

**Effectiveness of Hyperthermia as Monotherapy and Adjuvant Therapy Approaches Against an *In Vitro* Model of Colorectal Carcinoma**

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## Supplementary Material

**Table S1:** The effect of hyperthermia on cell viability (live) and death (apoptotic, necrotic) in CaCo2, HT29 and CCD841CoN cells. Cells were exposed to control (37°C) and hyperthermic (45°C) conditions for 120 min and monitored by means of flow cytometry. Data show percent (%) of CaCo2, HT29 and CCD841CoN cells being either live, apoptotic or necrotic at 24 h post treatment. Data are expressed as means  $\pm$  standard error of the mean and are representative of two independent experiments.

	Live (%)	Apoptotic (%)	Necrotic (%)
CaCo2 Control 37°C	90.3 $\pm$ 0.2	1.45 $\pm$ 0.15	8.15 $\pm$ 0.05
CaCo2 45°C (120 min)	92.8 $\pm$ 0.1	0.8 $\pm$ 0.1	6.15 $\pm$ 0.05
HT29 Control 37°C	87.85 $\pm$ 0.15	2.8 $\pm$ 0.0	9.35 $\pm$ 0.15
HT29 45°C (120 min)	88.4 $\pm$ 0.1	2.8 $\pm$ 0.1	8.8 $\pm$ 0.2
CCD841 CoN Control 37°C	90.8 $\pm$ 0.1	0.9 $\pm$ 0.1	8.25 $\pm$ 0.05
CCD841 CoN 45°C (120 min)	93.45 $\pm$ 0.05	0.7 $\pm$ 0.0	5.65 $\pm$ 0.05

**Table S2:** The effect of hyperthermia on cell cycle distribution in CaCo2, HT29 and CCD841CoN cells. Cells were exposed to control (37°C) and hyperthermic (45°C) conditions for 120 min and monitored by means of flow cytometry. Data show percent (%) of cells in sub-G1, G1, S and G2/M cell-cycle phases in CaCo2, HT29 and CCD841CoN cells at 24 h post treatment. Data are expressed as means  $\pm$  standard error of the mean and are representative of two independent experiments. \*Significantly different at  $p < 0.05$  from the control at 37°C.

	<b>Sub-G1 (%)</b>	<b>G1 (%)</b>	<b>S (%)</b>	<b>G2/M (%)</b>
<b>CaCo2 Control 37°C</b>	3.86 $\pm$ 0.31	49.16 $\pm$ 3.04	19.4 $\pm$ 1.5	27.56 $\pm$ 2.24
<b>CaCo2 45°C (120 min)</b>	3.2 $\pm$ 0.55	26.6 $\pm$ 1.6*	25.06 $\pm$ 0.46*	45.06 $\pm$ 2.54*
<b>HT29 Control 37°C</b>	2.5 $\pm$ 0.5	59.23 $\pm$ 5.3	16.7 $\pm$ 1.51	21.53 $\pm$ 3.5
<b>HT29 45°C (120 min)</b>	2.5 $\pm$ 0.2	38.73 $\pm$ 4.09*	13.2 $\pm$ 1.44	45.6 $\pm$ 4.9*
<b>CCD841 CoN Control 37°C</b>	3.03 $\pm$ 0.98	81.66 $\pm$ 2.2	3.76 $\pm$ 0.61	11.53 $\pm$ 1.91
<b>CCD841 CoN 45°C (120 min)</b>	4.46 $\pm$ 1.31	74.06 $\pm$ 2.88	4.4 $\pm$ 0.47	17.03 $\pm$ 1.24

**Table S3:** The combinational effect of hyperthermia with drugs on cell viability (live) and death (apoptotic, necrotic) in CaCo2 and HT29 cells. Cells were exposed to 3.25 µg/ml 5-fluorouracil (5-FU), 360 µg/ml capecitabine, 250 µg/ml bevacizumab or 250 µg/ml cetuximab either as single agents (37°C) or in combination with hyperthermia (45°C) for 120 min, and monitored by means of flow cytometry. Data show percent (%) of CaCo2 and HT29 cells being either live, apoptotic or necrotic at 24 h post treatment. Data are expressed as means ± standard error of the mean and are representative of two independent experiments. Significantly different at p<0.05 from the control at #37°C and at \*45°C.

CaCo2	Live (%)	Apoptotic (%)	Necrotic (%)
37°C (Control)	86.10 ± 2.7	2.4 ± 0.5	11.35 ± 2.05
45°C (120 min)	80.85 ± 0.85	3.15 ± 0.25	15.55 ± 0.85
5-FU - 37°C	79.25 ± 0.85	3.9 ± 2.3	16.6 ± 2.9
5-FU - 45°C	69.25 ± 7.95	9.0 ± 7.1	21.35 ± 1.25 <sup>*</sup>
Capecitabine - 37°C	88.05 ± 1.25	1.35 ± 0.05	10.3 ± 1.0
Capecitabine - 45°C	65.00 ± 9.7 <sup>*</sup>	10.65 ± 7.25	24.00 ± 2.8 <sup>*</sup>
Bevacizumab - 37°C	85.2 ± 3.1	2.15 ± 0.35	12.55 ± 2.75
Bevacizumab - 45°C	73.3 ± 7.0	3.1 ± 0.3	21.05 ± 5.55
Cetuximab - 37°C	84.5 ± 1	2.4 ± 0.4	12.90 ± 1.4
Cetuximab - 45°C	69.95 ± 5.65 <sup>*</sup>	7.2 ± 1.0 <sup>*</sup>	22.35 ± 6.55

HT29	Live (%)	Apoptotic (%)	Necrotic (%)
37°C (Control)	89.85 ± 0.35	1.65 ± 0.35	8.5 ± 0.0
45°C (120 min)	65.05 ± 5.05	16.15 ± 5.65	18.75 ± 0.55
5-FU - 37°C	79.0 ± 1.1 <sup>#</sup>	6.3 ± 0.1 <sup>#</sup>	14.65 ± 0.95 <sup>#</sup>
5-FU - 45°C	65.6 ± 4.3	13.65 ± 2.45	20.7 ± 1.9
Capecitabine - 37°C	90.85 ± 1.55	1.1 ± 0.2	7.95 ± 1.35
Capecitabine - 45°C	54.35 ± 0.95 <sup>*</sup>	20.2 ± 2.3	25.4 ± 1.4 <sup>*</sup>
Bevacizumab - 37°C	91.3 ± 0.1	1.15 ± 0.05	7.55 ± 0.05
Bevacizumab - 45°C	66.45 ± 1.65	13.05 ± 2.05	20.45 ± 0.35
Cetuximab - 37°C	90.5 ± 1.3	1.4 ± 0.3	8.05 ± 1.05
Cetuximab - 45°C	73.15 ± 5.65	8.95 ± 4.85	17.7 ± 1.0

**Table S4:** The combinational effect of hyperthermia with drugs on cell cycle distribution in CaCo2 and HT29 cells. Cells were exposed to 3.25 µg/ml 5-fluorouracil (5-FU), 360 µg/ml capecitabine, 250 µg/ml bevacizumab or 250 µg/ml cetuximab either as single agents (37°C) or in combination with hyperthermia (45°C) for 120 min, and monitored by means of flow cytometry. Data show percent (%) of cells in sub-G1, G1, S and G2/M cell-cycle phases in CaCo2 and HT29 cells at 24 h post treatment. Data are expressed as means ± standard error of the mean and are representative of two independent experiments. Significantly different at p<0.05 from the control at #37°C and at \*45°C.

CaCo2	Sub-G1 (%)	G1 (%)	S (%)	G2/M (%)
37°C (Control)	2.8 ± 0.3	51.6 ± 0.2	19.6 ± 0.3	26.0 ± 0.2
45°C (120 min)	7.05 ± 0.55	31.9 ± 3	14.35 ± 1.25	46.7 ± 3.7
5-FU - 37°C	2.9 ± 1.3	73.5 ± 3.35 <sup>#</sup>	9.8 ± 1.6 <sup>#</sup>	13.75 ± 0.55 <sup>#</sup>
5-FU - 45°C	3.2 ± 0.4 <sup>*</sup>	13.95 ± 1.55 <sup>*</sup>	10.05 ± 0.85	72.8 ± 0.3 <sup>*</sup>
Capecitabine - 37°C	5.0 ± 0.2 <sup>#</sup>	51.4 ± 0.2	19.8 ± 0.9	23.8 ± 1.3
Capecitabine - 45°C	4.8 ± 0.7 <sup>*</sup>	28.15 ± 3.25	12.25 ± 4.45	54.8 ± 8.4
Bevacizumab - 37°C	2.8 ± 0.1	54.5 ± 2.7	14.9 ± 0.5 <sup>#</sup>	27.8 ± 2.1
Bevacizumab - 45°C	6.4 ± 1.4	32.3 ± 2.6	11.65 ± 1.65	49.65 ± 0.45
Cetuximab - 37°C	3.35 ± 0.45	60.95 ± 0.35 <sup>#</sup>	12.5 ± 1.45 <sup>#</sup>	23.55 ± 0.55
Cetuximab - 45°C	8.15 ± 2.15	37.5 ± 1.9	10.3 ± 0.7	44.05 ± 0.95

HT29	Sub-G1 (%)	G1 (%)	S (%)	G2/M (%)
37°C (Control)	1.65 ± 0.45	69.8 ± 1.2	11.85 ± 1.05	16.7 ± 1.8
45°C (120 min)	13.4 ± 1.4	40.15 ± 8.55	17.95 ± 3.05	28.55 ± 10.25
5-FU - 37°C	9.4 ± 1.1 <sup>#</sup>	38.9 ± 2.1 <sup>#</sup>	32.9 ± 0.4 <sup>#</sup>	18.75 ± 1.98
5-FU - 45°C	15.9 ± 4.8	40.75 ± 10.95	11.7 ± 1.9	31.65 ± 13.85
Capecitabine - 37°C	2.3 ± 0.1	65.8 ± 2.7	15.05 ± 3.35	16.75 ± 0.55
Capecitabine - 45°C	14.05 ± 2.05	41.7 ± 5.0	18.1 ± 2.1	26.15 ± 9.15
Bevacizumab - 37°C	3.95 ± 1.05 <sup>#</sup>	68.65 ± 1.35	9.7 ± 2.4	17.7 ± 0.1
Bevacizumab - 45°C	15.95 ± 2.35	40.95 ± 7.75	14.05 ± 2.15	29.0 ± 12.2
Cetuximab - 37°C	2.15 ± 0.65	69.65 ± 0.75	9.05 ± 1.95	19.0 ± 1.9
Cetuximab - 45°C	13.65 ± 0.65	43.05 ± 9.55	13.25 ± 4.35	30.05 ± 13.25