilteritinib-G3	0.2474
Gilteritinib-G4	0.2125
Abemaciclib-G1	0.0267
Abemaciclib-G2	0.0169
Abemaciclib-G3	0.0211
Abemaciclib-G4	0.0485
Gilteritinib+Palbociclib-G1	0.0201
Gilteritinib+Palbociclib-G2	0.0245
Gilteritinib+Palbociclib-G3	0.0167
Gilteritinib+Palbociclib-G4	0.0212
Gilteritinib+Abemaciclib-G1	0.0224
Gilteritinib+Abemaciclib-G2	0.0269
Gilteritinib+Abemaciclib-G3	0.0251
Gilteritinib+Abemaciclib-G4	0.0177
Abemaciclib+Copanlisib-G1	0.0242
Abemaciclib+Copanlisib-G2	0.0264
Abemaciclib+Copanlisib-G3	0.0291
Abemaciclib+Copanlisib-G4	0.0184
Abemaciclib+Quizartinib-G1	0.0215
Abemaciclib+Quizartinib-G2	0.0209
Abemaciclib+Quizartinib-G3	0.0171
Abemaciclib+Quizartinib-G4	0.0244

Figure S8: The effect of combination treatments on CDK4 expression levels. MOLM-14 (a) and MV4-11 (b). Cells were cultured in the absence or presence of the indicated drugs for 24 h. Cell lysates were analysed by immunoblotting for the expression of CDK4. β -Actin was used as loading control. Adjusted densities based on (a) and (b) was calculated using the Fiji software and are shown in (c), in which x-axis positions from 1 to 11 represent the lanes in (a) and (b) from left to right.

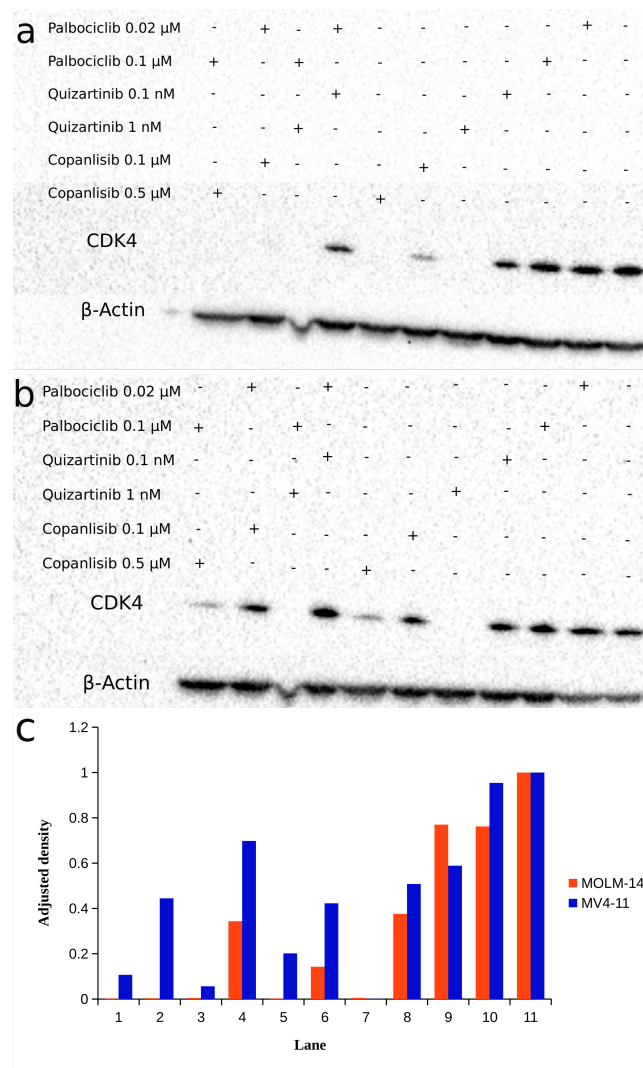
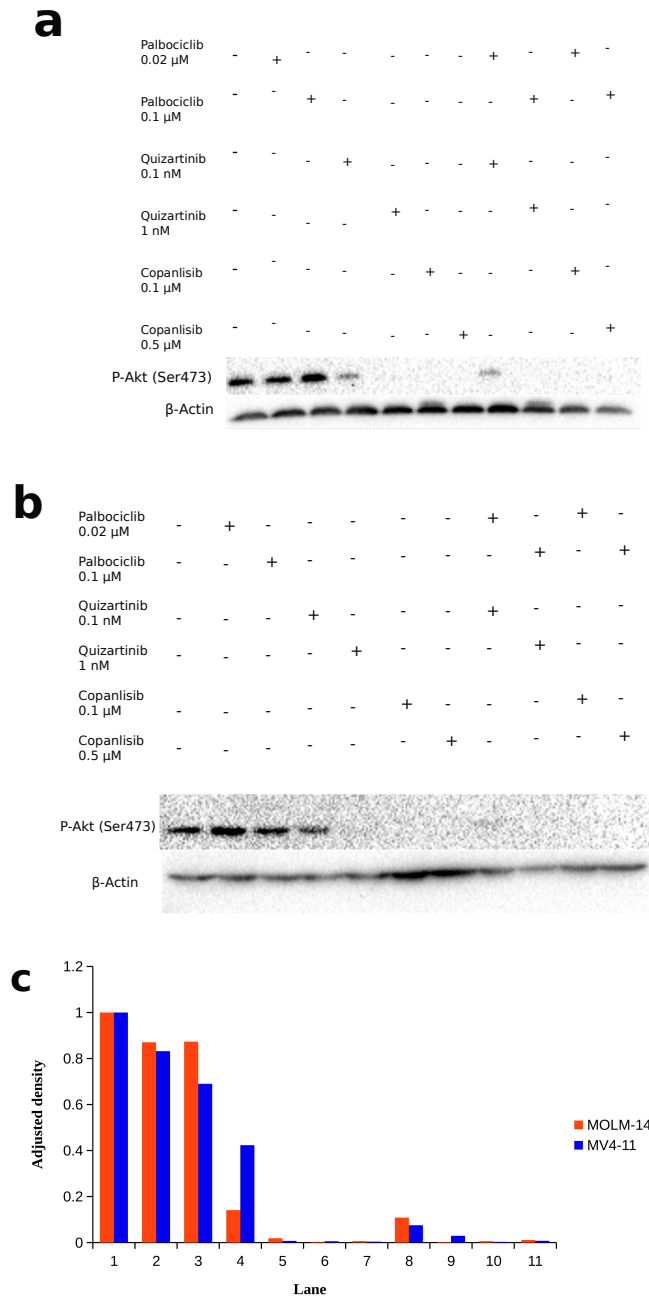
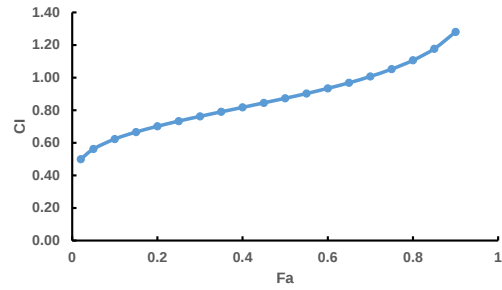
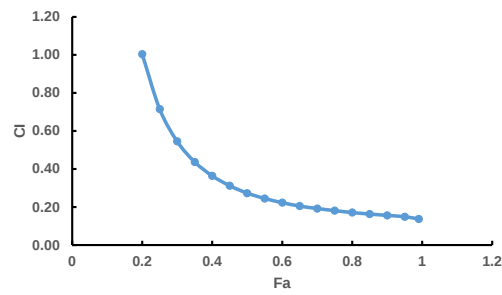


Figure S9: The effect of combination treatment on p-Akt expression levels. MOLM-14 (a) and MV4-11 (b). Cells were cultured in the absence or presence of the indicated drugs for 24 h. Cell lysates were analysed by immunoblotting for the expression of phosphorylated-Akt. β -Actin was used as loading control. Adjusted densities based on (a) and (b) were calculated using the Fiji software and showed on (c), in which, x-axis from 1 to 11 represented the lanes in (a) and (b) from left to right.

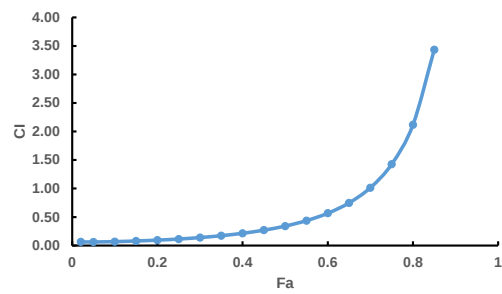




((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of idelalisib and palbociclib

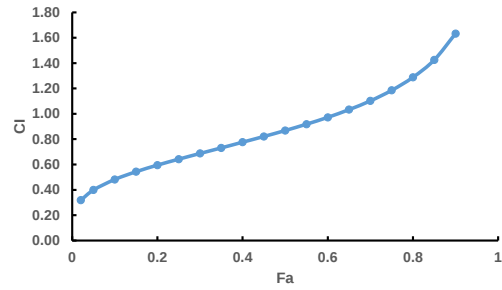


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of idelalisib and palbociclib

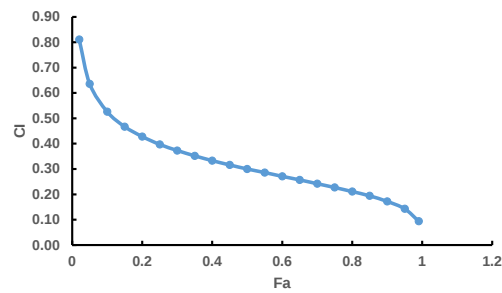


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of idelalisib and palbociclib

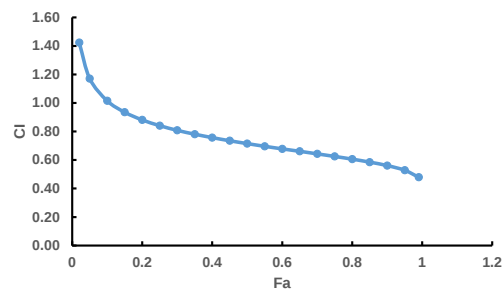
Figure S10: CI values measured in AML cell lines after 48 hours in the combination of idelalisib and palbociclib at different concentrations.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of alpelisib and palbociclib

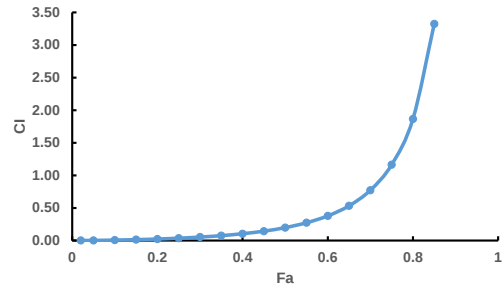


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of alpelisib and palbociclib

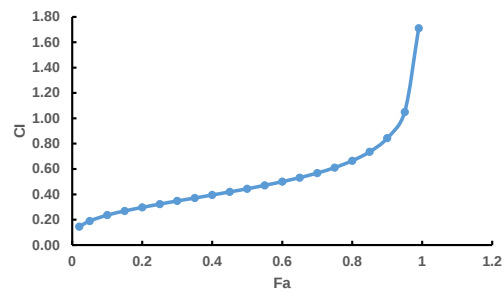


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of alpelisib and palbociclib

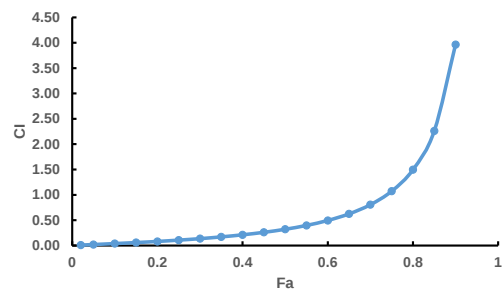
Figure S11: CI values measured in AML cell lines after 48 hours in the combination of alpelisib and palbociclib at different concentrations.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of Copanlisib and palbociclib

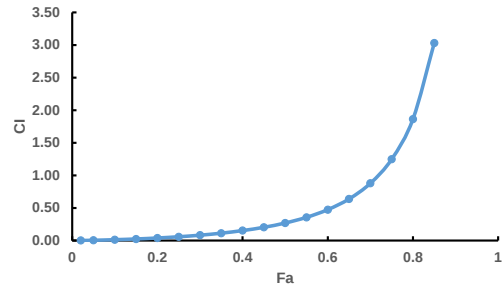


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of Copanlisib and palbociclib

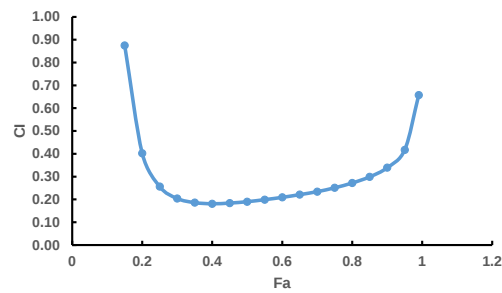


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of Copanlisib and palbociclib

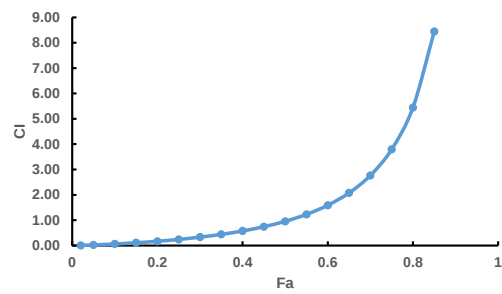
Figure S12: CI values measured in AML cell lines after 48 hours in the combination of Copanlisib and palbociclib at different concentrations.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of Duvelisib and palbociclib

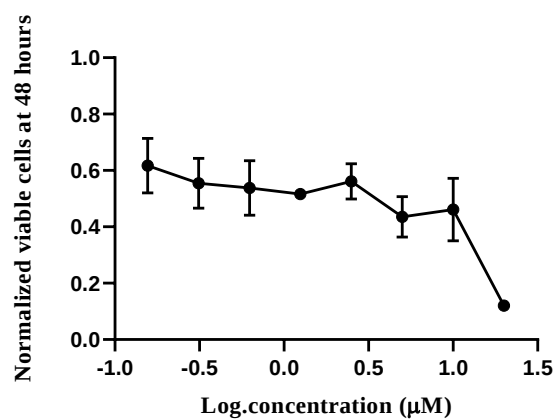


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of Duvelisib and palbociclib

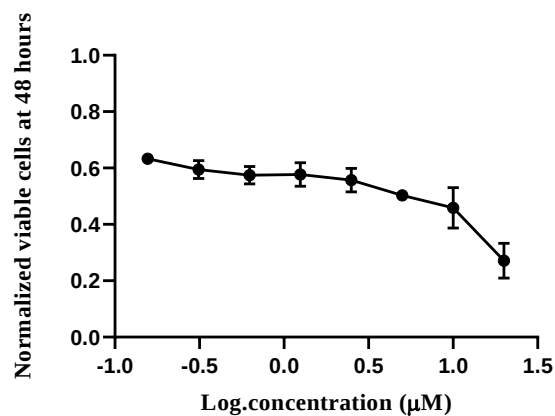


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of Duvelisib and palbociclib

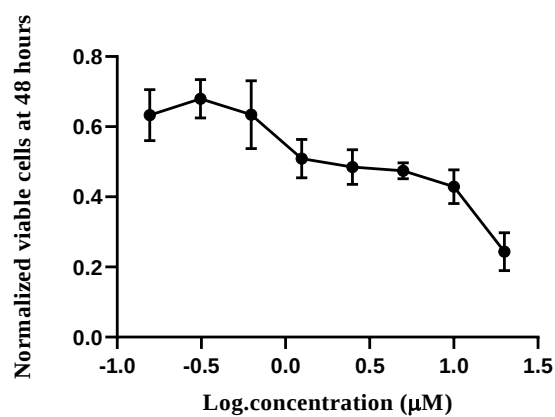
Figure S13: CI values measured in AML cell lines after 48 hours in the combination of Duvelisib and palbociclib at different concentrations.



((a)) Normalized cell viability of MOLM-13 after 48 hours in various concentrations of abemaciclib

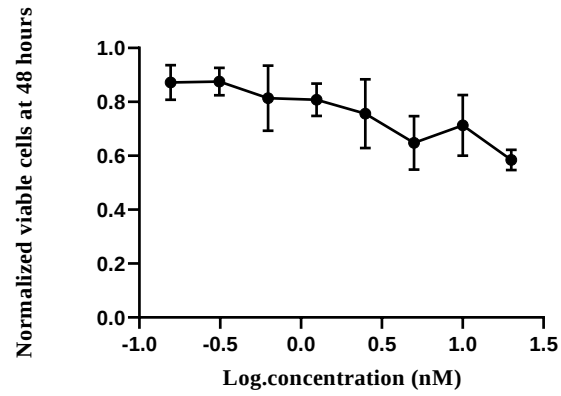


((b)) Normalized cell viability of MOLM-14 after 48 hours in various concentrations of abemaciclib

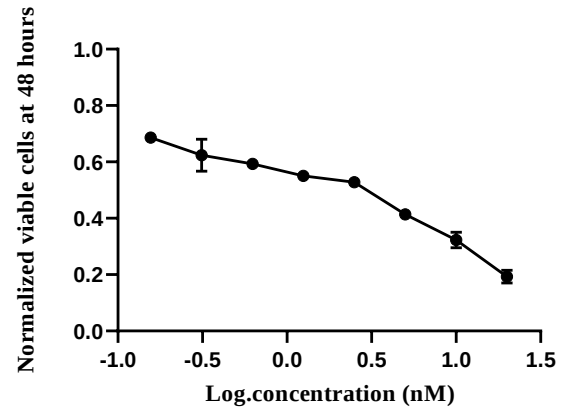


((c)) Normalized cell viability of MV4-11 after 48 hours in various concentrations of abemaciclib

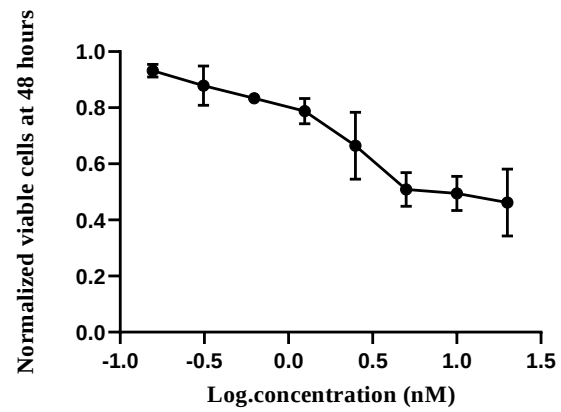
Figure S14: Dose-response curve with CDK6 inhibitor abemaciclib alone in the AML cell lines. Three independent experiments were carried out.



((a)) Normalized cell viability of MOLM-13 after 48 hours in various concentrations of gilteritinib

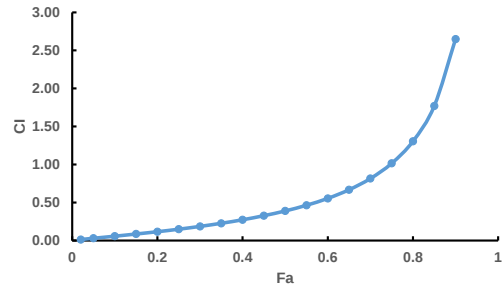


((b)) Normalized cell viability of MOLM-14 after 48 hours in various concentrations of gilteritinib

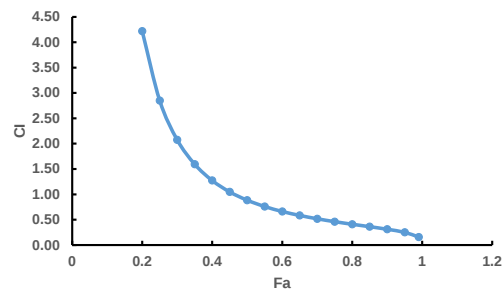


((c)) Normalized cell viability of MV4-11 after 48 hours in various concentrations of gilteritinib

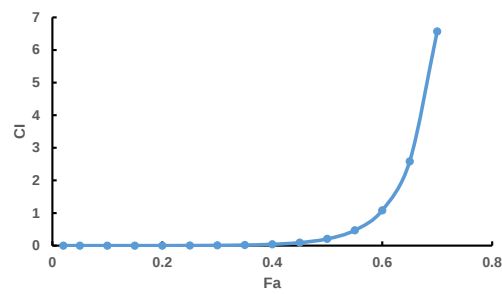
Figure S15: Dose-response curve with FLT3 inhibitor gilteritinib alone in the AML cell lines. Three independent experiments were carried out.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of abemaciclib and copanlisib

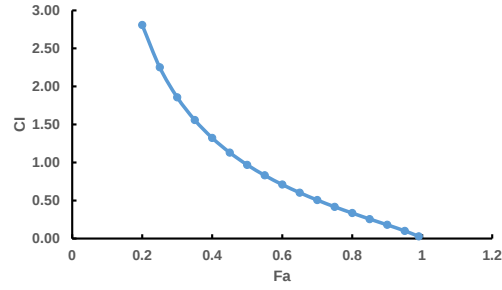


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of abemaciclib and copanlisib

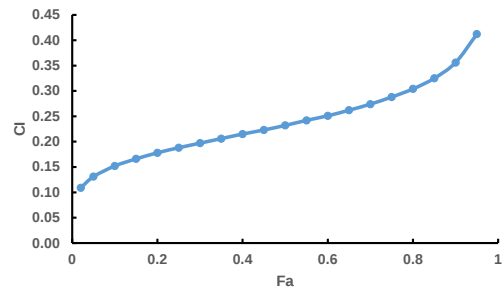


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of abemaciclib and copanlisib

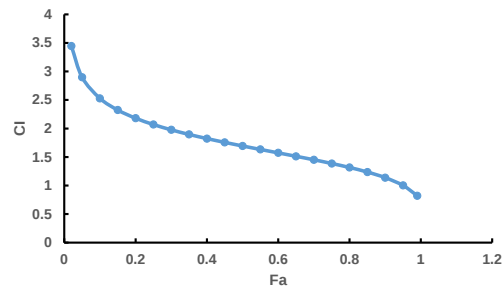
Figure S16: CI values measured in AML cell lines after 48 hours in the combination of abemaciclib and copanlisib at different concentrations.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of abemaciclib and gilteritinib

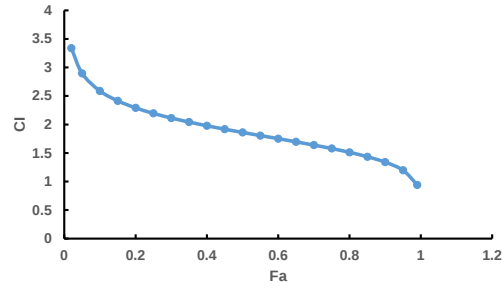


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of abemaciclib and gilteritinib

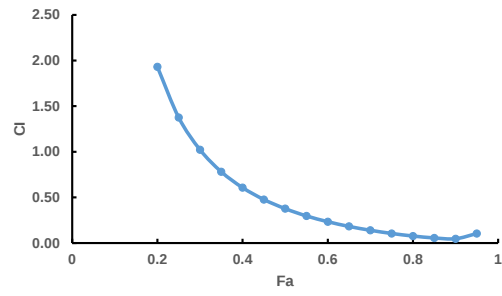


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of abemaciclib and gilteritinib

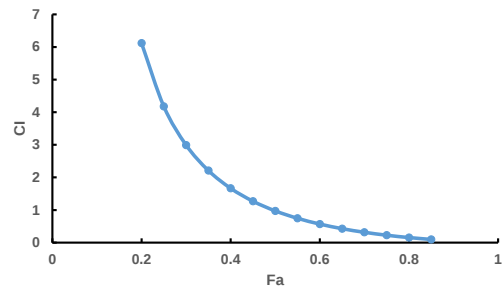
Figure S17: CI values measured in AML cell lines after 48 hours in the combination of abemaciclib and gilteritinib at different concentrations.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of abemaciclib and quizartinib

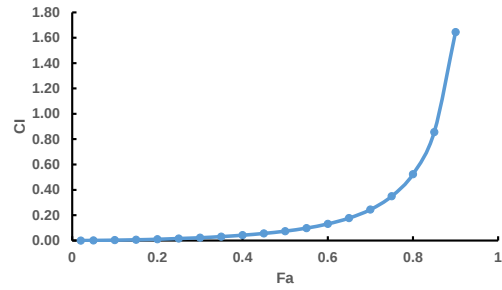


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of abemaciclib and quizartinib

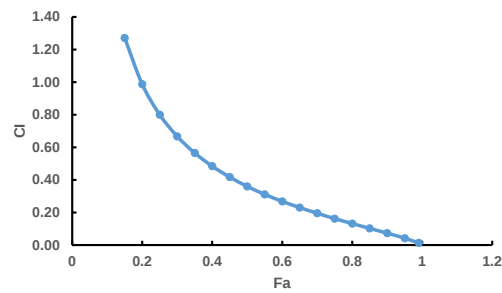


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of abemaciclib and quizartinib

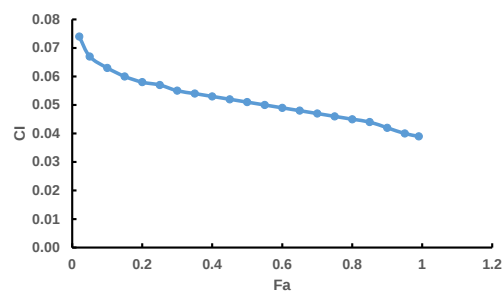
Figure S18: CI values measured in AML cell lines after 48 hours in the combination of abemaciclib and quizartinib at different concentrations.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of gilteritinib and copanlisib

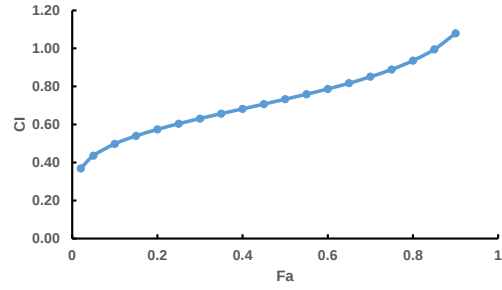


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of gilteritinib and copanlisib

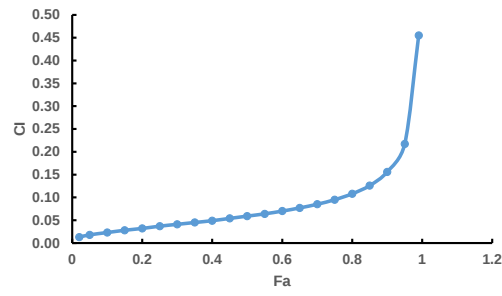


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of gilteritinib and copanlisib

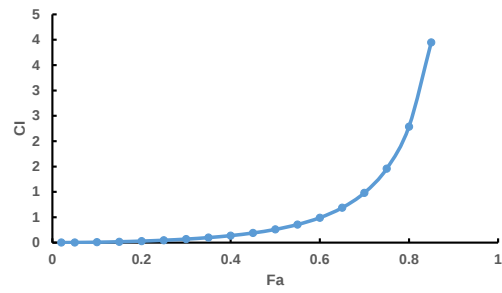
Figure S19: CI values measured in AML cell lines after 48 hours in the combination of gilteritinib and copanlisib at different concentrations.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of gilteritinib and palbociclib

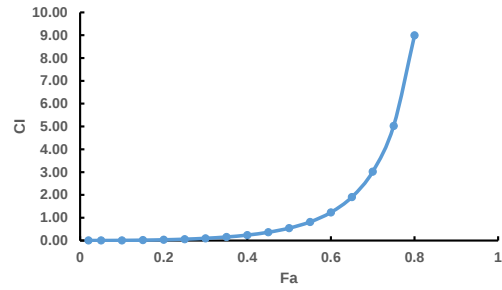


((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of gilteritinib and palbociclib

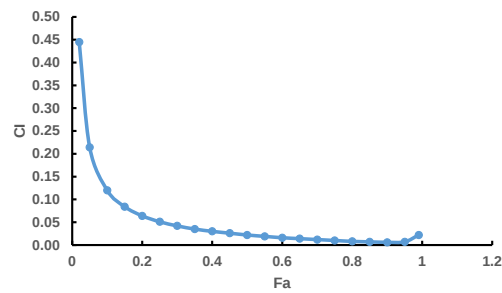


((c)) CI values measured in MV4-11 cells after 48 hours in the combination of gilteritinib and palbociclib

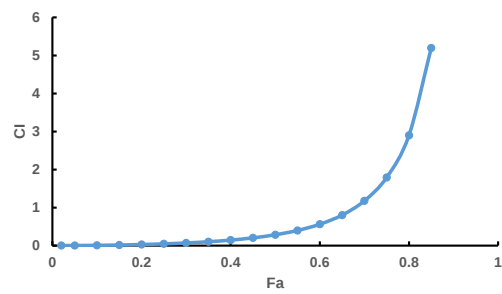
Figure S20: CI values measured in AML cell lines after 48 hours in the combination of gilteritinib and palbociclib at different concentrations.



((a)) CI values measured in MOLM-13 cells after 48 hours in the combination of quizartinib and palbociclib



((b)) CI values measured in MOLM-14 cells after 48 hours in the combination of quizartinib and palbociclib



((c)) CI values measured in MV4-11 cells after 48 hours in the combination of quizartinib and palbociclib

Figure S21: CI values measured in AML cell lines after 48 hours in the combination of quizartinib and palbociclib at different concentrations.

Figure S22: Normalised cell viabilities of MOLM-13 (a, d, g), MOLM-14 (b, e, h) and MV4-11 (c, f, i) cells after 48 hours of treatment with alpelisib (grey lines, a, b and c), duvelisib (grey lines, d, e, and f) or idelalisib (grey lines, g, h, and i). Dark blue lines in all subfigures represented the combination of palbociclib with the PI3K inhibitor and red lines represent cells treated with palbociclib. In all subfigures, the x-axis on the bottom shows the concentration of the PI3K inhibitor while the x-axis on the top shows the concentration of palbociclib. Error bars represent standard deviations calculated from three measurements.

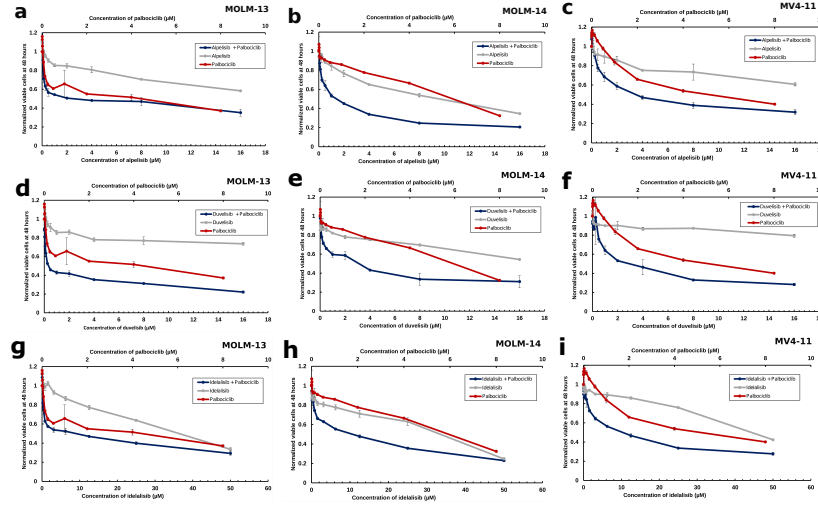


Figure S23: Cell growth of AML cell lines- MOLM-13 (a, d, g, j), MOLM-14 (b, e, h, k) and MV4-11 (c, f, i, l)- treated by palbociclib, idelalisib (a, b, c) or a combination thereof; palbociclib, copanlisib (d, e, f) or a combination thereof; palbociclib, duvelisib (g, h, i) or a combination thereof; palbociclib, alpelisib (j, k, l) or a combination thereof. Error bars were calculated from four experiments. ANOVA with Tukey's post hoc test: one asterisk indicates $p < 0.05$ between experimental group and control, two asterisks indicate $p < 0.01$, three asterisks indicate $p < 0.001$ while ns indicates $p > 0.05$. The concentrations of the inhibitors match their IC₃₀, Table 4.

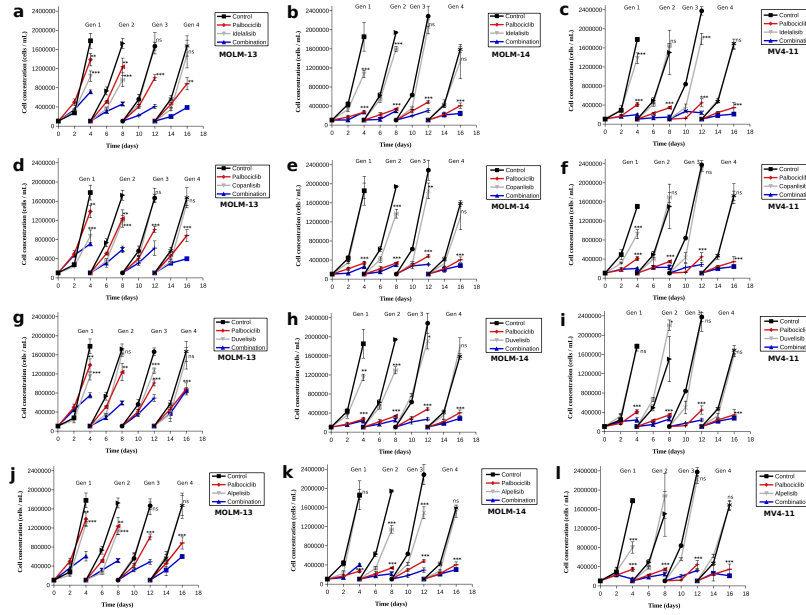


Figure S24: Normalised cell viabilities of MOLM-13 (a, b, c), MOLM-14 (d, e, f) and MV4-11 (g, h, i) cells after 48 hours treated with palbociclib (red lines, a, d and g), copanlisib (red lines, d, e, and h) or abemaciclib (red lines, c, f, and i). While dark blue lines in all subfigures represent the combination of gilteritinib with each different inhibitor and grey lines represent cells treated with gilteritinib. In all subfigures, the x-axis on the bottom shows the concentration of gilteritinib while the x-axis on the top shows the concentration of indicated inhibitor. Error bars represent standard deviations calculated from three measurements.

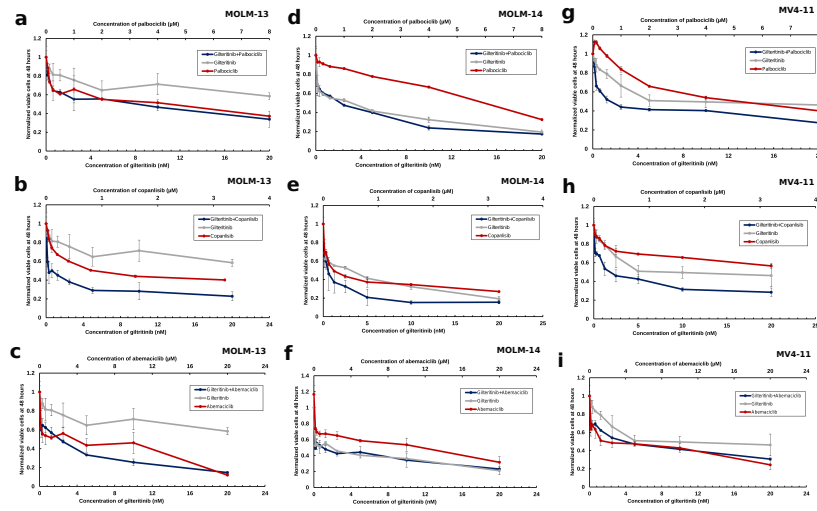


Figure S25: Normalised cell viabilities of MOLM-13 (a, b), MOLM-14 (c, d) and MV4-11 (e,f) cells after 48 hours of treatment with copanlisib (red lines, a, c and e), quizartinib (red lines, b, d and f) or their combinations with abemaciclib. Dark blue lines in all subfigures represented the combination of abemaciclib with different inhibitors and grey lines represented cells treated with abemaciclib. In all subfigures, the x-axis on the bottom shows the concentration of abemaciclib while the x-axis on the top shows the concentration of indicated inhibitor. Error bars represent standard deviations calculated from three measurements.

