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Supplemental Tables for:
Myocardial ischemia induced by 5-fluorouracil: a prospective electrocardiographic and cardiac biomarker study
Anne Dyhl-Polk et al.

Table S1: Treatment regimens

Treatment regimen	Administration
de Gramont	5-FU bolus (400 mg/m^2) + 46-hour continuous 5-FU (2400 mg/m^2) + Calcium folinate (400 mg/m^2) *
FOLFOX	5-FU bolus (400 mg/m^2) + 46-hour continuous 5-FU (2400 mg/m^2) + Calcium folinate (400 mg/m^2) + oxaliplatin (85 mg/m^2) +/- cetuximab (500 mg/m^2) and/or panitumumab (6 mg/kg) *
FOLFIRI	5-FU bolus (400 mg/m^2) + 46-hour continuous 5-FU (2400 mg/m^2) + Calcium folinate (400 mg/m^2) + irinotecan (180 mg/m^2) +/- cetuximab (500 mg/m^2) and/or panitumumab (6 mg/kg) *
Chemoradiation with 5-FU and cisplatin	96-hour continuous 5-FU (3200 mg/m^2) + cisplatin (75 mg/m^2) + radiotherapy **

*Two-week schedule. Antiemetics: prednisolone 50 mg day 1–3, ondansetron 16/24 mg day 1 and domperidone 10 mg prn up to three doses per day

**Four-week schedule. Radiotherapy was initiated on day 1 in the first cycle of chemotherapy and was given all weekdays for a total of 30 days. Antiemetics: prednisolone 50 mg day 1–4 and 25 mg day 5+6, aprepitant 125 mg day 1 and 80 mg day 2–3, ondansetron 16 mg day 1 and domperidone 20 mg prn up to three doses per day.

Table S2: Definition of cardiovascular risk factors

Hypertension	A medical history or self-reported diagnosis of hypertension or current intake of antihypertensive medications
Hypercholesterolemia	A medical history or self-reported diagnosis of hypercholesterolemia or current intake of cholesterol lowering medications and/or a non-fasting total cholesterol > 5.0 mmol/L[a]
Diabetes	A medical history or self-reported diagnosis of diabetes or current intake of anti-diabetic medications and/or a fraction of glycosylated hemoglobin (HbA1c) > 48 mmol/mol[b]
Smoking	Self-reported smoking habits Categorized as current smoker, former smoker or never smoked
Body mass index	Calculated from height and weight Categorized according to WHO's classification in: Underweight (< 18.5 kg/m^2) Normal (18.5–24.9 kg/m^2) Overweight (25.0–29.9 kg/m^2) Obese (> 29.9 kg/m^2)

[a] Nordestgaard BG, Langsted A, Mora S et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points—a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. European Heart Journal (2016) 37, 1944–1958. doi:10.1093/eurheartj/ehw152

[b] The Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. European Heart Journal (2013) 34, 3035–3087. doi:10.1093/eurheartj/eht108

Table S3: Intraobserver variability of Holter variables evaluated by re-analyses of 1 day of recording for 10 randomly selected patients

Holter parameters	**Intra-observer agreement / repeatability:**
ST elevations (yes/no)	NA
ST depressions (yes/no)	$\kappa = 1.000$ $p = 0.002$
Fluctuating negative T waves (yes/no)	$\kappa = 0.615$ $p = 0.035$
Number of ST elevations	NA
Number of ST depressions	ICC = 0.995 (95% CI 0.983 – 0.999)
Number of minutes with ST deviations	ICC = 0.971 (95% CI 0.896 – 0.993)
Average duration ischemic episodes	ICC = 0.990 (95% CI 0.962 – 0.997)
Ischemic burden	ICC = 0.980 (95% CI 0.927 – 0.995)
VT (yes/no)	$\kappa = 1.000$ $p = 0.002$
Number of VT episodes	ICC = 0.602 (95% CI 0.030 - 0.882)
HF max	ICC = 1.000
HF min	ICC = 1.000
Time to first occurrence of VT or ST deviations	ICC = 1.000

Intraobserver variability for continuous variables are expressed as intraclass correlation coefficients (ICC) with 95% confidence intervals and intraobserver variability for categorical variables are expressed as kappa values (κ). For ICC the absolute agreement of single measures was estimated using a two-way mixed model.

Table S4: Endpoint definitions

Clinical events	Including acute coronary syndromes, symptomatic tachyarrhythmias and cardiac arrest. Acute coronary syndromes and myocardial infarction were defined according to current guidelines from the European Society of Cardiology.[a, b]
Myocardial ischemia on Holter recording	ST elevation of ≥ 1 mV measured in the J-point lasting at least 1 minute or downsloped or horizontal ST depression of ≥ 1 mV measured 60 ms after the J-point lasting at least one minute. An interval of ≥ 1 minute of recording with no ST deviations should be present before a new discrete episode was counted. The PR-segment was used as reference point, but we corrected for baseline ST-abnormalities.[c]
Myocardial ischemia on 12-lead ECG	Significant ST elevation or significant ST depression in at least two adjacent leads or negative T-waves of ≥ 0.1 mV in two adjacent leads with prominent R or R/S > 1. ST elevation was measured in the J-point and considered significant if ≥ 0.25 mV in V2-V3 for men < 40 years old, ≥ 0.20 mV in V2-V3 for men > 40 years old, ≥ 0.15 mV in V2-V3 for women and ≥ 0.10 mV in all other leads. ST depression was measured 60 ms after the J-point and horizontal or downsloped depressions of ≥ 0.05 mV in at least two adjacent leads were considered significant.[d, e]
Troponin I, elevations and fluctuations	Elevations: Defined as values above the upper 99th percentile cut-off of 40 ng/L.[f] Fluctuations: Increases in troponin I plasma concentrations larger than the assay variation but below the 99th percentile. According to the assay specifications an increase in troponin of 44.4% is considered clinically significant.
Copeptin	Copeptin was analyzed as a continuous variable. Secondly, the number of patients with co-peptin levels above the suggested cut-off for myocardial infarction (10 pmol/L) is given.
Ventricular tachyarrhythmia (both 12 lead ECG and Holter)	≥ 3 complexes with a QRS interval > 120 ms and ≥ 100 beats per minute.[h]
QTc	QT was measured on resting 12-lead ECG and corrected by use of Bazett's formula[i]

[a] Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA et al. Fourth universal definition of myocardial infarction (2018). Eur Heart J 2018.

[b] Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F et al. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). Eur Heart J 2016;37:267-315.

[c] Bjerregaard P, El-Shafei A, Kotar SL, Labovitz AJ. ST segment analysis by Holter Monitoring: methodological considerations. Ann Noninvasive Electrocardiol. 2003 Jul;8(3):200-7.

[d] Guidelines from The Danish Society of Cardiology: https://www.nbv.cardio.dk/aks. Assessed 15th of January 2019.

[e] ESC Scientific Document Group; 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC), Eur Heart J 2018 Jan 7;39(2):119-177. doi: 10.1093/eurheartj/ehx393.

[f] Apple FS, Sandoval Y, Jaffe AS, et al: Cardiac Troponin Assays: Guide to Understanding Analytical Characteristics and Their Impact on Clinical Care. Clin Chem 63:73-81, 2017

[g] Mueller C, Möckel M, Giannitsis E et al. Study Group on Biomarkers in Cardiology of the Acute Cardiovascular Care Association. Use of copeptin for rapid rule-out of acute myocardial infarction. Eur Heart J Acute Cardiovasc Care. 2018 Sep;7(6):570-576. doi: 10.1177/2048872617710791.

[h] Katritsis DG, Zareba W, Camm AJ. Nonsustained ventricular tachycardia. J Am Coll Cardiol. 2012 Nov 13;60(20):1993-2004. doi: 10.1016/j.jacc.2011.12.063. Epub 2012 Oct 17.

[i] QT interval and drug therapy. Bmj 2016;353:i2732.

Table S5: Details concerning patients with acute coronary syndromes

Case	Treatment	Dose intensity of 5-FU	Cycle of onset (day)	Symptoms	Type of event	ECG changes on Holter recording	ECG changes on 12-lead ECG	Elevated troponin or CK-MB	Other findings	Initiated cardiac therapy	Retreatment with 5-FU, dose intensity	Symptoms at retreatment
1	Adj. FOLFOX	100 %	1 (3)	Chest pain radiating to left arm, palpitations	Unstable angina	No	No	No	No	NTG, ASA, Brillique, Arixtra, Statin, Isosorbide mononitrate	Yes, 100%	Chestpain cycle 3
2	Chemoradiation, 5-FU + cisplatin	100 %	1 (4)	Chest pain	Unstable angina	ST↑ (day 4)	No	No	ECHO: Normal	No	No	-
3	Met. FOLFIRI + cetuximab	75%	2 (2)	Chest pain, dyspnea	Unstable angina	-	ST ↑	No	ECHO: Normal CAG: No stenoses	ASA, Brillique	No	-
4	Met. FOLFOX + panitumumab	75%	1 (1)	Severe nausea, dyspnea	STEMI	ST ↑ (day 1+2)	ST ↑, negative T-waves	TnI ↑ CK-MB ↑	ECHO: LVEF 35-40%, regional wall-motion abnormalities CAG: no stenoses	NTG, Isosorbide mononitrate	No	-
5	Adj. FOLFOX	100 %	1 (1)	Severe nausea	NSTEMI	ST ↑ (day 1+2), ST ↓ (day 2) NSVT (day 2)	Non-significant ST ↑ in II, III, aVF, V4-V6	TnI ↑	No	NaCl infusion	No	-

Case	Treatment	Dose intensity of 5-FU	Cycle of onset (day)	Symptoms	Type of event	ECG changes on Holter recording	ECG changes on 12-lead ECG	Elevated troponin or CK-MB	Other findings	Initiated cardiac therapy	Retreatment with 5-FU, dose intensity	Symptoms at retreatment
6	Adj. FOLFOX	100 %	1 (3)	Cardiac arrest	Cardiac arrest	ST ↑ (day 2) ST↓ (day 2)	Sustained VF After ROSC: ST ↑, atrial fibrillation	TnI ↑ CK-MB ↑	ECHO: biventricular failure, LVEF 15-20% CAG: No stenosis	CPR, defibrillation, therapeutic hypothermia, ASA, Heparin	No	-

Adj., adjuvant; Met., metastatic; FOLFOX, 5-FU + oxaliplatin; FOLFIRI, 5-FU + irinotecan; ECG, electrocardiogram; STEMI, ST-elevation myocardial infarction; NSVT, non-sustained ventricular tachycardia; VF, ventricular fibrillation; TnI, troponin I; CK-MB, creatine kinase MB; ECHO, echocardiography; LVEF, left ventricular ejection fraction; CAG, coronary angiography; CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; ASA, ; NTG, nitroglycerin.

Table S6: Patients with silent myocardial ischemia

Case (continued from Table A5)	Treatment	Dose intensity of 5-FU	Ischemia before 1st cycle	Ischemia during 1st cycle	Ischemia before $2^{nd}/3^{rd}/4^{th}$ cycle	Ischemia during $2^{nd}/3^{rd}/4^{th}$ cycle	Other findings	Initiated cardiac therapy	Retreatment with 5-FU, dose intensity
7	Chemoradiation, 5-FU + cisplatin	100%	ST↓	ST↓ (day 1+2+3+4)	No	ST↓ (day 3+4)	-	No	Yes, 100%
8	Chemoradiation, 5-FU + cisplatin	100%	ST↓, negative T-waves	ST↓ (day 1+2+3+4), negative T-waves	-	-	NSVT (baseline + day 1+2+3+4)	No	No
9	Adj. FOLFOX	100%	ST↓	ST↓ (day 1+2+3+4)	-	-	Transient SA block grade 3 with atrial escape rhythm. CAG: No stenoses	Betablocker	No
10	Adj. FOLFOX	100%	ST ↑, ST↓, negative T-waves	ST↓ (day 1+2), negative T-waves, ST ↑ (day 2)	No	No	CAG: No stenoses	No	Yes, reduced to 75% after 1st cycle
11	Adj. FOLFOX	100%	No	-	ST↓	ST↓ (day 1+2)	No	No	Yes, reduced to 50% after 3^{rd} cycle
12	Adj. FOLFOX	100%	No	No	ST ↑	ST ↑ (day 1)	No	No	Yes, 100%
13	Chemoradiation, 5-FU + cisplatin	100%	No	ST↓ (day 1)	-	-	Excessive supraventricular activity	No	No
14	Adj. FOLFOX	100%	No	ST↓ (day 1)	No	No	NSVT (day 1)	Calcium antagonist	Yes, 100%
15	Adj. FOLFOX	100%	No	ST↓ (day 2), negative T-waves	No	No	No	No	Yes, 100%

16	Adj. FOLFOX	100%	No	ST↓ (day 2)	No	ST ↑ (day 2)	No	Calcium antagonist	Yes, reduced to 75% after 3rd cycle
17	Met. FOLFOX	100%	No	ST↓ (day 2), negative T-waves (day 1)	-	-	No	No	No
18	Met. FOLFIRI	100%	No	Day 2+3 ST↓ (day 2+3), negative T-waves	-	-	No	No	No
19	Chemoradiation, 5-FU + cisplatin	100%	No	ST↑ (day 3)	No	No	Transient SA block grade 3 with atrial escape rhythm (cycle 1, day 3)	No	Yes, 100%
20	Chemoradiation, 5-FU + cisplatin	75%	No	No	No	ST↓ (day 2)	No	No	No, completed treatment
21	Adj. FOLFOX	100%	No	No	No	ST↓ (day 2)	No	No	Yes, 100%

Adj., adjuvant; Met., metastatic; FOLFOX, 5-FU + oxaliplatin; FOLFIRI, 5-FU + irinotecan; NSVT, non-sustained ventricular tachycardia; SA, sinus atrial; CAG, coronary angiography.

Day-to-day variation of ischemic episodes before 5-FU treatment

Among the 107 patients evaluable for myocardial ischemia before and during 1st 5-FU infusion, 65 had ≥ 2 days of Holter recording before 1st 5-FU infusion, allowing for the assessment of day-to-day variability. The variability in ischemic episodes was calculated by subtracting the "best day" from the "worst day".

Of the 65 patients evaluable for day-to-day variability before 1st 5-FU infusion, 62 patients had no episodes of myocardial ischemia and thus a day-to-day variability of 0, while three had myocardial ischemia. For these three patients, the variability in number of episodes was four, four and 19 (corresponding to 44%, 49% and 60%, respectively), while the variability in total duration of ischemic episodes was 4.5, 44.4 and 65.3 minutes (corresponding to 13%, 18% and 56%, respectively), and the variability in ischemic burden was 8.8, 102.3 and 188.4 mm*min (corresponding to 20%, 26% and 58%, respectively).

In 2nd/3rd or 4th cycle, 84 patients were evaluable for ischemia analyses, and among these, 45 had ≥ 2 days of Holter recording before 5-FU infusion. Only two patients had myocardial ischemia before infusion and both patients had a variability in number of episodes (1 and 3), total duration of episodes (9.1 and 42.0) and ischemic burden (25.9 and 73.5) of 100%, since myocardial ischemia was observed on only 1 day before treatment (one episode in one patient and three in the other) The other 43 patients had a variability of 0%.

Day-to day variation in ischemia during 5-FU treatment

The day-to-day variation in ischemic burden during 5-FU infusion is shown graphically for patients with myocardial ischemia in Figures A2a and b and Figures A3a and b:

Table S7: Median recording time

	Before 5-FU infusion (hours)	During 46-hour 5-FU infusion (hours)	During 96-hour 5-FU infusion (hours)
First cycle	53.3 (range 10.5–99.2)	47.5 (range 26.0–96.0)	93.2 (range 89.8–120.0)
Second recording (2nd, 3rd or 4th cycle)	48.0 (range 2.1–100.4)	47.7 (range 21.6–131.6)	93.3 (range 32.7–143.2)

Table S8: The number and duration of ischemic episodes and the total ischemic burden per patient per 24 hours

	Before 1st 5-FU infusion (n = 107)	During 1st 5-FU infusion (n = 106)	Before 2nd, 3rd or 4th 5-FU infusion (n = 84)	During 2nd, 3rd or 4th 5-FU infusion (n = 84)
Total ischemic burden[a] (mm*min)	8.05	39.53	0.38	3.00
Total duration of episodes with ST-depression[a] (min)	4.23	9.20	0.16	1.09
Total duration of episodes with ST-elevation[a] (min)	0.40	8.18	0.03	0.20
Number of episodes with ST-depression[a]	0.42	0.74	0.004	0.10
Number of episodes with ST-elevation[a]	0.01	0.35	0.01	0.01
Number of patients with both symptomatic and silent ischemic episodes	0	4	0	0

[a]per patient per 24 hours

Table S9: Myocardial ischemia according to the different days in cycle 1: Results obtained with Friedman's test

	Row Mean Scores Differ (Degrees of freedom)	p-value
Bolus plus 46-hour infusion		
Ischemic burden	12.41 (2)	**0.002**
Total duration of ischemia	11.57 (2)	**0.003**
Number of ST depression episodes	12.48 (2)	**0.002**
Number of ST elevation episodes	2.71 (2)	0.257
96-hour continuous infusion		
Ischemic burden	0.86 (4)	0.931
Total duration of ischemia	1.00 (4)	0.910
Number of ST depression episodes	1.12 (4)	0.891
Number of ST elevation episodes	3.00 (4)	0.558

P values in bold are significant

Table S10: Myocardial ischemia according to the different days in cycle 1: Bonferroni corrected p-values from Wilcoxon signed rank test for patients receiving 46-hour infusion

	p-value (Bonferroni corrected)
Ischemic burden	
Day 1 *v* 0	0.180
Day 2 *v* 0	**0.027**
Day 2 *v* 1	0.097
Total duration of ischemia	
Day 1 *v* 0	0.130
Day 2 *v* 0	**0.023**
Day 2 *v* 1	0.085
Number of ST-depression episodes	
Day 1 *v* 0	0.540
Day 2 *v* 0	**0.042**
Day 2 *v* 1	0.052

P values in bold are significant