# Supplementary Information

## Diverse "just-right" levels of chromosomal instability and their clinical implications in neoadjuvant treated gastric cancer

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## Supplementary Methods

### DNA Isolation

DNA from paired tumour and non-tumorous formal-fixed paraffin-embedded (FFPE) tissues was isolated after microdissection from 8µm thick sections after deparaffinization and proteinase K digestions using the Maxwell extraction system according to the instructions of the manufacturer (Promega, Madison, WI, USA) or using a FFPE DNA purification kit (Qiagen, Hilden, Germany). The tumour areas for DNA isolation were marked by experienced pathologists (M.J., J.S.-H.). Tissue samples with only small amounts were re-suspended after manual microdissection in 200 µl of 50 mmol/L Tris-HCl pH 8.5, 1 mmol/L of EDTA, 0.5% of Tween 20, 0.2 mg/ml of proteinase K and incubated at 55°C for 3 hours. Proteinase K was inactivated by boiling for 10 minutes and a 1:3 – 1:10 dilution was directly used for PCR. Non-tumorous and tumour DNA concentrations were determined by measuring the absorbance at 260 nm using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) or the Qubit DNA quantitation assay (Thermo Fisher Scientific).

### Analysis for microsatellite instability (MSI) using mono- and dinucleotide repeats

MSI was analysed using the five markers BAT25, BAT26, D2S123, D5S346 and D17S250 recommended by the National Cancer Institute.[1] A multiplex PCR with fluorescence-tagged primers was performed on non-tumorous and tumour DNA using the Type-it Microsatellite PCR kit according to the instructions of the manufacturer (Qiagen, Hilden, Germany). Tumours that showed instabilities exclusively at dinucleotide repeats were additionally analysed using the three mononucleotide repeat markers NR-21, NR-24 and NR-27 as described.[2] If no instabilities were observed at these mononucleotide repeats, the tumours were reclassified as MSI-L. This additional analysis leads to the reclassification of three resected tumours initially classified as MSI-H to MSI-L.[2]

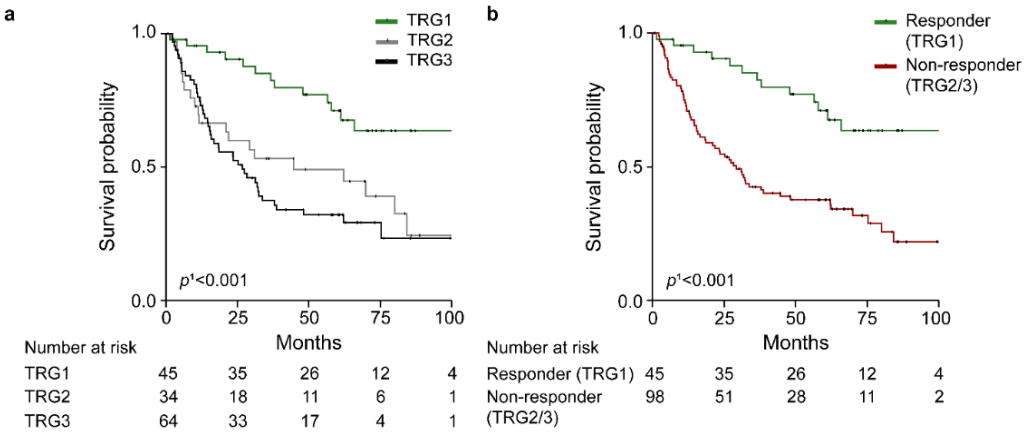
**Cycle conditions of microsatellite based multiplex PCRs and fragment analysis**

The cycle conditions were as follows: after an initial step of 95°C for 5 min, 32 cycles were performed consisting of denaturation at 95°C for 30 sec, annealing at 58°C for 90 sec and extension at 72°C for 30 sec and final extension at 60°C for 30 min. Separation and detection of the PCR products were performed in a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) loaded with POP-7 polymer (Applied Biosystems) and using ROX-500 Genescan (Thermo Fisher Scientific) as size standard. Samples were analysed with the GeneMapper Software 5 (Applied Biosystems).

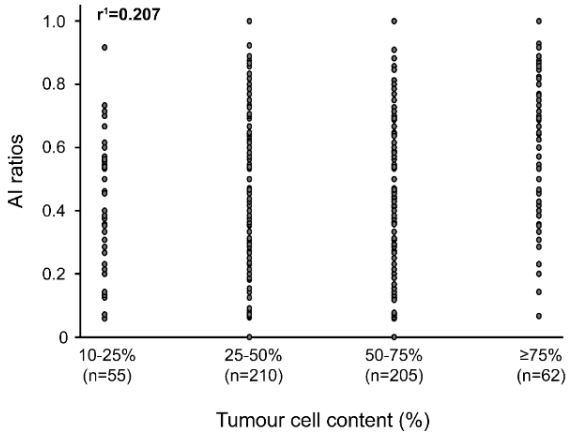
**Statistical analysis**

Comparison between the AI ratios determined by the microsatellite based multiplex PCR assays and the tumour cell contents was performed using the Pearson correlation coefficient (*r*). For the statistical analysis regarding OS and clinical characteristics of the patients, the following tests were used: Kaplan-Meier estimates of survival probabilities were compared by log rank tests. Relative risks were estimated by hazard ratios (HRs) from univariable Cox proportional hazard models. Two-sided Chi-squared tests or Fisher's exact tests were used for hypothesis testing of differences between the relative frequencies. Multivariable analysis was performed by stepwise forward variable selection using Wald-tests of pre- and post-therapeutically available clinical factors and the CIN status was also included as a variable in the model. The pre-therapeutic clinical factors included sex, age (continuous variable), histological Laurén subtypes (intestinal versus non-intestinal), tumour localization (proximal versus non-proximal) and clinical tumour stage (cT2 versus cT3/cT4). The post-therapeutic clinical factors included sex, age (continuous variable), histological Laurén subtypes (intestinal versus non-intestinal), tumour localization (proximal versus non-proximal), (y)pT (pT1-pT4), (y)pN, M-category and R-category. Overall, exploratory 5% significance levels (two-tailed) were used for hypothesis testing. All statistical analyses were performed using SPSS Statistics 25 (IBM corp., Armonk, NY, USA).

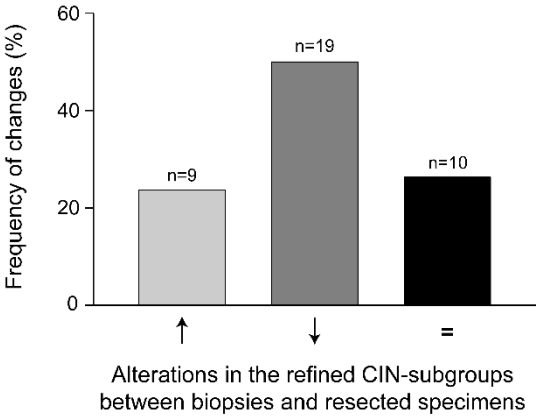
# Supplementary Figures



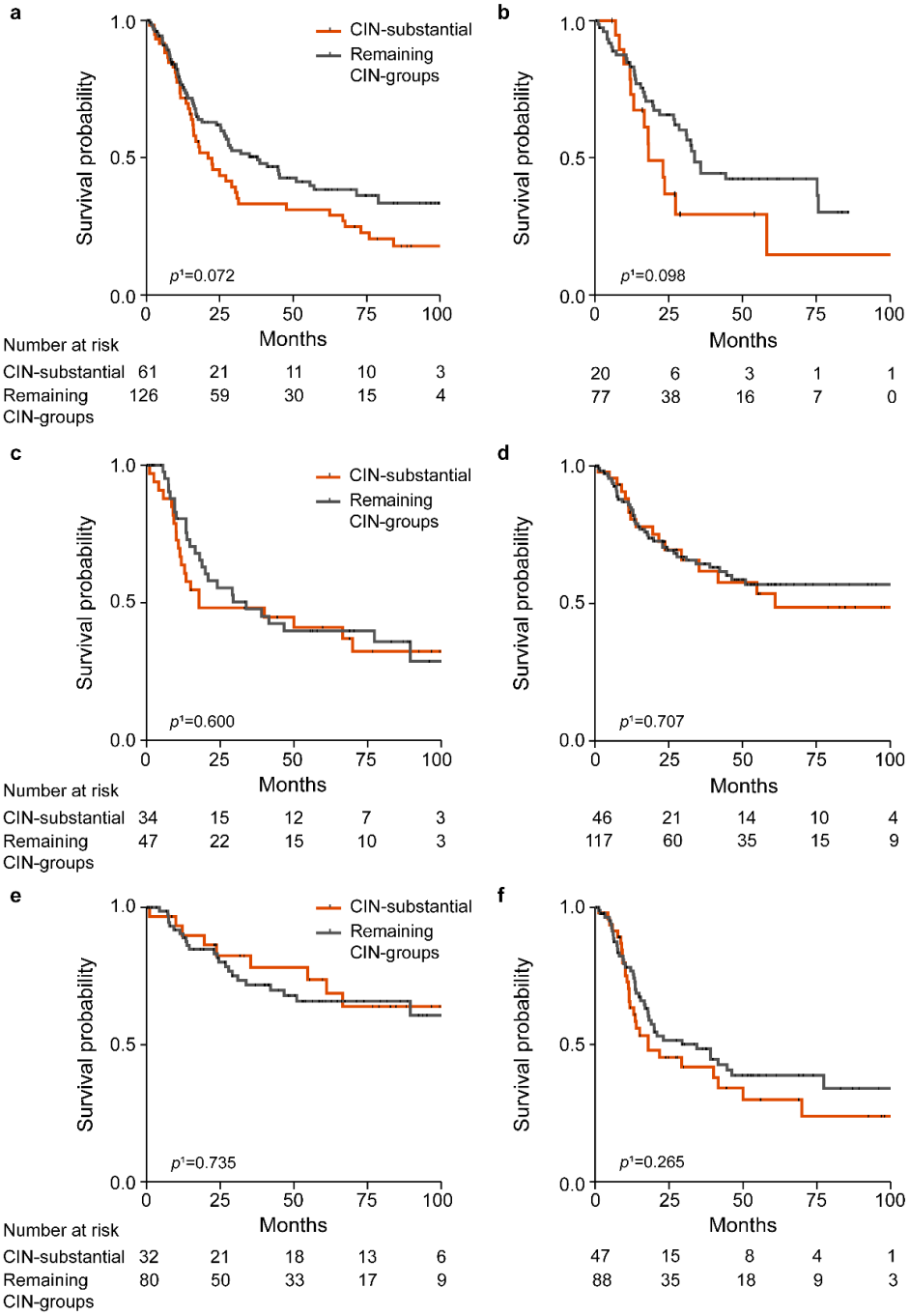

**Supplementary Figure S1: Response to neoadjuvant CTx and association with overall survival in the tumour biopsy cohort before CTx (n=143).** Patients' survival is discriminated by the three tumour regression grades (TRG1, TRG2, TRG3) (a) and by responders (TRG1) and non-responders (TRG2/3) respectively (b). [1]log-rank test




**Supplementary Figure S2: Correlation of tumour cell contents and AI ratios of the resected tumours with or without CTx analysed for the refined CIN classification (n=532).** AI, allelic imbalance; CIN, chromosomal instability; [1]Pearson correlation coefficient




**Supplementary Figure S3. Alterations in the four refined CIN-subgroups between corresponding biopsies before and resected tumours after CTx.** Change from lower CIN-group in the biopsies before CTx to higher one in the corresponding resected tumours after CTx (↑); change from higher CIN-group in the tumour biopsies to lower one in the corresponding resected tumours (↓); identical CIN classification between tumour biopsies and resected tumours (=). CIN, chromosomal instability




**Supplementary Figure S4. Two-tiered CIN classification and association with patients' survival stratified according to tumour localization and clinical tumour stage.** Kaplan-Meier curves are shown for patients with resected tumours with CTx harbouring proximal located tumours (a) and non-proximal located tumours (b), for patients with resected tumours without CTx harbouring proximal located tumours (c) and non-proximal located tumours (d) and for patients with resected tumours without CTx with clinical tumour stage cT2 (e) and cT3/4 (f). AI, allelic imbalance; CIN, chromosomal instability; [1]log-rank test

# Supplementary Tables

**Supplementary Table S1.** Neoadjuvant chemotherapy regimens of the patients included for the refined CIN classification

| **Neoadjuvant chemotherapy** | **Resected tumours after CTx** | | **Tumour biopsies before CTx** | |
|---|---|---|---|---|
| | ***n*** | **%** | ***n*** | **%** |
| Total | 284 | 100 | 122 | 100 |
| Cis + 5-FU or Cap | 113 | 39.8 | 101 | 82.8 |
| Ox + 5-FU or Cap | 39 | 13.7 | 15 | 12.3 |
| Cis + 5-FU + Doc or Pac | 25 | 8.8 | 1 | <1 |
| Ox + 5-FU + Doc | 16 | 5.6 | 0 | 0 |
| Cis or Ox + 5-FU or Cap + Epi | 71 | 25 | 4 | 3.3 |
| Others | 19 | 6.7 | 1 | <1 |
| n/a | 1 | <1 | 0 | 0 |

Cis, cisplatin; Ox, oxaliplatin; 5-FU, 5-fluorouracil; Cap, capecitabine; Doc, docetaxel; Pac, paclitaxel; Epi, epirubicin; Others, combination of Cis/Ox with other agents as small molecule inhibitors or monoclonal antibodies (Imatinib, Panitumumab, Cetuximab) or cross over between different treatment regimens; No patient received preoperative radiotherapy; n/a, no data available.

**Supplementary Table S2.** Chromosomal regions covered by microsatellite markers included in the multiplex PCR assays for the detection of AI and CIN

| **Chromosomal region** | **Microsatellite marker** |
|---|---|
| 2p21 | D2S123[a] |
| 4q22 | D4S423 |
| 5q11 | D5S624 |
| 5q21 | D5S346[a] |
| 6p25 | D6S1617 |
| 7q21 | D7S492 |
| 7q31 | D7S486 |
| 8p23 | D8S552 |
| 8q24[b] | D8S1720 |
| 8q24[b] | D8S1793 |
| 9p21[b] | D9S171 |
| 9p21[b] | D9S157 |
| 12p12[b] | D12S1682 |
| 12p12[b] | D12S1631 |
| 16q23 | D16S507 |
| 17p13 | D17S796 |
| 17q12[b] | D17S1861 |
| 17q12[b] | D17S1872 |
| 17q21 | D17S250[a] |
| 18q21[b] | D18S1119 |
| 18q21[b] | D18S487 |
| 19q12 | D19S875 |

[a]Dinucleotide markers included in Bethesda panel for MSI analysis. [b]Chromosomal regions covered with two microsatellite markers.

**Supplementary Table S3.** Frequency of the molecular subgroups according to TCGA and the refined CIN-groups

| | Resected tumour cohorts | | | Tumour biopsy cohort before CTx n (%) |
|---|---|---|---|---|
| | All n (%) | With CTx n (%) | Without CTx n (%) | |
| **Molecular classification according to TCGA** | | | | |
| EBV(+) | 24 (3.9) | 16 (5) | 8 (2.8) | 6 (4.2) |
| MSI-H | 56[a] (9.2) | 21[a] (6.5) | 35 (12) | 15 (10.5) |
| GS | 56 (9.2) | 30 (9.3) | 26 (8.9) | 7 (4.9) |
| CIN | 476 (77.7) | 254 (79.2) | 222 (76.3) | 115 (80.4) |
| Total | 612[b] (100) | 321[b] (100) | 291 (100) | 143 (100) |
| **Refined CIN classification** | | | | |
| CIN-low | 97 (18.2) | 51 (18) | 46 (18.5) | 10 (8.2) |
| CIN-moderate | 214 (40.2) | 121 (42.6) | 93 (37.5) | 40 (32.8) |
| CIN-substantial | 161 (30.3) | 81 (28.5) | 80 (32.3) | 48 (39.3) |
| CIN-high | 60 (11.3) | 31 (10.9) | 29 (11.7) | 24 (19.7) |
| Total[c] | 532 (100) | 284 (100) | 248 (100) | 122 (100) |

MSI-H, high microsatellite instability; GS, genomic stable; CIN, chromosomal instability; [a]Three resected tumours with instabilities only at dinucleotide repeats and classified initially as MSI-H were reclassified as MSS/MSI-L as specified[2]; [b]From five resected tumours of initially included 617 tumours, no CIN data were available. [c]Only EBV and MSI-H negative tumours were analysed for CIN.

**Supplementary Table S4.** Refined CIN classification and association with clinical characteristics of the resected tumour cohort with CTx (n=284)

| **Category** | **Value** | **CIN low** ***n*** | **CIN moderate** ***n*** | **CIN substantial** ***n*** | **CIN high** ***n*** | ***p*-value[a]** |
|---|---|---|---|---|---|---|
| **Cases** | Total | 51 | 121 | 81 | 31 | |
| **Age Median [yr]** | <64.3 | 29 | 69 | 52 | 16 | 0.604 |
| | ≥64.3 | 22 | 52 | 29 | 15 | |
| **Sex** | Male | 38 | 90 | 70 | 28 | 0.061 |
| | Female | 13 | 31 | 11 | 3 | |
| **Tumour localization** | Proximal | 27 | 76 | 61 | 23 | 0.037 |
| | Non-proximal | 24 | 45 | 20 | 8 | |
| **Laurén classification** | Intestinal | 22 | 64 | 53 | 26 | 0.001 |
| | Non-intestinal | 29 | 57 | 28 | 5 | |
| **Tumour grade** | G1/2 | 9 | 18 | 8 | 7 | 0.459 |
| | G3/4 | 31 | 68 | 50 | 18 | |
| | n/a | 11 | 35 | 23 | 6 | |
| **Clinical tumour stage** | cT2 | 3 | 5 | 6 | 0 | 0.435 |
| | cT3/4 | 48 | 116 | 75 | 30 | |
| | n/a | - | - | - | 1 | |
| **ypT[b]** | ypT1/2 | 7 | 15 | 9 | 7 | 0.435 |
| | ypT3/4 | 44 | 106 | 72 | 24 | |
| **ypN[b]** | Negative | 16 | 35 | 12 | 8 | 0.086 |
| | Positive | 35 | 86 | 69 | 23 | |
| **Metastasis status** | No | 44 | 96 | 63 | 21 | 0.256 |
| | Yes | 7 | 25 | 18 | 10 | |
| **Resection category** | R0 | 40 | 81 | 59 | 21 | 0.394 |
| | R1 | 11 | 41 | 22 | 10 | |
| **Tumour regression grade** | TRG2 | 29 | 56 | 44 | 12 | 0.284 |
| | TRG3 | 22 | 65 | 37 | 19 | |

AI, allelic imbalance; CTx, chemotherapy; CIN, chromosomal instability; n/a, not available; TRG, tumour regression grade; [a]Chi-squared or Fisher's exact test; [b]Classification according to 7th Edition UICC 2007.

**Supplementary Table S5.** Refined CIN classification and association with clinical characteristics of the resected tumour cohort without CTx (n=248)

| Category | Value | CIN low *n* | CIN moderate *n* | CIN substantial *n* | CIN high *n* | *p*-value[a] |
|---|---|---|---|---|---|---|
| **Cases** | Total | 46 | 93 | 80 | 29 | |
| **Age Median [yr]** | <64.3 | 19 | 44 | 27 | 9 | 0.223 |
| | ≥64.3 | 27 | 49 | 53 | 20 | |
| **Gender** | Male | 30 | 62 | 54 | 19 | 0.994 |
| | Female | 16 | 31 | 26 | 10 | |
| **Tumour localization** | Proximal | 3 | 30 | 34 | 14 | <0.001 |
| | Non-proximal | 43 | 60 | 46 | 14 | |
| | n/a | - | 3 | - | - | |
| **Laurén classification** | Intestinal | 7 | 47 | 51 | 22 | <0.001 |
| | Non-intestinal | 39 | 46 | 29 | 7 | |
| **Tumour grade** | G1/2 | 4 | 23 | 33 | 11 | 0.001 |
| | G3/4 | 42 | 69 | 47 | 18 | |
| | n/a | - | 1 | - | - | |
| **Clinical tumour stage** | cT2 | 30 | 40 | 32 | 10 | 0.022 |
| | cT3/4 | 16 | 53 | 47 | 19 | |
| | n/a | - | - | 1 | - | |
| **pT[b]** | pT1/2 | 18 | 31 | 24 | 7 | 0.551 |
| | pT3/4 | 28 | 62 | 56 | 22 | |
| **pN[b]** | Negative | 17 | 36 | 26 | 8 | 0.669 |
| | Positive | 29 | 57 | 54 | 21 | |
| **Metastasis status** | No | 40 | 87 | 74 | 28 | 0.506 |
| | Yes | 6 | 6 | 6 | 1 | |
| **Resection category** | R0 | 40 | 68 | 64 | 26 | 0.122 |
| | R1 | 6 | 25 | 16 | 3 | |

AI, allelic imbalance; CTx, chemotherapy; CIN, chromosomal instability; n/a, not available; [a]Chi-squared or Fisher's exact test; [b]Classification according to 7th Edition UICC 2007.

**Supplementary Table S6.** Survival data of the patients with resected tumours with and without CTx in association with the refined CIN classification in four or two subgroups

| **Resected tumours with CTx (n=284)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Refined CIN classification** | **No.** | **Events** | **Survival probability [%]** | | | **Median survival [months]** | **HR** | ***p*-value[a]** |
| | | | **1 yr** | **3 yr** | **5 yr** | **(95% CI)** | **(95% CI)** | |
| CIN-low | 51 | 23 | 82.5 | 43.5 | 38.7 | 31.1 (22.0-40.3) | 1 ref. | 0.097 - |
| CIN-moderate | 121 | 65 | 78.7 | 48.5 | 38.0 | 35.1 (21.1-49.1) | 1.08 (0.67-1.75) | 0.741 |
| CIN-substantial | 81 | 55 | 73.6 | 32.0 | 28.6 | 21.0 (15.5-36.5) | 1.53 (0.94-2.49) | 0.087 |
| CIN-high | 31 | 13 | 74.8 | 54.0 | 48.6 | 38.7 (-) | 0.84 (0.42-1.66) | 0.611 |
| CIN-substantial | 81 | 55 | 73.6 | 32.0 | 28.6 | 21.0 (15.5-26.5) | 1.49 (1.07-2.08) | 0.017 |
| Remaining CIN-groups | 203 | 101 | 79.1 | 48.4 | 39.8 | 35.1 (23.9-46.3) | 1 ref. | |
| Total | 284 | 156 | 77.5 | 43.7 | 36.6 | 30.3 (25.2-35.4) | - | - |
| **Resected tumours without CTx (n=248)** | | | | | | | | |
| **Refined CIN classification** | **No.** | **Events** | **Survival probability [%]** | | | **Median survival [months]** | **HR** | ***p*-value[a]** |
| | | | **1 yr** | **3 yr** | **5 yr** | **(95% CI)** | **(95% CI)** | |
| CIN-low | 46 | 14 | 87.7 | 73.3 | 58.7 | nr | 1 ref. | 0.379 - |
| CIN-moderate | 93 | 39 | 81.9 | 56.5 | 51.6 | 77.3 (25.9-128.7) | 1.46 (0.79-2.69) | 0.227 |
| CIN-substantial | 80 | 40 | 74.4 | 56.2 | 48.3 | 54.8 (23.7-85.9) | 1.69 (0.92-3.11) | 0.091 |
| CIN-high | 29 | 15 | 82.1 | 52.3 | 45.7 | 51.0 (0.96-101.04) | 1.68 (0.81-3.47) | 0.165 |
| CIN-substantial | 80 | 40 | 74.4 | 56.2 | 48.3 | 54.8 (23.7-85.9) | 1.24 (0.84-1.83) | 0.288 |
| Remaining CIN-groups | 168 | 68 | 83.4 | 59.9 | 52.3 | 89.5 (40.6-138.4) | 1 ref. | |
| Total | 248 | 108 | 80.5 | 58.7 | 50.8 | 61.1 (27.5-94.7) | - | - |

CIN; chromosomal instability; CTx, neoadjuvant chemotherapy; No., number of patients; CI, confidence interval; HR, Hazard ratio; nr, not reached; ref., reference. [a]Cox's regression compared to reference.

**Supplementary Table S7.** Multivariable analysis of survival including the two-tiered CIN status and pre- and post-therapeutically available clinical factors in the resected tumour cohort with CTx

| | HR | 95% CI | *p*-value[a] |
|---|---|---|---|
| **Resected tumours with CTx: Pretherapeutic factors** | | | |
| **CIN status** | | | |
| CIN-substantial | 1.48 | 1.07-2.06 | 0.019 |
| Remaining CIN-groups | 1 ref. | - | |
| **Resected tumours with CTx: Posttherapeutic factors** | | | |
| **R-category** | | | |
| R0 | 0.54 | 0.38-0.78 | 0.001 |
| R1 | 1 ref. | - | |
| **ypN[b]** | | | |
| ypN0 | 0.40 | 0.26-0.63 | <0.001 |
| ypN1 | 1 ref. | - | |
| **ypT[b]** | | | 0.003 |
| ypT1 | 0.43 | 0.10-1.81 | 0.252 |
| ypT2 | 0.43 | 0.21-0.88 | 0.021 |
| ypT3 | 0.53 | 0.37-0.75 | <0.001 |
| ypT4 | 1 ref. | - | - |
| **Metastasis status** | | | |
| Negative | 0.56 | 0.39-0.81 | 0.002 |
| Positive | 1 ref. | - | |
| **CIN status** | | | |
| CIN-substantial | 1.48 | 1.05-2.08 | 0.026 |
| Remaining CIN-groups | 1 ref. | - | |

ref., reference; CIN, chromosomal instability; HR; Hazard ratio; CI, confidence interval. [a]Wald-Test of Hazard Ratio; [b]TNM classification according to 7th Edition UICC

**Supplementary Table S8.** Multivariable analysis of survival including the two-tiered CIN status and pre- and post-therapeutically available clinical factors in the resected tumour cohort without CTx

| | HR | 95% CI | *p*-value[a] |
|---|---|---|---|
| **Resected tumours without CTx: Pretherapeutic factors** | | | |
| **cT** | | | |
| cT2 | 0.34 | 0.23-0.52 | <0.001 |
| cT3/4 | 1 ref. | - | |
| **Age** | 1.03 | 1.01-1.05 | <0.001 |
| **Resected tumours without CTx: Posttherapeutic factors** | | | |
| **pN[b]** | | | |
| pN0 | 0.27 | 0.16-0.46 | <0.001 |
| pN1 | 1 ref. | - | |
| **Age** | 1.03 | 1.01-1.04 | 0.002 |
| **Metastasis status** | | | |
| Negative | 0.40 | 0.20-0.80 | 0.010 |
| Positive | 1 ref. | - | |
| **Localization** | | | |
| Proximal | 1.56 | 1.06-2.30 | 0.025 |
| Non-proximal | 1 ref. | - | |

ref., reference; CIN, chromosomal instability; HR; Hazard ratio; CI, confidence interval. [a]Wald-Test of Hazard Ratio; [b]TNM classification according to 7th Edition UICC

**Supplementary Table S9.** Survival data of the patients with tumour biopsies before CTx in association with the refined CIN classification and response to neoadjuvant CTx

| **Refined CIN classification** | **No.** | **Events** | **Survival probability [%]** | | | **Median survival [months]** | **HR** | ***p*-value[a]** |
|---|---|---|---|---|---|---|---|---|
| | | | **1 yr** | **3 yr** | **5 yr** | **(95% CI)** | **(95% CI)** | |
| | | | | | | | | 0.674 |
| CIN-low | 10 | 5 | 90.0 | 64.3 | 64.3 | 62.1 (4.7-119.5) | 1 ref. | - |
| CIN-moderate | 40 | 24 | 73.2 | 47.9 | 44.7 | 33.8 (4.3-63.3) | 1.35 (0.51-3.54) | 0.546 |
| CIN-substantial | 48 | 24 | 75.0 | 55.1 | 50.5 | 62.2 (-) | 0.98 (0.37-2.59) | 0.973 |
| CIN-high | 24 | 15 | 73.9 | 55.9 | 37.3 | 37.9 (27.0-48.8) | 1.32 (0.48-3.64) | 0.592 |
| | | | | | | | | 0.004 |
| CIN-high, responder | 12 | 6 | 81.8 | 63.6 | 45.5 | 57.8 (-) | 0.62 (0.26-1.44) | 0.226 |
| CIN-high, non-responder | 12 | 9 | 66.7 | 48.6 | 29.2 | 31.1 (1.9-60.2) | 1.10 (0.54-2.26) | 0.786 |
| non-CIN-high, responder | 26 | 5 | 100 | 90.9 | 86.1 | nr | 0.19 (0.08-0.48) | <0.001 |
| non-CIN-high, non-responder | 72 | 48 | 67.5 | 40.7 | 37.2 | 27.4 (16.7-38.1) | 1 ref. | - |
| Total | 122 | 68 | 75.6 | 54.1 | 47.1 | 44.6 (18.5-70.8) | - | - |

CIN; chromosomal instability; CTx, neoadjuvant chemotherapy; No., number of patients; CI, confidence interval; HR, Hazard ratio; nr, not reached; ref., reference. [a]Cox's regression compared to reference.

**Supplementary Table S10.** Sequence variants identified by targeted sequencing using a custom designed GC related gene panel

| Gene | Exon | Ref Seq Number | cDNA description | Protein description | Type of mutation | Entry in database[a] |
|---|---|---|---|---|---|---|
| APC | 17 | NM_001127510.3 | c.4348C>T | p.R1450* | Nonsense | COSV57321313 |
| ARID1A | 5 | NM_006015.6 | c.2077C>T | p.R693* | Nonsense | COSV61375361 |
| ARID1A | 20 | NM_006015.6 | c.5965C>T | p.R1989* | Nonsense | COSV61370535 |
| ARID1A | 20 | NM_006015.6 | c.6020_6036del | p.L2007Qfs*20 | Frameshift deletion | - |
| ATM | 29 | NM_000051.4 | c.4385C>G | A1462G | Missense | - |
| CCND1 | 5 | NM_053056.3 | c.775_790del | p.L259Rfs*91 | Frameshift deletion | - |
| CDH1 | 1 | NM_004360.5 | c.11G>A | p.W4* | Nonsense | - |
| CDH1 | 3 | NM_004360.5 | c.220C>T | p.R74* | Nonsense | COSV55732282 |
| CDH1 | 5 | NM_004360.5 | c.641T>C | p.L214P | Missense | COSV55734778 |
| CDH1 | 7 | NM_004360.5 | c.948_956del | p.M316_T318del | Nonframeshift deletion | - |
| CDH1 | 7 | NM_004360.5 | c.975_998del | p.I326_L333del | Nonframeshift deletion | - |
| CDH1 | 8 | NM_004360.5 | c.1009-2A>G | p.? | Splice acceptor variant | cBioPortal |
| CDH1 | 8 | NM_004360.5 | c.1088T>A | p.I363N | Missense | - |
| CDH1 | 10 | NM_004360.5 | c.1765-1_1765-4del | p.? | Splice region variant | - |
| CDH1 | 13 | NM_004360.5 | c.2145_2164del | p.G716Sfs*25 | Frameshift deletion | - |
| CDKN2A | 1 | NM_058197.4 | c.148_164del | p.Q50Cfs*23 | Frameshift del. | - |
| CDKN2A | 2 | NM_000077.5 | c.160_162del | p.M54del | Nonframeshift deletion | COSV58699795 |
| CTNNA1 | 6 | NM_001903.5 | c.643C>T | p.Q215* | Nonsense | - |
| CTNNB1 | 3 | NM_001904.4 | c.134C>T | p.S45F | Missense | COSV62687872 |
| ERBB2 | 8 | NM_004448.4 | c.929C>T | p.S310F | Missense | COSV54062198 |
| ERBB2 | 20 | NM_004448.4 | c.2329G>C | p.V777L | Missense | COSV54062385 |
| ERBB3 | 7 | NM_001982.4 | c.734C>T | p.A245V | Missense | COSV57248597 |
| ERBB3 | 7 | NM_001982.4 | c.850G>A | p.G284R | Missense | COSV57246350 |
| ERBB4 | 23 | NM_005235.3 | c.2762A>T | p.Y921F | Missense | - |
| FBXW7 | 9 | NM_033632.3 | c.1322G>A | p.R441Q | Missense | COSV55902900 |
| FBXW7 | 10 | NM_033632.3 | c.1513C>T | p.R505C | Missense | COSV55891274 |
| FGFR1 | 8 | NM_023110.3 | c.1010G>A | p.G337E | Missense | rs1064793122 |
| FGFR1 | 8 | NM_023110.3 | c.1042G>A | p.G348R | Missense | rs886037634 |
| FGFR1 | 10 | NM_023110.3 | c.1334G>A | p.R445Q | Missense | - |
| KRAS | 2 | NM_033360.4 | c.34G>A | p.G12S | Missense | COSV55497461 |
| KRAS | 2 | NM_033360.4 | c.35G>T | p.G12V | Missense | COSV55497419 |
| NRAS | 2 | NM_002524.5 | c.34G>T | p.G12C | Missense | COSV54736487 |
| PIK3CA | 10 | NM_006218.4 | c.1624G>A | p.E542K | Missense | COSV55873227 |
| PIK3CA | 10 | NM_006218.4 | c.1634A>C | p.E545A | Missense | COSV55873209 |
| PIK3CA | 10 | NM_006218.4 | c.1633G>A | p.E545K | Missense | COSV55873239 |
| PIK3R1 | 13 | NM_181523.3 | c.1709_1714delinsGGA | p.L570_Q572delinsRK | Nonframeshift substitution | - |
| PREX2 | 2 | NM_024870.4 | c.148T>G | p.L50V | Missense | COSV55751655 |
| PREX2 | 2 | NM_024870.4 | c.190A>C | p.N64H | Missense | - |
| PREX2 | 24 | NM_024870.4 | c.2738G>T | p.R913M | Missense | COSV99909747 |
| PTEN | 5 | NM_000314.8 | c.335T>A | p.L112Q | Missense | COSV64304040 |
| PTPRT | 8 | NM_133170.4 | c.1317G>T | p.E439D | Missense | COSV61984828 |
| RHOA | 2 | NM_001664.4 | c.125A>G | p.Y42C | Missense | COSV69041523 |
| RNF43 | 8 | NM_017763.6 | c.935G>A | p.C312Y | Missense | - |
| SMAD4 | 9 | NM_005359.6 | c.1049T>G | p.V350G | Missense | COSV61684103 |
| SMAD4 | 9 | NM_005359.6 | c.1067C>A | p.P356H | Missense | COSV61689602 |
| TGFBR2 | 5 | NM_001024847.2 | c.1243G>A | p.D415N | Missense | COSV55447128 |
| TGFBR2 | 5 | NM_001024847.2 | c.1228_1235dup | p.L413Sfs*3 | Frameshift insertion | - |
| TGFBR2 | 5 | NM_001024847.2 | c.1259T>A | p.L420Q | Missense | - |
| TGFBR2 | 5 | NM_001024847.2 | c.1217A>G | p.K406R | Missense | - |
| TGFBR2 | 6 | NM_001024847.2 | c.1406_1409del | p.Q469Pfs*13 | Frameshift deletion | - |
| TGFBR2 | 8 | NM_001024847.2 | c.1649C>G | p.P550R | Missense | - |
| TLR4 | 3 | NM_138554.5 | c.1459T>G | p.F487V | Missense | COSV62923063 |
| TP53 | 4 | NM_000546.5 | c.338dup | p.H115Afs*34 | Frameshift insertion | COSV53530722 |
| TP53 | 5 | NM_000546.5 | c.524G>A | p.R175H | Missense | COSV52661038 |
| TP53 | 5 | NM_000546.5 | c.541C>T | p.R181C | Missense | COSV52689134 |

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|---|---|---|---|---|---|---|
| TP53 | 5 | NM_000546.5 | c.404G>A | p.C135Y | Missense | COSV52675774 |
| TP53 | 5 | NM_000546.5 | c.527G>A | p.C176Y | Missense | COSV52660760 |
| TP53 | 5 | NM_000546.5 | c.392A>T | p.N131I | Missense | COSV52718021 |
| TP53 | 5 | NM_000546.5 | c.437G>A | p.W146* | Nonsense | COSV52661900 |
| TP53 | 6 | NM_000546.5 | c.567_577del | p.P190S*15 | Frameshift deletion | - |
| TP53 | 6 | NM_000546.5 | c.659A>G | p.Y220C | Missense | COSV52661282 |
| TP53 | 6 | NM_000546.5 | c.560_562-7del | p.? | Splice region variant | cBioPortal |
| TP53 | 7 | NM_000546.5 | c.764_766del | p.I255del | Nonframeshift deletion | COSV52737696 |
| TP53 | 7 | NM_000546.5 | c.743G>A | p.R248Q | Missense | COSV52661580 |
| TP53 | 7 | NM_000546.5 | c.722C>T | p.S241F | Missense | COSV52661688 |
| TP53 | 7 | NM_000546.5 | c.673-1G>C | p.? | Splice acceptor variant | COSV52707963 |
| TP53 | 8 | NM_000546.5 | c.824G>A | p.C275Y | Missense | COSV52661919 |
| TP53 | 8 | NM_000546.5 | c.892G>T | p.E298* | Nonsense | COSV52661551 |
| TP53 | 8 | NM_000546.5 | c.817C>T | p.R273C | Missense | COSV52662066 |
| TP53 | 8 | NM_000546.5 | c.818G>A | p.R273H | Missense | COSV52660980 |
| TP53 | 8 | NM_000546.5 | c.818G>T | p.R273L | Missense | COSV52664805 |
| TP53 | 8 | NM_000546.5 | c.844C>T | p.R282W | Missense | COSV52662048 |
| TP53 | 8 | NM_000546.5 | c.916C>T | p.R306* | Nonsense | COSV52662281 |
| TP53 | 9 | NM_000546.5 | c.920-2A>G | p.? | Splice acceptor variant | COSV52706655 |
| TP53 | 9 | NM_000546.5 | c.989T>G | p.L330R | Missense | COSV52677086 |
| TP53 | 10 | NM_000546.5 | c.1043T>G | p.L348W | Missense | COSV52736234 |
| TP53 | 10 | NM_000546.5 | c.1009C>T | p.R337C | Missense | COSV52669243 |
| XIRP2 | 9 | NM _152381.5 | c.4131_4133del | p.E1377del | Nonframeshift deletion | COSV54683582 |

[a]Databases: Catalogue Of Somatic Mutations In Cancer (COSMIC): Genomic Mutation Identifier (COSV)[3], cBioPortal[4,5], NCBI dbSNP database[6].

## Supplementary References


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