

# Targeted therapy guided by circulating tumor DNA analysis in advanced gastrointestinal tumors

Received: 25 March 2024

Accepted: 9 August 2024

Published: 16 September 2024

 Check for updates

A list of authors and their affiliations appears at the end of the paper

Although comprehensive genomic profiling has become standard in oncology for advanced solid tumors, the full potential of circulating tumor DNA (ctDNA)-based profiling in capturing tumor heterogeneity and guiding therapy selection remains underexploited, marked by a scarcity of evidence on its clinical impact and the assessment of intratumoral heterogeneity. The GOZILA study, a nationwide, prospective observational ctDNA profiling study, previously demonstrated higher clinical trial enrollment rates using liquid biopsy compared with tissue screening. This updated analysis of 4,037 patients further delineates the clinical utility of ctDNA profiling in advanced solid tumors, showcasing a significant enhancement in patient outcomes with a 24% match rate for targeted therapy. Patients treated with matched targeted therapy based on ctDNA profiling demonstrated significantly improved overall survival compared with those receiving unmatched therapy (hazard ratio, 0.54). Notably, biomarker clonality and adjusted plasma copy number were identified as predictors of therapeutic efficacy, reinforcing the value of ctDNA in reflecting tumor heterogeneity for precise treatment decisions. These new insights into the relationship between ctDNA characteristics and treatment outcomes advance our understanding beyond the initial enrollment benefits. Our findings advocate for the broader adoption of ctDNA-guided treatment, signifying an advancement in precision oncology and improving survival outcomes in advanced solid tumors.

Comprehensive genomic profiling is the standard of clinical care for advanced solid tumors in modern oncology<sup>1</sup>. Circulating tumor DNA (ctDNA)-based genomic profiling is widely used because of its minimally invasive nature and rapid turnaround time, both of which have been demonstrated to increase the identification of patients eligible for targeted therapy and improve survival<sup>2,3</sup>. Despite these advantages, ctDNA genotyping is still not universally adopted and is often used as an alternative to tissue-based testing<sup>4,5</sup>.

Another, less-studied advantage of ctDNA analysis with similar potential to improve the quality of precision oncology care is its ability to capture both temporal and geographic heterogeneity within tumors. In brief, ctDNA represents a composite of DNA released from

all tumor subclones, some of which may comprise genomic alterations that differ from the original dominant tumor clone and that influence optimal therapy selection<sup>6–8</sup>. Moreover, the relative prevalence of clones bearing such nascent alterations (herein referred to as clonality) may influence their biological relevance, with rare clones potentially having less clinical relevance than more common ones. Despite this potential relevance, evidence regarding both methods for assessing intratumoral heterogeneity and its clinical impact is scant.

To address this knowledge gap and investigate ctDNA-guided treatment in general, we launched the GOZILA study, a large, nationwide, prospective observational study designed to profile ctDNA genomic alterations for patients with advanced solid tumors in which

✉ e-mail: [tyoshino@east.ncc.go.jp](mailto:tyoshino@east.ncc.go.jp)

patients are treated based on identified biomarkers in affiliated clinical trials or clinical practice<sup>9,10</sup>. The GOZILA study previously showed the superiority of ctDNA genotyping in patient screening for clinical trials versus tissue genotyping<sup>11</sup>. Here, we evaluate the efficacy of targeted therapies matched to biomarkers detected by ctDNA in over 4,000 patients with advanced cancer and use this dataset to define the influence of biomarker clonality on outcomes in response to genomically targeted therapy. These data describe a rubric by which ctDNA may more informedly be integrated into clinical practice.

## Results

### Patient characteristics

Of the 5,280 patients enrolled in the GOZILA study from January 2018 to August 2022, 4,037 underwent subsequent systemic therapy after blood sampling and were included in this analysis (Extended Data Fig. 1). Median duration of follow-up was 10.8 months (range 0 to 56.1). The demographics and baseline characteristics of patients are shown in Extended Data Fig. 2. Median age was 64 years (range 20 to 91) with 39% of the patients being female. Major cancer types included colorectal ( $n = 1,820$ , 45%), pancreatic ( $n = 899$ , 22%), esophageal ( $n = 363$ , 9%), gastric ( $n = 327$ , 8%) and biliary tract cancer ( $n = 291$ , 7%). The most common metastatic sites were liver ( $n = 2,082$ , 52%), lymph node ( $n = 1,475$ , 37%), lung ( $n = 1,270$ , 31%) and peritoneum ( $n = 954$ , 24%). This large-scale cohort serves as a robust representation of patients commonly diagnosed with advanced solid tumors, with an emphasis on gastrointestinal cancers.

### Biomarker-matched therapy guided by ctDNA genotyping

Similar to previous reports on such tumor types and published compendia, *TP53* was the most frequently altered gene (33%), followed by *APC* (19%), *KRAS* (13%) and *EGFR* (11%) (Fig. 1a). Supplementary Table 1 presents the identified biomarkers. No variant was detected in 333 (8%) patients. As of the data cutoff date, 952 (24%) patients had been treated with at least one targeted therapy matching biomarkers identified by ctDNA genotyping (Fig. 1b). By contrast, 2,479 (61%) patients received no matched therapy despite having targetable biomarkers identified.

Among the more common cancer types with  $\geq 50$  patients, colorectal cancer (38%) was most likely to be treated with biomarker-matched therapy, whereas patients with pancreatic (4%), esophageal (8%) and neuroendocrine tumor or carcinoma (10%) received matched therapy less often. Biomarker-matched treatments primarily served as the first, second or third line of therapy (58%) (Fig. 1c). Of 1,106 biomarker-matched treatments, 362 (33%) were investigational, provided under clinical trials affiliated with GOZILA, with the remainder supplied as part of routine clinical practice (Fig. 1d). As expected from the targeted therapy approval landscape, the majority of patients with biliary tract cancer (76%), neuroendocrine tumor or carcinoma (63%), esophageal cancer (61%), gastric cancer (50%) and hepatocellular carcinoma (50%) received their matched therapy through clinical trials.

### Efficacy of biomarker-matched therapy guided by ctDNA

To understand the impact of biomarker-matched therapy on patient outcomes, treatments were curated according to distinct molecular biomarkers. The primary biomarkers guiding matched therapy included *RAS* wild-type ( $n = 473$ ), *ERBB2* amplification and mutations

( $n = 163$ ), *BRAF* mutations and amplification ( $n = 113$ ), high tumor mutational burden (TMB,  $n = 77$ ) and microsatellite instability (MSI,  $n = 52$ ) (Fig. 2a). Biomarkers leading to targeted therapy varied across cancer types, suggesting that the prevalence of actionable biomarkers and the availability of targeted therapies may differ among tumor types (Extended Data Fig. 3a). In targetable biomarkers for which targeted therapy could be recommended, MSI high (76%) most frequently led to actual treatment with matched agents, followed by *ERBB2* (51%), *KIT* (48%), TMB high (28%), *BRAF* (22%) and *MET* (20%) (Fig. 2b). MSI or TMB high, and *ERBB2* alterations consistently led to the administration of targeted therapy across various cancer types (Extended Data Fig. 3b). The drug targets for matched therapies provided were epidermal growth factor receptor (EGFR) ( $n = 480$ ), human epidermal growth factor receptor 2 (HER2) ( $n = 150$ ), *BRAF* ( $n = 101$ ), programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1) ( $n = 90$ ) and MEK ( $n = 78$ ) (Extended Data Fig. 4a).

Classifying efficacy of targeting individual biomarkers when at least 10 patients were treated, therapies for *ERBB2* amplification and mutations demonstrated the highest objective response rate (ORR) (50%), followed by MSI high (47%), *RAS* wild-type (36%), TMB high (34%), *KRAS* G12C (27%), *FGFR1-3* alterations (26%), and *BRAF* alterations (24%) (Fig. 2c). Progression-free survival (PFS) was the most favorable with therapy for MSI high (median, 8.0 months), followed by *RAS* wild-type (median, 6.0 months), *ERBB2* (median, 5.6 months), TMB high (median, 4.4 months), *KRAS* G12C (median, 4.1 months), *BRAF* (median, 2.9 months) and *MET* (median, 2.9 months) (Fig. 2d and Extended Data Fig. 4b). Targeted therapies directed against MSI or TMB high, or *ERBB2* demonstrated consistently high efficacy across different cancer types (Extended Data Fig. 5a,b).

Recent advancements in cancer therapy have focused on the development of various drug classes for precise targeted treatment. We then assessed the classes of drugs used in the GOZILA study. These included monoclonal antibodies (54%), trailed by small-molecule inhibitors (29%), and antibody–drug conjugates (ADCs) (7%) (Extended Data Fig. 4c). When we examined the ORR for each class of drug in biomarker-matched therapy, ADCs showed the highest efficacy, with an ORR of 57%, compared with 36% for monoclonal antibodies ( $P < 0.001$  versus ADCs) and 20% for small-molecule inhibitors ( $P < 0.001$  versus ADCs) (Extended Data Fig. 4d). Median PFS was 6.0 months (95% confidence interval (CI), 4.8 to 10.1 months) with ADCs and 5.6 months (95% CI, 5.2 to 6.4 months) with monoclonal antibodies. Both of these drug classes significantly outperformed small-molecule inhibitors (ADCs versus small-molecule inhibitors, hazard ratio (HR) 0.46, 95% CI 0.34 to 0.65; monoclonal antibodies versus small-molecule inhibitors, HR 0.51, 95% CI 0.44 to 0.60), which showed median a PFS of 2.8 months (95% CI 2.6 to 3.0 months) (Fig. 2e).

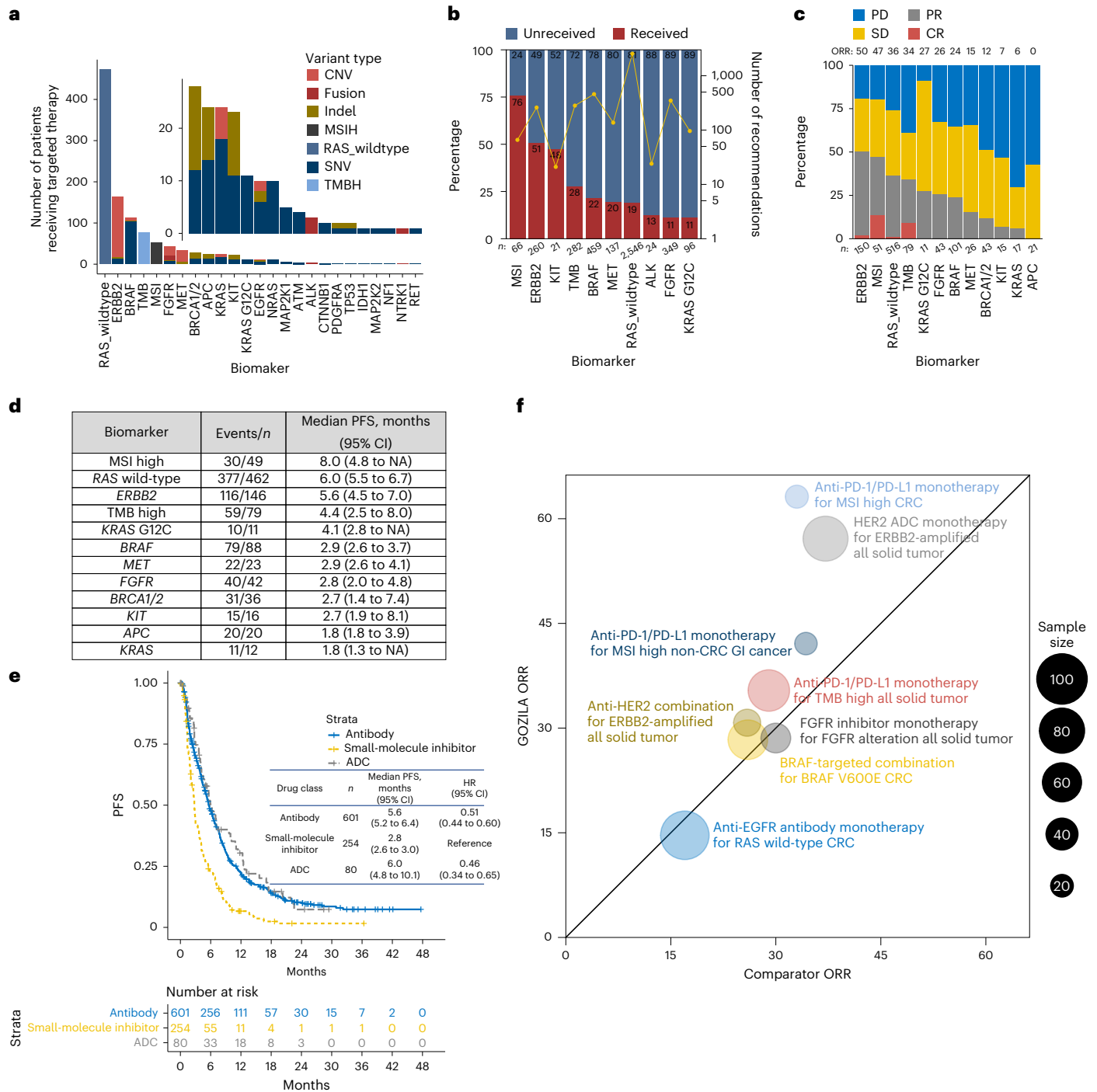
Further analysis compared the ORR in our study's cohort with those reported in previous tissue-based studies, stratified by biomarkers, treatments and cancer types<sup>12–18</sup>. Anti-PD-1 or anti-PD-L1 therapies for MSI or TMB high disease and HER2-targeted therapies based on ctDNA genotyping exhibited higher ORRs compared with those derived from tissue analysis, whereas the effectiveness was comparable in therapies targeting *RAS* wild-type colorectal cancer (CRC) and *BRAF* V600E and *FGFR*-altered solid tumors (Fig. 2f). Although there were differences in patient characteristics, particularly in the proportion of gastrointestinal cancers, the GOZILA study had a

### Fig. 1 | Overview of matched therapy based on ctDNA genotyping in GOZILA.

**a**, Pathogenic genomic alterations across cancer types. Genes identified in at least 10 patients are displayed. Bars indicate the number of patients harboring different classes of genomic alterations. The inset bar plot provides a zoomed-in view of the genes with lower frequencies of alterations, using a different scale on the x axis to enhance visibility. **b**, Proportion of patients receiving ctDNA-guided matched therapy by cancer types with at least 20 patients enrolled. The yellow bar indicates the percentage of patients receiving matched therapy

within each cancer type. The gray bar represents the proportion of patients with no actionable biomarker. The total number of patients per cancer type is shown above the abbreviation for the cancer type. The red line represents the number of patients with each cancer type. **c**, Number of patients receiving matched therapies by treatment lines. **d**, Proportion of patients treated in clinical trials among those receiving matched therapy for tumor types with at least 20 patients enrolled. Abbreviations for cancer types are according to Extended Data Fig. 2.





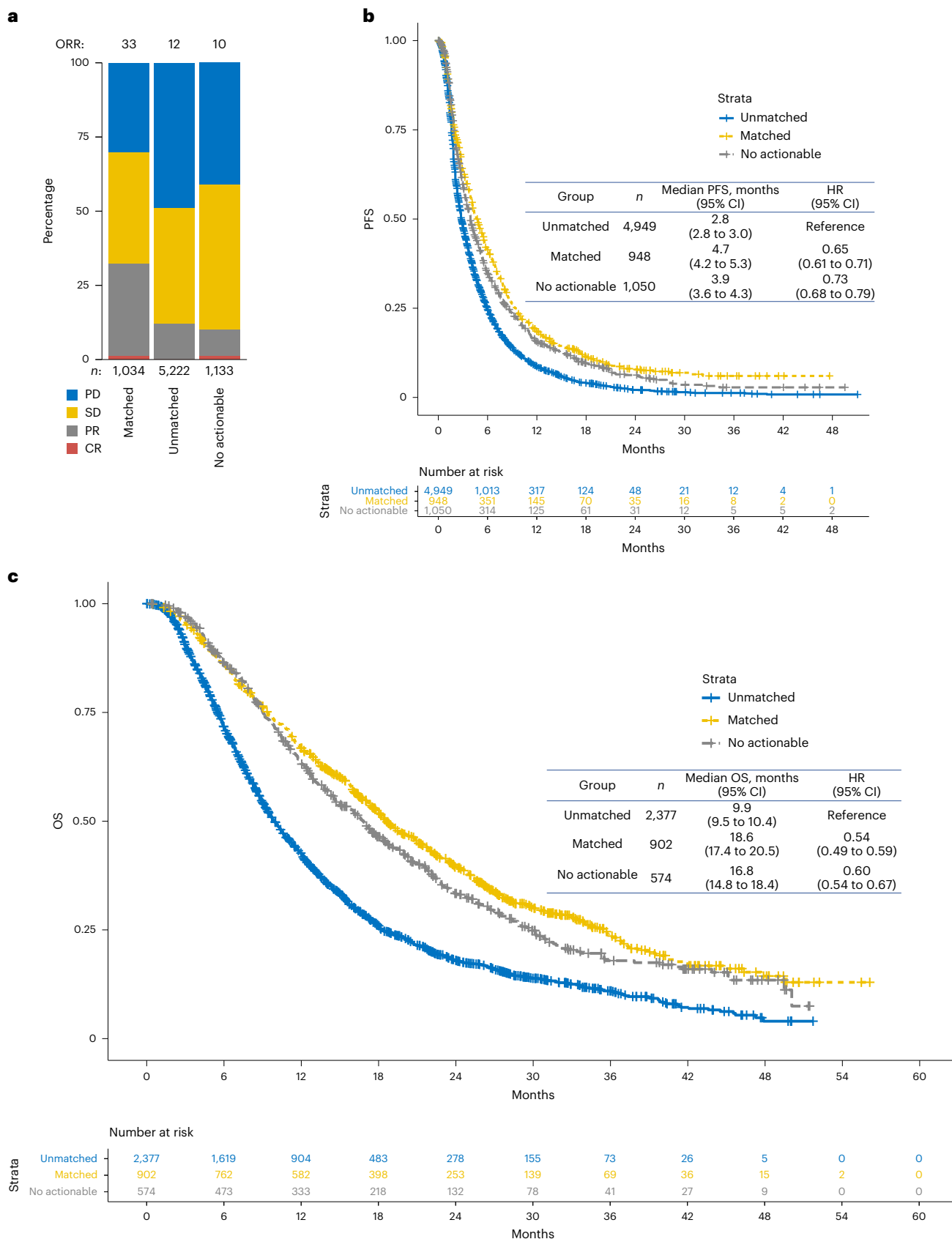
**Fig. 2 | Efficacy of matched targeted therapy by biomarkers. a**, Number of patients receiving matched therapy by biomarkers. The inset bar plot provides a zoomed-in view of the genes with lower frequencies of alterations, using a different scale on the y-axis to enhance visibility. **b**, Top 10 biomarkers with the highest percentage of patients receiving matched therapy among those with biomarkers identified in at least 10 patients, for which a targeted treatment was recommended. The yellow line represents the number of patients with biomarkers that had a recommendation. **c**, Percentage of each tumor response

of matched therapy by biomarkers. **d**, Number of events and median PFS of matched therapy targeting each biomarker that led to treatment in at least 10 patients. **e**, Kaplan–Meier plots of PFS for patients treated with matched therapy by drug class. **f**, Comparison of ORR between GOZILA and previous studies. The x and y axes indicate ORR of targeted therapies in previous studies and GOZILA, respectively. CR, complete response; MSIH, MSI high; NA, not applicable; PD, progressive disease; PR, partial response; SD, stable disease; TMBH, TMB high.

similar or higher number of previous treatment lines across all groups (Supplementary Tables 2–9). In summary, the study demonstrates that treatments aligned with biomarkers identified through ctDNA profiling can achieve therapeutic efficacy that is on par with, or in some cases superior to, that of traditional tissue-based genotyping. This finding strengthens the argument for the utilization of ctDNA

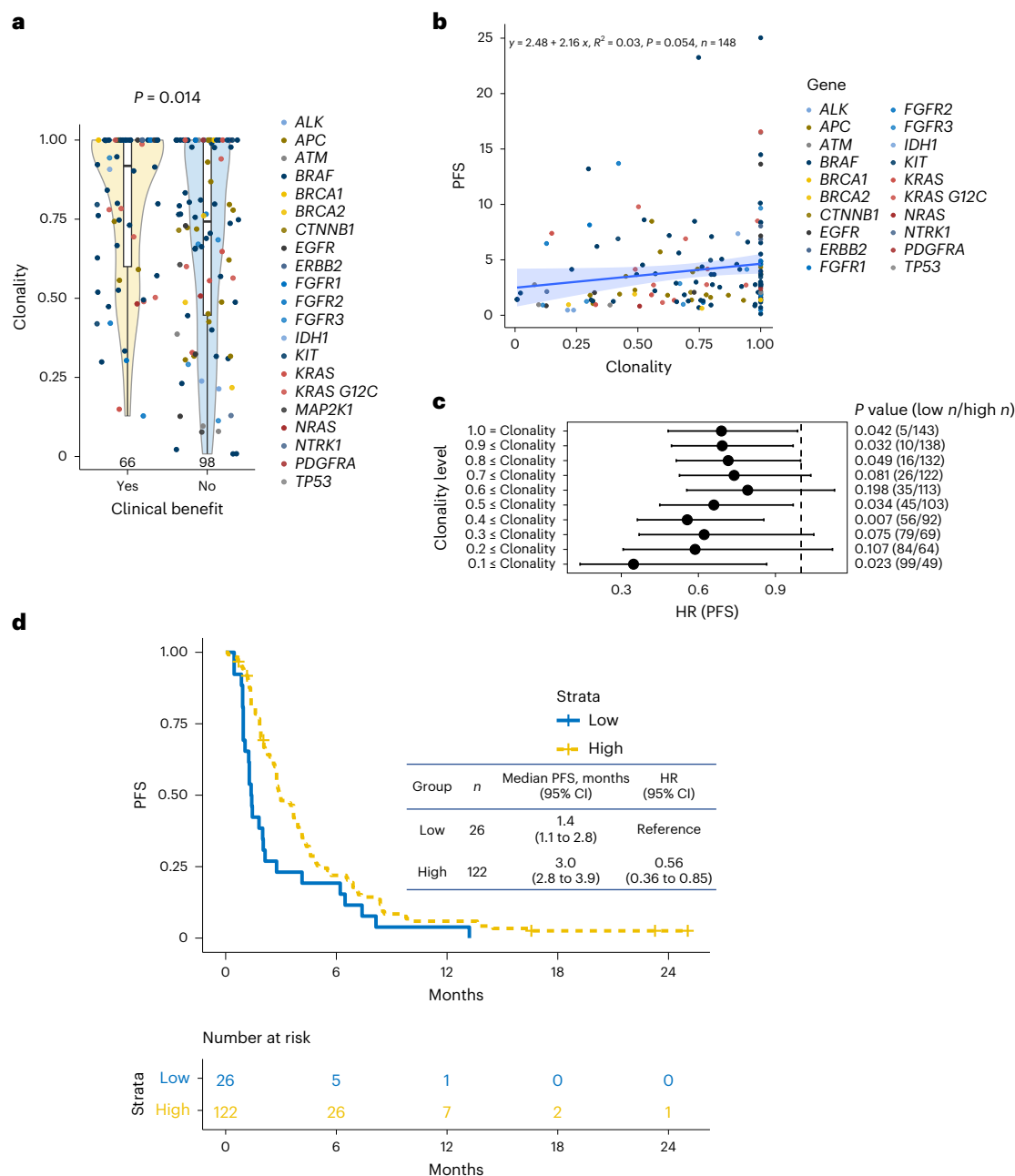
genotyping as a viable and effective approach in precision medicine and targeted therapy.

**Survival benefit of ctDNA-guided biomarker-matched therapy**  
Despite the advanced line of therapy of most of the enrollment and the investigational nature of many of the interventions, patients receiving



**Fig. 3 | Clinical outcomes by biomarker-treatment matching status. a,** Tumor response by biomarker-treatment matching status. **b,** Kaplan–Meier plots of PFS by biomarker-treatment matching status. The PFS analysis is performed per treatment, with each treatment classified as matched, unmatched or no actionable biomarkers, based on the biomarker status. **c,** Kaplan–Meier plots

of OS by biomarker-treatment matching status. The OS analysis is performed per patient, with each patient classified as having received matched therapy if at least one of their treatments was matched to their biomarker status, as having received unmatched therapy if none of their treatments was matched to identified actionable biomarkers, or as having no actionable biomarkers.



**Fig. 4 | Association of clonality with therapeutic efficacy.** **a**, Clonality of variants targeted in patients with clinical benefit and no benefit. The clonality was significantly higher in patients with clinical benefit (two-sided  $P = 0.014$ , Mann–Whitney  $U$ -test). Boxes represent the 25th–75th percentiles, center lines indicate the median, whiskers extend to the maximum and minimum values within  $1.5 \times$  the interquartile range and dots indicate outliers. **b**, Correlation of

PFS with clonality. Shaded area represents 95% CI.  $P$  values were calculated using the two-sided Wald test. **c**, Forest plots of HR for PFS with each threshold of clonality. HR (circles) and 95% CI (horizontal lines) are shown for each threshold of clonality.  $P$  values were calculated using the two-sided Wald test. The vertical dashed line indicates 1.0. **d**, Kaplan–Meier plots of PFS on therapies targeting variants with clonality  $\geq 0.4$  versus  $< 0.4$ . 6E.

ctDNA-matched therapy were more than twice as likely to respond (ORR 33%, 95% CI 30% to 36%) compared with those not harboring targetable biomarkers (ORR 12%, 95% CI 11% to 13%,  $P < 0.001$ ) (Fig. 3a). Matched therapy was similarly associated with extended median PFS (4.7 months, 95% CI 4.2 to 5.3 months) relative to unmatched (2.8 months, 95% CI 2.8 to 3.0 months; HR 0.65, 95% CI 0.61 to 0.71) (Fig. 3b) as well as overall survival (OS) (18.6 months, 95% CI 17.4 to 20.5 months versus 9.9 months, 95% CI 9.5 to 10.4 months; HR 0.54, 95% CI 0.49 to 0.59) (Fig. 3c). Compared with the unmatched group, patients harboring no actionable biomarkers with recommended targeted therapies had similar ORR (10%,  $P = 0.08$ ) and longer PFS

(median 3.9 months; HR 0.73, 95% CI 0.68 to 0.79) and OS (median 16.8 months; HR 0.60, 95% CI 0.54 to 0.67). We further investigated the efficacy of matched therapy versus unmatched therapy for patients with *APC*, *KRAS*, *KIT* and *BRCA1/2* alterations associated with low ORR but found no significant differences between the two groups (Supplementary Tables 10 and 11).

To address the heterogeneity of the study population and potential confounding factors, we performed multivariate analyses for ORR, PFS and OS, including cancer types, the type of therapy and line of therapy as variables (Extended Data Fig. 6). Despite the adjustment for these factors, matched therapy remained significantly associated with better



treatment outcomes compared with unmatched therapy, strengthening our finding that ctDNA-guided matched therapy provides clinical benefit to patients with advanced solid tumors. The benefit from biomarker-matched therapy in OS was not different by sex (Extended Data Fig. 7a) and the greatest benefit was observed when used as a first-line treatment (Extended Data Fig. 7b). Statistically significant benefits for PFS and OS were observed in patients with colorectal, pancreatic, gastric and biliary tract cancers with the largest sample size (Extended Data Fig. 7c,d).

### Therapeutic efficacy by ctDNA clonality

Using this dataset of patients receiving matched therapy, we next investigated whether the clonality of targeted biomarkers, calculated as the variant of interest's allelic fraction (VAF) divided by the maximum VAF detected in that sample, influenced the observed therapeutic efficacy. We first plotted the observed efficacy as measured by both clinical benefit and PFS against VAF of genomic mutations of fusions targeted by matched therapy (Extended Data Fig. 8a,b), which demonstrated no consistent association and a trend towards an inverse correlation of benefit with VAF, potentially because of the confounding of clonal and subclonal variants and the confounding prognostic effect of high tumor fraction<sup>19</sup>. To correct for the differences in ctDNA shedding, we estimated the clonality of the targeted sequence variants as previously described<sup>11</sup>. Clinical benefit and PFS were significantly associated with clonality (Fig. 4a,b). Patients who experienced clinical benefits exhibited a significantly higher median clonality of targeted mutations or fusions (median, 92% versus 74%;  $P = 0.014$ ) (Fig. 4a). PFS was also correlated with the clonality (Fig. 4b).

Because threshold selection is critical for therapeutic decision-making, we investigated the sensitivity of the association between clonality and patient benefit from biomarker-targeted therapies to variation in clonality threshold. As shown in Fig. 4c, HR values for PFS were consistently below 1.0 with any threshold level; however, because the clonality distribution was skewed to the extremes, the enrichment for clinical benefit was relatively robust to changes in threshold above 0.4.

At the optimal clonality threshold of 0.4, patients whose therapy was selected based on clonal biomarkers had a markedly increased rate of clinical benefit as measured by both radiographic response (26% versus 7%,  $P = 0.055$ ) and PFS (median 3.0 months versus 1.4 months; HR 0.56, 95% CI 0.36 to 0.85) (Fig. 4d).

### Therapeutic efficacy by plasma copy number

Similar to the effect observed for sequence variant VAF, absolute observed plasma copy number (pCN) had minimal association with either clinical benefit or PFS, with a trend toward reduced benefit in higher pCN, compatible with a confounding effect from tumor fraction (Extended Data Fig. 8c,d). However, when corrected for tumor fraction as previously described<sup>20,21</sup>, the adjusted pCN (ApCN) was significantly associated with clinical benefit and PFS (Fig. 5a,b). Patients with clinical benefit had higher ApCN (36 versus 13,  $P < 0.001$ ) (Fig. 5a) and PFS was significantly correlated with ApCN (Fig. 5b). Both ORR (54% versus 31%,  $P = 0.003$ ) and PFS (median, 5.9 versus 3.7 months; HR 0.64, 95% CI 0.47 to 0.89) were significantly improved in patients with high ApCN versus low when stratified by the median value of 22.42 (Fig. 5c). A consistent trend with higher ApCN in patients who experienced clinical benefit was observed across the top five cancer types (colorectal, pancreatic, esophageal, gastric and biliary tract cancers) (Extended Data Fig. 9).

To further investigate the effect of *ERBB2* amplification, we conducted separate analyses for *ERBB2* and other genes (Extended Data Fig. 10a,b). In the *ERBB2* group ( $n = 127$ ), patients with clinical benefit had significantly higher ApCN compared with those without clinical benefit (median, 48 versus 18;  $P < 0.001$ ). Although not statistically significant, a similar trend was observed in the other genes group,

which included therapies targeting fibroblast growth factor receptor (FGFR) ( $n = 24$ ), multi-tyrosine kinase ( $n = 22$ ), MEK ( $n = 6$ ), EGFR ( $n = 2$ ) and MET ( $n = 2$ ) (median, 13 versus 8;  $P = 0.23$ ). Separate correlation analyses between ApCN and PFS for *ERBB2* and other genes revealed positive trends in both groups, although the correlations were no longer statistically significant (Extended Data Fig. 10c,d).

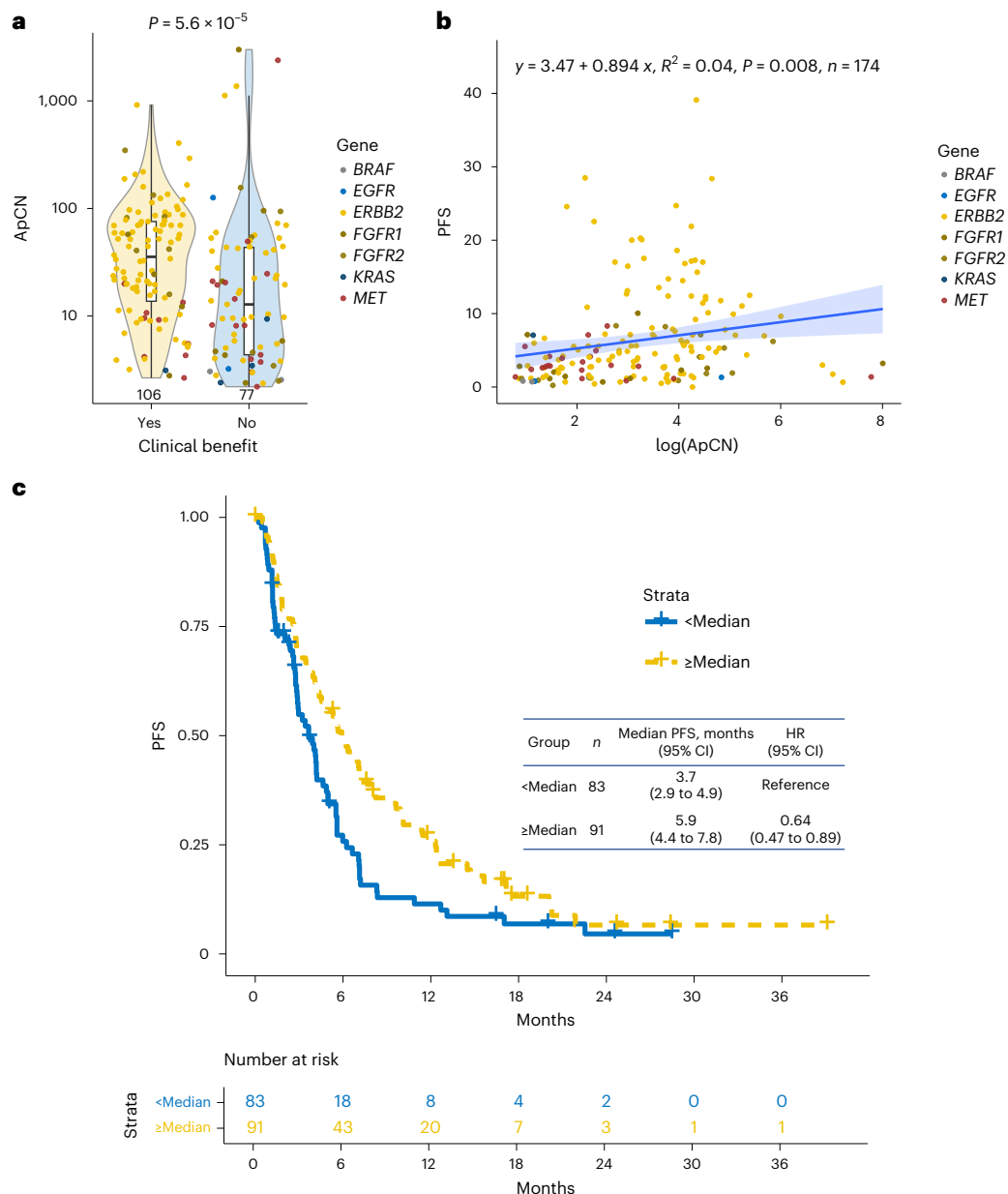
These findings highlight the importance of considering both clonality and ApCN when interpreting ctDNA results and making treatment decisions based on targeted biomarkers.

## Discussion

The GOZILA study has established a new benchmark in the field of precision oncology by demonstrating that patients treated with matched targeted therapy based on biomarkers identified by ctDNA genotyping can significantly extend OS compared with those receiving unmatched therapy. Although previous research has indicated a survival advantage with therapies aligned to tissue genotyping<sup>22–29</sup>, we illustrated the survival benefits of ctDNA-based treatment matching across cancer types. Furthermore, patients with clonal sequence variants and high ApCN amplifications were shown to enjoy improved efficacy, suggesting a clinical decision-making framework critical for proper deployment of ctDNA in patient care. These findings underscore the value of ctDNA genotyping as an important tool for biomarker identification in precision oncology.

Perhaps the greatest challenge in precision oncology is the timely identification of eligible patients. In GOZILA, 24% of patients were treated with therapies matched to ctDNA-based biomarkers, much higher than in other published studies examining the utility of tissue-based genomic profiling<sup>30–37</sup> and congruent with the published literature regarding the advantages of ctDNA genotyping relative to tissue-based testing. Indeed, the initial report of the GOZILA enrollment<sup>11</sup> specifically demonstrated that the increase in matched therapy with ctDNA screening was largely a because of ctDNA's faster turnaround time resulting in more patients having genotyping results at the time of therapy selection relative to tissue testing. In addition, the recent PRISM study underscores the importance of access to advanced biomolecular technologies for the successful implementation of precision oncology. The proportion of patients receiving matched therapy based on identified biomarkers varied, potentially influenced by the availability of relevant clinical trials. Notably, biomarkers more frequently associated with the administration of matched treatments included *ERBB2*, *BRAF*, *RAS* wild-type, *MET*, *FGFR*, *TMB* and *BRCA1/2*, which were targeted in GOZILA-affiliated trials<sup>10</sup>.

In addition to increasing the proportion of patients receiving targeted therapy, ctDNA-directed therapy in GOZILA also demonstrated widespread efficacy, with patients more than twice as likely to respond to matched therapy and a concomitant reduction in their risk of death by more than 40% regardless of confounding baseline characteristics. As expected from their own literature, survival effects did vary between specific therapies, reflecting the heterogeneity of the treatments and patient populations included in the study. This heterogeneity is also possibly associated with a favorable prognosis in the no actionable biomarker group. The absence of actionable biomarkers is associated with better prognosis in colorectal cancer, possibly because of the lack of genomic alterations related to the resistance against anti-EGFR therapy (for example, *RAS*, *BRAFV600E* and *ERBB2* amplification)<sup>38,39</sup>. By contrast, in lung and pancreatic cancers, patients without actionable biomarkers have been reported to have similar outcomes as those with actionable biomarkers who do not receive matched therapy<sup>23,28</sup>. Moreover, the favorable prognosis of patients with no actionable biomarkers may be attributed to patients with the absence of detectable variants in ctDNA, which is known to be associated with significantly better prognosis<sup>19,40</sup>. Despite the favorable prognosis of patients with no actionable biomarkers, our study still demonstrates a trend towards better outcomes for patients with actionable biomarkers who received



**Fig. 5 | Association of ApCN with therapeutic efficacy. a**, ApCN of gene amplifications targeted in patients with clinical benefit and no benefit. The ApCN was significantly higher in patients with clinical benefit (two-sided  $P < 0.001$ , Mann–Whitney  $U$ -test). Boxes represent 25th–75th percentiles, center lines indicate the median, whiskers extend to the maximum and minimum values

within 1.5× the interquartile range and dots indicate outliers. **b**, Correlation of PFS with ApCN. Shaded area represents 95% CI.  $P$  values were calculated using the two-sided Wald test. **c**, Kaplan–Meier plots of PFS on therapies targeting amplifications with ApCN equal to or greater than the median (22.42) versus less than the median.

matched therapy compared with those with no actionable biomarkers. This finding further highlights the importance of matched therapy in precision oncology.

One observation of particular importance in this report is that the survival advantage associated with ctDNA-guided therapy diminishes after first-line treatment. This is compatible with commonly held theories of cancer evolution under therapeutic selective pressure, which have progressively shifted targeted therapy application to earlier lines of treatment to improve survival<sup>41,42</sup>. However, realization of this survival benefit has been hampered by access to and use of comprehensive genomic profiling to guide first-line treatment. In part, this is due to reliance on tissue-only testing practices; tissue testing’s lengthy processing time often leads to the initiation of empirical systemic therapy before the availability of genomic results, whereas ctDNA genomic

profiles are typically returned quickly enough to inform first-line treatment decision-making. Moreover, in other healthcare systems, access to comprehensive genomic profiling is limited to later lines of therapy or even after the exhaustion of all other therapeutic options. The data in this report and others<sup>3</sup> demonstrate that such practices reduce patient survival.

To fully access the survival benefits of ctDNA-based testing, clinical guidelines are necessary to ensure evidence-driven implementation, particularly in diagnostic scenarios unique to ctDNA testing such as decision-making regarding subclonal biomarkers. The initial publication of GOZILA<sup>11</sup> highlighted the high prevalence of subclonal biomarkers, and the current work underscores the clinical relevance of treating them appropriately. Specifically, absolute VAF and pCN did not accurately predict benefit from targeted therapy; however, variant



clonality and ApCN did, suggesting these are more appropriate metrics for clinical decision-making. This finding is compatible with previous research regarding the effectiveness of HER2-targeted therapies in relation to ApCN in patients with HER2-positive colorectal cancer<sup>20,21</sup>.

An important caveat in this study is the heterogeneity of the cancer types and therapeutic regimens, which includes subsets with relatively few patients and investigational therapies with variable efficacy. Although ctDNA-guided therapy demonstrated significant therapeutic benefit for the overall cohort and for cancer types with adequate sample sizes, additional investigation is necessary for cancers represented here by smaller numbers of patients. Furthermore, the majority of cancers included in this study are of gastrointestinal, hepatobiliary and pancreatic origin. The high mutational heterogeneity of these cancers raises concerns about the generalizability of our results to other tumor types with different genetic landscapes. Future studies involving a broader spectrum of cancer types are needed to validate the efficacy of ctDNA-guided therapy across diverse oncological conditions. The analysis by therapeutic classes also has limitations, such as the heterogeneity of targets and therapies, pharmacokinetic and pharmacodynamic variability, tumor tissue of origin, and other potential imbalances between the groups. Another limitation is the lack of tissue profiling data as a comparator. Without this comparison, it remains uncertain how much incremental clinical value ctDNA analysis provides over traditional tissue-based methods, including the extent to which low ctDNA shedding may affect the detection of biomarkers compared with tissue testing. This gap highlights the need for integrated studies that compare the outcomes of ctDNA and tissue profiling side by side. Nevertheless, the efficacy of ctDNA-guided therapy was comparable to or better than one reported in pivotal clinical trials, although such comparisons must be interpreted with caution because of inherent biases related to different patient populations and therapies.

Together, these data describe the relevance and survival benefit of comprehensive genomic profiling using ctDNA across solid tumors and therapeutic interventions. Furthermore, the predictive ability of clonality describes an effective, data-driven rubric by which clinical decisions may be made in the common scenario of subclonal biomarkers with therapeutic relevance. ctDNA is already broadly used in genotyping patients with advanced cancer to select therapies, but guidelines have not provided comprehensive recommendations. For example, the European Society for Medical Oncology recommendation refers to the use of ctDNA for limited variants, such as *PIK3CA*, *ESR1* and *BRCA1/2* in breast cancer, *ERBB2* in gastric cancer, *IDH1* and *FGFR2* in cholangiocarcinoma, *RAS*, *BRAF V600E*, *EGFR* and *MSI* in colorectal cancer, and DNA damage repair genes in prostate cancer<sup>5</sup>. In our study, we leverage data from over 4,000 patients with advanced cancers representing multiple tumor types. We provide both survival-based evidence supporting the use of ctDNA and, critically, outcome-based data clarifying the interpretation of VAF and variant clonality in clinical practice. These findings warrant the adoption of comprehensive genomic profiling using ctDNA as a standard-of-care approach to first-line patients with advanced solid tumors.

## Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-024-03244-8>.

## References

- Mosele, F. et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann. Oncol.* **31**, 1491–1505 (2020).
- Nakamura, Y. & Yoshino, T. Clinical utility of analyzing circulating tumor DNA in patients with metastatic colorectal cancer. *Oncologist* **23**, 1310–1318 (2018).
- Aggarwal, C. et al. Association between availability of molecular genotyping results and overall survival in patients with advanced nonsquamous non-small-cell lung cancer. *JCO Precis. Oncol.* **7**, e2300191 (2023).
- Merker, J. D. et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *J. Clin. Oncol.* **36**, 1631–1641 (2018).
- Pascual, J. et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann. Oncol.* **33**, 750–768 (2022).
- Siravegna, G. et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat. Med.* **21**, 795–801 (2015).
- Pectasides, E. et al. Genomic heterogeneity as a barrier to precision medicine in gastroesophageal adenocarcinoma. *Cancer Discov.* **8**, 37–48 (2018).
- Parikh, A. R. et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nat. Med.* **25**, 1415–1421 (2019).
- Nakamura, Y. et al. SCRUM-Japan GI-SCREEN and MONSTAR-SCREEN: path to the realization of biomarker-guided precision oncology in advanced solid tumors. *Cancer Sci.* **112**, 4425–4432 (2021).
- Bando, H., Nakamura, Y., Kotani, D. & Yoshino, Y. Comparing GOZILA and COLOMATE: ongoing umbrella/basket trials examining genetic testing in gastrointestinal malignancies. *Oncology (Williston Park)* **35**, 382–389 (2021).
- Nakamura, Y. et al. Clinical utility of circulating tumor DNA sequencing in advanced gastrointestinal cancer: SCRUM-Japan GI-SCREEN and GOZILA studies. *Nat. Med.* **26**, 1859–1864 (2020).
- Patterson, S. D. et al. Comprehensive analysis of *KRAS* and *NRAS* mutations as predictive biomarkers for single agent panitumumab (pmab) response in a randomized, phase III metastatic colorectal cancer (mCRC) study (20020408). *J. Clin. Oncol.* **31**, 3617 (2013).
- Kopetz, S. et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer. *N. Engl. J. Med.* **381**, 1632–1643 (2019).
- Meric-Bernstam, F. et al. Efficacy and safety of trastuzumab deruxtecan in patients with HER2-expressing solid tumors: primary results from the DESTINY-PanTumor02 phase II trial. *J. Clin. Oncol.* **42**, 47–58 (2024).
- Marabelle, A. et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol.* **21**, 1353–1365 (2020).
- Marabelle, A. et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: results from the phase II KEYNOTE-158 study. *J. Clin. Oncol.* **38**, 1–10 (2020).
- Sweeney, C. J. et al. MyPathway human epidermal growth factor receptor 2 basket study: pertuzumab + trastuzumab treatment of a tissue-agnostic cohort of patients with human epidermal growth factor receptor 2-altered advanced solid tumors. *J. Clin. Oncol.* **42**, 258–265 (2024).
- Andre, T. et al. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. *N. Engl. J. Med.* **383**, 2207–2218 (2020).

19. Reichert, Z. R. et al. Prognostic value of plasma circulating tumor DNA fraction across four common cancer types: a real-world outcomes study. *Ann. Oncol.* **34**, 111–120 (2023).
20. Siravegna, G. et al. Plasma HER2 (*ERBB2*) copy number predicts response to HER2-targeted therapy in metastatic colorectal cancer. *Clin. Cancer Res.* **25**, 3046–3053 (2019).
21. Nakamura, Y. et al. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer: a phase 2 trial. *Nat. Med.* **27**, 1899–1903 (2021).
22. Tsimberidou, A. M. et al. Personalized medicine in a phase I clinical trials program: the MD Anderson Cancer Center initiative. *Clin. Cancer Res.* **18**, 6373–6383 (2012).
23. Kris, M. G. et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA* **311**, 1998–2006 (2014).
24. Schwaederle, M. et al. Impact of precision medicine in diverse cancers: a meta-analysis of phase II clinical trials. *J. Clin. Oncol.* **33**, 3817–3825 (2015).
25. Schwaederle, M. et al. Precision oncology: the UC San Diego Moores Cancer Center PREDICT experience. *Mol. Cancer Ther.* **15**, 743–752 (2016).
26. Sunami, K. et al. Clinical practice guidance for next-generation sequencing in cancer diagnosis and treatment (edition 1.0). *Cancer Sci.* **109**, 2980–2985 (2018).
27. Sicklick, J. K. et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nat. Med.* **25**, 744–750 (2019).
28. Pishvaian, M. J. et al. Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. *Lancet Oncol.* **21**, 508–518 (2020).
29. Naito, Y. et al. Clinical practice guidance for next-generation sequencing in cancer diagnosis and treatment (edition 2.1). *Int. J. Clin. Oncol.* **26**, 233–283 (2021).
30. Park, H. S. et al. Pilot study of a next-generation sequencing-based targeted anticancer therapy in refractory solid tumors at a Korean institution. *PLoS ONE* **11**, e0154133 (2016).
31. Tanabe, Y. et al. Comprehensive screening of target molecules by next-generation sequencing in patients with malignant solid tumors: guiding entry into phase I clinical trials. *Mol. Cancer* **15**, 73 (2016).
32. Zehir, A. et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat. Med.* **23**, 703–713 (2017).
33. Hamblin, A. et al. Clinical applicability and cost of a 46-gene panel for genomic analysis of solid tumours: retrospective validation and prospective audit in the UK National Health Service. *PLoS Med.* **14**, e1002230 (2017).
34. Kou, T. et al. Clinical sequencing using a next-generation sequencing-based multiplex gene assay in patients with advanced solid tumors. *Cancer Sci.* **108**, 1440–1446 (2017).
35. Sunami, K. et al. Feasibility and utility of a panel testing for 114 cancer-associated genes in a clinical setting: a hospital-based study. *Cancer Sci.* **110**, 1480–1490 (2019).
36. Cobain, E. F. et al. Assessment of clinical benefit of integrative genomic profiling in advanced solid tumors. *JAMA Oncol.* **7**, 525–533 (2021).
37. O'Dwyer, P. J. et al. The NCI-MATCH trial: lessons for precision oncology. *Nat. Med.* **29**, 1349–1357 (2023).
38. Yaeger, R. et al. Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. *Cancer Cell* **33**, 125–136 (2018).
39. Nakamura, Y. et al. Efficacy of targeted trials and signaling pathway landscape in advanced gastrointestinal cancers from SCRUM-Japan GI-SCREEN: a nationwide genomic profiling program. *JCO Precis. Oncol.* **7**, e2200653 (2023).
40. Jee, J. et al. Overall survival with circulating tumor DNA-guided therapy in advanced non-small-cell lung cancer. *Nat. Med.* **28**, 2353–2363 (2022).
41. Douillard, J. Y. et al. Panitumumab–FOLFOX4 treatment and RAS mutations in colorectal cancer. *N. Engl. J. Med.* **369**, 1023–1034 (2013).
42. Ramalingam, S. S. et al. Overall survival with osimertinib in untreated, EGFR-mutated advanced NSCLC. *N. Engl. J. Med.* **382**, 41–50 (2020).

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2024

**Yoshiaki Nakamura**<sup>1,2,3,29</sup>, **Hiroshi Ozaki**<sup>2,29</sup>, **Makoto Ueno**<sup>4</sup>, **Yoshito Komatsu**<sup>5</sup>, **Satoshi Yuki**<sup>6</sup>, **Taito Esaki**<sup>7</sup>, **Hiroya Taniguchi**<sup>8</sup>, **Yu Sunakawa**<sup>9</sup>, **Kensei Yamaguchi**<sup>10</sup>, **Ken Kato**<sup>11</sup>, **Tadamichi Denda**<sup>12</sup>, **Tomohiro Nishina**<sup>13</sup>, **Naoki Takahashi**<sup>14</sup>, **Taroh Satoh**<sup>15</sup>, **Hisateru Yasui**<sup>16</sup>, **Hironaga Satake**<sup>17,18</sup>, **Eiji Oki**<sup>19</sup>, **Takeshi Kato**<sup>20</sup>, **Takashi Ohta**<sup>21</sup>, **Nobuhisa Matsuhashi**<sup>22</sup>, **Masahiro Goto**<sup>23</sup>, **Naohiro Okano**<sup>24</sup>, **Koushiro Ohtsubo**<sup>25</sup>, **Kentaro Yamazaki**<sup>26</sup>, **Riu Yamashita**<sup>27</sup>, **Naoko Iida**<sup>2</sup>, **Mihoko Yuasa**<sup>2</sup>, **Hideaki Bando**<sup>2,3</sup> & **Takayuki Yoshino**<sup>3,28</sup> ✉

<sup>1</sup>International Research Promotion Office, National Cancer Center Hospital East, Kashiwa, Japan. <sup>2</sup>Translational Research Support Office, National Cancer Center Hospital East, Kashiwa, Japan. <sup>3</sup>Department of Gastrointestinal Oncology, National Cancer Center Hospital East, Kashiwa, Japan. <sup>4</sup>Department of Gastroenterology, Kanagawa Cancer Center, Yokohama, Japan. <sup>5</sup>Department of Cancer Center, Hokkaido University Hospital, Sapporo, Japan. <sup>6</sup>Department of Gastroenterology and Hepatology, Hokkaido University Hospital, Sapporo, Japan. <sup>7</sup>Department of Gastrointestinal and Medical Oncology, NHO Kyushu Cancer Center, Fukuoka, Japan. <sup>8</sup>Department of Clinical Oncology, Aichi Cancer Center, Nagoya, Japan. <sup>9</sup>Department of Clinical Oncology, St. Marianna University School of Medicine, Kawasaki, Japan. <sup>10</sup>Department of Gastroenterological Chemotherapy, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan. <sup>11</sup>Department of Head and Neck, Esophageal Medical Oncology, National Cancer Center

Hospital, Tokyo, Japan. <sup>12</sup>Division of Gastroenterology, Chiba Cancer Center, Chiba, Japan. <sup>13</sup>Gastrointestinal Medical Oncology, NHO Shikoku Cancer Center, Matsuyama, Japan. <sup>14</sup>Department of Gastroenterology, Saitama Cancer Center, Ina, Japan. <sup>15</sup>Center for Cancer Genomics and Precision Medicine, Osaka University Hospital, Suita, Japan. <sup>16</sup>Department of Medical Oncology, Kobe City Medical Center General Hospital, Kobe, Japan. <sup>17</sup>Cancer Treatment Center, Kansai Medical University Hospital, Hirakata, Japan. <sup>18</sup>Department of Medical Oncology, Kochi Medical School, Nankoku, Japan. <sup>19</sup>Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. <sup>20</sup>Department of Surgery, NHO Osaka National Hospital, Osaka, Japan. <sup>21</sup>Department of Clinical Oncology, Kansai Rosai Hospital, Amagasaki, Japan. <sup>22</sup>Department of Gastroenterological Surgery and Pediatric Surgery, Center for One Medicine Innovative Translational Research (COMIT), Gifu University Graduate School of Medicine, Gifu, Japan. <sup>23</sup>Cancer Chemotherapy Center, Osaka Medical and Pharmaceutical University Hospital, Takatsuki, Japan. <sup>24</sup>Department of Medical Oncology, Kyorin University Faculty of Medicine, Tokyo, Japan. <sup>25</sup>Department of Medical Oncology, Kanazawa University Hospital, Kanazawa, Japan. <sup>26</sup>Division of Gastrointestinal Oncology, Shizuoka Cancer Center, Nagaizumi, Japan. <sup>27</sup>Division of Translational Informatics, Exploratory Oncology Research and Clinical Trial Center, National Cancer Center, Kashiwa, Japan. <sup>28</sup>Kindai University Faculty of Medicine, Osakasayama, Japan. <sup>29</sup>These authors contributed equally: Yoshiaki Nakamura, Hiroshi Ozaki. ✉ e-mail: [tyoshino@east.ncc.go.jp](mailto:tyoshino@east.ncc.go.jp)

## Methods

### Study design and patients

SCRUM-Japan GOZILA is a nationwide plasma genomic profiling study involving 31 core cancer institutions in Japan (National Cancer Center Hospital East, Aichi Cancer Center, National Cancer Center Hospital, NHO Kyushu Cancer Center, Hokkaido University Hospital, Saitama Cancer Center, Kanagawa Cancer Center, Kansai Rosai Hospital, NHO Shikoku Cancer Center, NHO Osaka National Hospital, University of Tsukuba Hospital, Chiba Cancer Center, Kyorin University Hospital, Kindai University Hospital, Kyushu University, St. Marianna University School of Medicine, Osaka University, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Kobe City Medical Center General Hospital, Osaka Medical and Pharmaceutical University Hospital, Gifu University, Kanazawa University, Shizuoka Cancer Center, Kagawa University Hospital, Keio University Hospital, Saitama Medical University International Medical Center, Shimane Prefectural Central Hospital, Kansai Medical University Hospital, Kyoto Katsura Hospital, Osaka International Cancer Institute, Osaka General Medical Center). The key eligibility criteria include: (1) histopathologically confirmed unresectable or metastatic gastrointestinal cancers; (2) patient aged  $\geq 20$  years; and (3) a life expectancy of at least 12 weeks. Although the study initially focused on gastrointestinal cancers, the eligibility expanded to include all solid tumors as the study progressed. To avoid the suppression of ctDNA shedding due to chemotherapy, patients are required to have received no previous systemic therapy or to have disease progression during systemic therapy and should not have started subsequent therapy at the time of blood sampling. Eligible patients provide written informed consent, and ctDNA genotyping was performed using Guardant360 (Guardant Health).

The study is conducted in accordance with the Declaration of Helsinki and the Japanese Ethical Guidelines for Medical and Biological Research Involving Human Subjects. The study protocol was approved by the institutional review board of each participating institution and registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN000029315), and the study was launched in January 2018. All patients provided written informed consent before participating in the study.

### ctDNA genotyping

Next-generation sequencing analysis of ctDNA was performed using Guardant360 (Guardant Health), an assay approved by the US Food and Drug Administration and Japan Pharmaceuticals and Medical Devices Agency and performed at a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, laboratory approved by New York State Department of Health, as previously described<sup>43</sup>. Guardant360 interrogates 74 genes and detects single nucleotide variants, indels, fusions and copy number alterations as well as MSI. For the GOZILA study,  $2 \times 10$  ml of whole blood was collected in a cell-free DNA blood collection tube (Streck, Inc.) from enrolled patients and sent to Guardant Health. Then, 5–30 ng of cell-free DNA isolated from plasma was labeled with nonredundant oligonucleotides ('molecular barcoding'), enriched using targeted hybridization capture, and sequenced on the NextSeq 550 platform (Illumina). Base call files generated using RTA software v.2.12 (Illumina) were demultiplexed using bcl2fastq (v.2.19) and processed using a custom pipeline for molecular barcode detection, sequencing adapter trimming and base quality trimming. Processed reads were aligned to hg19 using the Burrows–Wheeler aligner-MEM algorithm<sup>44</sup>. Somatic ctDNA alterations were detected by comparing read and consensus molecule characteristics to sequencing platform- and position-specific reference error noise profiles determined independently for each position in the panel<sup>43</sup>. ctDNA fraction was measured by the maximum VAF. To estimate the cell-free DNA clonality for somatic single nucleotide variants, indels and fusions, relative clonality was initially defined as alteration VAF divided by maximum somatic VAF in the sample. The ApCN

was calculated as follows:  $\text{ApCN} = (\text{observed pCN} - 2 \times [1 - T\%]) / T\%$ , where  $T\% = 2 \times \text{maximum VAF} / 100$ . TMB score was calculated by adjustment of somatic mutation count by tumor fraction and TMB score of  $\geq 10$  was defined as TMB high.

### Therapeutic selection and clinical data collection

Patients were treated according to the guidelines for their cancer type, with enrollment in clinical trials of genotype-matched targeted agents based on biomarker results identified by ctDNA genotyping. The affiliated trials included company-sponsored and investigator-initiated trials. If the biomarker identified in the patient could be a target of the drug used to treat the patient, then the patient was considered to have received matched therapy. Patients with no biomarker that can be targeted by available therapies were grouped as having no actionable biomarker.

Clinicopathological information and efficacy data for systemic therapy were collected using an electronic data capture system, TrialMaster v.5.0 (Anju Software). The clinical data were updated annually. All data were finalized by combining auto-generated and manually added queries from the SCRUM-Japan Data Center. Clinical data and biomarker results were stored in a clinical grade database and used for integrated clinico-genomic analysis.

### Statistical analysis

Tumor response to systemic therapy was assessed according to the Response Evaluation Criteria in Solid Tumors v.1.1. Clinical benefit was defined as tumor response or stable disease for four or more months. PFS was estimated as the time from the date of treatment initiation to the date of disease progression according to the investigator's assessment or the date of death from any cause. OS was estimated from the date of enrollment to the date of death from any cause.

ORR, PFS and OS were compared among patients who received and did not receive matched therapy and those with no actionable biomarker leading to targeted therapy. The Kaplan–Meier method was used to estimate survival rates, and treatment groups were compared using the log-rank test. HR values and 95% CI were calculated using the Cox proportional hazards model. Statistical comparisons of ORR were performed using the chi-squared test or Fisher's exact test. Clonality and pCN were compared using the Mann–Whitney test between patients with and without clinical benefit. All statistical tests were two-sided without adjustment for multiple comparisons. Statistical analyses were performed using R v.4.2.1. *P* values  $< 0.05$  were considered statistically significant. The data cutoff date for the analyses was 31 March 2023.

### Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

### Data availability

The authors declare that all variant data used in the conduct of the analyses are available within the article and its Supplementary Information. To protect the privacy and confidentiality of patients in this study, clinical data are not made publicly available in a repository or the supplementary material of the article, but can be requested at any time from the corresponding author (T.Y.: tyoshino@east.ncc.go.jp). Any requests will be reviewed within a time frame of 2 to 3 weeks by the corresponding author to verify whether the request is subject to any intellectual property or confidentiality obligations. All data shared will be de-identified under an agreement.

### References

- Odegaard, J. I. et al. Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. *Clin. Cancer Res.* **24**, 3539–3549 (2018).



44. Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Preprint at <https://arxiv.org/abs/1303.3997> (2013).

## Acknowledgements

We thank all of the patients and their families who participated in this study; all SCRUM-Japan GOZILA investigators and site personnel; and Translational Research Support Office, National Cancer Center Hospital East, Kashiwa, Japan for study management and data center support. This work was supported by grants from the National Cancer Center Research and Development Fund (grant no. 2021-A-6), the Japan Agency for Medical Research and Development (grant nos 19ck0106445h0002, 19lk0201084h0002, 19ck0106447h0002, 19lk0201065h0003, 19lk0201067h0003 and 20lk0201126h0001) and SCRUM-Japan Funds (<http://www.scrum-japan.ncc.go.jp/index.html>).

## Author contributions

Y.N. contributed to planning and conducting studies, recruiting patients, acquisition of data, analyzing and interpreting data, statistical analysis and writing the manuscript. H.O. contributed to planning and conducting studies, analyzing and interpreting data, statistical analysis and writing the manuscript. M.U., Y.K., S.Y., T.E., H.T., Y.S., K. Yamaguchi, K.K., T.D., T.N., N.T., T.S., H.Y., H.S., E.O., T.K., T.O., N.M., M.G., N.O., K.O. and K. Yamazaki contributed to recruiting patients and acquisition of data. R.Y. and N.I. contributed to the acquisition of data and analyzing the data. T.Y. contributed to conducting studies. T.Y. contributed to planning and conducting studies, interpreting data and the writing manuscript. All authors agree to be accountable for all aspects of the work and will ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Competing interests

Y.N. reports advisory roles with Guardant Health Pte Ltd, Natera, Inc., Roche Ltd, Seagen, Inc., Premo Partners, Inc., Daiichi Sankyo Co., Ltd, Takeda Pharmaceutical Co., Ltd, Exact Sciences Corporation and Gilead Sciences, Inc.; speakers' bureau from Guardant Health Pte Ltd, MSD K.K., Eisai Co., Ltd, Zeria Pharmaceutical Co., Ltd, Miyarisan Pharmaceutical Co., Ltd, Merck Biopharma Co., Ltd, CareNet, Inc., Hisamitsu Pharmaceutical Co., Inc, Taiho Pharmaceutical Co., Ltd, Daiichi Sankyo Co., Ltd, Chugai Pharmaceutical Co., Ltd and Becton, Dickinson and Company, Guardant Health Japan Corp; research funding from Seagen Inc., Genomedia Inc., Guardant Health AMEA, Inc., Guardant Health, Inc., Tempus Labs, Inc., Roche Diagnostics K.K., Daiichi Sankyo Co., Ltd and Chugai Pharmaceutical Co., Ltd. H.O. declares no competing interests. M.U. reports honoraria from Taiho Pharmaceutical Co., Ltd, AstraZeneca, K.K., Yakult Honsha Co., Ltd, MSD K.K., Nihon Servier Co., Ltd, Ono Pharmaceutical Co., Ltd, Incyte Biosciences Japan GK, Chugai Pharmaceutical Co., Ltd, Boehringer Ingelheim GmbH, J-pharma Co., Ltd, Daiichi Sankyo Co., Ltd, Eisai Co., Ltd, Takeda Pharmaceutical Co., Ltd and Novartis Pharma K.K.; research funding from Taiho Pharmaceutical Co., Ltd, AstraZeneca, K.K., MSD K.K., Nihon Servier Co., Ltd, Ono Pharmaceutical Co., Ltd, Incyte Biosciences Japan GK, Chugai Pharmaceutical Co., Ltd, Boehringer Ingelheim GmbH, J-pharma Co., Ltd, Eisai Co., Ltd, Novartis Pharma K.K., Astellas Pharma Inc., J-pharma Co., Ltd, DFP (Delta Fly Pharma), Inc., Novocure GmbH and Chiome Bioscience Inc. Y.K. reports research funding from Ono Pharmaceutical Co., Ltd, Chugai Pharmaceutical Co., Ltd, Taiho Pharmaceutical Co., Ltd, Shionogi & Co., Ltd, Nippon Zoki Pharmaceutical Co., Ltd, Asahi Kasei Pharma Corporation, Nippon Kayaku Co. Ltd, Daiichi Sankyo Co., Ltd, IQVIA Services Japan K.K., MSD, Astellas Pharma Inc., National Cancer Center Japan, Syneos Health Clinical K.K., ShiftZero K.K., Parexel International Inc., Japan Clinical Cancer Research Organization, EPS Holdings, Inc., Sysmex Corporation, Public Health Research

Foundation, Aichi Cancer Center and Kyushu Study Group of Clinical Cancer; honoraria from Ono Pharmaceutical Co., Ltd, Taiho Pharmaceutical Co., Ltd, Chugai Pharmaceutical Co., Ltd, Daiichi Sankyo Co., Ltd, Eli Lilly and Company, Alfresa Pharma Corporation, Astellas Pharma Inc., EA Pharma Co., Ltd, Nippon Kayaku Co. Ltd, Pfizer, Nippon Zoki Pharmaceutical Co., Ltd, Sanofi K.K., Nipro, Moroo, Boehringer Ingelheim, Hakodate National Hospital, Asahi Kasei Pharma Corporation, MSD, Zeria Pharmaceutical Co., Ltd, Bayer Yakuhin, Ltd, Yakult Honsha Co., Ltd, Sumitomo Dainippon Pharma Co., Ltd, Incyte Corporation, Merck Biopharma Co., Ltd, the Japanese Gastroenterological Association, Sapporo Minami Tokushukai Hospital and Pancan Japan. S.Y. reports honoraria from Eli Lilly Japan K.K., Chugai Pharmaceutical Co., Ltd, Taiho Pharmaceutical Co., Ltd, Daiichi Sankyo Co., Ltd, Takeda Pharmaceutical Co., Ltd, Bristol-Myers Squibb K.K., Ono Pharmaceutical Co., Ltd, Bayer Yakuhin, Ltd, MSD K.K., Merck Biopharma Co., Ltd and Nippon Boehringer Ingelheim Co., Ltd. T.E. reports research funding from MSD, Daiichi Sankyo, Pfizer, Chugai, Amgen, Ono, Astellas Amgen Biopharma, Asahikasei Pharma, ALX Oncology, Seagen, Taiho and Jazz Pharmaceuticals; honoraria from Chugai, Daiichi Sankyo and Taiho. H.T. reports research funding from Takeda Pharmaceutical Co., Ltd, Daiichi Sankyo Co., Ltd and Ono Pharmaceutical Co., Ltd; honoraria from Takeda Pharmaceutical Co., Ltd, Ono Pharmaceutical Co., Ltd, Eli Lilly Japan K.K., Merck Biopharma Co., Ltd and Chugai Pharmaceutical Co., Ltd. Y.S. reports research funding from Chugai Pharmaceutical Co., Ltd and Taiho Pharmaceutical Co., Ltd; honoraria from Eli Lilly Japan K.K., Bristol-Myers Squibb K.K., Chugai Pharmaceutical Co., Ltd, Takeda Pharmaceutical Co., Ono Pharmaceutical Co., Ltd, Merck Biopharma Co., Ltd, Taiho Pharmaceutical Co., Ltd, Bayer Yakuhin, Ltd, Daiichi Sankyo Co., Ltd, MSD K.K., Novartis Pharmaceuticals, Astellas Pharma Inc., Sysmex, and Guardant Health; participation on a Data Safety Monitoring Board or Advisory Board for Merck Biopharma Co., Ltd., Ono Pharmaceutical Co., Ltd., and Guardant Health. K. Yamaguchi reports consulting or advisory role from Bristol Myers Squibb Japan, Daiichi Sankyo; Speakers' Bureau from Chugai Pharma, Bristol Myers Squibb Japan, Takeda, Taiho Pharmaceutical, Lilly, Ono Pharmaceutical, Daiichi Sankyo, Merck; research funding from Ono Pharmaceutical, Taiho Pharmaceutical, Daiichi Sankyo, Lilly, Gilead Sciences, Yakult Honsha, Chugai Pharma, Boehringer Ingelheim, Eisai, MSD Oncology, Sanofi, and Bristol Myers Squibb. K.K. reports consulting fees from ONO, Bristol Myers Squibb, Beigene/Novartis, AstraZeneca, Roche, BAYER, Merck & Co., Merck bio, and Janssen; honoraria from ONO and Bristol Myers Squibb, and expert testimony from Ono Pharmaceutical, Amgen, MSD, Pfizer, and Bristol Myers Squibb Foundation; honoraria from Daiichi-Sankyo, Ono Pharmaceutical, and Sysmex. T.N. reports honoraria from Daiichi Sankyo Co., Ltd., Ono Pharmaceutical Co., Ltd., Bristol-Myers Squibb K.K., Eli Lilly Japan K.K., Takeda Pharmaceutical Co., Ltd., Merck Biopharma Co., Ltd., Chugai Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., and Yakult Honsha Co., Ltd. N.T. reports research funding from Ono Pharmaceutical Co.; honoraria for lectures from Ono Pharmaceutical Co., Ltd., Taiho Pharmaceutical Co., Ltd., Bristol-Myers Squibb K.K. T.S. reports research funding from Ono Pharmaceutical, Chugai-Pharmaceutical, and Yakult-Honsha; honoraria from Elli-Lilly, Daiichi-Sankyo, Ono Pharmaceutical, and Bristol-Myers. H.Y. reports research funding from MSD, Daiichi Sankyo, Ono Pharmaceutical and Astellas Pharma; honoraria from Daiichi Sankyo, Ono Pharmaceutical, Taiho Pharmaceutical, Chugai Pharma, Bristol-Myers Squibb Japan, TERUMO, Eli Lilly Japan, Merck Biopharma, Yakult Honsha, Bayer Yakuhin, and Takeda Pharmaceutical. H.S. reports research funding from Ono Pharmaceutical Co. Ltd., Taiho Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., Asahi KASEI, and honoraria from Bayer Co., Ltd., Bristol-Myers Squibb Co., Ltd., Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Eli Lilly



Japan Co., Ltd., Merck Bio Pharma Co., Ltd., MSD Co., Ltd., Ono Pharmaceutical Co., Ltd., Sanofi Co., Ltd., Taiho Pharmaceutical Co., Ltd., Takeda Co., Ltd. and Yakult Honsha Co., Ltd. E.O. reports honoraria from Ono Pharm., Chugai Pharm., Eli Lilly, Bayer, and Takeda Pharm. T.K. reports honoraria from Chugai Pharmaceutical Co., Ltd., Eli Lilly and Company, Takeda Pharmaceutical Company Limited, and Ono Pharmaceutical Co. T.O. reports research funding from Takeda Pharmaceutical Co., Ltd.; honoraria for lectures from Eli Lilly Japan K.K., Taiho Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., EA Pharma Co., Ltd., Bristol-Myers Squibb K.K., Ono Pharmaceutical Co., Ltd., Eisai Co., Ltd., Yakult Honsha Co., Ltd., MSD K.K., AstraZeneca K.K., Merck Biopharma Co., Ltd., Novartis Pharma K.K., and Otsuka Pharmaceutical Co., Ltd. N.M. reports honoraria from Abbott, AMCO, Asahi Kasei Pharma, AstraZeneca, Bayer Yakuhin, Bristol-Myers Squibb, Chugai Pharm., Covidien Japan, Daiichi Sankyo, EA Pharma, Eisai, Eli Lilly Japan, Gunze Medical Limited, Johnson & Johnson, Kaken Pharm., Kyowa Kirin, MC Medical, Merck Biopharma Japan, Miyarisan Pharm., MSD, Novartis, Olympus Marketing, Ono Pharm., Stryker, Taiho Pharm., Takeda Pharm., TERUMO, Tsumura, Viatris, Yakult Honsha; research funding from Daiichi Sankyo, EP-CRSU, EPS Corporation, MSD, Ono Pharm., ShiftZero K.K., Taiho Pharm. M.G. reports research funding from Taiho Pharmaceutical Co., Ltd, Chugai Pharmaceutical Co., Ltd and Nippon Kayaku Co., Ltd; honoraria for lectures from Ono Pharmaceutical Co., Ltd, Daiichi Sankyo Co., Ltd and MSD K.K. N.O. reports honoraria from Taiho Pharmaceutical, Bayer Yakuhin, Lilly Japan, Chugai Pharma, Ono Pharmaceutical, Eisai, Daiichi Sankyo, AstraZeneca, MSD, Incyte, Nihon Servier. K.O. declares no competing interests. K. Yamazaki reports honoraria from Chugai, Takeda, Yakult, Daiichi Sankyo, Merck Biopharma, Lilly, Sanofi, Ono, MSD and Bristol. R.Y. reports expert

testimony from Takeda Pharmaceutical Co., Ltd. N.I. declares no competing interests. M.Y. declares no competing interests. H.B. reports honoraria from Ono Pharmaceutical, Eli Lilly Japan and Taiho Pharmaceutical. T.Y. reports honoraria from Chugai Pharmaceutical, Takeda Pharmaceutical, Merck Biopharma, Bayer Yakuhin, Ono Pharmaceutical and MSD K.K.; consulting fees from Sumitomo Corp.; and research funding from Amgen, Bristol-Myers Squibb, Chugai Pharmaceutical, Daiichi Sankyo, Eisai, FALCO Biosystems, Genomedia, Medical & Biological Laboratories, Merus N.V., Molecular Health GmbH, MSD, Nippon Boehringer Ingelheim, Ono Pharmaceutical, Pfizer Japan, Roche Diagnostics, Sanofi, Sysmex, Taiho Pharmaceutical and Takeda Pharmaceutical.

### Additional information

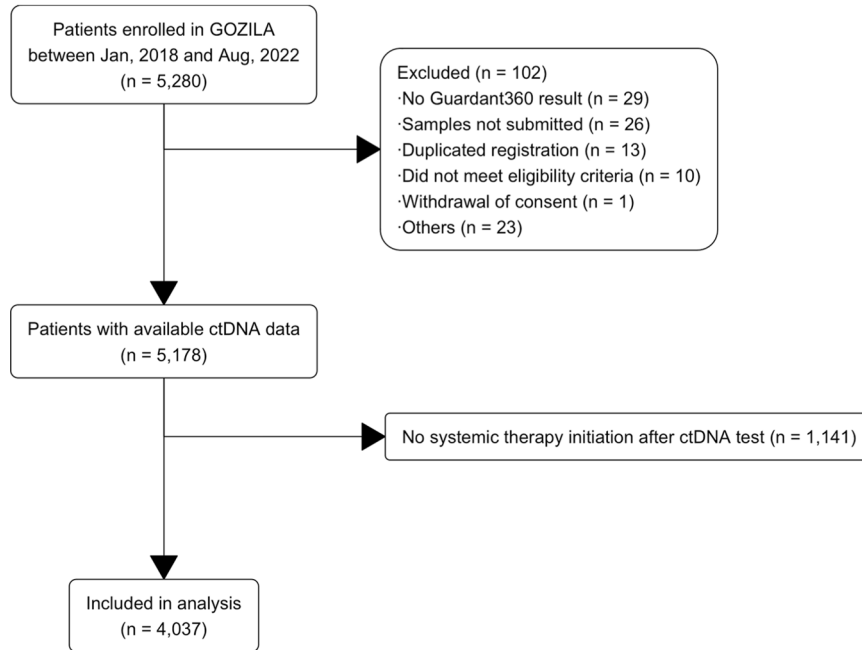
**Extended data** is available for this paper at <https://doi.org/10.1038/s41591-024-03244-8>.

**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41591-024-03244-8>.

**Correspondence and requests for materials** should be addressed to Takayuki Yoshino.

**Peer review information** *Nature Medicine* thanks the anonymous reviewers for their contribution to the peer review of this work. Primary Handling Editors: Jean Nakhle and Saheli Sadanand, in collaboration with the *Nature Medicine* team.

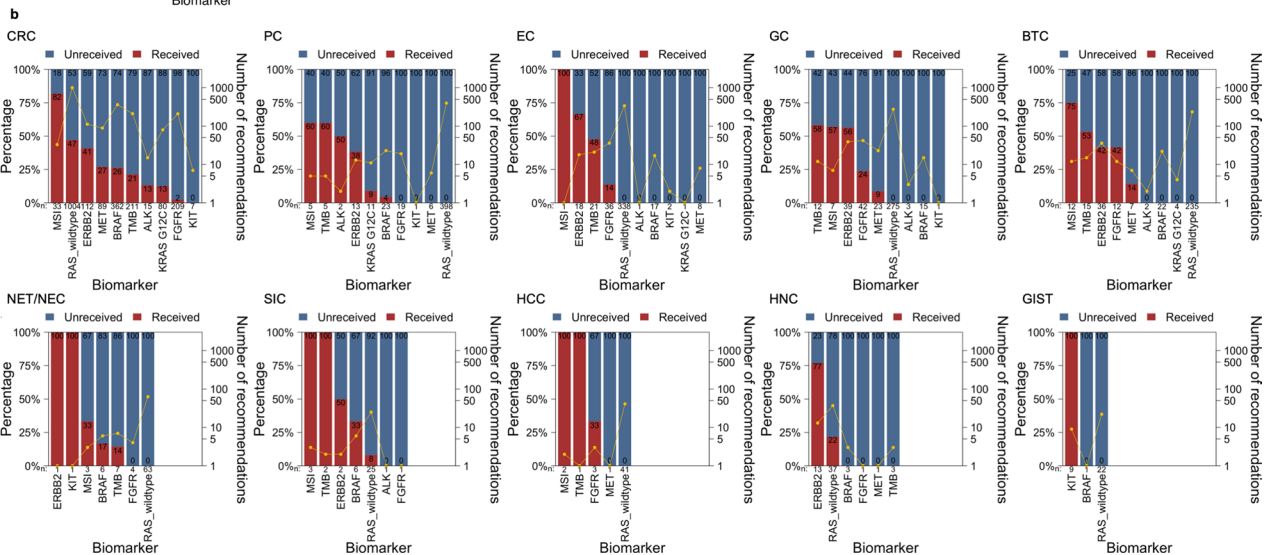
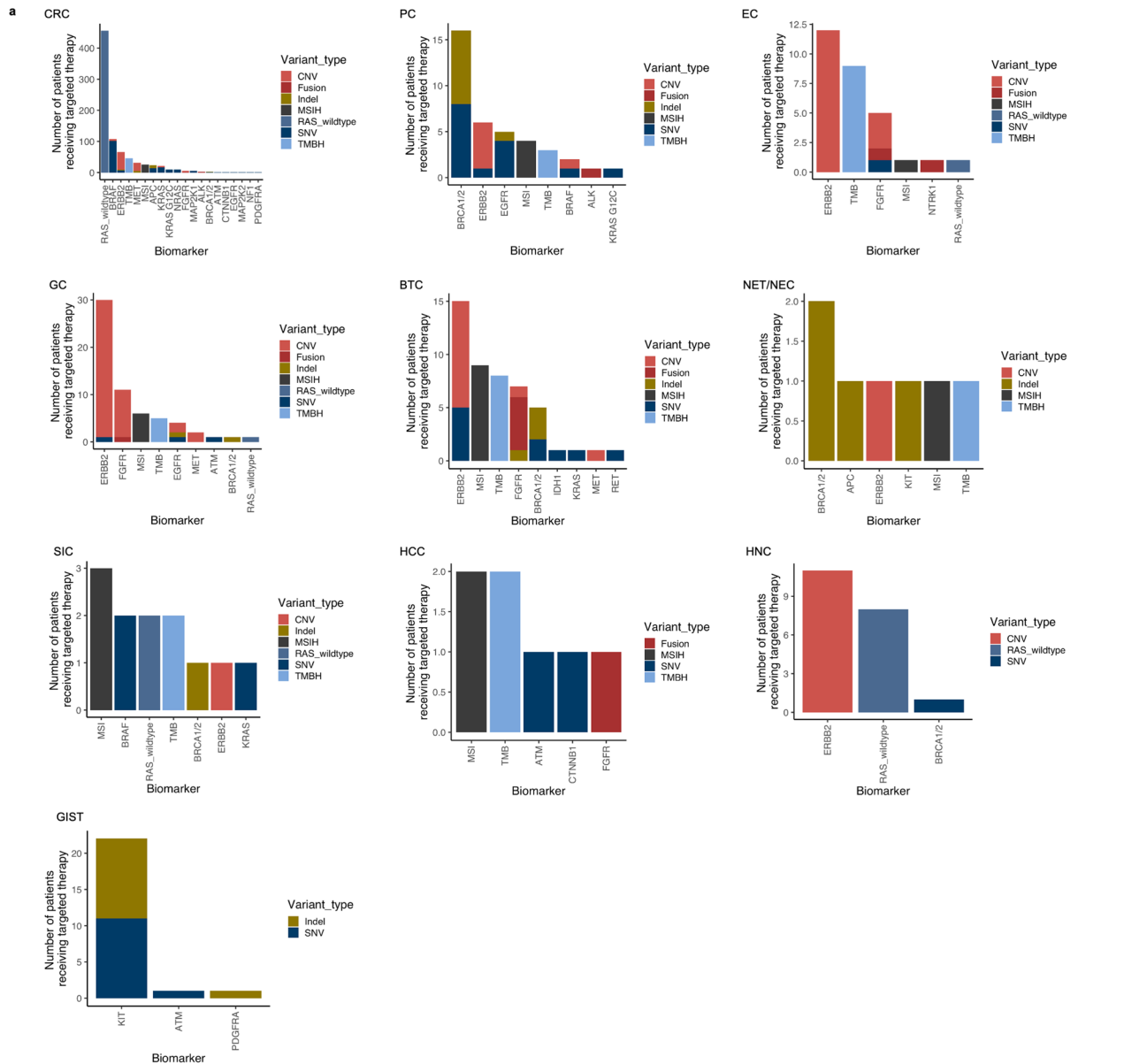
**Reprints and permissions information** is available at [www.nature.com/reprints](http://www.nature.com/reprints).



**Extended Data Fig. 1 | Patient flow.** Of the 5,280 patients enrolled in the GOZILA study from January 2018 to August 2022, 5,178 had available ctDNA data. Among them, 1,141 patients with no systemic therapy initiation after ctDNA test were excluded. Consequently, 4,037 were included in the analysis.

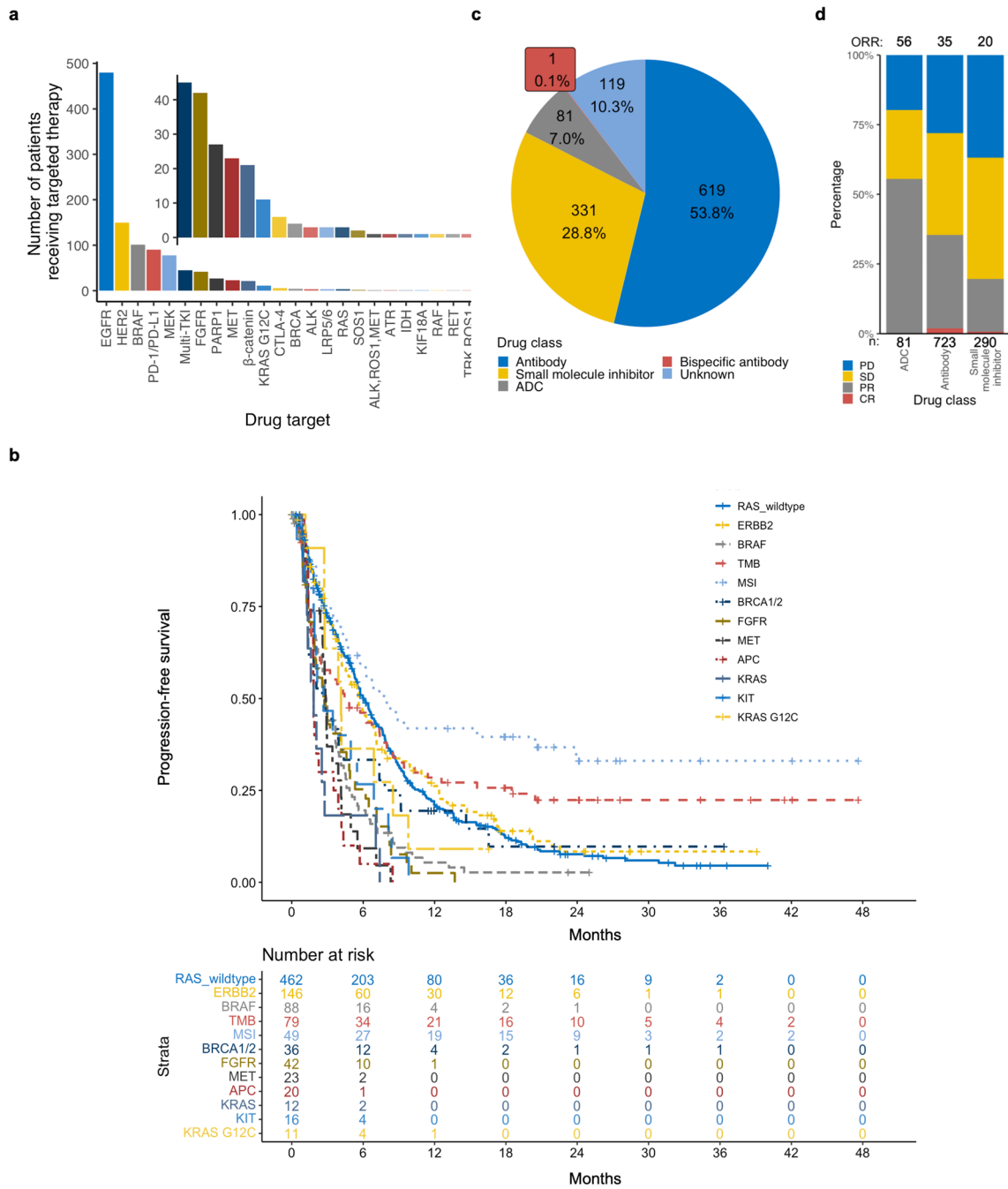
Characteristic	N = 4,037
Median age, years (range)	64 (20, 91)
Sex, n (%)	
Female	1,583 (39%)
Male	2,454 (61%)
Cancer type, n (%)	
Colorectal cancer (CRC)	1,820 (45%)
Pancreatic cancer (PC)	899 (22%)
Esophageal cancer (EC)	363 (9%)
Gastric cancer (GC)	327 (8%)
Biliary tract cancer (BTC)	291 (7%)
Neuroendocrine tumor/carcinoma (NET/NEC)	71 (2%)
Small intestine cancer (SIC)	47 (1%)
Hepatocellular carcinoma (HCC)	42 (1%)
Head and neck cancer (HNC)	40 (1%)
Gastrointestinal stromal tumor (GIST)	23 (0.6%)
Appendiceal cancer (AC)	18 (0.4%)
Uterine corpus endometrial carcinoma (UCEC)	14 (0.3%)
Ovarian cancer (OC)	13 (0.3%)
Anal canal cancer (ACC)	12 (0.3%)
Cervical squamous cell carcinoma (CESC)	12 (0.3%)
Breast cancer (BRCA)	11 (0.3%)
Lung cancer (LC)	11 (0.3%)
Urothelial carcinoma (UC)	10 (0.2%)
Not classified	8 (0.2%)
Cancer of unknown primary (CUP)	2 (< 0.1%)
Prostate adenocarcinoma (PRAD)	2 (< 0.1%)
Malignant melanoma (MM)	1 (< 0.1%)
Number of metastatic organs (range)	1 (0, 7)
Metastatic site, n (%)	
Liver	2,082 (52%)
Lymph node	1,475 (37%)
Lung	1,270 (31%)
Peritoneum	954 (24%)
Bone	316 (8%)

**Extended Data Fig. 2 | Patient baseline characteristics.** Summary of baseline characteristics of patients included in the analysis. Data are n (%) or median (range).



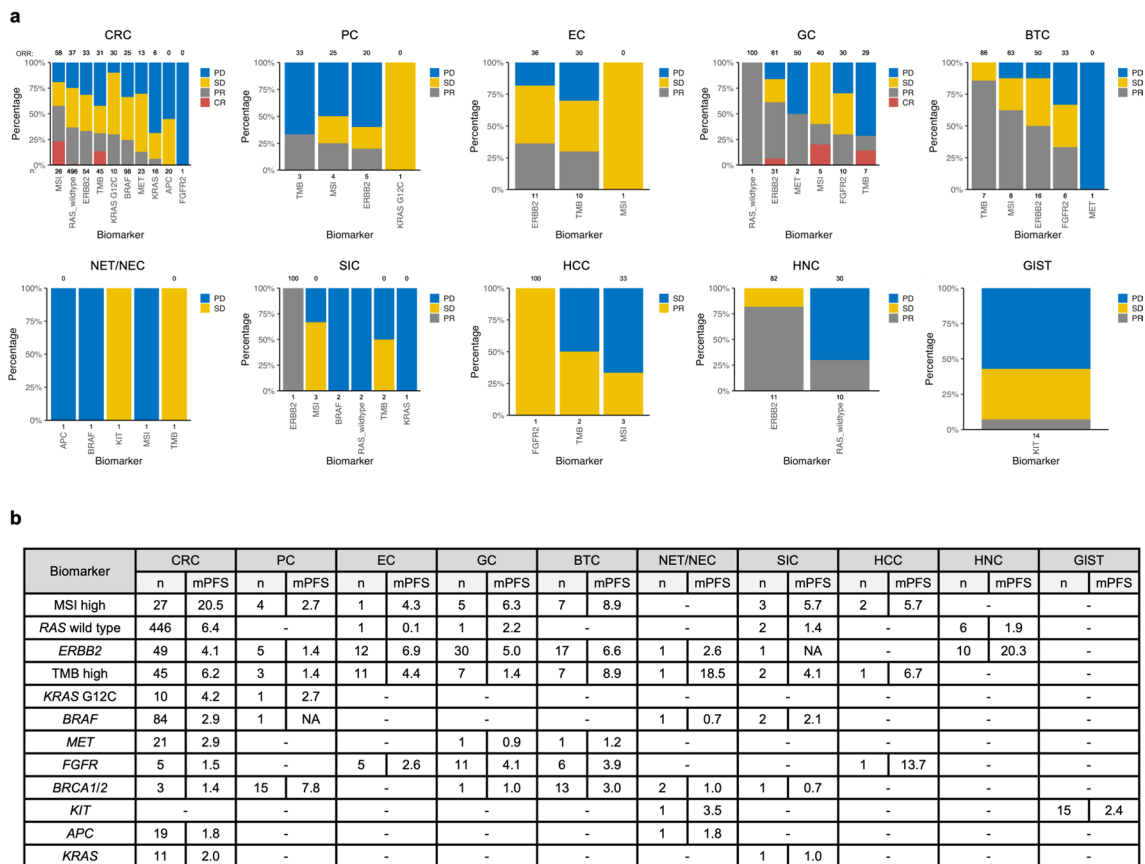
**Extended Data Fig. 3 | Number of patients receiving matched therapy by drug targets. a.** Number of patients receiving matched therapy by biomarkers in each cancer type with at least 20 patients enrolled. **b.** Top 10 biomarkers with the highest percentage of patients receiving matched therapy, for which a targeted

treatment was recommended. Yellow line represents the number of patients with biomarkers that had a recommendation. Data are shown by each cancer type with at least 20 patients enrolled.



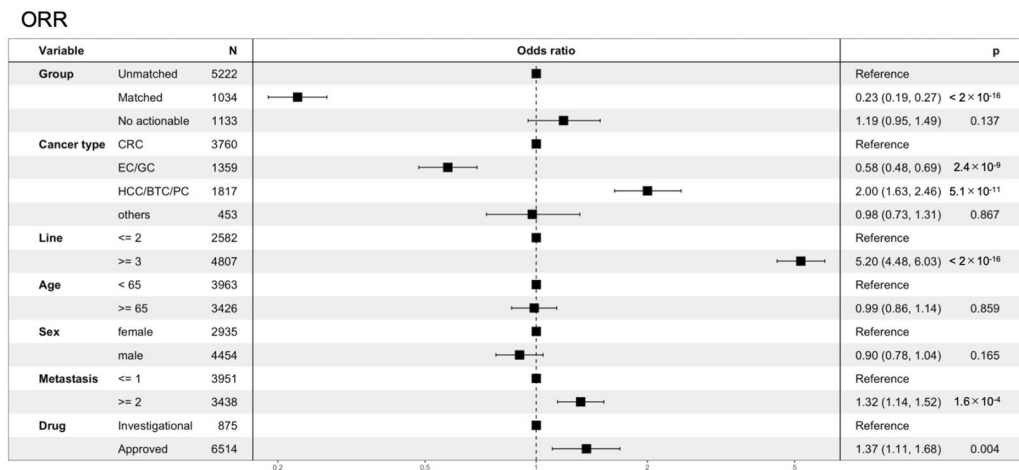
**Extended Data Fig. 4 | Efficacy of matched targeted therapy by biomarkers and drug class. a.** Number of patients receiving matched therapy by drug targets. **b.** Kaplan-Meier plots of progression-free survival (PFS) of matched therapy by biomarkers. **c.** Number of patients receiving matched therapy by drug class. **d.** Tumor response to matched therapy by drug class.



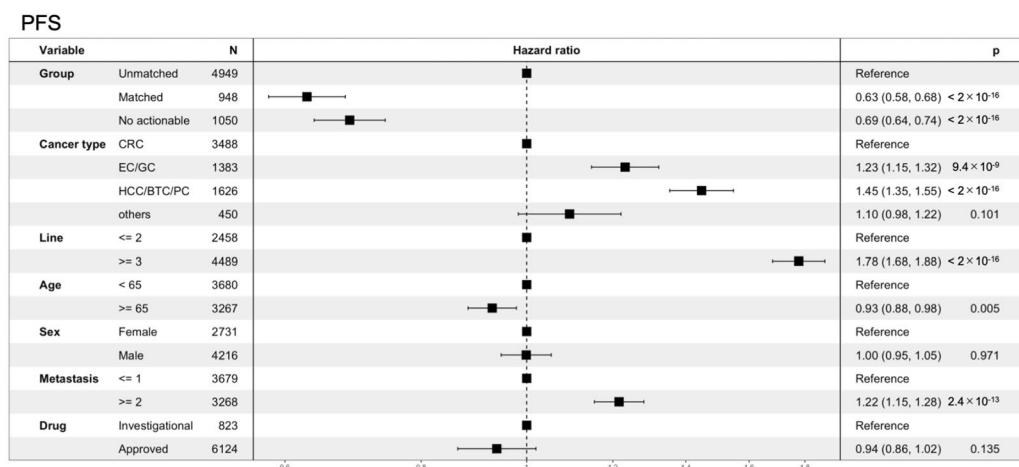


**Extended Data Fig. 5 | Objective response rate (ORR) and progression-free survival (PFS) of matched targeted therapy by biomarkers in each cancer type with at least 20 patients enrolled. a.** Percentage of each tumor response of matched therapy by biomarkers. **b.** Median PFS (mPFS) of matched therapy targeting each biomarker. NA, not available.

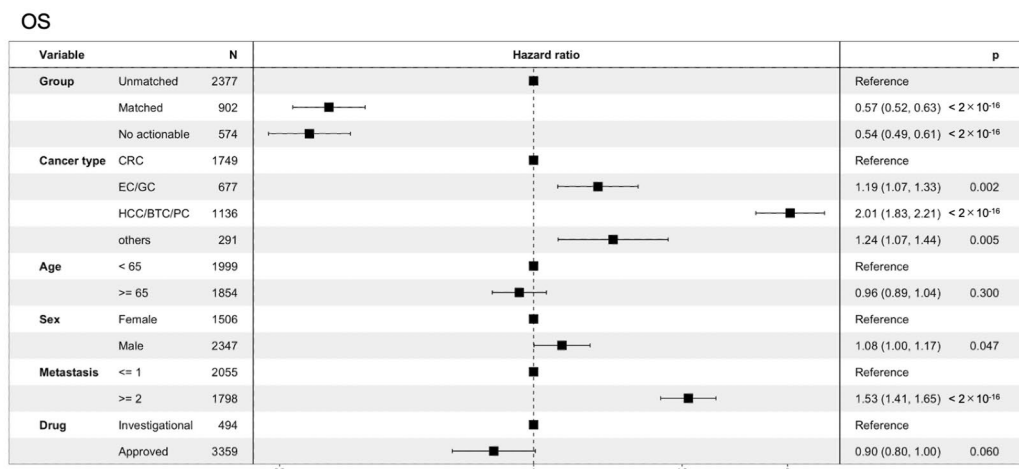
**a**



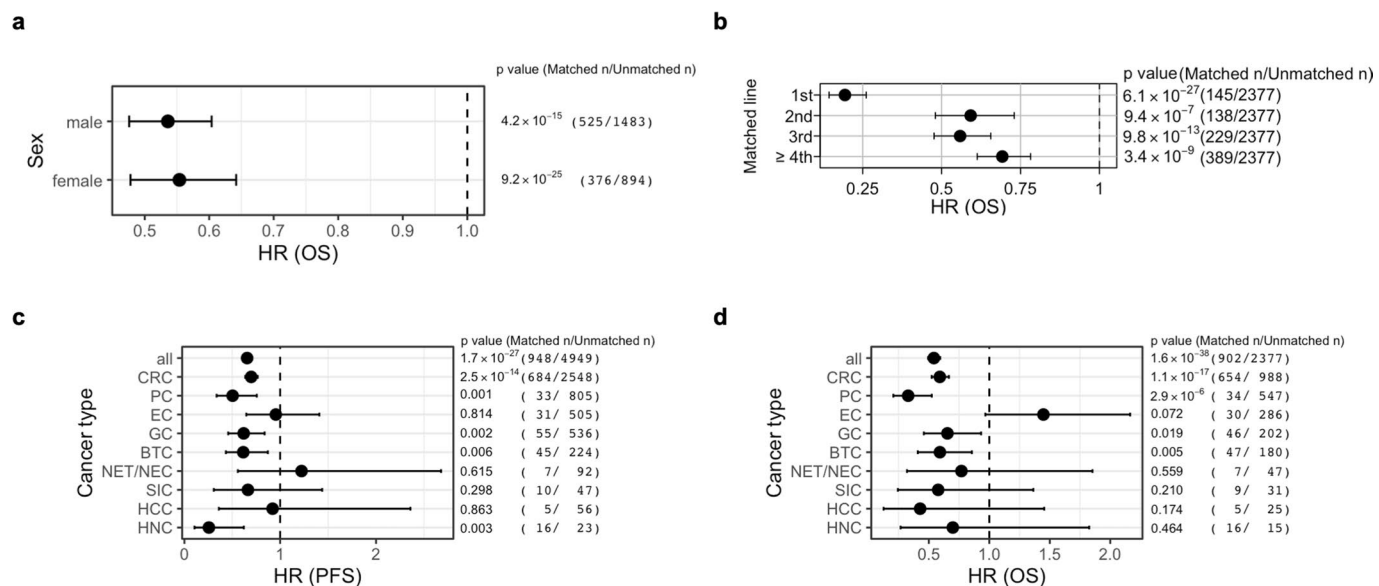
**b**



**c**

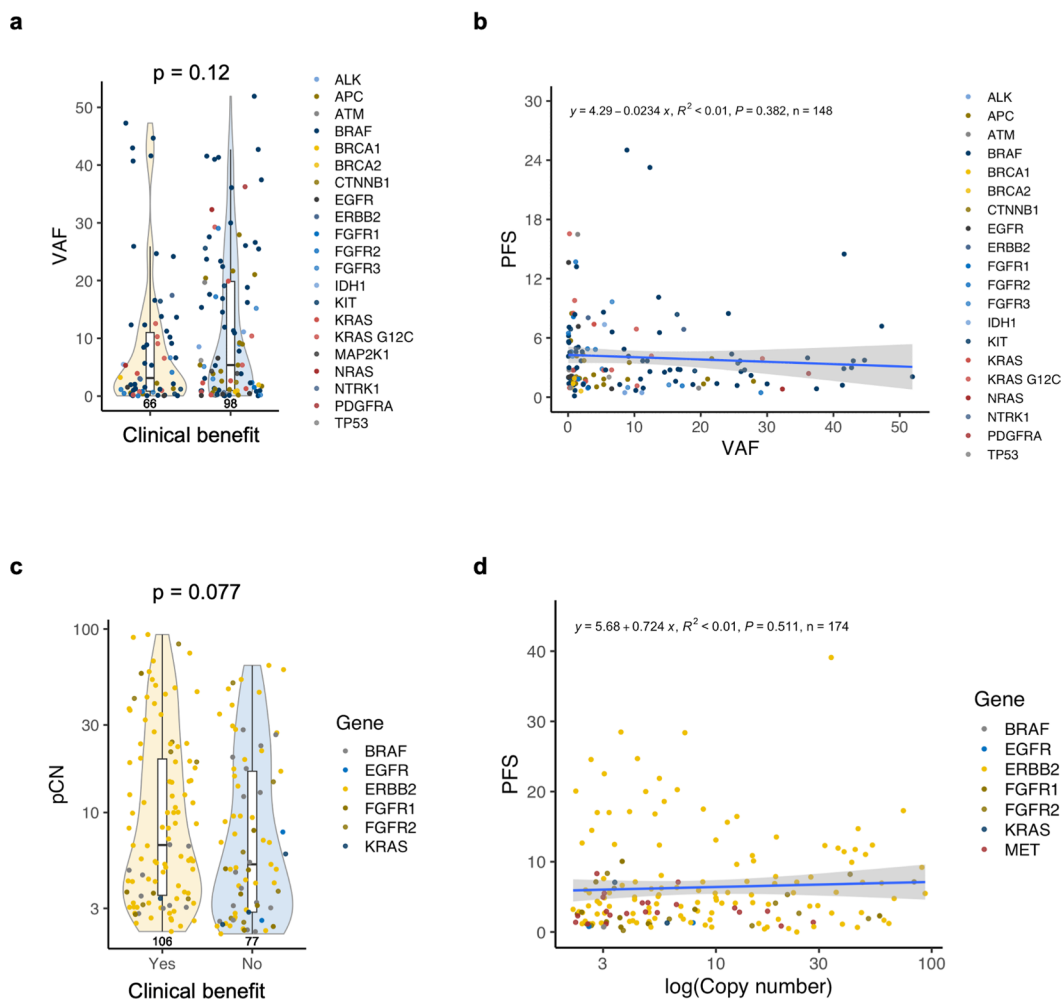


**Extended Data Fig. 6 | Multivariate analysis for efficacy endpoints.** The odds ratio (boxes) for ORR (a) and hazard ratios (boxes) for PFS (b) and OS (c) and 95% confidence intervals (horizontal lines) are shown for each subgroup. P values were calculated using the two-sided Wald test.



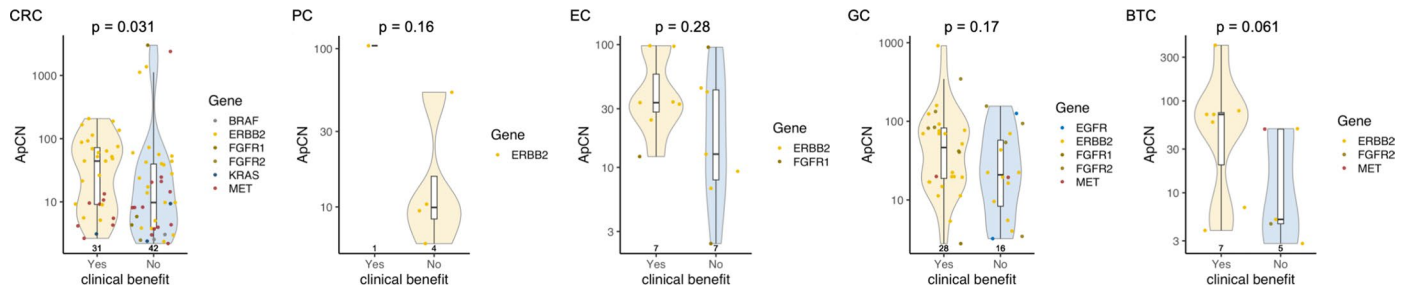
**Extended Data Fig. 7 | Benefit of matched therapy by subgroup. a.** Forest plots of hazard ratio (HR) for overall survival (OS) of matched therapy by sex. The HR (circles) and 95% confidence intervals (CIs, horizontal lines) are shown for each subgroup. P values were calculated using the two-sided Wald test. **b.** Forest plots of HR for OS of matched therapy by treatment line. The HR (circles) and 95% CIs (horizontal lines) are shown for each subgroup. P values were calculated using

the two-sided Wald test. **c.** Forest plots of HR for progression-free survival (PFS) in each cancer type including at least 40 patients. The HR (circles) and 95% CIs (horizontal lines) are shown for each subgroup. P values were calculated using the two-sided Wald test. **d.** Forest plots of HR for OS in each cancer type including at least 40 patients. The HR (circles) and 95% CIs (horizontal lines) are shown for each subgroup. P values were calculated using the two-sided Wald test.



**Extended Data Fig. 8 | Association of variant allelic fraction (VAF) or plasma copy number (pCN) with therapeutic efficacy. a.** VAF of variants targeted in patients with clinical benefit and no benefit. The clonality was significantly higher in patients with clinical benefit (two-sided  $p = 0.001$ , Mann–Whitney U-test). The boxes represent 25th–75th percentiles; center lines indicate the median; whiskers extend to the maximum and minimum values within  $1.5\times$  of the interquartile range; and dots indicate outliers. **b.** Correlation of progression-free survival (PFS) with VAF. Shaded area represents 95% confidence interval. P values

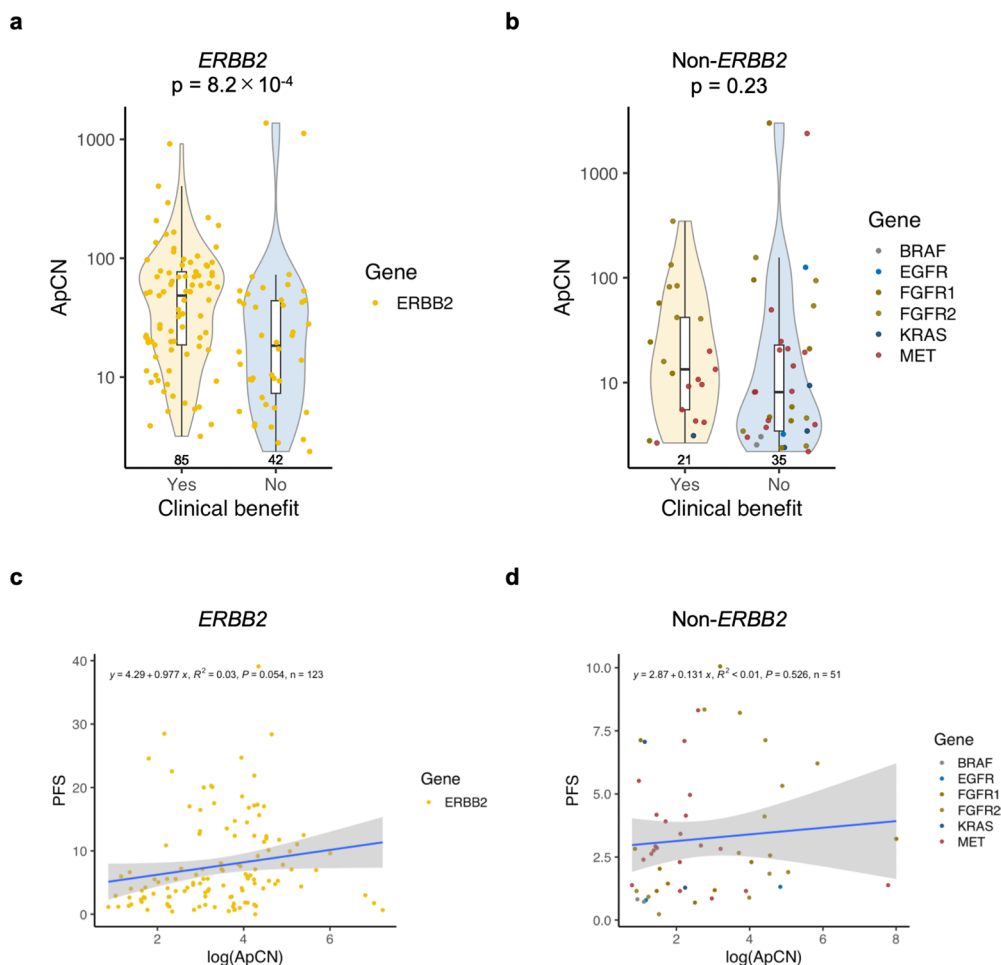
were calculated using the two-sided Wald test. **c.** pCN of gene amplification targeted in patients with clinical benefit and no benefit. The pCN was significantly higher in patients with clinical benefit (two-sided  $p = 0.077$ , Mann–Whitney U-test). The boxes represent 25th–75th percentiles; center lines indicate the median; whiskers extend to the maximum and minimum values within  $1.5\times$  of the interquartile range; and dots indicate outliers. **d.** Correlation of PFS with pCN. Shaded area represents 95% confidence interval. P values were calculated using the two-sided Wald test.



**Extended Data Fig. 9 | Association of adjusted plasma copy number (ApCN) with therapeutic efficacy by cancer types.** ApCN of gene amplifications targeted in patients with clinical benefit and no benefit in top 5 cancer types.

The boxes represent 25th–75th percentiles; center lines indicate the median; whiskers extend to the maximum and minimum values within 1.5× of the interquartile range; and dots indicate outliers.





**Extended Data Fig. 10 | Association of adjusted plasma copy number (ApCN) with therapeutic efficacy in *ERBB2* and non-*ERBB2* genes.** **a.** ApCN of *ERBB2* gene amplifications targeted in patients with clinical benefit and no benefit. The ApCN of *ERBB2* was significantly higher in patients with clinical benefit (two-sided  $p < 0.001$ , Mann–Whitney U-test). The boxes represent 25th–75th percentiles; center lines indicate the median; whiskers extend to the maximum and minimum values within  $1.5\times$  of the interquartile range; and dots indicate outliers. **b.** ApCN of non-*ERBB2* gene amplifications targeted in patients with clinical benefit and no benefit. The ApCN of non-*ERBB2* tended to be higher in

patients with clinical benefit (two-sided  $p = 0.23$ , Mann–Whitney U-test). The boxes represent 25th–75th percentiles; center lines indicate the median; whiskers extend to the maximum and minimum values within  $1.5\times$  of the interquartile range; and dots indicate outliers. **c.** Correlation of progression-free survival (PFS) with ApCN of *ERBB2*. Shaded area represents 95% confidence interval. P values were calculated using the two-sided Wald test. **d.** Correlation of progression-free survival (PFS) with ApCN of non-*ERBB2* genes. Shaded area represents 95% confidence interval. P values were calculated using the two-sided Wald test.

## Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided  
*Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
*Give  $P$  values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's  $d$ , Pearson's  $r$ ), indicating how they were calculated

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

Policy information about [availability of computer code](#)

Data collection Data collection was done by input into an EDC systems; the EDC was TrialMaster version 5.0. RTA software version 2.12, bcl2fastq version 2.19., BWA-MEM version 0.7.15 (arXiv:1303.3997v2).

Data analysis R version 4.2.1

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

### Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Processed reads were aligned to hg19.

The authors declare that all variant data used in the conduct of the analyses are available within the article and its Supplementary Information. To protect the privacy and confidentiality of patients in this study, clinical data are not made publicly available in a repository or the supplementary material of the article, but can

be requested at any time to the Corresponding author (tyoshino@east.ncc.go.jp). Any requests will be reviewed within a time frame of 2 to 3 weeks by the Corresponding Author to verify whether the request is subject to any intellectual property or confidentiality obligations. All data shared will be de-identified under an agreement.

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Our study is a clinical study with human participants who self-reported their biological sex on the requisition form upon enrollment. Information on the breakdown of biological sex in our cohort is detailed in Extended Fig. 2. where 39% (1,583/4,037) of the cohort were female and the remainder were male. The sex of participants were determined based on self-report during the enrollment process. The benefit from biomarker-matched therapy in OS was not different by sex.
Reporting on race, ethnicity, or other socially relevant groupings	This study is conducted in Japan, and all participants are Japanese.
Population characteristics	Population characteristics are provided in Extended Fig. 2.
Recruitment	Participants were recruited by each investigator at the sites based on the study eligibility criteria. To standardize the recruitment process, all investigators followed a common protocol for patient approach and information provision. Patients were given detailed information about the study aims, procedures, and potential risks/benefits. Those who expressed interest were then thoroughly assessed against the full eligibility criteria. The possibility of bias in the selection by researchers cannot be ruled out. However, since enrollment was prospective and each site enrolled most of patients who fit the criteria, we believe that bias was suppressed as much as possible.
Ethics oversight	The study is conducted in accordance with the Declaration of Helsinki and the Japanese Ethical Guidelines for Medical and Biological Research Involving Human Subjects. The study protocol was approved by the institutional review board of each participating institution (National Cancer Center Hospital East; Aichi Cancer Center; National Cancer Center Hospital; NHO Kyushu Cancer Center; Hokkaido University Hospital; Saitama Cancer Center; Kanagawa Cancer Center; Kansai Rosai Hospital; NHO Shikoku Cancer Center; NHO Osaka National Hospital; University of Tsukuba Hospital; Chiba Cancer Center; Kyorin University Hospital; Kindai University Hospital; Kyushu University; St. Marianna University School of Medicine; Osaka University; Cancer Institute Hospital of Japanese Foundation for Cancer Research; Kobe City Medical Center General Hospital; Osaka Medical and Pharmaceutical University Hospital; Gifu University; Kanazawa University; Shizuoka Cancer Center; Kagawa University Hospital; Keio University Hospital; Saitama Medical University International Medical Center; Shimane Prefectural Central Hospital; Kansai Medical University Hospital; Kyoto Katsura Hospital; Osaka International Cancer Institute; Osaka General Medical Center) and registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN000046220), and the study was launched in January 2018. All patients provided written informed consent before participating in the study.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Life sciences  Behavioural & social sciences  Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	As this study was observational, a sample size calculation based on the formal statistical testing was not performed. 5,280 patients enrolled in the GOZILA study from January 2018 to August 2022 were analyzed.
Data exclusions	Data on patients who did not meet the eligibility criteria, had duplicate registration, had samples not submitted, failed blood test, and withdrew consent were excluded.
Replication	This study was a clinical study with human participants. Hence, replication of data set is not applicable for our study.
Randomization	This is an observational study and is not a randomized trial.
Blinding	Since this study is not a randomized trial, no blinding was used. In an observational study of this nature, blinding participants to their own medical condition or treatment would be ethically problematic and could interfere with standard care.

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

- |                                     |                                     |                               |
|-------------------------------------|-------------------------------------|-------------------------------|
| n/a                                 | <input checked="" type="checkbox"/> | Involvement in the study      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Antibodies                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Palaeontology and archaeology |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Animals and other organisms   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Clinical data                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Dual use research of concern  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Plants                        |

## Methods

- |                                     |                                     |                          |
|-------------------------------------|-------------------------------------|--------------------------|
| n/a                                 | <input checked="" type="checkbox"/> | Involvement in the study |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | ChIP-seq                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Flow cytometry           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | MRI-based neuroimaging   |

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<input type="text" value="UMIN000029315"/>
Study protocol	<input type="text" value="The protocol has been submitted along with the manuscript."/>
Data collection	<input type="text" value="Data collection was performed using an EDC system, specifically TrialMaster version 5.0. The data were collected and entered directly into the EDC system by authorized personnel at each participating study site. Patient recruitment and enrollment occurred from January 2018 to August 2022. Data collection began with the enrollment of the first patient in January 2018 and continued through the follow-up period. The final data cutoff date was set as March 31, 2023."/>
Outcomes	<input type="text" value="Prevalence of genomic alterations in 74 cancer-associated genes detected by Guardant360 as well as TMB, and MSI in blood samples and clinicopathological and molecular biological profiles, clinical course (treatments received and clinical outcomes such as RR, PFS, and TTF), and cancer-associated genomic alterations in cancer tissue. For this study, objective response rate, progression-free survival, and overall survival were assessed as endpoints. Tumor response to systemic therapy was assessed according to the Response Evaluation Criteria in Solid Tumors version 1.1. Clinical benefit was defined as tumor response or stable disease for four or more months. Progression-free survival (PFS) was estimated as the time from the date of treatment initiation to the date of disease progression according to the investigator's assessment or the date of death from any cause. Overall survival (OS) was estimated from the date of enrollment to the date of death from any cause."/>

## Plants

Seed stocks	<input type="text" value="NA"/>
Novel plant genotypes	<input type="text" value="NA"/>
Authentication	<input type="text" value="NA"/>