Supplementary Materials: Ramucirumab and GSK1838705A Enhance the Inhibitory Effects of Low Concentration Sorafenib and Regorafenib Combination on HCC Cell Growth and Motility

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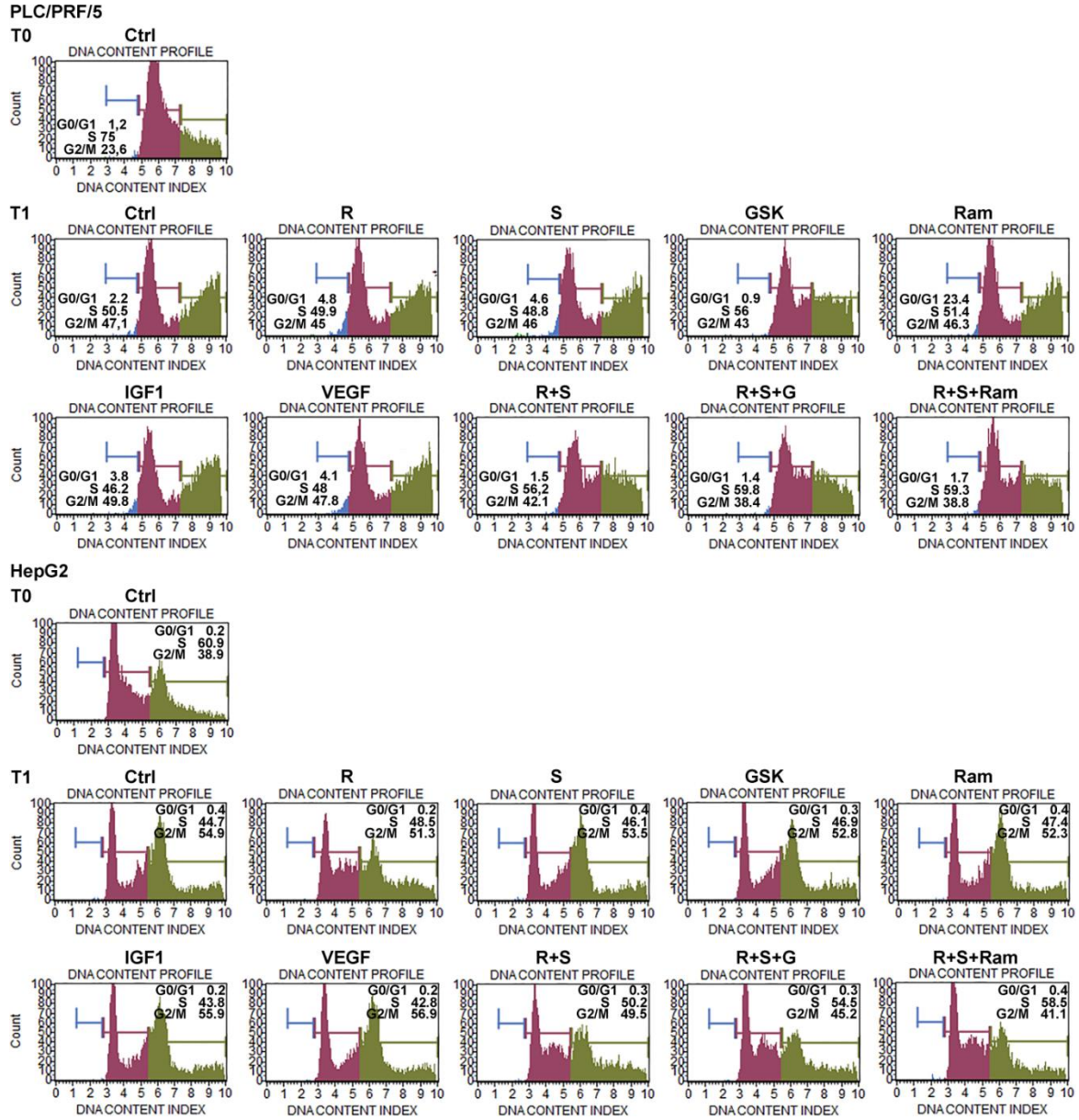

Figure S1. Cell distribution in the cell cycle. Cell cycle analysis performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. IGF1 75 ng/mL and VEGF 20 ng/mL recombinant molecules were used as single treatment. Cells were synchronized with 0.2 M thymidine in the S phase of the cell cycle (T0), after 3 h from block release (T1), the cells were processed and examined with Muse Cell Analyzer to evaluate the percentage of cell distribution in G0/G1, S and G2/M phases. An example of cell cycle progression after different treatments was shown in the panels.

Table S1. Clonogenic Assay. Clonogenic assay performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. IGF1 75 ng/mL and VEGF 20 ng/mL recombinant molecules were used as single treatment. The average value of number of colonies derived from three independent experiments, the *p* value and the percentage decreases for the interest group are reported.

PLC/PRF/5 Samples (Num of Colonies)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
94 vs. 64	0.0001	31.9
Ctrl vs. S	***	
94 vs. 68.5	0.0001	27.1
Ctrl vs. GSK	***	
94 vs. 73	0.0001	22.3
Ctrl vs. Ram	***	
94 vs. 76	0.0001	19.4
Ctrl vs. IGF	ns	
94 vs. 96		2.1
Ctrl vs. VEGF	***	
94 vs. 101	0.0001	+7.4
R vs. R+S	***	
64 vs. 54	0.0001	15.6
R vs. R+S+GSK	***	
64 vs. 42	0.0001	34.4
R vs. R+S+Ram	***	
64 vs. 32.5	0.0001	49.2
S vs. R+S	***	
68.5 vs. 54	0.0001	21.2
S vs. R+S+GSK	***	
68.5 vs. 42	0.0001	38.7
S vs. R+S+Ram	***	
68.5 vs. 32.5	0.0001	52.6
GSK vs. R+S+GSK	***	
73 vs. 42	0.0001	42.5
Ram vs. R+S+Ram	***	
76 vs. 32.5	0.0001	57.2
R+S vs. R+S+GSK	***	
54 vs. 42	0.0001	22.2
R+S vs. R+S+Ram	***	
54 vs. 32.5	0.0001	39.8
HepG2 Samples (Num of Colonies)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
65 vs. 43	0.0001	33.8
Ctrl vs. S	***	
65 vs. 49	0.0001	24.6
Ctrl vs. GSK	***	
65 vs. 51	0.0001	21.5
Ctrl vs. Ram	***	
65 vs. 45	0.0001	30.8
Ctrl vs. IGF	***	
65 vs. 70	0.0001	+7.7
Ctrl vs. VEGF	***	
65 vs. 59.5	0.0001	8.5
R vs. R+S	***	
43 vs. 32.5	0.0001	24.2

R vs. R+S+GSK	***	
43 vs. 24	0.0001	44.2
R vs. R+S+Ram	***	
43 vs. 18.5	0.0001	57
S vs. R+S	***	
49 vs. 32.5	0.0001	33.7
S vs. R+S+GSK	***	
49 vs. 24	0.0001	51
S vs. R+S+Ram	***	
49 vs. 18.5	0.0001	62.2
GSK vs. R+S+GSK	***	
51 vs. 24	0.0001	52.9
Ram vs. R+S+Ram	***	
45 vs. 18.5	0.0001	58.9
R+S vs. R+S+GSK	***	
32.5 vs. 24	0.0001	26.2
R+S vs. R+S+Ram	***	
32.5 vs. 18.5	0.0001	43.1

Table S2. KI67 immunofluorescent staining. KI67 immunofluorescent staining performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. IGF1 75 ng/mL and VEGF 20 ng/mL recombinant molecules were used as single treatment. The average value of IF intensity signals derived from three independent experiments, the *p* value and the percentage decreases for the interest group are reported.

PLC/PRF/5 Samples (IF Intensity Signal)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	**	
2.3 vs. 2	0.001	13
Ctrl vs. S	**	
2.3 vs. 1.7	0.001	26.1
Ctrl vs. GSK	***	
2.3 vs. 1.4	0.0001	39.1
Ctrl vs. Ram	***	
2.3 vs. 0.9	0.0001	60.9
Ctrl vs. IGF	ns	
2.3 vs. 2.6		+13
Ctrl vs. VEGF	ns	
2.3 vs. 2.4		+4.3
R vs. R+S	***	
2 vs. 1.3	0.0001	35
R vs. R+S+GSK	***	
2 vs. 0.5	0.0001	75
R vs. R+S+Ram	***	
2 vs. 0.4	0.0001	80
S vs. R+S	**	
1.7 vs. 1.3	0.001	23.5
S vs. R+S+GSK	***	
1.7 vs. 0.5	0.0001	70.6
S vs. R+S+Ram	***	
1.7 vs. 0.4	0.0001	76.5
GSK vs. R+S+GSK	***	
1.4 vs. 0.5	0.0001	64.3
Ram vs. R+S+Ram	**	
0.9 vs. 0.4	0.001	55.6
R+S vs. R+S+GSK	***	

1.3 vs. 0.5	0.0001	61.5
R+S vs. R+S+Ram	***	
1.3 vs. 0.4	0.0001	69.2
HepG2 Samples (IF Intensity Signal)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
7.9 vs. 6.5	0.0001	17.7
Ctrl vs. S	ns	
7.9 vs. 7.5		5.1
Ctrl vs. GSK	ns	
7.9 vs. 7.5		5.1
Ctrl vs. Ram	***	
7.9 vs. 6.6	0.0001	16.5
Ctrl vs. IGF	ns	
7.9 vs. 8		+1.3
Ctrl vs. VEGF	***	
7.9 vs. 11.5	0.0001	+45.6
R vs. R+S	**	
6.5 vs. 5.6	0.001	13.8
R vs. R+S+GSK	ns	
6.5 vs. 6.3		3.1
R vs. R+S+Ram	***	
6.5 vs. 5	0.0001	23.1
S vs. R+S	***	
7.5 vs. 5.6	0.0001	25.3
S vs. R+S+GSK	***	
7.5 vs. 6.3	0.0001	16
S vs. R+S+Ram	***	
7.5 vs. 5	0.0001	33.3
GSK vs. R+S+GSK	***	
7.5 vs. 6.3	0.0001	16
Ram vs. R+S+Ram	***	
6.6 vs. 5	0.0001	24.2
R+S vs. R+S+GSK	**	
5.6 vs. 6.3	0.001	+12.5
R+S vs. R+S+Ram	**	
5.6 vs. 5	0.001	10.7

Table S3. Muse Annexin V Cell Assay. Muse Annexin V Cell Assay performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. The average value of the percentage of apoptotic cells derived from three independent experiments, the *p* value and the percentage increases for the interest group are reported.

PLC/PRF/5 Samples (% of Apoptotic Cells)	**"Mann Whitney Test" *p* Value**	**Increase (%)**
Ctrl vs. R	***	
17.32 vs. 21.67	0.0001	25
Ctrl vs. S	***	
17.32 vs. 19.15	0.0001	10.6
Ctrl vs. GSK	**	
17.32 vs. 18.16	0.001	4.8
Ctrl vs. Ram	***	
17.32 vs. 25.75	0.0001	48.7
R vs. R+S	***	
21.67 vs. 23.63	0.0001	9
R vs. R+S+GSK	***	

21.67 vs. 30.15	0.0001	39.1
R vs. R+S+Ram	***	
21.67 vs. 39.77	0.0001	83.5
S vs. R+S	***	
19.15 vs. 23.63	0.0001	23.4
S vs. R+S+GSK	***	
19.15 vs. 30.15	0.0001	57.4
S vs. R+S+Ram	***	
19.15 vs. 39.77	0.0001	107.7
GSK vs. R+S+GSK	***	
18.16 vs. 30.15	0.0001	66
Ram vs. R+S+Ram	***	
25.75 vs. 39.77	0.0001	54.4
R+S vs. R+S+GSK	***	
23.63 vs. 30.15	0.0001	27.6
R+S vs. R+S+Ram	***	
23.63 vs. 39.77	0.0001	68.3
HepG2 Samples (% of Apoptotic Cells)	**"Mann Whitney Test" *p* Value**	**Increase (%)**
Ctrl vs. R	***	
15.95 vs. 21.55	0.0001	35.1
Ctrl vs. S	***	
15.95 vs. 21.17	0.0001	32.7
Ctrl vs. GSK	***	
15.95 vs. 21.52	0.0001	34.9
Ctrl vs. Ram	***	
15.95 vs. 28.23	0.0001	77
R vs. R+S	***	
21.55 vs. 27.88	0.0001	29.4
R vs. R+S+GSK	***	
21.55 vs. 26.17	0.0001	21.4
R vs. R+S+Ram	***	
21.55 vs. 38.53	0.0001	78.8
S vs. R+S	***	
21.17 vs. 27.88	0.0001	31.7
S vs. R+S+GSK	***	
21.17 vs. 26.17	0.0001	23.6
S vs. R+S+Ram	***	
21.17 vs. 38.53	0.0001	82
GSK vs. R+S+GSK	***	
21.52 vs. 26.17	0.0001	21.6
Ram vs. R+S+Ram	***	
28.23 vs. 38.53	0.0001	36.5
R+S vs. R+S+GSK	ns	
27.88 vs. 26.17		-0.9
R+S vs. R+S+Ram	***	
27.88 vs. 38.53	0.0001	38.2

Table S4. Cell Migration Assay. Cell Migration Assay performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. The average value of the percentage of migration derived from three independent experiments, the *p* value and the percentage decreases for the interest group are reported.

PLC/PRF/5 Samples (% of Migration)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R 57.09 vs. 29.17	*** 0.0001	48.9
Ctrl vs. S 57.09 vs. 19.65	*** 0.0001	65.6
Ctrl vs. GSK 57.09 vs. 45.59	*** 0.0001	20.1
Ctrl vs. Ram 57.09 vs. 27.19	*** 0.0001	52.4
R vs. R+S 29.17 vs. 13.59	*** 0.0001	53.4
R vs. R+S+GSK 29.17 vs. 8.06	*** 0.0001	72.4
R vs. R+S+Ram 29.17 vs. 3.59	*** 0.0001	87.7
S vs. R+S 19.65 vs. 13.59	*** 0.0001	30.8
S vs. R+S+GSK 19.65 vs. 8.06	*** 0.0001	59
S vs. R+S+Ram 19.65 vs. 3.59	*** 0.0001	81.7
GSK vs. R+S+GSK 45.59 vs. 8.06	*** 0.0001	82.3
Ram vs. R+S+Ram 27.19 vs. 3.59	*** 0.0001	99.9
R+S vs. R+S+GSK 13.59 vs. 8.06	*** 0.0001	40.7
R+S vs. R+S+Ram 13.59 vs. 3.59	*** 0.0001	73.6
HepG2 Samples (% of Migration)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R 17.40 vs. 6.59	*** 0.0001	62.1
Ctrl vs. S 17.40 vs. 5.93	*** 0.0001	65.9
Ctrl vs. GSK 17.40 vs. 10.55	*** 0.0001	39.4
Ctrl vs. Ram 17.40 vs. 0.94	*** 0.0001	94.6
R vs. R+S 6.59 vs. 5.91	ns	10.3
R vs. R+S+GSK 6.59 vs. 3.11	*** 0.0001	52.8
R vs. R+S+Ram 6.59 vs. 0.56	*** 0.0001	91.5
S vs. R+S 5.93 vs. 5.91	ns	0.3
S vs. R+S+GSK 5.93 vs. 3.11	*** 0.0001	47.6
S vs. R+S+Ram 5.93 vs. 0.56	*** 0.0001	90.6

GSK vs. R+S+GSK	***	
10.55 vs. 3.11	0.0001	70.5
Ram vs. R+S+Ram	***	
0.94 vs. 0.56	0.0001	40.4
R+S vs. R+S+GSK	***	
5.91 vs. 3.11	0.0001	47.4
R+S vs. R+S+Ram	***	
5.91 vs. 0.56	0.0001	90.5

Table S5. DyLight 554 Phalloidin immunofluorescent staining. DyLight 554 Phalloidin immunofluorescent staining performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. IGF1 75 ng/mL and VEGF 20 ng/mL recombinant molecules were used as single treatment. The average value of IF intensity signals derived from three independent experiments, the *p* value and the percentage decreases for the interest group are reported.

PLC/PRF/5 Samples (IF Intensity Signal)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
13.8 vs. 6.1	0.0001	55.8
Ctrl vs. S	***	
13.8 vs. 7.2	0.0001	47.8
Ctrl vs. GSK	***	
13.8 vs. 5.8	0.0001	58
Ctrl vs. Ram	***	
13.8 vs. 8.5	0.0001	38.4
Ctrl vs. IGF	ns	
13.8 vs. 13.9		+0.8
Ctrl vs. VEGF	**	
13.8 vs. 11	0.001	20.8
R vs. R+S	***	
6.1 vs. 0.9	0.0001	85.2
R vs. R+S+GSK	***	
6.1 vs. 0.2	0.0001	96.7
R vs. R+S+Ram	***	
6.01 vs. 0.9	0.0001	85
S vs. R+S	***	
7.2 vs. 0.9	0.0001	87.5
S vs. R+S+GSK	***	
7.2 vs. 0.2	0.0001	97.2
S vs. R+S+Ram	***	
7.2 vs. 0.9	0.0001	87.5
GSK vs. R+S+GSK	***	
5.8 vs. 0.2	0.0001	96.6
Ram vs. R+S+Ram	***	
8.5 vs. 0.9	0.0001	89.4
R+S vs. R+S+GSK	***	
0.9 vs. 0.2	0.0001	77.8
R+S vs. R+S+Ram	ns	
0.9 vs. 0.9		0
HepG2 Samples (IF Intensity Signal)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
20 vs. 12.2	0.0001	39
Ctrl vs. S	***	
20 vs. 14	0.0001	30

Ctrl vs. GSK	***	
20 vs. 14.2	0.0001	29
Ctrl vs. Ram	***	
20 vs. 15	0.0001	25
Ctrl vs. IGF	ns	
20 vs. 19.2		4
Ctrl vs. VEGF	ns	
20 vs. 19.2		4
R vs. R+S	ns	
12.2 vs. 10.1		17.2
R vs. R+S+GSK	***	
12.2 vs. 7.1	0.0001	41.8
R vs. R+S+Ram	***	
12.2 vs. 6.2	0.0001	49.2
S vs. R+S	**	
14 vs. 10.1	0.001	27.9
S vs. R+S+GSK	***	
14 vs. 7.1	0.0001	49.3
S vs. R+S+Ram	***	
14 vs. 6.2	0.0001	55.7
GSK vs. R+S+GSK	***	
14.2 vs. 7.1	0.0001	50
Ram vs. R+S+Ram	***	
15 vs. 6.2	0.0001	58.7
R+S vs. R+S+GSK	**	
10.1 vs. 7.1	0.001	29.7
R+S vs. R+S+Ram	**	
10.1 vs. 6.2	0.001	38.6

Table S6. (A) AFP levels. Chemiluminescent immunometric assay performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. The average value of AFP/cell derived from three independent experiments, the *p* value and the percentage decreases for the interest group are reported.

PLC/PRF/5 Samples (AFP/Cell)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
1 vs. 0.82	0.0001	18
Ctrl vs. S	***	
1 vs. 0.72	0.0001	28
Ctrl vs. GSK	***	
1 vs. 0.79	0.0001	21
Ctrl vs. Ram	***	
1 vs. 0.73	0.0001	27
R vs. R+S	***	
0.82 vs. 0.53	0.0001	35.4
R vs. R+S+GSK	***	
0.82 vs. 0.29	0.0001	64.6
R vs. R+S+Ram	***	
0.82 vs. 0.49	0.0001	40.2
S vs. R+S	***	
0.72 vs. 0.53	0.0001	26.4
S vs. R+S+GSK	***	
0.72 vs. 0.29	0.0001	59.7
S vs. R+S+Ram	***	
0.72 vs. 0.49	0.0001	31.9

GSK vs. R+S+GSK	***	
0.79 vs. 0.29	0.0001	63.3
Ram vs. R+S+Ram	***	
0.73 vs. 0.49	0.0001	32.9
R+S vs. R+S+GSK	***	
0.53 vs. 0.29	0.0001	45.3
R+S vs. R+S+Ram	***	
0.53 vs. 0.49	0.0001	7.5
HepG2 Samples (AFP/Cell)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
1 vs. 0.63	0.0001	37
Ctrl vs. S	**	
1 vs. 0.79	0.001	21
Ctrl vs. GSK	***	
1 vs. 0.81	0.0001	19
Ctrl vs. Ram	***	
1 vs. 0.79	0.0001	21
R vs. R+S	ns	
0.63 vs. 0.66		+4.8
R vs. R+S+GSK	***	
0.63 vs. 0.56	0.0001	11.1
R vs. R+S+Ram	***	
0.63 vs. 0.41	0.0001	34.9
S vs. R+S	***	
0.79 vs. 0.66	0.0001	16.5
S vs. R+S+GSK	***	
0.79 vs. 0.56	0.0001	29.1
S vs. R+S+Ram	***	
0.79 vs. 0.41	0.0001	48.1
GSK vs. R+S+GSK	***	
0.81 vs. 0.56	0.0001	30.9
Ram vs. R+S+Ram	***	
0.79 vs. 0.41	0.0001	48.1
R+S vs. R+S+GSK	***	
0.66 vs. 0.56	0.0001	15.2
R+S vs. R+S+Ram	***	
0.66 vs. 0.41	0.0001	37.9

Table S6. (B). DCP levels. Chemiluminescent immunometric assay performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. The average value of DCP/cell derived from three independent experiments, the *p* value and the percentage decreases for the interest group are reported.

PLC/PRF/5 Samples (DCP/Cell)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
1 vs. 0.69	0.0001	31
Ctrl vs. S	***	
1 vs. 0.61	0.0001	39
Ctrl vs. GSK	***	
1 vs. 0.64	0.0001	36
Ctrl vs. Ram	ns	
1 vs. 1.18		+18
R vs. R+S	***	
0.69 vs. 0.19	0.0001	72.5

R vs. R+S+GSK	***	
0.69 vs. 0.12	0.0001	82.6
R vs. R+S+Ram	***	
0.69 vs. 0.23	0.0001	66.7
S vs. R+S	***	
0.61 vs. 0.19	0.0001	68.9
S vs. R+S+GSK	***	
0.61 vs. 0.12	0.0001	80.3
S vs. R+S+Ram	***	
0.61 vs. 0.23	0.0001	62.3
GSK vs. R+S+GSK	***	
0.64 vs. 0.12	0.0001	81.3
Ram vs. R+S+Ram	***	
1.18 vs. 0.23	0.0001	80.5
R+S vs. R+S+GSK	***	
0.19 vs. 0.12	0.0001	36.8
R+S vs. R+S+Ram	ns	
0.19 vs. 0.23		+21.1
HepG2 Samples (DCP/Cell)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	**	
1 vs. 0.82	0.001	18
Ctrl vs. S	ns	
1 vs. 0.99		1
Ctrl vs. GSK	ns	
1 vs. 1.01		+1
Ctrl vs. Ram	***	
1 vs. 0.72	0.0001	28
R vs. R+S	ns	
0.82 vs. 0.79		3.7
R vs. R+S+GSK	ns	
0.82 vs. 0.79		3.7
R vs. R+S+Ram	***	
0.82 vs. 0.59	0.0001	28
S vs. R+S	***	
0.99 vs. 0.79	0.0001	20
S vs. R+S+GSK	***	
0.99 vs. 0.79	0.0001	20
S vs. R+S+Ram	***	
0.99 vs. 0.59	0.0001	40.4
GSK vs. R+S+GSK	***	
1.01 vs. 0.79	0.0001	21.8
Ram vs. R+S+Ram	***	
0.72 vs. 0.59	0.0001	18.1
R+S vs. R+S+GSK	ns	
0.79 vs. 0.79		0
R+S vs. R+S+Ram	***	
0.79 vs. 0.59	0.0001	25.3

Table S7. (A) Muse MAPK Activation Dual Detection Assay. Muse MAPK Activation Dual Detection Assay performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. The average value of percentage of P-Erk activated cells derived from three independent experiments, the *p* value and the percentage decreases for the interest group are reported.

PLC/PRF/5 Samples (% P-Erk Activated Cells)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
86.67 vs. 77.67	0.0001	10.4
Ctrl vs. S	***	
86.67 vs. 79.67	0.0001	8
Ctrl vs. GSK	***	
86.67 vs. 68	0.0001	21.5
Ctrl vs. Ram	***	
86.67 vs. 75	0.0001	13.5
R vs. R+S	***	
77.67 vs. 68.33	0.0001	12
R vs. R+S+GSK	***	
77.67 vs. 63	0.0001	18.9
R vs. R+S+Ram	***	
77.67 vs. 54.33	0.0001	30.1
S vs. R+S	***	
79.67 vs. 68.33	0.0001	14.2
S vs. R+S+GSK	***	
79.67 vs. 68	0.0001	14.6
S vs. R+S+Ram	***	
79.67 vs. 54.33	0.0001	31.8
GSK vs. R+S+GSK	**	
68 vs. 63	0.001	4.4
Ram vs. R+S+Ram	***	
75 vs. 54.33	0.0001	27.6
R+S vs. R+S+GSK	**	
68.33 vs. 63	0.001	7.8
R+S vs. R+S+Ram	***	
68.33 vs. 54.33	0.0001	20.5
HepG2 Samples (% P-Erk Activated Cells)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
97.03 vs. 85.63	0.0001	11.7
Ctrl vs. S	***	
97.03 vs. 89.27	0.0001	8
Ctrl vs. GSK	***	
97.03 vs. 91.90	0.0001	5.3
Ctrl vs. Ram	***	
97.03 vs. 90.53	0.0001	6.7
R vs. R+S	***	
85.63 vs. 77.97	0.0001	8.9
R vs. R+S+GSK	***	
85.63 vs. 65.07	0.0001	24
R vs. R+S+Ram	***	
85.63 vs. 63.83	0.0001	25.5
S vs. R+S	***	
89.27 vs. 77.97	0.0001	12.7
S vs. R+S+GSK	***	
89.27 vs. 65.07	0.0001	27.1

S vs. R+S+Ram	***	
89.27 vs. 63.83	0.0001	28.5
GSK vs. R+S+GSK	***	
91.90 vs. 65.07	0.0001	29.2
Ram vs. R+S+Ram	***	
90.53 vs. 63.83	0.0001	29.5
R+S vs. R+S+GSK	***	
77.97 vs. 65.07	0.0001	16.5
R+S vs. R+S+Ram	***	
77.97 vs. 63.83	0.0001	18.1

Table S7. (**B**) Muse PI3K Activation Dual Detection Assay. Muse PI3K Activation Dual Detection Assay performed in cells cultured with 2.5 μM (PLC/PRF/5) or 1 μM (HepG2) Sorafenib (S), 1 μM (PLC/PRF/5) or 0.1 μM (HepG2) Regorafenib (R), 3 μM GSK1838705A (GSK) and 400 μM Ramucirumab (Ram) alone or in combination. The average value of percentage of P-Akt activated cells derived from three independent experiments, the *p* value and the percentage decreases for the interest group are reported.

PLC/PRF/5 Samples (% P-Akt Activated Cells)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
86.33 vs. 81.97	0.0001	5.1
Ctrl vs. S	*	
86.33 vs. 84	0.05	2.7
Ctrl vs. GSK	***	
86.33 vs. 68.13	0.0001	21.1
Ctrl vs. Ram	***	
86.33 vs. 68.47	0.0001	20.7
R vs. R+S	***	
81.97 vs. 69.83	0.0001	14.7
R vs. R+S+GSK	***	
81.97 vs. 56.70	0.0001	30.8
R vs. R+S+Ram	***	
81.97 vs. 52.60	0.0001	35.8
S vs. R+S	***	
84 vs. 69.83	0.0001	16.9
S vs. R+S+GSK	***	
84 vs. 56.70	0.0001	32.5
S vs. R+S+Ram	***	
84 vs. 52.60	0.0001	37.4
GSK vs. R+S+GSK	***	
68.13 vs. 56.70	0.0001	16.8
Ram vs. R+S+Ram	***	
68.47 vs. 52.60	0.0001	23.2
R+S vs. R+S+GSK	***	
69.83 vs. 56.70	0.0001	18.8
R+S vs. R+S+Ram	***	
69.83 vs. 52.60	0.0001	24.7
HepG2 Samples (% P-Akt Activated Cells)	**"Mann Whitney Test" *p* Value**	**Decrease (%)**
Ctrl vs. R	***	
92.17 vs. 84.07	0.0001	8.8
Ctrl vs. S	***	
92.17 vs. 84.97	0.0001	7.8
Ctrl vs. GSK	***	
92.17 vs. 64.70	0.0001	29.8
Ctrl vs. Ram	***	

92.17 vs. 55.53	0.0001	39.8
R vs. R+S	***	
84.07 vs. 76.07	0.0001	9.5
R vs. R+S+GSK	***	
84.07 vs. 44.93	0.0001	46.6
R vs. R+S+Ram	***	
84.07 vs. 37.10	0.0001	55.9
S vs. R+S	***	
84.97 vs. 76.07	0.0001	10.5
S vs. R+S+GSK	***	
84.97 vs. 44.93	0.0001	47.1
S vs. R+S+Ram	***	
84.97 vs. 37.10	0.0001	56.3
GSK vs. R+S+GSK	***	
64.70 vs. 44.93	0.0001	30.6
Ram vs. R+S+Ram	***	
55.53 vs. 37.10	0.0001	33.2
R+S vs. R+S+GSK	***	
76.07 vs. 44.93	0.0001	40.9
R+S vs. R+S+Ram	***	
76.07 vs. 37.10	0.0001	51.2