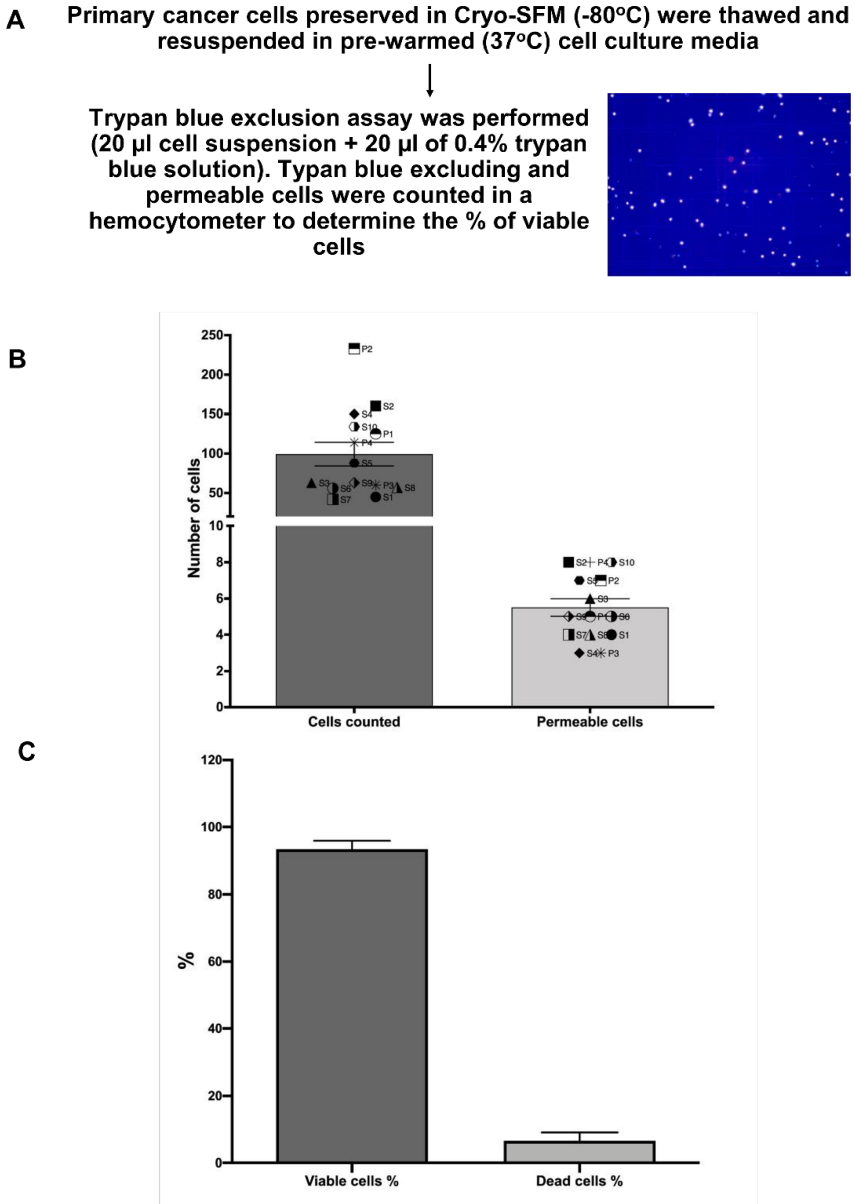
Supplementary Figures

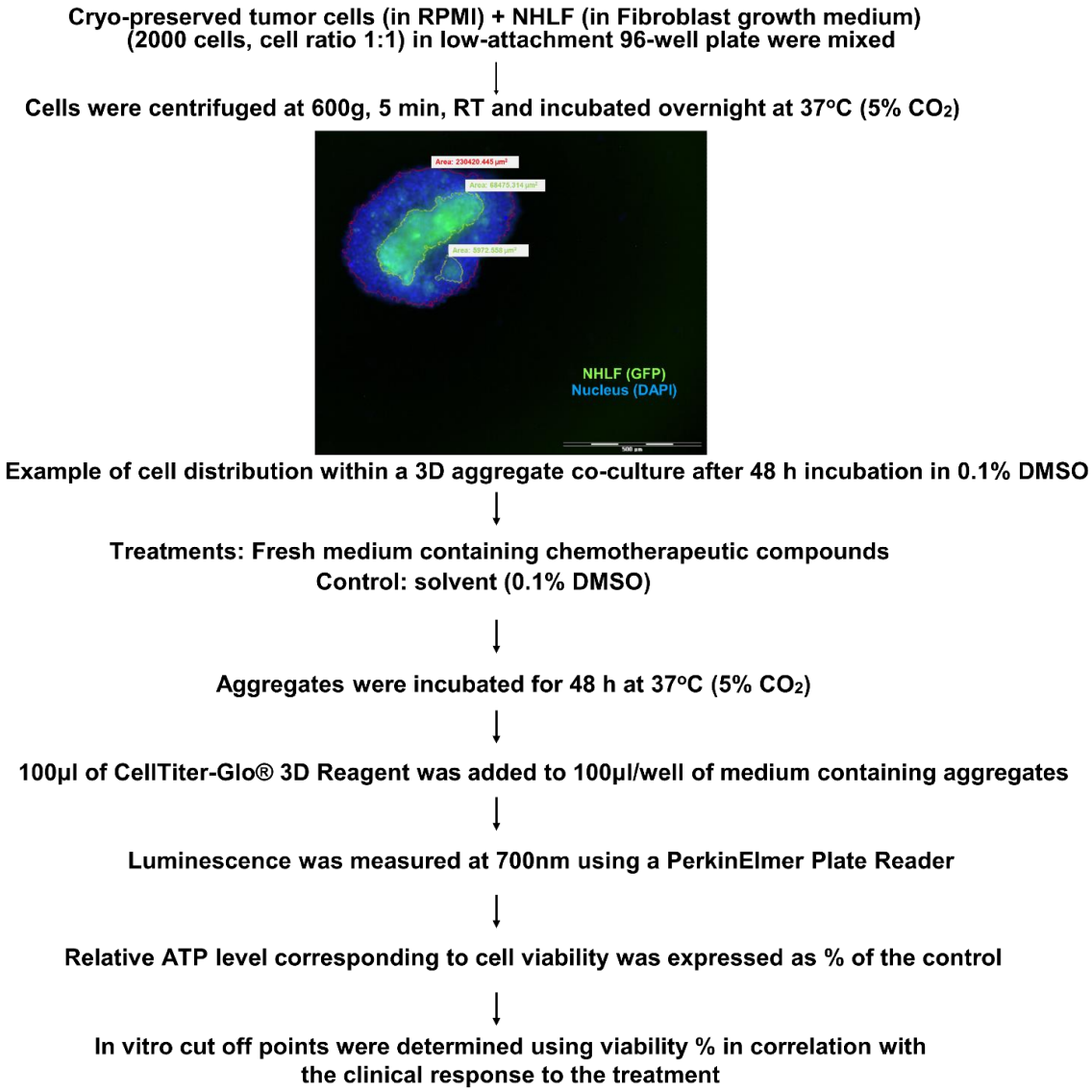
S. Figure 1.



S. Figure 1. Assessment of viable cells by Trypan blue exclusion. A) The main steps of the protocol using 0.4% trypan blue ((Invitrogen, Paisley, UK). Image of a hemocytometer was acquired with EVOS® FL Cell Imaging (Thermo Fisher Scientific, USA); 10x magnification. B) The graph shows the total number of counted cells in individual samples and the corresponding

number of permeable cells. C) The first column represents the average of cellular viability while the second column represents the average of permeable cells in the samples before aggregation.

S. Figure 2.

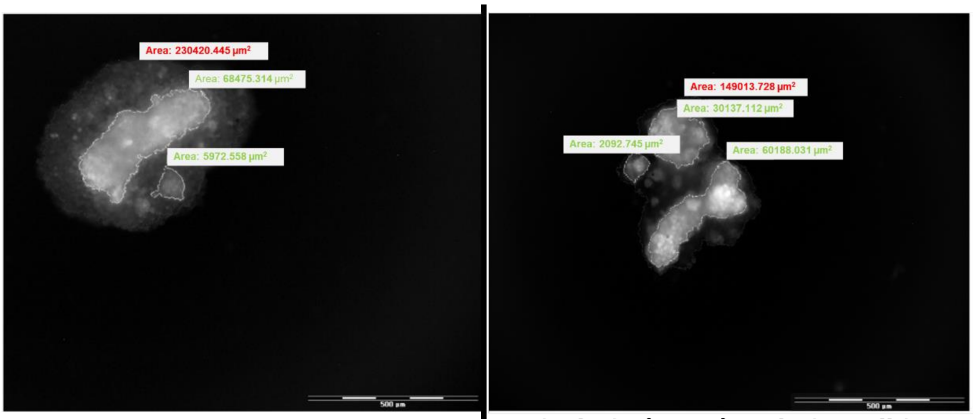


S. Figure 2. Step-by-step protocol of generating tumor aggregate co-cultures and testing their drug response. Cancer and NHLF (GFP) cells were aggregated in equal numbers. The nuclei of all cell types were stained with DAPI. Distribution of different cell types within the aggregate was determined using an Olympus IX83-ZDC ScanR High-content Screening Station (OLYMPUS Corporation, Tokyo, Japan). The core of the aggregate was formed by the NHLF cells containing GFP (green fluorescent protein). The rest of the cells marked only by DAPI (blue) are tumor cells. Size bar 500 nm, 10x magnification. Aggregates were then treated with chemotherapeutic compounds or DMSO as solvent control. Following 48 h incubation, ATP levels were determined

using the CellTiter-Glo® reagent as detailed above. Luminescence was measured at 700 nm using a Perkin Elmer plate reader. ATP levels were compared to the control.

S. Figure 3.

A



B

	Control (0.1% DMSO final cc)	Cisplatin (7 μM) and Vinorelbine (150 nM) (in 0.1% DMSO final cc)	Tumor area compared to tumor area in control (%)	Viability (%) (based on ATP level)
Total area (μm^2)	230420	149013	-	-
NHLF cell area (μm^2)	74444	90553	-	-
Tumor cell area (μm^2)	155976	58460	37.5	-
ATP (luminescence at 700 nm)	4495767.75	2090840.5	-	46.5

C

RECIST	In vitro ATP levels												
	Controls	Various treatments (compared to controls)										Average 1.	Average 2.	
SD	1	0.48	0.47	0.80	0.92	0.79	0.79	0.36	0.74	0.74	-	0.68	0.69	8 out of 9 are at or below 0.8
PD	1	1.02	0.72	0.89	0.85	0.86	0.92	0.99	0.86	0.83	0.87	0.88	0.88	9 out of 10 are above 0.8

S Figure 3. Drug response and cut-off threshold determination. A) **Drug treatment affects primarily the tumor cells.** Cancer and NHLF (GFP) cells were aggregated, then distribution of different cell types within the control and the drug treated aggregates was determined after 48 h incubation at 37°C, 5% CO_2, using an Olympus IX83-ZDC ScanR High-content Screening Station (OLYMPUS Corporation, Tokyo, Japan) at the cross section of each

aggregate. ATP levels were determined by adding the CellTiter-Glo® reagent detailed in S Figure 2, then luminescence was measured at 700 nm in a Perkin Elmer plate reader. ATP levels in individual patient samples were compared to their solvent controls. B) **Loss of tumor cells calculated by the cell area and corresponding ATP levels**. Example of area measurement (μm^2) and ATP luminescence (raw data average of four parallels) in a control (0.1% DMSO) and a cisplatin (7 µM) and vinorelbine (150 nM) (in 0.1% DMSO final cc) treated tumor sample. C) **Cut-off threshold determination**. RECIST1.1 information was collected and compared to in vitro ATP levels. In Average 1. in vitro ATP levels were averaged using all the values, while in Average 2. the highest and the lowest values were disregarded. The last column shows how many individual sample met the 0.8 cut-off criteria.

Supplementary Table 1 (S. Table 1).

Sample number	Mutation	T	N	M	Clinical treatment	RECIST	In vitro treatment	1	2	3	4	Average	Relative viability compared to Control	% of viability compared to control
Solid 1	KRAS	pT1b	N3	M1a	Cisplatin+Pemetrexed	SD	DMSO CTRL	4392962	5766420	4382986	5794422	5084197.5	1	100
							Cisplatin + Pemetrexed	2663124	2347727	2587908	2209790	2452137.25	0.482305664	48.2305664
		T3	N3	M1a	Pemetrexed mono	PD	DMSO CTRL	4392962	5766420	4382986	5794422	5084197.5	1	100
							Pemetrexed	5563192	5180897	5291057	4709620	5186191.5	1.020060983	102.0060983
Solid 2	WT	pT2a	N1	Mx	Cisplatin+Vinorelbine	SD	DMSO CTRL	4592481	4343604	4593782	4453204	4495767.75	1	100
							Cisplatin + Vinorelbine	2138330	2027245.5	1820175	2377611.5	2090840.5	0.465068619	46.5068619
Solid 3	KRAS	T3	N2	M1c	Cisplatin + Pemetrexed	PD	DMSO CTRL	5070243	5035584	5063629	5045554	5053752.5	1	100
							Cisplatin + Vinorelbine	3512207.75	3625248.25	3618955	3741931	3624585.5	0.717206769	71.7206769
Solid 4	KRAS	pT3	N0	M1b	Cisplatin + Gemcitabine	SD	DMSO CTRL	3498680.67	3926307	4204942	3766030	3965759.667	1	100
							Cisplatin + Gemcitabine	3284114	3198635	3141463	3192319	3204132.75	0.807949301	80.7949301
Solid 5	KRAS	pT3a	N1	Mx	Cisplatin + Vinorelbine	SD	DMSO CTRL	2067778	2589611	2204682	2424082	2321538.25	1	100
							Cisplatin + Vinorelbine	1887211	2238472.5	2263340.5	2117668	2126673	0.916062012	91.6062012
Solid 6	KRAS	pT2a	N2	M1b	Carboplatin+Gemcitabine	PD	DMSO CTRL	5744883	6060057	6279357	6216119	6075104	1	100
							Carboplatin + Gemcitabine	5084919.5	5521594.5	5553229.5	5364647.5	5381097.75	0.885762244	88.5762244
Solid 7	KRAS	pT2	pN1	Mx	Cisplatin + Pemetrexed	SD	DMSO CTRL	2656031	2488488	2610351	2389062	2535983	1	100
							Cisplatin + Pemetrexed	2007198	2001356	1960374	2007398	1994081.5	0.786315011	78.6315011
Solid 8	unknown	pT2a	pNx	Mx	Carboplatin-Paclitaxel	SD	DMSO CTRL	2002612	2121004	2191382	2210329	2131331.75	1	100
							Carboplatin + Paclitaxel	1650474	1732825	1707375	1640088	1682690.5	0.789501916	78.9501916
Solid 9	KRAS	pT2a	N1	Mx	Cisplatin + Vinorelbine	SD	DMSO CTRL	4290171	4120942	4158087	4025557	4148689.25	1	100
							Cisplatin + Vinorelbine	552420.5	1797248	1831414	1830274	1502839.125	0.362244322	36.2244322
Solid 10	EGFR	pT2a	pN2	Mx	Cisplatin + Vinorelbine	SD	DMSO CTRL	2807750	2996326	3090362	3092174	2996653	1	100
							Cisplatin + Vinorelbine	2192971	2273866	2200168.5	2158476.5	2206370.5	0.736278274	73.6278274
		pT2	N2	M1b	Giotrif	PD	DMSO CTRL	2807750	2996326	3090362	3092174	2996653	1	100
							Afatinib	2495188.5	2513675.5	2686283.5	2477770	2543229.375	0.84868998	84.868998
PE 1	EGFR	T3	Nx	M1b	Gefitinib	PD	DMSO CTRL	3744748	3919521	3894455	3978613	3884334.25	1	100
							Gefitinib	3210945	3564969	3152655	3370455	3324756	0.855939728	85.5939728
PE 2	KRAS	T2a	N2	M1a	Cisplatin + Pemetrexed	SD	DMSO CTRL	3027400	3460874	2927300	3560974	3244137	1	100
							Cisplatin + Pemetrexed	2431636	2319520	2390011	2442401	2395892	0.738529846	73.8529846
		T2a	N2	M1a	Pemetrexed mono	PD	DMSO CTRL	1598518	1289989	1486734	1461095	1459084	1	100
							Pemetrexed	1322760	1454081	1232542	1332515	1335474.5	0.915282808	91.5282808
PE 3	EGFR	T4	N2	M1	Erlotinib mono	PD	DMSO CTRL	4767514	4605768	4182015	4574209	4532376.5	1	100
							Erlotinib	4204466	4382013	4113864	5286366	4496677.25	0.992123503	99.2123503
		T4	N3	M1c	Pemetrexed mono	PD	DMSO CTRL	4767514	4605768	4182015	4574209	4532376.5	1	100
							Pemetrexed	5132654	4869754	5283504	389019	3918732.75	0.864608832	86.4608832
PE 4	WT	T4	N2	M1b	Carboplatin + Paclitaxel	PD	DMSO CTRL	12701750	13091369	13581975	11969724	13437680	1	100
							Carboplatin + Paclitaxel	12052465	11597837	10830728	10153731	11158690.25	0.830403035	83.0403035
		T4	N3	M1b	Erlotinib mono	PD	DMSO CTRL	12701750	13091369	13581975	11969724	13437680	1	100
							Erlotinib	10874383	12285676	11564835	12305259	11757538.25	0.87496787	87.496787

S. Table 1. In vitro cell viability drug response in individual patient samples compared to clinical treatment and RECIST assessment. Table shows clinical patient number, mutation data, TNM status, clinically used drug combination and RECIST1.1 assessment. The table also contains raw read-out data corresponding to ATP levels in individual controls and drug response in primary cell aggregates. Data is expressed both as average of four parallels, % to the controls as well as relative to individual controls.