Personalized analysis of minimal residual cancer cells in peritoneal lavage fluid predicts peritoneal dissemination of gastric cancer

Dongbin Zhao
Chinese Academy of Medical Sciences and Peking Union Medical College
Pinli Yue
Chinese Academy of Medical Sciences and Peking Union Medical College
Tongbo Wang
National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College
Pei Wang
Chinese Academy of Medical Sciences and Peking Union Medical College
Qianqian Song
Chinese Academy of Medical Sciences and Peking Union Medical College
Jingjing Wang
Jinchenjunchuang Clinical Laboratory
Yuchen jiao (jiaoyuchen@126.com)
Chinese Academy of Medical Sciences and Peking Union Medical College https://orcid.org/0000-0001-7917-8810

Article

Keywords: Gastric Cancer, Peritoneal lavage fluid, Peritoneal dissemination, Personalized mutation assay, Minimal residual disease

Posted Date: March 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-275520/v1

Personalized analysis of minimal residual cancer cells in peritoneal lavage fluid predicts peritoneal dissemination of gastric cancer

Author information

Dongbing Zhao,[1,4] Pinli Yue,[2,4] Tongbo Wang,[1,4] Pei Wang,[2] Qianqian Song,[2] Jingjing Wang,[3] Yuchen Jiao[2]

Author Affiliations

[1]Department of Pancreatic and Gastric Surgical Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100021, China. [2]State Key Laboratory of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100021, China. [3]Jinchenjunchuang Clinical Laboratory, Hangzhou, Zhejiang, China. [4]These authors contributed equally: Dongbing Zhao, Pinli Yue and Tongbo Wang.

Address correspondence to: Yuchen Jiao, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Panjiayuan nanli 17, Chaoyang District, Beijing 100021, China. Phone: 86.10.87787662; E-mail: jiaoyuchen@cicams.ac.cn

ABSTRACT

Peritoneal dissemination (PD) is the major type of gastric cancer (GC) recurrence and leads to rapid death. Current approach cannot precisely determine which patients are at high risk of PD. In this study, we developed a technology to detect minimal residual cancer cells in peritoneal lavage fluid (PLF) samples by parallel profiling tumor-specific mutations. We applied the technology to a prospective cohort of 110 GC patients. The technology showed ultra-high sensitivity by successfully detecting all the 27 cases that developed PD. The minimal residual cancer cells in PLF was associated with an increased risk of PD (HR = 145.13; 95%CI = 20.20-18435.79; $p < 0.001$) and significantly shorter overall survival. In pathologically high-risk (T4) patients, the PLF mutation profiling model exhibited even greater specificity of 91% and positive predictive value of 88%, while retaining sensitivity of 100%. PLF cancer cell fraction remained the strongest independent predictor of PD and recurrence-free survival over pathologic diagnosis and cytological diagnosis in GC patients. This approach may help in the postsurgical management of GC patients by detecting PD far before the metastatic lesions grow to significant size detectable by conventional methods such as MRI and CT scanning.

Key words: Gastric Cancer; Peritoneal lavage fluid; Peritoneal dissemination; Personalized mutation assay; Minimal residual disease

INTRODUCTION

Gastric Cancer (GC) is the fifth most common and the third leading cause of cancer death in the world[1]. Peritoneal dissemination (PD) is the most common form of GC recurrence and a strong indicator of poor prognosis with a 13% overall 5-year survival rate[2]. Although multiple therapeutic solutions, such as hyperthermic intraperitoneal chemotherapy (HIPEC) and extensive intraoperative peritoneal lavage plus intraperitoneal chemotherapy (EIPL-IPC), have been developed as prophylactic strategies targeting PD[3,4], the preventive effect is compromised due to the difficulty to precisely identify the patients who will develop PD. Many studies have demonstrated that HIPEC and IPC could effectively prevent PD and improve the survival rate of patients with advanced gastric cancer[5-7]. However, once the peritoneal metastasis reaches a stage detectable by imaging, subsequent chemotherapy is not effective because of the complex intraperitoneal environment, and the advanced PD leads to many complications and rapid death (5-16 months)[8-11]. Thus, prediction or early detection of PD would help identify the GC patients who need intensive therapy to prevent PD.

Several studies shed light on the prediction of PD by the detection of cancer cells in peritoneal lavage fluid (PLF) samples[12]. Previous studies of peritoneal metastasis focused on conventional cytology. However, the sensitivity to detect minimal residual cancer cells to predict PD reached no more than 30%[13-15]. With the development of reverse transcriptase PCR (RT-PCR) technology, several studies began to examine cancer tissue specific messenger RNAs (mRNA) in PLF, such as carcinoembryonic antigen (CEA)[16-18], cytokeratin-20 (CK-20)[19], and matrix metalloproteinase-7 (MMP-7)[20]. None of these mRNAs is an ideal marker due to low sensitivity or specificity. The main source of such results is thought

63 to be the amplification of low-level CEA from peritoneal inflammatory cells, and the aberrant
64 expression of mRNA originating from granulocytes[21]. In recent years, there have been several
65 minimal residual disease (MRD) studies using exosomes and methylation to predict PD[22,23].
66 Some studies detected MRD by profiling GC-specific methylation alterations with bisulfite
67 treatment and qPCR[24,25]. The clinical application of such MRD detection from PLF is limited
68 primarily for two reasons. First, GC is a highly heterogeneous disease, rendering it difficult to
69 identify highly tumor-specific and prevalent biomarkers among all GC cases. Second, the ratio
70 of residual cancer cells among normal cells in PLF can be very low and undetectable with
71 standard qPCR-based methods.

72 Recent developments in next generation sequencing (NGS) enable genome-wide
73 profiling of tumor tissues to identify somatic mutations which are highly tumor-specific
74 biomarkers to track MRD[26]. With recent developments in experimental and bioinformatic
75 technologies, high throughput sequencing can also be applied to detect low frequency
76 mutations in cell free DNA (cfDNA), providing a basis to improve MRD detection with high
77 sensitivity[27]. Several recent studies have profiled tumor samples for somatic mutations and
78 tracked the mutations in the cfDNA from matched blood samples to detect MRD[28-31]. The
79 mutation-based MRD detection showed strong performance in the prediction of recurrence in
80 multiple tumor types.

81 In this study, we applied Mutation Capsule, a mutation profiling technology, to profile up to
82 20 mutations on the genomic DNA of cell pellets of PLF samples collected after abdominal
83 exploration and before any manipulation of the stomach. We developed a model to evaluate
84 the fraction of cancer cells among normal cells based on the number, fraction and sequencing

depth of the mutations detected. The assay precisely predicted all PD cases with 100% sensitivity, 85% specificity, 71% positive predictive value (PPV) and 100% negative predictive value (NPV). For stage T4 GC patients, the approach exhibited higher specificity (91%) and PPV (88%), with sensitivity and NPV at 100%.

To our knowledge, this study is the first to apply the personalized and mutation-based assay to profile MRD in cells pelleted from PLF. Our study demonstrates that PLF cancer cell fraction is a powerful biomarker for prognosis and early detection of PD.

RESULTS

Patient enrollment and study design

A total of 110 patients with stages I to III GC (mean [SD] age, 61 [9.6] years; 79 [76%] male) were enrolled in the study. Six patients were excluded from the study for palliative surgery (n = 4) or the presence of other types of cancer (n = 2). Finally, a total of 104 patients were included in the analysis (Fig. 1). Recurrence of PD was defined as lesions detected in one of the following ways: MRI, CT scan, PET-CT scan visualized [^{18}F]fluorodeoxyglucose (^{18}F-FDG) uptake over peritoneum and bowel with or without ascites >50 mL, or tumor cells detected in ascites or biopsy of peritoneal lesions. The median follow-up period was 20 months (6-41 months). Clinicopathological characteristics of the 104 patients are listed in Supplementary Table 1. Surgery consisted of a radical resection of the primary tumor and at least D2 lymph node dissection.

We performed exome sequencing on the DNA from tumor tissue and matched white blood cell (WBC) samples to identify somatic mutations. A median of 101 mutations were detected in the primary tumor samples of each patient. We designed customized primers to profile up to 20 somatic mutations in the peritoneal lavage samples with the Mutation Capsule technology (Supplementary Table 2)[32].

Construction and validation of the cancer cell fraction model

Due to the low fraction of residual cancer cells among normal cells in PLF, the number of molecules present for a given mutation site in the sequencing (distinct coverage) might be insufficient to precisely detect the mutation. Thus, the detected frequency of one mutation

might not precisely represent the ratio of cancer cells[33,34]. Here, we set up a model to estimate the cancer cell ratio based on allele frequency and sequencing depth of somatic mutations in tumor tissue and paired PLF samples (Fig. 2a). For each traced somatic mutation, we determined the mutation frequency in the PLF sample and compared with the frequency in the primary tumor. The sample-level estimated cancer cell fraction was determined with the Maximum Likelihood Estimation.

To validate the accuracy of the cancer cell fraction model, we serially diluted cells from the cell line PLC/PRF/5 with cells from a second cell line A549 to generate 9 dilutions (PLC/PRF/5 cell fraction = 0%, 0.0001%, 0.0003%, 0.001%, 0.005%, 0.05%, 0.5%, 5% and 33%). We selected 20 unique SNPs in PLC/PRF/5, and designed a customized assay targeting the SNPs. In each diluted sample, we profiled the 20 SNPs to calculate the estimated dilution ratio with the model (Supplementary Table 3). The data exhibited a strong linear correlation between the theoretical and estimated dilution ratios up to the dilution of 1:100,000 (R^2 = 0.9998) (Fig. 2b). Background noise observed at 0% PLC/PRF/5 cell input was 0.0007% (maximum value) among the 20 independent replicates (Fig. 2c). Based on the analytical validation results, the cancer cell fraction model exhibited 100% sensitivity at 0.001% spiked-in cell fraction, 67% sensitivity at 0.0003% cell fraction, and 33% sensitivity at 0.0001% cell fraction (Supplementary Table 4 and Supplementary Fig. 1). The data indicated a limit of detection (LOD) at a 0.001% cancer cell fraction based on the multi target site model.

To evaluate the biological noise of random mutations from non-tumor cells in PLF samples, we determined the fraction of 20 mutations that were not detected in the matched tumor sample. We calculated a set of cancer cell fractions based on these non-tumor-specific

mutations, and found the fractions from background noise in all PLF samples to be lower than 0.01% (Fig. 2d, mean = 0.0006%, maximum = 0.0082%). To achieve a high specificity, we classified the cancer cell fraction as positive or negative with a cutoff of 0.01% in further analyses.

Association of peritoneal lavage cancer cell fraction with risk of peritoneal dissemination

For the 104 patients, we chose a total of 1,717 mutations in the analysis (Supplementary Table 5). On average, 17 somatic mutations (3-23 mutations per person) were tracked for each patient (Fig. 3a), and 25.7% (441/1,717) of the tracked mutations were detected in matched PLF samples. We calculated the cancer cell fraction based on the frequency and distribution of the tracked mutations with the model. The cancer cell fraction ranged from 0 to 23.41% (mean 0.48%, median 0.0074%). Forty-two out of the 104 (40%) patients were positive ($\geq$ 0.01% cancer cell fraction in PLF sample), and 62 (60%) were negative in the PLF mutation profiling model (Fig. 3a and Supplementary Table 6). Among the 104 patients, 27 patients experienced peritoneal dissemination, and all (27/27, 100%) of them tested positive in the PLF mutation profiling model. Six patients developed lymphatic metastasis, and 4 (67%) tested positive in the PLF mutation profiling model. In addition, 15% (11/71) of the non-recurrence patients were positive. The differences in cancer cell fraction between recurrence and non-recurrence groups were significant (PD, mean [SD], 0.0169 [0.04706] versus non-recurrence, mean [SD], 0.0004 [0.00294], $p < 0.001$; lymphatic metastasis, mean [SD], 0.0020 [0.00433] versus non-recurrence, mean [SD], 0.0004 [0.00294], $p = 0.013$) (Fig. 3b).

Patients that were positive in the PLF mutation profiling model showed an increased risk of PD (HR = 145.13; 95%CI = 20.20-18435.79; $p < 0.001$) (Fig. 4a). Twenty-seven of the 38 (71%) test-positive patients and none of the 60 (0%) test-negative patients experienced PD. Kaplan-Meier estimates of recurrence free interval (RFI) at 3 years for test-positive patients were 17.60% (95%CI = 0%-35.24%), and for test-negative patients, 100%. In receiver operating characteristic (ROC) analysis, cancer cell fraction (AUC = 0.92) achieved 100% sensitivity and 85% specificity, with a PPV of 71% (Table 1).

Comparison of the cancer cell fraction and clinical risk factors on the prediction of PD

We further compared the predictive value of the PLF mutation profiling model with clinical risk factors including cytological diagnosis and pathologic diagnosis, which was defined as high (T4) or low (T0-3) according to the standard criteria. The RFI outcomes of cytological and pathologic diagnosis risk assessments were the following, respectively: HR = 4.92; 95%CI = 2.10-11.51; $p < 0.001$ (Supplementary Fig. 2a); and HR = 5.04; 95%CI = 1.74-14.58; $p = 0.001$ (Supplementary Fig. 2b). Cytological diagnosis exhibited high specificity (94%, 67/71) but low sensitivity (30%, 8/27). On the other hand, pathologic diagnosis showed high sensitivity (85%, 23/27) but low specificity (54%, 38/71) (Table 1). The performance of cytological diagnosis and pathologic diagnosis were consistent with previous reports[35-37].

Interestingly, we found that 8 of the 11 false positive cases by the PLF mutation profiling model occurred in stage T1-3 cases, while only 4 cases in this group developed PD. Among the pathologically high-risk patients of stage T4 ($n = 56$), the PLF mutation profiling model exhibited a lower false positive rate, delivering 100% (23/23) sensitivity, 91% (30/33) specificity,

and 88% (23/26) PPV. The RFI outcomes of PLF cancer cell fraction assessment in stage T4 cases was HR = 132.43; 95%CI = 17.55-17006.37; $p < 0.001$ (Fig. 4b).

To adjust for multiple variables in a single model, we used a cox proportional hazard model. PLF cancer cell fraction remained the strongest independent predictor of recurrence-free survival (RFS) (HR = 141.27, 95%CI = 17.37-18633.39; $p < 0.001$), followed by pathologic diagnosis (HR = 3.82, 95%CI = 1.21-17.27; $p = 0.02$) (Supplementary Table 7).

Prediction of recurrence with the PLF mutation profiling model

Six of the 104 patients experienced lymphatic metastasis during the 41-month follow-up. Of these 6 samples, 4 were positive in the PLF mutation profiling test. When we combined peritoneal dissemination and lymphatic metastasis patients as a recurrence group, the test-positive patients by the PLF mutation profiling model were 40 times more likely to relapse than the test-negative patients (HR = 39.97; 95%CI = 9.50-168.10; $p < 0.001$) (Supplementary Fig. 2c). Kaplan-Meier estimates of RFI at 3 years for test-positive patients was 15.80% (95% CI = 0%-31.68%), and for those test-negative was 96.50% (95% CI = 91.80%-100%). The PLF mutation profiling model achieved 94% (31/33) sensitivity, 85% (60/71) specificity, 74% (31/42) PPV and 97% (60/62) NPV (Table 1).

During the follow-up period, 10 patients had developed disease recurrence and died from metastatic disease, and all of them were test-positive in the PLF mutation profiling model. A significantly shorter overall survival (OS) was associated with a positive result in the model (HR = 50.35; 95%CI = 6.41-6492.15; $p < 0.001$) (Figure 4C). The median survival of these 10 patients was 22 months (6-39 months).

DISCUSSION

PD accounts for 50% of recurrence in GC patients and is associated with exceptionally poor prognosis[38]. CT and other imaging modalities are used to diagnose PD, but the sensitivity to predict or detect PD in early and curable stage is inadequate as such metastatic lesions could be small and easily missed. When GC patients show PD-associated symptoms such as ascites and intestinal obstruction, or when metastases can be observed by imaging, the PD lesions are often in significant size and advanced stage, with no effective treatments available. In addition to peritoneal cytology, multiple biomarkers including mRNA, methylation and protein markers have been used to profile PLF to predict PD among GC patients[39-41]. However, the accuracy of these approaches for the early detection of PD is still limited[13,42]. A potential reason is that GC is a highly heterogeneous disease, rendering it difficult to find a marker suitable for all GC cases. In addition, the fraction of cancer cells among PLF cell pellets could be very low and beyond the limit of detection.

MRD detection based on a customized assay targeting tumor-specific mutations in plasma cfDNA have shown promising performance in prognostic prediction and disease monitoring in several tumor types, including breast, colorectal and lung cancers[28,30,43]. Other non-blood samples have also been used for specific clinical scenarios. For example, cell pellets in urine have been used to monitor the development of bladder cancers[44]. In this study, we developed a customized assay to profile up to 20 tumor-specific mutations in PLF DNA. We also set up a model to evaluate the fraction of cancer cells. Using a standard reference of mixed cancer cell lines, we found that the approach confidently detected cancer cells when the fraction was as low as 0.001%. In clinical PLF samples, the biological noise was close to

225 0.01%, possibly due to random mutations in epithelial cells or white blood cells[45].

226 We applied the pipeline to detect MRD in PLF samples from GC patients. The approach
227 exhibited a strong performance in the prediction of PD with 100% sensitivity, 85% specificity
228 and 71% PPV. The sensitivity of the mutation profiling model is higher than that of peritoneal
229 cytology or pathology based on a direct comparison in the same cohort. Mutation profiling also
230 exhibited increased performance relative to other previously reported PLF-based detection
231 assays[46-48]. Furthermore, the combination with clinical risk factors (stage T4) further improved
232 the performance. In the subgroup of T4 high risk individuals, the mutation profiling model
233 showed an even stronger performance with 100% sensitivity, 91% specificity and 88% PPV.
234 Therefore, the PLF mutation profiling should be especially helpful for stage T4 patients to
235 predict the development of PD. An interventional clinical trial targeting T4 and
236 PLF-mutation-positive patients might answer the question as to whether intensive
237 post-operational treatment targeting PD might help to prevent recurrence and improve overall
238 survival.

239 Our study suggests that mutation-based MRD profiling in PLF samples is effective in
240 predicting PD at the time of surgery, especially in stage T4 patients.

241

METHODS

Patients and samples

From June, 2017, to December, 2019, 110 gastric cancer patients scheduled for surgery in National Cancer Center, Cancer Hospital, Chinese Academy of Medical Sciences, were enrolled in our study. Eligibility criteria of the study included a diagnosis of stage I to III resectable gastric cancer determined by chest-abdominal-pelvic enhanced computed tomography-scan (CT-scan), MRI or upper gastrointestinal endoscopy ± endoscopic ultrasound (EUS); no metastatic disease evident on staging computed tomography (CT); age < 80 years; no preoperative radiotherapy or chemotherapy; and a treatment plan for resection. In accordance with NCCN guidelines, postoperative chemotherapy was recommended after surgery. Patients with another malignant neoplasm diagnosed within the last 3 years were excluded. After therapy, surveillance was performed according to the standard of care, with clinical assessment every 3 months for the first 2 years and every 6 months for the next years. TNM stages were determined according to the 8th edition of the Union for International Cancer Control.

For collection of PLF samples, 300-400 mL of normal saline was used to wash the upper abdominal cavity after abdominal exploration and before any manipulation of the tumor during surgery. A total of 200 mL of PLF was collected. PLF (100 mL) was examined through conventional cytological diagnosis with Papanicolaou and Giemsa staining. The remaining 100 mL of PLF was used in mutation profiling. PLF samples were centrifuged first at 2500 rpm (1098g) for 10 min to remove the supernatant, and further centrifuged at 12,000 rpm for 10 min to obtain the PLF pellet. Blood samples were collected before surgery through a standard

blood draw protocol. Fresh frozen tumor tissue was collected from surgery. Tumor tissues, matched white blood cells (WBC) and PLF pellets were stored at -80ºC until DNA extraction. In total, PLF, frozen tumor tissue and blood samples were collected for analysis from 104 patients.

Cell lines and standard reference

The two cancer cell lines hepatocarcinoma PLC/PRF/5 cells and lung carcinoma A549 cells used to construct the cancer cell fraction model were both obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). In the model, PLC cells were used as the "tumor cells" and diluted with A549 cells which were used as the "normal cells". The two cell lines were counted with FACSAriaSORP flow cytometry (Becton Dickinson; San Diego, CA,USA) and serially diluted for a total of 9 dilutions (PLC cell fraction = 0%, 0.0001%, 0.0003%, 0.001%, 0.005%, 0.05%, 0.5%, 5% and 33%). The details of the model construction and number of cells are shown in Supplementary Table 4.

DNA extraction and library preparation

Genomic DNAs were extracted from PLF, frozen tumor tissue, WBC and cell line samples using the QIAamp DNA Mini Kit (Qiagen; Hilden, Germany). Exome sequencing was performed on genomic DNA (1000 ng) from frozen tumor tissue and WBC, and customized panel targeting 20 mutations was performed on genomic DNA (200 ng) from cell lines or PLF. Genomic DNA was sheared into small fragments (mean length ~ 200 bp) with the Covaris E220 instrument (Covaris; Woburn, MA, USA). The sheared DNA was prepared for genomic

libraries using the KAPA Hyper Prep Kit (Roche; Basel, Switzerland) through a series of enzymatic steps including, end-repair, dA tailing, ligation of adaptors and amplification following standard protocols except that for the use of a customized adaptor with barcodes for PLF and cell line samples as previously described[32,49].

Whole-exome sequencing and identification of somatic mutations

Whole genome libraries of tumor and matched WBC DNAs were enriched for exome regions with Agilent SureSelectXT Human All Exon V5 probe and reagents (Agilent; Santa Clara, CA, USA). The captured and amplified libraries were sequenced on the Illumina HiSeqX Ten with 150-bp paired-end sequencing to a median depth of 183× for tumor tissue samples and 105× for WBC samples after removing duplicate molecules. The sequencing raw data (FASTQ file) were aligned to the UCSC human reference genome hg19 using the Burrows-Wheeler aligner software (BWA, v0.7.15). Basic processing, marking duplicates, local realignments and score recalibration were analyzed using The Genome Analysis Toolkit (GATK, v3.6), Picard (v2.7.1) and Samtools (v1.3.1). Candidate somatic mutations were detected by comparing sequencing data from tumor tissue samples with MuTect1 and Strelka. All selected mutations were further validated with manual inspection using Integrated Genome Viewer (IGV)[50].

Targeted sequencing in cell line samples

The whole genome libraries generated from cell line DNA were captured using a 63-gene panel (Supplementary Table 8, 202 kb) and Agilent SureSelect hybrid capture reagents. The sequencing and analysis flow of the captured library is consistent with that of whole exome

sequencing (WES). PLC cells were used as the tumor sample and A549 cells were used as the normal cell sample, with a median depth of 8221× distinct high quality reads for PLC samples and 6721× for A549 samples. 20 SNPs specific for PLC were chosen and compared with A459. These SNPs were assessed in the diluted cell line samples where the mutation frequency was 100% in the PLC cell line and 0% in the A549 cell line using the previously described method[32]. Experiments were repeated three times on samples from each dilution and 20 times for the 0% PLC cell fraction. Targeted sequencing achieved median pre-deduplication sequencing depths of 415,165× and 20,074× after removing duplicate molecules for each detection SNP.

Customized assay to profile multiple mutations

Genomic DNAs (200 ng) from cell lines or PLF were used for the customized panel targeting 20 mutations. The ligation products with customized adaptor were amplified for a whole genome library and used as template to profile mutations identified in the matched tumor tissue. The customized adaptor contained sufficient unique DNA barcodes identifying each original molecule[32]. We selected 20 tumor-specific somatic mutations from the WES results of tumor tissue for primer design. For each patient, the 20 tumor-specific variants were selected according to the potential to be a driver mutation (Supplementary Table 9), the confidence of the mutation by IGV and the frequency of the mutation. Oligo software (v7.53) was used to design multiplex-PCR primer pairs for the two rounds of nested amplification and verified uniqueness in the human genome (http://genome.ucsc.edu/) to ensure amplification efficiency. In the first round of amplification, the target regions were amplified in 9 cycles of PCR using a

target-specific primer and a primer matching the adapter sequence. A second round of 14 cycles of amplification was performed with one pair of nested primers matching the adapter and the target region to further enrich the target region and add the Illumina adapter sequences to the construct. This method can amplify more than 200 target regions in parallel. The targeted sequencing libraries were sequenced on the Illumina Novaseq 6000 with a median depth of 101,703× before de-duplication and 11,760× after removing duplicate molecules. The on-target ratio of reads mapped to the target region was 80% in median.

Bioinformatics pipeline and SNV calling

With the DNA barcode in the customized adapter, redundant reads can be tracked from an original DNA molecule to minimize false positive calling due to PCR amplification and sequencing errors. Sequencing reads were mapped to the hg19 reference genome using 'bwa mem' with the default parameters after extracting tags and removing sequence adapters. The SNV analysis mutation frequency calculation method was used as previously described[32]. Briefly, reads with the same tags and start and end coordinates were grouped into Unique Identifier families (UID families). If > 80% of the reads in a UID family harbor the same mutation identified in the matched tumor tissue, the UID family was defined as an Effective Unique Identifier family (EUID family) with the mutation. The mutant EUID families were further confirmed by manual inspection (IGV) and used to calculate the frequency of the mutation with the total number of UID families covering the mutant site.

PLF mutation profiling model

Estimated cancer cell fraction analysis was performed in the R statistical environment version 3.6.3.

Assumption: 1. Because of the low fraction of residual cancer cells among normal cells in PLF, there are chances that some mutations present in the tumor tissue are not detected in the corresponding PLF sample. The algorithm is also based on the assumption that this tumor-PLF mismatch results from the low concentration of mutant molecules and/or the randomness in sampling. 2. The estimation model is based on the assumption that mutant allele reads and non-mutant wild-type allele reads fit well with the binomial distribution.

Let $(m_1, m_2, \dots, m_{n-1}, m_n)$ be N mutations in PLF, each with a mutation frequency of Pc_i, sequencing depth of D_i, mutation reads number of A_i. And Pt_i indicates the mutation frequency in the corresponding solid tumor tissue. If R is the overall cancer cell concentration of this sample, then for mutation m_i, the mutational frequency in PLF, Pc_i=$Pt_i * R$, and the distribution of observing X_i reads with mutation m_i out of D_i reads in PLF follow the binomial distribution:

$$X_i \sim B(D_i, Pc_i)$$

Then the probability of observing A_i reads with mutation m_i out of D_i reads at position m_i is:

$$P_i(X_i = A_i) = \binom{D_i}{A_i} {Pc_i}^{A_i} (1 - Pc_i)^{D_i - A_i}$$

Assuming that the events of observing $(A_1, A_2, \dots, A_{n-1}, A_n)$ reads supports that mutations $(m_1, m_2, \dots, m_{n-1}, m_n)$ are independent, then the probability of observing a sequence of $(A_1, A_2, \dots, A_{n-1}, A_n)$ is the product of $P_i(X_i = A_i)$ for each mutation m_i. Let P be the likelihood function of cancer cell concentration R:

$$L(R) = P = \prod_{i=1}^{n} P_i = \prod_{i=1}^{n} \binom{D_i}{A_i} (Pt_i * R)^{A_i} (1 - Pt_i * R)^{D_i - A_i}$$

And P is maximized to obtain the predicted R, namely $\hat{R}$:

$$\hat{R} = \arg\max_{R} \prod_{i=1}^{n} P_i = \prod_{i=1}^{n} \binom{D_i}{A_i} (Pt_i * R)^{A_i} (1 - Pt_i * R)^{D_i - A_i}$$

$$= \arg\max_{R} \ln \left[\prod_{i=1}^{n} \binom{D_i}{A_i} (Pt_i * R)^{A_i} (1 - Pt_i * R)^{D_i - A_i} \right]$$

$$= \arg\max_{R} \sum_{i=1}^{n} \left[ln \binom{D_i}{A_i} + A_i * ln(Pt_i * R) + (D_i - A_i) * \ln(1 - Pt_i * R) \right]$$

A search through a grid (0.00001, 1, step=0.00001) for R in the above formula yields the optimized $\hat{R}$. Maximum mutation frequencies in tumor tissues were used for normalization of tumor sample purity.

Statistics

Recurrence-free survival (RFS) was calculated as the time from surgery to the date of recurrence, last visit or death, whichever comes first. Overall survival (OS) was measured from surgery to the date of cause-specific death or last visit. Survival curves were plotted and analyzed with the Kaplan–Meier method, and the log-rank test was used to test the significance of the difference between survival curves.

In addition to the cancer cell fraction, the prognostic impact of other factors, such as Lauren classification, pathology T/N stage, cytology, lymphovascular and nerve invasion were also analyzed. HRs were first estimated using univariate Cox proportional hazard models. Thereafter, factors with statistical significance ($p < 0.05$) were further assessed in multivariate

388 analysis using the Cox proportional hazard model. Whenever there was no event in one group
389 of categorical variables, Firth's penalized likelihood was adopted to allow for monotone
390 likelihood (R package "coxphf"). All statistical analysis was performed using the survival
391 package or coxphf package from the R software (V.3.6.3). Statistical comparison of the cancer
392 cell fraction distribution in patients with no recurrence, peritoneal dissemination or lymphatic
393 metastasis was performed using 2-tailed Wilcoxon Mann-Whitney U test with significance level
394 set at 5% and results are expressed as mean ± SD. $P < 0.05$ was considered statistically
395 significant.
396
397 **Data availability**
398 The sequencing data from tumor tissue, WBC and PLF samples have been deposited at the
399 Genome Sequence Archive for Human under the accession code HRA000528
400 (https://bigd.big.ac.cn/gsa-human/).
401
402 **Study approval**
403 All patients provided written informed consent, and the study was approved by Ethics
404 Committee of National Cancer Center, Cancer Hospital, Chinese Academy of Medical
405 Sciences and Peking Union Medical College (approval number: 17-093/1349).
406

Author Contributions

D.Z.: designed the study, organized sample collection, acquired data, interpreted data, and provided study supervision. P.Y.: performed experiments, analysed data, interpreted data, and wrote the manuscript. T.W.: Organized patient enrollment, sample collection, and clinical data curation. P.W.: developed and optimized experimental protocols, and analysed data. Q.S.: analyzed data. J.W.: interpreted data and wrote the manuscript. Y.J.: designed the study, acquired, analyzed and interpreted data, wrote the manuscript, and provided study supervision. All authors critically reviewed the manuscript.

Acknowledgments: This study was supported by National Key R&D Program of China (2018YFC1312100), the Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Sciences (CIFMS) (2016-I2M-1-001, 2019-I2M-1-003 and 2017-I2M-4-002).

Correspondence and requests for materials should be addressed to Yuchen Jiao.

Conflict of interest: Y.J. is one of the cofounders and have owner interest in Genetron Holdings, and receives royalties from Genetron. Y.J., D.Z., P.W., Q.S., and P.Y. have filed patents/patent applications based on the technology and data generated from this work. The remaining authors disclose no conflicts.

REFERENCES

1 Bray, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* **68**, 394-424 (2018).

2 Badgwell, B. et al. Long-term survival in patients with metastatic gastric and gastroesophageal cancer treated with surgery. *J Surg Oncol* **111**, 875-881 (2015).

3 Kuramoto, M. et al. Extensive intraoperative peritoneal lavage as a standard prophylactic strategy for peritoneal recurrence in patients with gastric carcinoma. *Ann Surg* **250**, 242-246 (2009).

4 Desiderio, J. et al. The 30-year experience-A meta-analysis of randomised and high-quality non-randomised studies of hyperthermic intraperitoneal chemotherapy in the treatment of gastric cancer. *Eur J Cancer* **79**, 1-14 (2017).

5 Yonemura, Y. et al. Intraoperative chemohyperthermic peritoneal perfusion as an adjuvant to gastric cancer: final results of a randomized controlled study. *Hepatogastroenterology* **48**, 1776-1782 (2001).

6 Kim, J. Y. & Bae, H. S. A controlled clinical study of serosa-invasive gastric carcinoma patients who underwent surgery plus intraperitoneal hyperthermo-chemo-perfusion (IHCP). *Gastric Cancer* **4**, 27-33 (2001).

7 Roviello, F., Caruso, S., Neri, A. & Marrelli, D. Treatment and prevention of peritoneal carcinomatosis from gastric cancer by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy: overview and rationale. *Eur J Surg Oncol* **39**, 1309-1316 (2013).

8 Gill, R. S. et al. Treatment of gastric cancer with peritoneal carcinomatosis by cytoreductive surgery and HIPEC: a systematic review of survival, mortality, and morbidity. *J Surg Oncol* **104**, 692-698 (2011).

9 Mizrak Kaya, D. et al. Risk of peritoneal metastases in patients who had negative peritoneal staging and received therapy for localized gastric adenocarcinoma. *J Surg Oncol* **117**, 678-684 (2018).

10 Chen, Y. et al. Predicting Peritoneal Dissemination of Gastric Cancer in the Era of Precision Medicine: Molecular Characterization and Biomarkers. *Cancers (Basel)* **12** (2020).

11 Yang, X. J., Li, Y. & Yonemura, Y. Cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy to treat gastric cancer with ascites and/or peritoneal carcinomatosis: Results from a Chinese center. *J Surg Oncol* **101**, 457-464 (2010).

12 Lisiecki, R., Kruszwicka, M., Spychała, A. & Murawa, D. Prognostic significance, diagnosis and treatment in patients with gastric cancer and positive peritoneal washings. A review of the literature. *Rep Pract Oncol Radiother* **22**, 434-440 (2017).

13 Wang, J.-Y. et al. Gastric cancer cell detection in peritoneal lavage: RT-PCR for carcinoembryonic antigen transcripts versus the combined cytology with peritoneal carcinoembryonic antigen levels. *Cancer Letters* **223**, 129-135 (2005).

14 Yonemura, Y. et al. Inhibition of peritoneal dissemination in human gastric cancer by MMP-7-specific antisense oligonucleotide. *J Exp Clin Cancer Res* **20**, 205-212 (2001).

15 Tokuda, K. et al. Clinical significance of CEA-mRNA expression in peritoneal lavage fluid from patients with gastric cancer. *Int J Mol Med* **11**, 79-84 (2003).

16 Kodera, Y. et al. Prognostic significance of intraperitoneal cancer cells in gastric carcinoma: analysis of real time reverse transcriptase-polymerase chain reaction after 5 years of followup. *J Am Coll Surg* **202**, 231-236 (2006).

17 Nakanishi, H. et al. Rapid quantitative detection of carcinoembryonic antigen-expressing free

tumor cells in the peritoneal cavity of gastric-cancer patients with real-time RT-PCR on the lightcycler. *Int J Cancer* **89**, 411-417 (2000).
18 Kodera, Y. et al. Quantitative detection of disseminated free cancer cells in peritoneal washes with real-time reverse transcriptase-polymerase chain reaction: a sensitive predictor of outcome for patients with gastric carcinoma. *Ann Surg* **235**, 499-506 (2002).
19 Kodera, Y. et al. Prognostic significance of intraperitoneal cancer cells in gastric carcinoma: detection of cytokeratin 20 mRNA in peritoneal washes, in addition to detection of carcinoembryonic antigen. *Gastric Cancer* **8**, 142-148 (2005).
20 Yonemura, Y. et al. Prediction of peritoneal micrometastasis by peritoneal lavaged cytology and reverse transcriptase-polymerase chain reaction for matrix metalloproteinase-7 mRNA. *Clin Cancer Res* **7**, 1647-1653 (2001).
21 Kim, Y. J. et al. The Detection of Messenger RNA for Carcinoembryonic Antigen and Cytokeratin 20 in Peritoneal Washing Fluid in Patients with Advanced Gastric Cancer. *Korean J Gastroenterol* **69**, 220-225 (2017).
22 Tokuhisa, M. et al. Exosomal miRNAs from Peritoneum Lavage Fluid as Potential Prognostic Biomarkers of Peritoneal Metastasis in Gastric Cancer. *PLoS One* **10**, e0130472 (2015).
23 Ohzawa, H. et al. Reduced expression of exosomal miR-29s in peritoneal fluid is a useful predictor of peritoneal recurrence after curative resection of gastric cancer with serosal involvement. *Oncol Rep* **43**, 1081-1088 (2020).
24 Hiraki, M. et al. Aberrant gene methylation is a biomarker for the detection of cancer cells in peritoneal wash samples from advanced gastric cancer patients. *Ann Surg Oncol* **18**, 3013-3019 (2011).
25 Ushiku, H. et al. DNA diagnosis of peritoneal fluid cytology test by CDO1 promoter DNA hypermethylation in gastric cancer. *Gastric Cancer* **20**, 784-792 (2017).
26 McDonald, B. R. et al. Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. *Sci Transl Med* **11** (2019).
27 Leal, A. et al. White blood cell and cell-free DNA analyses for detection of residual disease in gastric cancer. *Nat Commun* **11**, 525 (2020).
28 Abbosh, C. et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature* **545**, 446-451 (2017).
29 Azad, T. D. et al. Circulating Tumor DNA Analysis for Detection of Minimal Residual Disease After Chemoradiotherapy for Localized Esophageal Cancer. *Gastroenterology* **158**, 494-505.e496 (2020).
30 Reinert, T. et al. Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. *JAMA Oncol* **5**, 1124-1131 (2019).
31 Christensen, E. et al. Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma. *J Clin Oncol* **37**, 1547-1557 (2019).
32 Qu, C. et al. Detection of early-stage hepatocellular carcinoma in asymptomatic HBsAg-seropositive individuals by liquid biopsy. *Proc Natl Acad Sci U S A* **116**, 6308-6312 (2019).
33 Patel, J. P. et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med* **366**, 1079-1089 (2012).
34 Schuurhuis, G. J. et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood* **131**, 1275-1291 (2018).
35 Thomassen, I. et al. Peritoneal carcinomatosis of gastric origin: a population-based study on

incidence, survival and risk factors. *Int J Cancer* **134**, 622-628 (2014).
36 Jung, Y. J., Seo, H. S., Kim, J. H., Park, C. H. & Lee, H. H. Cross-Sectional Location of Gastric Cancer Affects the Long-Term Survival of Patients as Tumor Invasion Deepens. *Ann Surg Oncol* **24**, 3947-3953 (2017).
37 La Torre, M. et al. Peritoneal wash cytology in gastric carcinoma. Prognostic significance and therapeutic consequences. *Eur J Surg Oncol* **36**, 982-986 (2010).
38 Coccolini, F. et al. Peritoneal carcinomatosis. *World J Gastroenterol* **19**, 6979-6994 (2013).
39 Katsuragi, K. et al. Prognostic impact of PCR-based identification of isolated tumour cells in the peritoneal lavage fluid of gastric cancer patients who underwent a curative R0 resection. *Br J Cancer* **97**, 550-556 (2007).
40 Bentrem, D., Wilton, A., Mazumdar, M., Brennan, M. & Coit, D. The value of peritoneal cytology as a preoperative predictor in patients with gastric carcinoma undergoing a curative resection. *Ann Surg Oncol* **12**, 347-353 (2005).
41 Hiraki, M. et al. Aberrant gene methylation in the peritoneal fluid is a risk factor predicting peritoneal recurrence in gastric cancer. *World J Gastroenterol* **16**, 330-338 (2010).
42 Yonemura, Y. et al. Diagnostic value of preoperative RT-PCR-based screening method to detect carcinoembryonic antigen-expressing free cancer cells in the peritoneal cavity from patients with gastric cancer. *ANZ J Surg* **71**, 521-528 (2001).
43 Coombes, R. C. et al. Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. *Clin Cancer Res* **25**, 4255-4263 (2019).
44 Dudley, J. C. et al. Detection and Surveillance of Bladder Cancer Using Urine Tumor DNA. *Cancer Discov* **9**, 500-509 (2019).
45 Razavi, P. et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. *Nat Med* **25**, 1928-1937 (2019).
46 Li, J. K. et al. Peritoneal lavage cytology and carcinoembryonic antigen determination in predicting peritoneal metastasis and prognosis of gastric cancer. *World J Gastroenterol* **11**, 7374-7377 (2005).
47 Oyama, K., Terashima, M., Takagane, A. & Maesawa, C. Prognostic significance of peritoneal minimal residual disease in gastric cancer detected by reverse transcription-polymerase chain reaction. *Br J Surg* **91**, 435-443 (2004).
48 Leake, P. A. et al. A systematic review of the accuracy and utility of peritoneal cytology in patients with gastric cancer. *Gastric Cancer* **15 Suppl 1**, S27-37 (2012).
49 Zhang, W. et al. Genetic Features of Aflatoxin-Associated Hepatocellular Carcinoma. *Gastroenterology* **153**, 249-262.e242 (2017).
50 Fujikura, K. et al. Multiregion whole-exome sequencing of intraductal papillary mucinous neoplasms reveals frequent somatic KLF4 mutations predominantly in low-grade regions. *Gut* (2020).

Fig. 1 Patient enrollment, sample detection workflow and the prognosis prediction of patients.

Fig. 1 Patient enrollment, sample detection workflow and the prognosis prediction of patients.

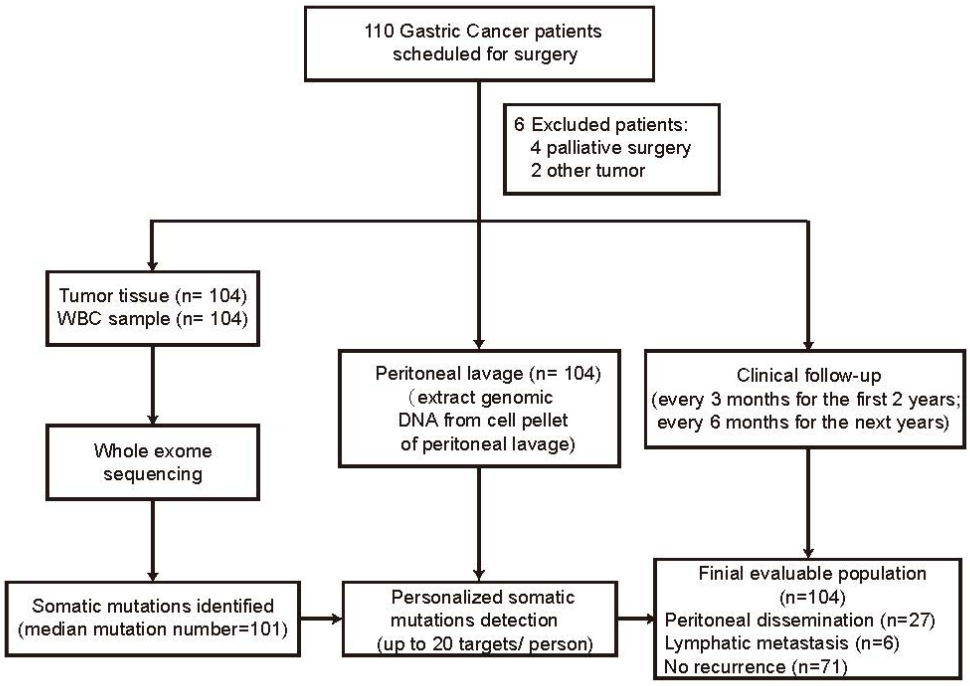
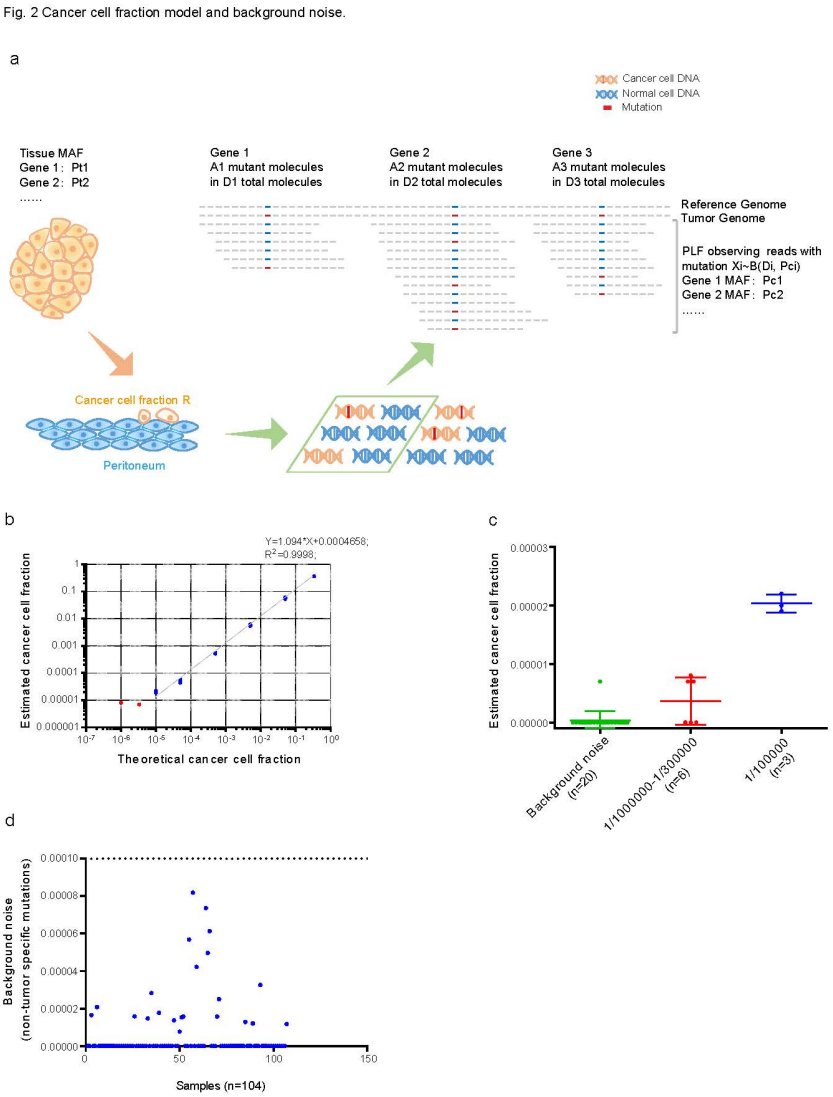


Fig. 2 Cancer cell fraction model and background noise.



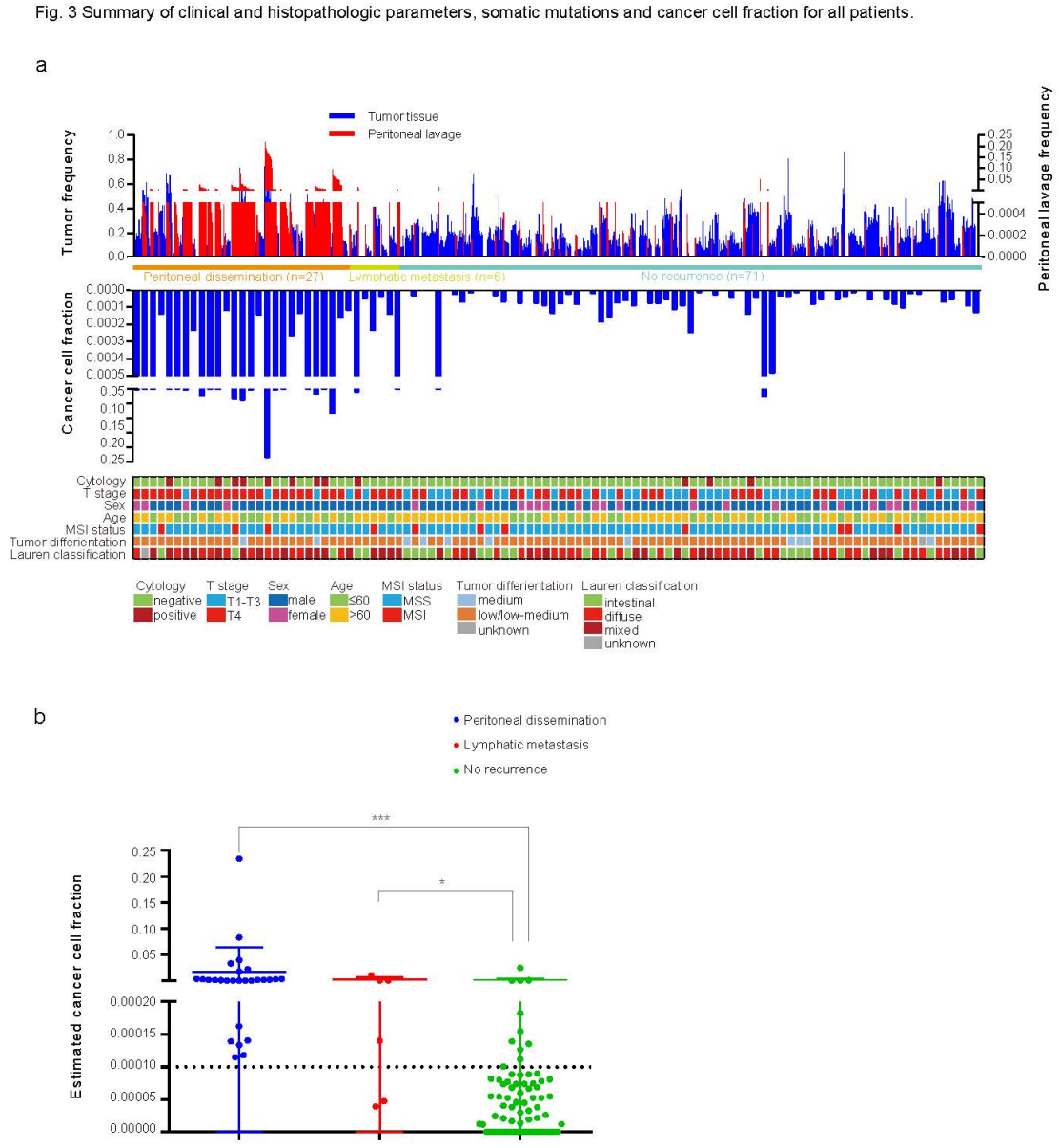
(a). Cancer cell fraction model. A model to estimate the cancer cell fraction based on allele frequency and sequencing depth of somatic mutations in tumor tissue and paired PLF samples. MAF: mutant allele frequency; Pt_i: MAF in solid tumor tissue; Pc_i: MAF in corresponding peritoneal lavage fluid (PLF); D_i: sequencing depth in PLF; A_i: mutation reads number in PLF; X_i: observing reads with mutation in PLF; and R: overall cancer cell concentration.

(b). The linear correlation between theoretical and estimated cancer cell fraction up to the dilution of $1:10^{-5}$. Each dilution was repeated three times. The blue dots highlight the fractions above the limit of detection (PLC/PRF/5 cell fraction = 0.001%, 0.005%, 0.05%, 0.5%, 5% and 33%). The red dots highlight the fractions under the limit of detection (PLC/PRF/5 cell fraction = 0.0001%, 0.0003%).

(c). Background noise observed in the cancer cell fraction model at 0% PLC/PRF/5 cell input among the 20 independent replicates (green dots). Experiments performed on different cell line dilutions (0.0001% and 0.0003%, red dots; 0.001%, blue dots) were repeated three times.

(d). Biological noise of the 104 PLF samples from patients. The cancer cell fraction for each sample was calculated based on non-tumor-specific mutations.

Fig. 3 Summary of clinical and histopathologic parameters, somatic mutations and cancer cell fraction for all patients.

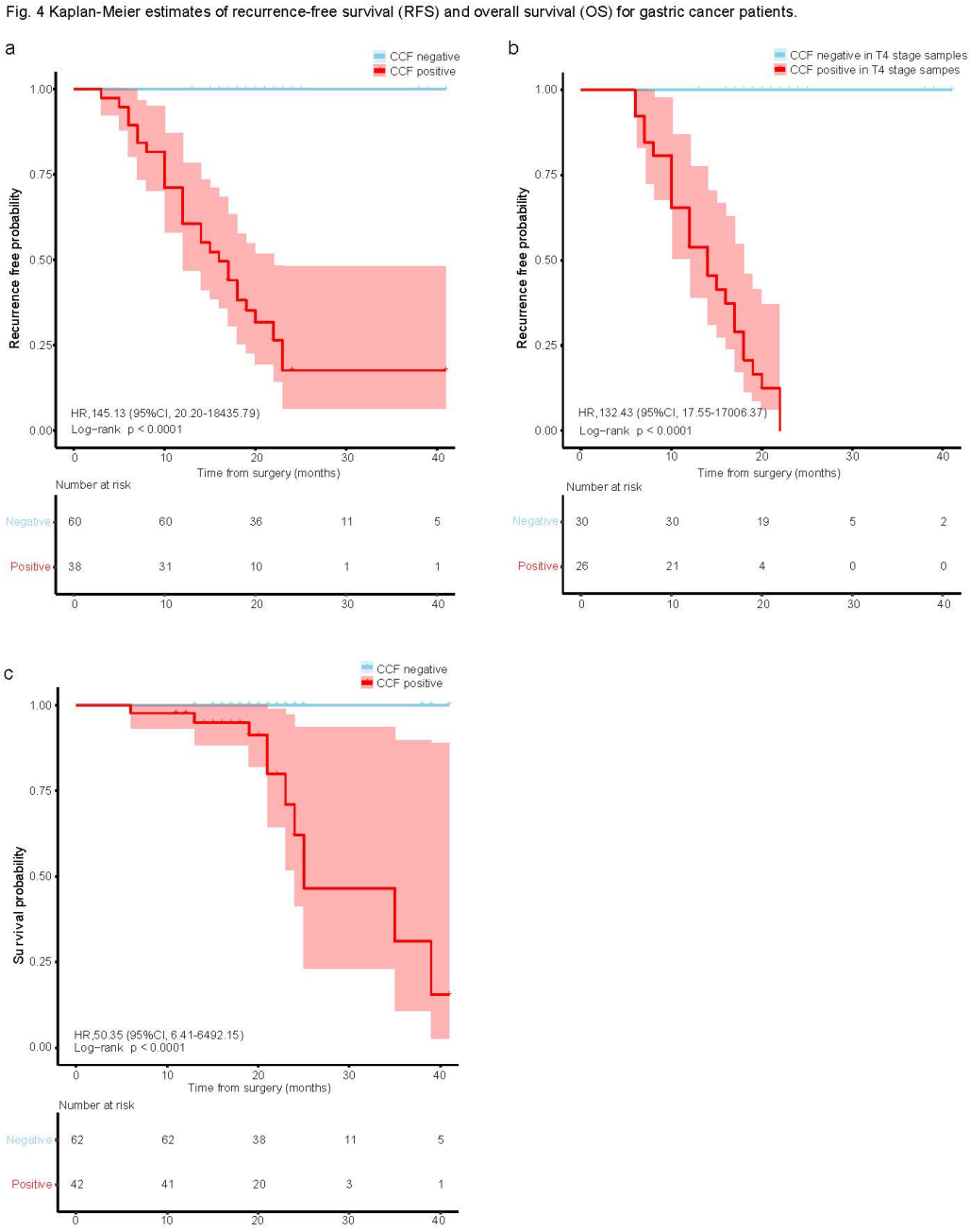


(a). Top panel, the summary of the frequencies of the tracked mutations in tumor and matched peritoneal lavage fluid samples from 104 patients. Blue bar, the tumor frequency of each tracked mutation. Frequency values are shown on the left vertical axis. Red bar, the detected peritoneal lavage fluid frequency of each tracked mutation. Frequency values are shown on the right vertical axis. The clinical outcome of patients is indicated under the bar. Middle panel, the summary of the cancer cell fractions for each patient. Bottom panel, clinical and histopathological characteristics.

(b). The cancer cell fraction distribution in patients with peritoneal dissemination (n = 27), lymphatic metastasis (n = 6) or no recurrence (n = 71). Reported p values are computed using 2-tailed Wilcoxon Mann-Whitney U test.

***, $p < 0.001$; *, $p < 0.05$

Fig. 4 Kaplan-Meier estimates of recurrence-free survival (RFS) and overall survival (OS) for gastric cancer patients.



(a) and (b). Kaplan-Meier survival analysis shows probability of recurrence-free survival (RFS) as determined by A. cancer cell fraction detected in peritoneal lavage fluid (*n* = 98); and B. cancer cell fraction detected in peritoneal lavage fluid in stage T4 patients (*n* = 56) (for peritoneal dissemination). A patient was classified as testing positive if the cancer cell fraction detected in peritoneal lavage fluid was ≥ 0.01%;

(c) Kaplan-Meier estimates of overall survival (OS) for 104 gastric cancer patients based on the estimated cancer cell fraction in peritoneal lavage fluid.

Shaded areas in the Kaplan-Meier plots indicate 95%CIs. HR: hazard ratio; CCF: cancer cell fraction.

Table 1 Binary results of the PLF mutation profiling model and clinical risk factors

		Estimated cancer cell fraction		Cytological diagnosis		Pathologic diagnosis	
Risk of peritoneal dissemination (*n* = 98)		true PD	true noPD	true PD	true noPD	true PD	true noPD
	predict PD	27	11	8	4	23	33
	predict noPD	0	60	19	67	4	38
	Sensitivity	100%		30%		85%	
	Specificity		85%		94%		54%
	PPV	71%		67%		41%	
	NPV		100%		78%		90%
Risk of recurrence (*n* = 104)		true RE	true noRE	true RE	true noRE	true RE	true noRE
	predict RE	31	11	9	4	28	33
	predict noRE	2	60	24	67	5	38
	Sensitivity	94%		27%		85%	
	Specificity		85%		94%		54%
	PPV	74%		69%		46%	
	NPV		97%		74%		88%

PD, peritoneal dissemination; RE, recurrence; PPV, positive predictive value; NPV, negative predictive value.

Estimated cancer cell fraction was defined as positive (CCF ≥ 0.01%) or negative (CCF < 0.01%).

Pathologic diagnosis was defined as high (T4) or low (T0-3) according to the standard criteria.

693

Figures

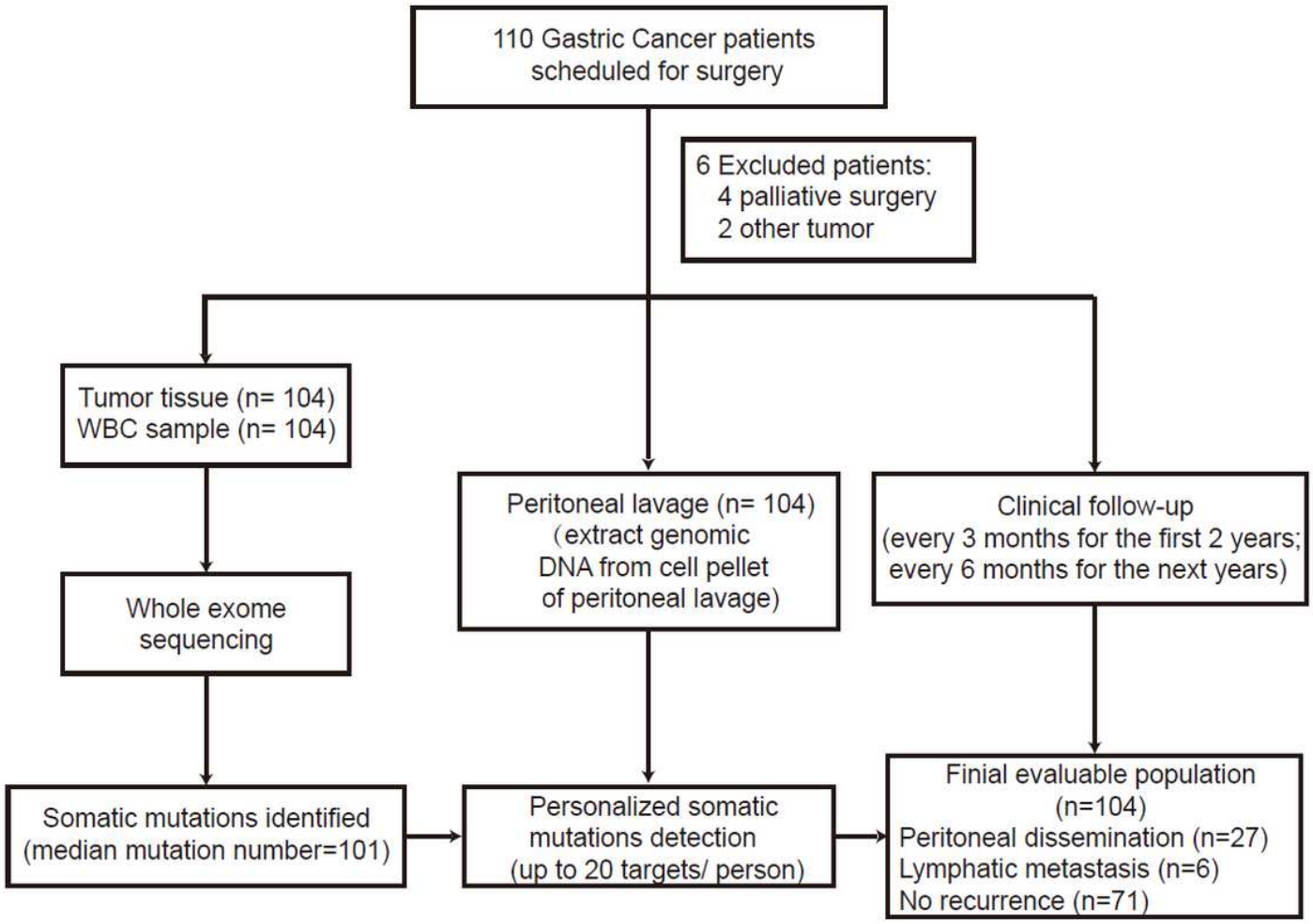


Figure 1

Patient enrollment, sample detection workflow and the prognosis prediction of patients.

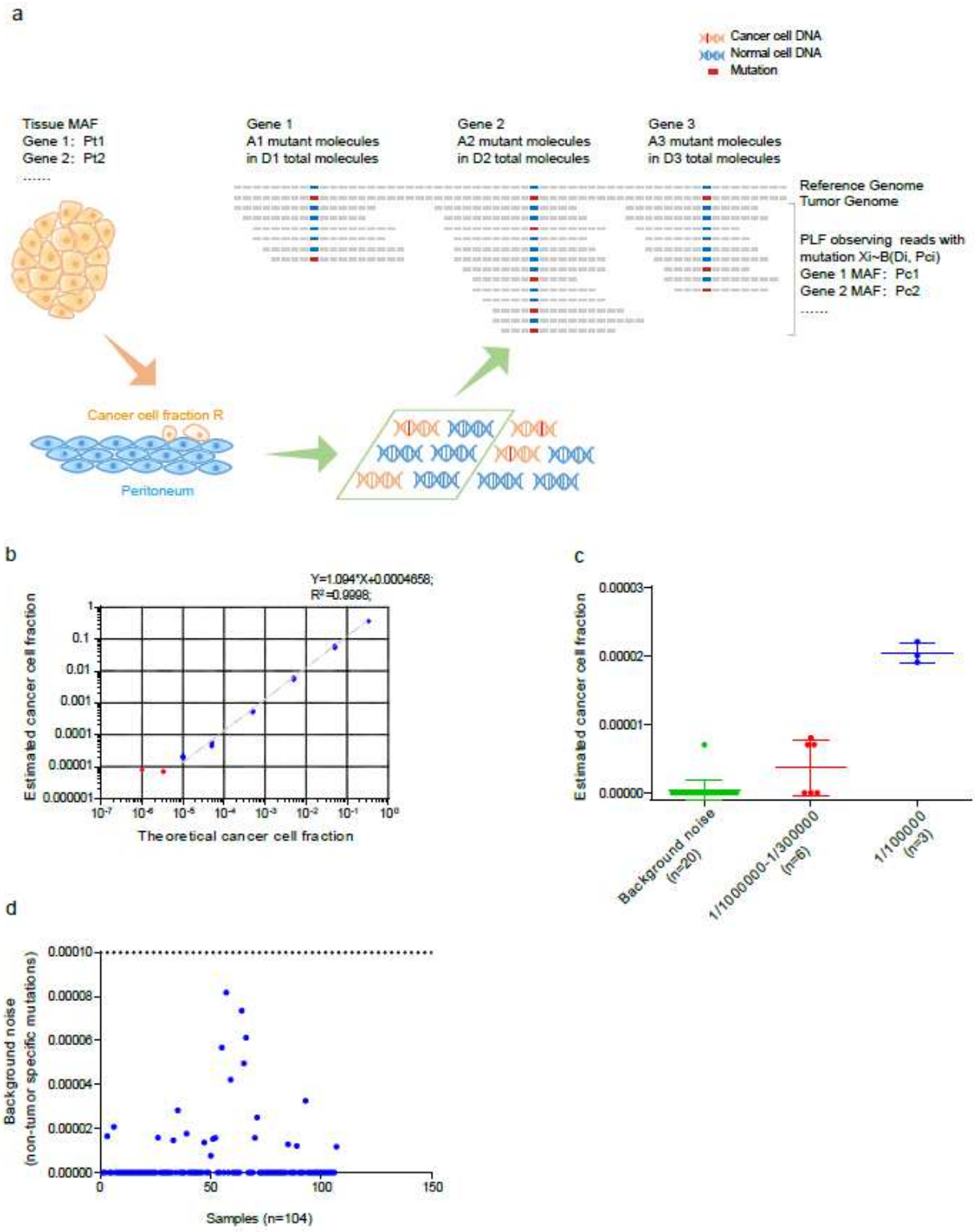


Figure 2

Cancer cell fraction model and background noise. (a). Cancer cell fraction model. A model to estimate the cancer cell fraction based on allele frequency and sequencing depth of somatic mutations in tumor tissue and paired PLF samples. MAF: mutant allele frequency; t: MAF in solid tumor tissue; c: MAF in corresponding peritoneal lavage fluid (PLF); : sequencing depth in PLF; : mutation reads number in PLF; : observing reads with mutation in PLF; and R: overall cancer cell concentration. (b). The linear

correlation between theoretical and estimated cancer cell fraction up to the dilution of 1:10-5. Each dilution was repeated three times. The blue dots highlight the fractions above the limit of detection (PLC/PRF/5 cell fraction = 0.001%, 0.005%, 0.05%, 0.5%, 5% and 33%). The red dots highlight the fractions under the limit of detection (PLC/PRF/5 cell fraction = 0.0001%, 0.0003%). (c). Background noise observed in the cancer cell fraction model at 0% PLC/PRF/5 cell input among the 20 independent replicates (green dots). Experiments performed on different cell line dilutions (0.0001% and 0.0003%, red dots; 0.001%, blue dots) were repeated three times. (d). Biological noise of the 104 PLF samples from patients. The cancer cell fraction for each sample was calculated based on non-tumor-specific mutations.

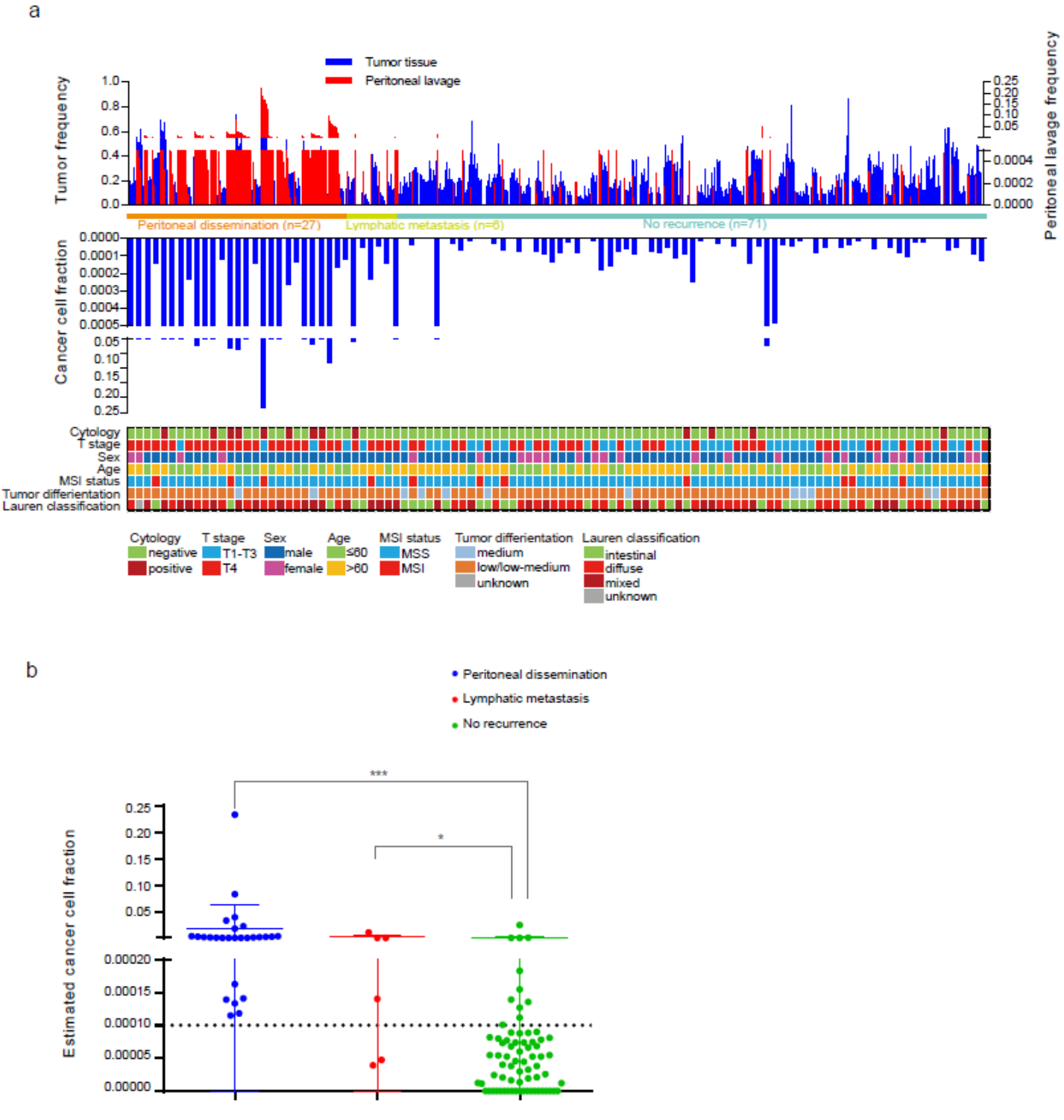


Figure 3

Summary of clinical and histopathologic parameters, somatic mutations and cancer cell fraction for all patients. (a). Top panel, the summary of the frequencies of the tracked mutations in tumor and matched peritoneal lavage fluid samples from 104 patients. Blue bar, the tumor frequency of each tracked mutation. Frequency values are shown on the left vertical axis. Red bar, the detected peritoneal lavage fluid frequency of each tracked mutation. Frequency values are shown on the right vertical axis. The clinical outcome of patients is indicated under the bar. Middle panel, the summary of the cancer cell fractions for each patient. Bottom panel, clinical and histopathological characteristics. (b). The cancer cell fraction distribution in patients with peritoneal dissemination (n = 27), lymphatic metastasis (n = 6) or no recurrence (n = 71). Reported p values are computed using 2-tailed Wilcoxon Mann-Whitney U test. ***, $p < 0.001$; *, $p < 0.05$

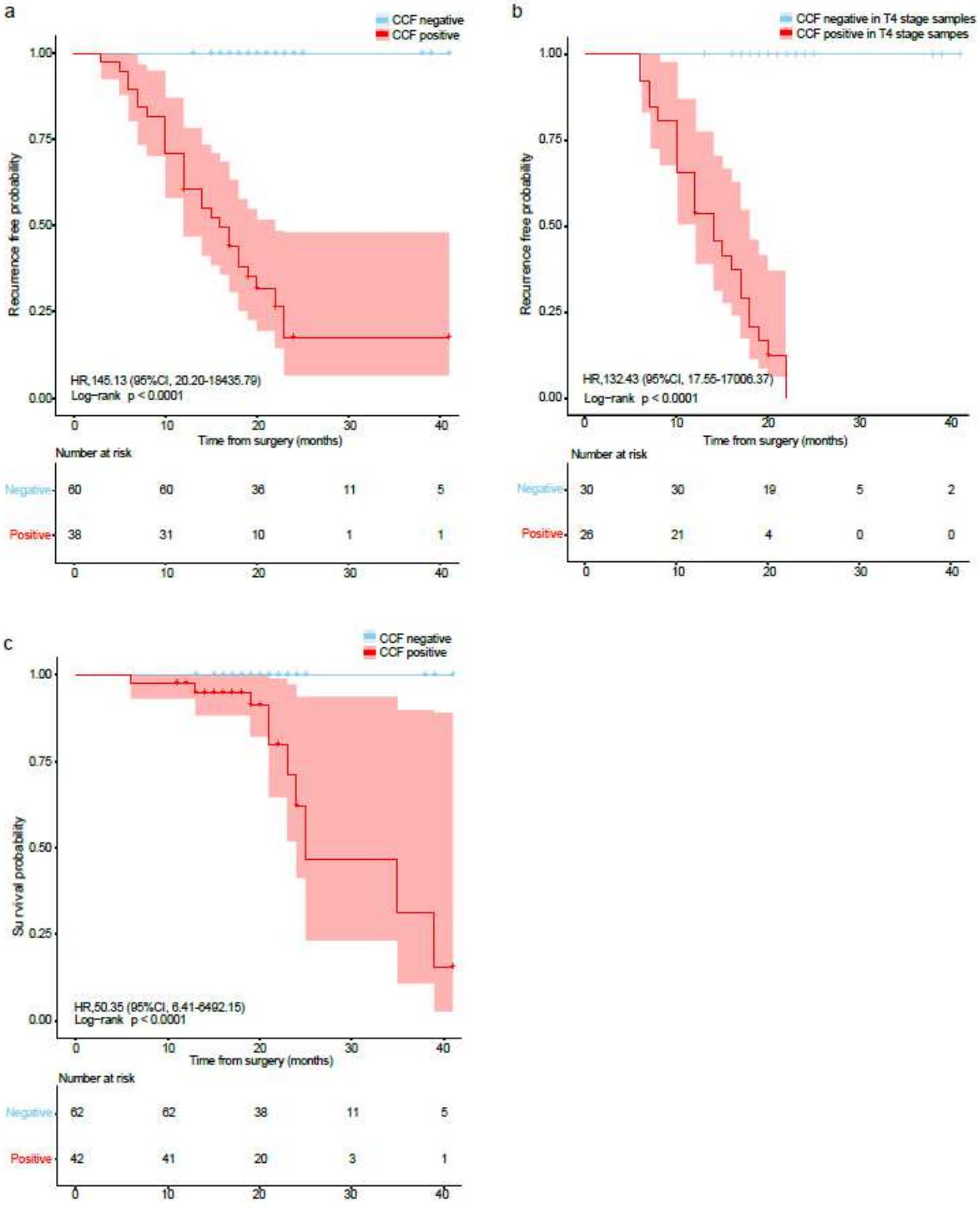


Figure 4

Kaplan-Meier estimates of recurrence-free survival (RFS) and overall survival (OS) for gastric cancer patients. (a) and (b). Kaplan-Meier survival analysis shows probability of recurrence-free survival (RFS) as determined by A. cancer cell fraction detected in peritoneal lavage fluid (n = 98); and B. cancer cell fraction detected in peritoneal lavage fluid in stage T4 patients (n = 56) (for peritoneal dissemination). A patient was classified as testing positive if the cancer cell fraction detected in peritoneal lavage fluid was

> 0.01%; (c) Kaplan-Meier estimates of overall survival (OS) for 104 gastric cancer patients based on the estimated cancer cell fraction in peritoneal lavage fluid. Shaded areas in the Kaplan-Meier plots indicate 95%CIs. HR: hazard ratio; CCF: cancer cell fraction.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.pdf
- SupplementaryTables.xlsx