Supplementary Figures

S. Figure 1.

A. Primary cancer cells preserved in Cryo-SFM (-80°C) were thawed and resuspended in pre-warmed (37°C) cell culture media. Trypan blue exclusion assay was performed (20 μl cell suspension + 20 μl of 0.4% trypan blue solution). Typan blue excluding and permeable cells were counted in a hemocytometer to determine the % of viable cells.

B. The graph shows the total number of counted cells in individual samples and the corresponding

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.

Cryo-preserved tumor cells (in RPMI) + NHLF (in Fibroblast growth medium) (2000 cells, cell ratio 1:1) in low-attachment 96-well plate were mixed

Cells were centrifuged at 600g, 5 min, RT and incubated overnight at 37°C (5% CO₂)

Example of cell distribution within a 3D aggregate co-culture after 48 h incubation in 0.1% DMSO

Treatments: Fresh medium containing chemotherapeutic compounds
Control: solvent (0.1% DMSO)

Aggregates were incubated for 48 h at 37°C (5% CO₂)

100μl of CellTiter-Glo® 3D Reagent was added to 100μl/well of medium containing aggregates

Luminescence was measured at 700nm using a PerkinElmer Plate Reader

Relative ATP level corresponding to cell viability was expressed as % of the control

In vitro cut off points were determined using viability % in correlation with the clinical response to the treatment

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.
**Figure 3.**

### A

**Control (0.1% DMSO final cc)**

**Cisplatin (7 μM) and Vinorelbine (150 nM) (in 0.1% DMSO final cc)**

### B

<table>
<thead>
<tr>
<th></th>
<th>Control (0.1% DMSO final cc)</th>
<th>Cisplatin (7 μM) and Vinorelbine (150 nM) (in 0.1% DMSO final cc)</th>
<th>Tumor area compared to tumor area in control (%)</th>
<th>Viability (%) (based on ATP level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total area (μm²)</td>
<td>230420</td>
<td>149013</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NHLF cell area (μm²)</td>
<td>74444</td>
<td>90553</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tumor cell area (μm²)</td>
<td>155976</td>
<td>58460</td>
<td>37.5</td>
<td>-</td>
</tr>
<tr>
<td>ATP (luminescence at 700 nm)</td>
<td>449576.75</td>
<td>2090840.5</td>
<td>-</td>
<td>46.5</td>
</tr>
</tbody>
</table>

### C

<table>
<thead>
<tr>
<th>RECIST</th>
<th>In vitro ATP levels</th>
<th>Average 1.</th>
<th>Average 2.</th>
<th>8 out of 9 are at or below 0.8</th>
<th>9 out of 10 are above 0.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>Controls</td>
<td>Various treatments (compared to controls)</td>
<td>0.68</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>1</td>
<td>0.48 0.47 0.80 0.92 0.79 0.79 0.98 0.74 0.74</td>
<td>-</td>
<td>0.68</td>
<td>0.69</td>
</tr>
</tbody>
</table>

**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% CO₂, 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 (µm²) 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).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mutation</th>
<th>T</th>
<th>N</th>
<th>W</th>
<th>Clinical Treatment</th>
<th>RECIST</th>
<th>in vitro treatment</th>
<th>V</th>
<th>E</th>
<th>S</th>
<th>L</th>
<th>Average</th>
<th>Relative viability compared to Control</th>
<th>% of viability compared to control</th>
</tr>
</thead>
</table>
| **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.