

Real-world clinical utility of comprehensive genomic profiling in advanced solid tumors

Corresponding Author: Professor Keisuke Kataoka

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This is a very interesting and important paper that systemically looks at the real world experience captured in the Japanese data base. IT provides a clear sense of the evolution of the use and implications of CGP-guided decisions and provides some interesting examples (from rare to common issues in clinical oncology). The ability to look over several years is important and should be done in the future to best understand factors and utility. The authors should consider a few points:

1. The abstract might benefit from highlighting comparisons between years and other studies (see below) instead of starkly stating several observations of specific findings for rare tumors or situations. The value here is capacity to look systematically and thus the conclusions form the larger picture should dominate the abstract.....
2. The paper would benefit greatly from perhaps shortening the depictions of specific settings (restricting to a few examples in the text and/or boxes or supplemental docs) and instead provide analyses and discussion of how this experience differs from several of the large references provided. This is a key point and one that should be fully addressed.
3. Is it possible to retrieve outcome data (either disease status or death) for the earlier cases. One of the key issues is whether the use- presumably sooner of NGS guided therapy really does have an impact on outcome in the real world setting....An attempt to address this would be a major addition to this fine work.

Minor issue: in the provided manuscript, the text from lines 274-277 was not clear and perhaps should be rephrased.

(Remarks on code availability)

N/A

Reviewer #2

(Remarks to the Author)

In their manuscript, Saito et al describe the use and impact of comprehensive genomic profiling in patients with cancer using a large database.

Overall, the results are quite disapointing because patients who present Level A alterations present only a modest improvement on their overall survival. The paper should better investigate the reasons for such a modest improvement.

Most of the patients with Level A present a "TMB-H". The benefit observed in this population could therefore be explained by the use of anti-PD1 Antibodies, rather than the use of the CGP itself. Indeed, anti PD1 antibody can generate some benefit in patients with cancers irrespective of biomarker result.

A description of the outcome in other cohorts (ERBB2, BRCA, ALK etc) should be reported in the same way as patients with NTRK fusions

There is a general criticism surrounding the use of CGP as "only approach" for precision medicine. THe authors confirm this impression by reporting a high rate of false negative results for CGP as compared to standard testings (companion diagnostic). This observation would deserve a specific figure in the main document and a chapter of results.

Authors should better describe the use of off-label drugs since this is a major issue when CGP is used since it generates major costs without proven benefit

minor points:

why are level B not included in figure 1 ?

how are responses assessed ? it's written in the paper that authors use the data from hospital records. nevertheless, not all hospitals and investigators assess RECIST in their daily practice. This point should be more precise in the method section

HR should be multivariate

Figure 2 is difficult to understand, authors should make it more clear

Some disease present a low rate of Level A. As example, breast and BTC present <10% level A while their frequency is usually more important (PIK3CA for breast cancer for example). Authors should comment it

(Remarks on code availability)

I went to the github but there are many codes, I could not review all of them

Reviewer #3

(Remarks to the Author)

This manuscript analyzes comprehensive genomic profiling (CGP) in over 54,000 patients with advanced solid tumors in Japan, leveraging the national C-CAT registry. It provides a valuable real-world perspective on the implementation of precision oncology at scale, revealing important insights into the clinical uptake of CGP, treatment stratification based on biomarker evidence levels, and tumor-specific responses to targeted and immune therapies.

The study is especially impactful due to its size, nationwide scope, and outcomes analysis. It underscores the gap between identifying actionable alterations (present in 73% of cases) and actual delivery of CGP-guided therapies (only 8%) and explores the reasons behind such discrepancies across tumor types. Furthermore, the authors highlight important findings around tumor mutational burden (TMB), suggesting a revised threshold (≥ 20 mutations/Mb) may better predict the benefit of immune checkpoint inhibitors. These real-world findings have important implications for refining biomarker-driven therapy.

The dataset, derived from the Center for Cancer Genomics and Advanced Therapeutics (C-CAT), significantly contributes to the global research community. It complements previously published experiences, such as the MSK-IMPACT cohort (PMID: 28481359), which used paired tumor-normal sequencing in a single-institution setting. The comparison between a national registry and institutional datasets offers a unique opportunity to assess generalizability, capture rare cancers, and evaluate systemic implementation challenges. The figures are well done and provide a good summary of the data.

Comments:

1. Pre-CGP testing context: Many patients had received treatment before CGP. Can the authors explain what diagnostic or molecular testing was used before CGP? For example, were tumor-type specific panels (e.g., EGFR for lung, HER2 for breast) routinely used?

2. Data access and sharing: Given the high value of this dataset, it would be important to clarify the process for outside researchers to access C-CAT data. Are there plans to make a version of the dataset publicly accessible via platforms like cBioPortal or a comparable repository, as done with other large-scale datasets (e.g., MSK-IMPACT)?

3. Contextualization with prior CGP efforts: A brief comparison with earlier institutional CGP efforts—such as MSKCC's work—could further highlight the strengths of the current national approach and inform how a centralized registry enables broader insights into clinical implementation, biomarker variability, and real-world treatment patterns.

4. Could they address if the data, in part, could be included in C-Bioportal? This would provide a subset of the data to the research community. There may be restrictions preventing this, which would be understandable.

Overall, this is a well-written, highly valuable study representing a significant step forward in the real-world application of precision oncology. Minor additions and clarifications will significantly benefit clinicians, researchers, and health policymakers.

(Remarks on code availability)

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

thank you for providing a markedly improved and clearer manuscript. It now focuses on the key issues and leaves some of the distracting anecdotes to the supplementals. The new figures are better and the authors address the outcome as well as subset analyses adeptly.

Well done.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

the authors have addressed my comments

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)

The authors have fully addressed all of my queries, including clarification of pre-CGP testing practices, description of data access and sharing through C-CAT, and additional methodological details regarding concordance with non-CGP assays. They have also strengthened the manuscript by refining figures, expanding comparisons with other large datasets, and improving clarity around the prognostic and therapeutic impact of comprehensive genomic profiling.

Overall, I believe the revised manuscript is much improved and will provide an important data resource for the research community.

(Remarks on code availability)

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Response to referees

Reviewer #1 (Remarks to the Author):

This is a very interesting and important paper that systematically looks at the real-world experience captured in the Japanese data base. IT provides a clear sense of the evolution of the use and implications of CGP-guided decisions and provides some interesting examples (from rare to common issues in clinical oncology). The ability to look over several years is important and should be done in the future to best understand factors and utility. The authors should consider a few points:

We appreciate the reviewer for the time and expertise in reviewing the manuscript and are very pleased that the reviewer considered our paper to be very interesting and important. We have addressed each comment, as detailed in the point-by-point response below.

#1-1

The abstract might benefit from highlighting comparisons between years and other studies (see below) instead of starkly stating several observations of specific findings for rare tumors or situations. The value here is capacity to look systematically and thus the conclusions from the larger picture should dominate the abstract.

In response to the reviewer's suggestion, we have removed the sentences regarding *NTRK* fusions and added several sentences regarding (1) the differences across tumor types and temporal change in the proportion of cases receiving CGP-guided approved/experimental genomic biomarker-linked therapies, and (2) the strengths of the C-CAT database compared with clinical trials and prospective sequencing projects regarding CGP in **Abstract** as follows:

(Page 3, line 2)

Abstract

Comprehensive genomic profiling (CGP) is crucial in precision oncology, yet its real-world utility remains unclear. Through analyzing 54,185 advanced solid tumor cases receiving CGP with FoundationOne CDx from the Japanese C-CAT database, we demonstrate the prognostic value of CGP-guided clinical evidence level classification, with level A (16.6%) and B (8.1%) alterations associated with better prognosis. Only 8.0% of cases receive CGP-guided approved/experimental genomic biomarker-linked therapies, **although the proportion varies markedly across tumor types and has improved over time.** Tumor-agnostic biomarker analyses reveal that tumor mutation burden (TMB) ≥ 20 mutations/megabase predicts better outcome, regardless of microsatellite instability status, in TMB-high cases receiving pembrolizumab. Conversely, extramammary Paget disease is exceptionally resistant to pembrolizumab. Some cases with biomarkers negative by non-CGP diagnostics but positive by CGP respond to related therapies. **The large-scale nationwide database allows evaluating inter-tumor type differences and investigating evidence-scarce situations, delineating where CGP offers greater benefit. These findings**

complement those from clinical trials and prospective sequencing projects regarding CGP, providing valuable information for individualized treatment.

#1-2

The paper would benefit greatly from perhaps shortening the depictions of specific settings (restricting to a few examples in the text and/or boxes or supplemental docs) and instead provide analyses and discussion of how this experience differs from several of the large references provided. This is a key point and one that should be fully addressed.

We appreciate the reviewer's comment. As the reviewer suggested, we have shortened the description in the "Effectiveness of genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP" section in **Results** and moved the explanations regarding TMB-H, *NTRK* fusion, and discordant results between non-CGP CDx and CGP in **Discussion** to **Supplementary Discussion**. In addition, we have moved **Fig. 6** (*NTRK* fusions) to **Extended Data Fig. 7** and combined two main figures related to TMB-H (**Figs. 4 and 5**) into one figure (**Fig. 4**), although we have added some data and explanations regarding the effectiveness of targeted therapies for tumor type-specific genomic biomarkers in **Results** and **Extended Data Fig. 8** (in response to #2-3).

Instead, we have summarized clinical trials, large-scale clinical sequencing projects, and registry databases in precision oncology in **Supplementary Table 6** and have compared them with our study (also in response to #3-3). Specifically, large-scale clinical sequencing projects have demonstrated the value of CGP by showing that many patients harbor actionable alterations (refs. 1, 2, 8, 9 of the revised manuscript). Particularly, the initiatives at the Memorial Sloan Kettering Cancer Center laid the groundwork as a prospective clinical sequencing platform, clarifying the difference of clinical actionability across major tumor types and its temporal change using the OncoKB knowledge base (refs. 2, 8). The registry databases (e.g., AACR Project GENIE) have collected such real-world sequencing data on a larger scale, facilitating the discovery of rare alterations across diverse tumors and the characterization of alterations in rare tumors (refs. 3, 4, 37). Moreover, early non-randomized clinical trials, in which the definition and/or extent of matching were retrospectively determined, showed that patients receiving matched therapies (or CGP-guided genomic biomarker-linked therapies) achieve improved survival (refs. 15-18). On the other hand, several randomized controlled trials (RCT) in all solid tumors failed to reproduce these findings, while an RCT in HER2-negative metastatic breast cancer reported a positive result (refs. 19-21). Thus, there are differing views on whether CGP and related genomic biomarker-linked therapies improve the overall outcomes with advanced solid tumors. Meanwhile, multiple basket or platform trials (e.g., NCI-MATCH) recruiting hundreds to thousands of patients have yielded clinical evidence for the efficacy of biomarker-linked therapies within specific genomically defined subgroups, ultimately supporting the regulatory approval of tumor-agnostic indication (refs. 38-41).

In this context, the C-CAT database can bridge the knowledge gap not covered by previous studies. The nationwide coverage allows investigating a patient population that better reflects real-world

practice. The large sample size and detailed clinical information enables assessment of the efficacy of genomic biomarker-linked therapies targeting rare tumor types (such as ICIs for EMPD) and rare alterations (including *NTRK* fusions) as well as in contexts where evidence is limited, such as discordant results between non-CGP CDx and CGP. More importantly, through utilizing prospective annotation for genetic alterations, we demonstrate that CGP-guided clinical evidence level classification enables prognostic stratification in advanced solid tumors irrespective of the timing of CGP. Particularly, level A and B alterations can predict better outcomes, which are more prominent in NSCLC and pancreatobiliary cancers. Therefore, it is reasonable to assume that CGP-guided approved/experimental administration of genomic biomarker-linked therapies (potentially excluding off-label drug use) can more consistently improve clinical outcomes, although several alterations themselves affect prognosis. In addition, we leveraged adequate information on treatment details before and after CGP to evaluate whether to initiate CGP-guided approved/experimental genomic biomarker-linked therapies. We estimate that only a small proportion (8.0%) of cases benefit from CGP at present, despite clinically actionable alterations (with evidence levels A–D) being identified in 72.7% of cases. The large gap between the two proportions was compatible with those reported from prospective cohort studies conducted at selected institutions (refs. 8, 14, 17). Remarkably, our pan-cancer analysis identifies a significant difference in the proportion of cases benefitting from CGP testing across tumor types, which is greater than the difference in the proportion of cases harboring actionable alterations. Therefore, these findings provide more reliable estimates for determining where CGP provides the most clinical benefit at present.

Therefore, we have added these explanations in **Introduction**, **Discussion**, and **Supplementary Table 6** in the revised manuscript. The changes are as follows:

(Page 5, line 42)

Introduction

Large-scale clinical sequencing projects have demonstrated the utility of CGP by showing that a large proportion of patients harbor actionable alterations ^{1,2,8,9}. However, CGP has several drawbacks, such as long turn-around time, high cost, unavailability as CDx for many molecularly targeted therapies, and limitation of testing timing and hospitals capable of performing CGP ^{1,5}. Thus, although several guidelines recommend CGP early in the management of advanced solid tumors in multiple tumor types ^{10,11}, many patients undergo non-NGS-based CDx and related therapies before CGP ^{12,13}, resulting in a smaller proportion of patients receiving CGP-guided genomic biomarker-linked therapies. In addition, although the efficacy of specific genomic biomarker-linked therapies against **a subset of tumors with corresponding actionable alterations** has been demonstrated in clinical trials, **whether the availability of diverse genomic biomarker-linked therapies improves the clinical outcome of the overall patient population receiving CGP has remained controversial** ^{5,14-21}. Therefore, actual utility of NGS-based CGP and its difference among tumor types remains unknown in the real world.

(Page 21, line 309)

Effectiveness of genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP.

Three breast and one esophageal/stomach cancer cases with no or low HER2 expression by non-CGP CDx but positive for *ERBB2* amplification by CGP showed PR or CR to HER2-targeting antibody-drug conjugate (ADC, trastuzumab deruxtecan and trastuzumab emtansine) (**Table 1 and Supplementary Table 5**). Furthermore, breast cancer cases positive for *ERBB2* amplification only by CGP showed comparable responses to anti-HER2 antibody (trastuzumab and/or pertuzumab) to those positive by both tests (**Fig. 5b**). Similar results were obtained for EGFR inhibitors used for lung cancer (mostly NSCLC) cases that were negative for *EGFR* mutations by non-CGP CDx but positive by CGP (**Fig. 5c**). Taken together, genomic biomarker-linked therapies are worth administering even in cases having discordant results between non-CGP CDx and CGP (**Supplementary Discussion**).

(Page 22, line 320)

Discussion

Large-scale clinical sequencing projects have demonstrated the value of CGP by showing that many patients harbor actionable alterations (**Supplementary Table 6**)^{1,2,8,9}. Particularly, the initiatives at the Memorial Sloan Kettering Cancer Center laid the groundwork as a prospective clinical sequencing platform, clarifying the difference of clinical actionability across major tumor types and its temporal change using the OncoKB knowledge base^{2,8}. The registry databases (e.g., AACR Project GENIE) have collected such real-world sequencing data on a larger scale, facilitating the discovery of rare alterations across diverse tumors and the characterization of alterations in rare tumors^{3,4,37}. Moreover, early non-randomized clinical trials, in which the definition and/or extent of matching were retrospectively determined, showed that patients receiving matched therapies (or CGP-guided genomic biomarker-linked therapies) achieve improved survival¹⁵⁻¹⁸. On the other hand, several randomized controlled trials (RCT) in all solid tumors failed to reproduce these findings, while an RCT in HER2-negative metastatic breast cancer reported a positive result¹⁹⁻²¹. Thus, there are differing views on whether CGP and related genomic biomarker-linked therapies improve the overall outcomes with advanced solid tumors. Meanwhile, multiple basket or platform trials (e.g., NCI-MATCH) recruiting hundreds to thousands of patients have yielded clinical evidence for the efficacy of biomarker-linked therapies within specific genomically defined subgroups, ultimately supporting the regulatory approval of tumor-agnostic indication³⁸⁻⁴¹.

In this context, the C-CAT database can bridge the knowledge gap not covered by previous studies. The nationwide coverage allows investigating a patient population that better reflects real-world practice. The large sample size and detailed clinical information enables assessment of the efficacy of genomic biomarker-linked therapies targeting rare tumor types (such as ICIs for EMPD) and rare alterations (including *NTRK* fusions) as well as in contexts where evidence is limited, such as discordant results between non-CGP CDx and CGP. More importantly, through utilizing prospective annotation for genetic alterations, we demonstrate that CGP-guided clinical evidence level classification enables prognostic stratification in advanced solid tumors irrespective of the timing of CGP.

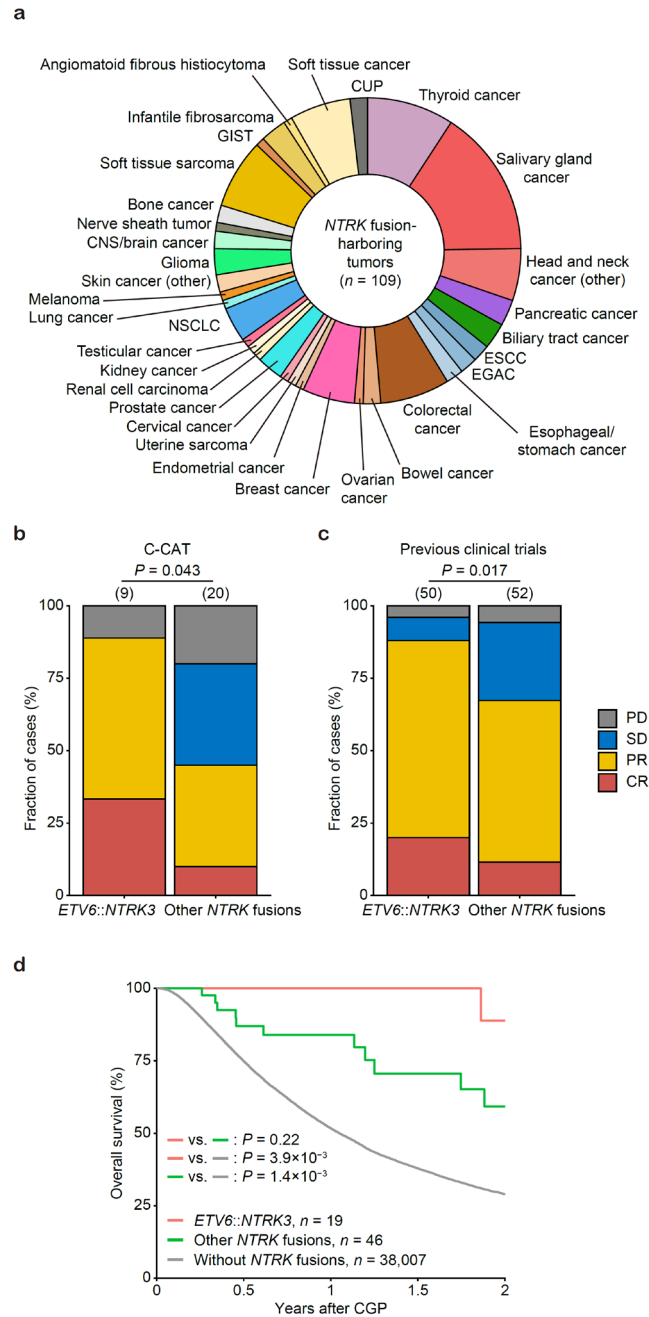
(Page 23, line 351)

Discussion

In addition, we leveraged adequate information on treatment details before and after CGP to evaluate whether to initiate CGP-guided approved/experimental genomic biomarker-linked therapies.

Supplementary Table 6: Summary of clinical trials, large-scale clinical sequencing projects, and registry databases in precision oncology.

Supplementary Table 6. Summary of clinical trials, large-scale clinical sequencing projects, and registry databases in precision oncology.								
Study type	Study name: registration number	Study design	Country	Tumor type	Study description and significance	Study results	Major publications	
Clinical sequencing project	MSK-IMPACT: NCT01775072	A prospective, single-center clinical sequencing project	USA	All solid tumors	The initiatives using MSK-IMPACT, the first CGP test authorized by FDA, laid the groundwork as a prospective clinical sequencing platform, clarifying the difference of clinical actionability across major tumor types and its temporal change using the OncoKB knowledge base. These initiatives have also contributed to cancer research, such as cancer genome, metastatic biology, germline pathogenicity, and clonal hematopoiesis. In addition, they have facilitated the application of artificial intelligence, such as natural language processing-based clinicogenomic data integration (Jee J., et al. <i>Nature</i> , 2024.).	Initially, data from 11,369 cases sequenced using MSK-IMPACT were reported. Of these, 11% of cases were enrolled in genomically matched clinical trials (Zehir A, et al. <i>Nat Med</i> . 2017.). Subsequent reports have included data on 47,211 samples (42,154 cases) detailing their clinical actionability over time. This study showed an increase from 8.9% to 31.6% in the fraction of tumors harboring an OncoKB level 1 or 2 biomarker between 2017 and 2022. Additionally, the fraction of tumors with a clinically actionable biomarker varied by tumor type, being highest in GIST and lowest in mesothelioma (Suehnholz SP, et al. <i>Cancer Discov</i> . 2024.). Key papers are summarized on the MSKCC website: https://www.mskcc.org/msk-impact .	Zehir A, et al. <i>Nat Med</i> . 2017. Coombs CC, et al. <i>Cell Stem Cell</i> . 2017. Srinivasan P, et al. <i>Nat Genet</i> . 2021. Nguyen B, et al. <i>Cell</i> . 2022. Suehnholz SP, et al. <i>Cancer Discov</i> . 2024. Jee J, et al. <i>Nature</i> . 2024.	
Clinical sequencing project	K-MASTER	A prospective, multi-center clinical sequencing project	South Korea	All solid tumors	K-MASTER, conducted in South Korea, performed CGP to identify targetable alterations and match them to appropriate clinical trials. The study provided a genomic overview of Korean patients, highlighting differences in molecular features and clinical actionability compared with Western populations.	A total of 4,028 cases were sequenced. Druggable alterations were identified in 28.7% of cases, with 440 cases enrolled into 20 different clinical trials. Comparison with TCGA revealed the differences of clinically actionable alterations based on ethnicity.	Park KH, et al. <i>Cancer Discov</i> . 2022.	
Clinical sequencing project	SCRUM-MONSTAR	A prospective, multi-center clinical sequencing project	Japan	All tumors (excluding lung cancer)	The SCRUM-Japan MONSTAR-SCREEN consortium is a large-scale molecular profiling project in Japan, employing artificial intelligence-driven multimomics analyses for cases with advanced malignancies. It is intricately linked to multiple clinical trials and observational studies, enabling enrollment in matched clinical trials.	More than 24,000 cases were enrolled, and 5.0% participated in matched clinical trials. Cases who received biomarker-linked therapies demonstrated significantly prolonged OS compared with those who did not (19.1 months versus 15.3 months).	Hashimoto T, et al. <i>Cancer Discov</i> . 2024.	
Registry database of clinical sequencing data	C-CAT	A registry database to collect clinical sequencing data	Japan	All solid tumors	C-CAT is a nationwide registry database in Japan, where almost all CGP tests conducted under the national insurance system have been registered. C-CAT includes not only genomic but also clinical information, including prognosis and treatment details. The large-scale database allows evaluating inter-tumor type differences and investigating evidence-scarce situations.	Data from 48,627 cases in C-CAT were analyzed to define the pan-cancer landscape of driver alterations and their clinical actionability in Japanese cases. Comparison with White populations revealed higher TP53 mutation frequencies in Asian cases across multiple tumor types (Horie S, et al. <i>Cancer Discov</i> . 2024.). Our subsequent analysis of 54,185 cases further demonstrates the prognostic value of CGP-guided evidence-level classification and identifies where CGP provides the greatest clinical benefit in real-world practice (this paper).	Kohno T, et al. <i>Cancer Discov</i> . 2022. Horie S, et al. <i>Cancer Discov</i> . 2024. This paper	
Registry database of clinical sequencing data	GENIE	A registry database to collect clinical sequencing data	USA and other countries	All tumors (including both solid and hematological malignancies)	AACR Project GENIE is an international, multi-institutional registry of clinical sequencing data from participating institutions. This is the largest genomic registry to date, enabling the assessment of prevalence of clinically actionable alterations across tumor types, including rare ones.	The latest release (version 17.0-public) includes more than 220,000 sequenced samples from over 190,000 cases. A list of AACR Project GENIE publications is available on the AACR website: https://www.aacr.org/professionals/research/aacr-project-genie/aacr-project-genie-publications/ .	AACR Project GENIE Consortium. <i>Cancer Discov</i> . 2017. Pugh TJ, et al. <i>Cancer Discov</i> . 2022.	
Registry database of clinical sequencing data	GENIE BPC	A registry database to collect clinical sequencing data	USA and Canada	Bladder, breast, colorectal, lung, pancreatic, and prostate cancers	GENIE Biopharma Collaborative (BPC) is an ongoing project that manually curates and annotates in-depth clinical data, including treatment information, with link with clinical sequencing data for a subset of cases from the GENIE project.	In phase 1, GENIE BPC integrated clinical data, including cancer treatments, with genomic data from nearly 8,000 cases across four institutions. Currently, datasets comprising 1,846 NSCLC cases and 1,485 colorectal cancer cases have been released.	Choudhury NJ, et al. <i>Clin Cancer Res</i> . 2023.	
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	MOSCATO-01: NCT01566019	A prospective, non-randomized single-center clinical trial	France	All solid tumors	The MOSCATO-01 trial evaluated whether clinical sequencing could identify actionable alterations and improve clinical outcomes when genomic biomarker-linked therapies were administered. This study was one of the earliest studies demonstrating that genomic biomarker-linked therapy could improve outcomes for a subset of cases using the "PFS2/PFS1 ratio".	A total of 1,035 cases were included, and 199 cases were treated with genomic biomarker-linked therapies. The PFS2/PFS1 ratio, defined as the PFS on biomarker-linked therapy divided by the PFS on the immediately preceding therapy, was greater than 1.3 in 33% of cases.	Massard C, et al. <i>Cancer Discov</i> . 2017.	
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	IMPACT: NCT00851032	A prospective, non-randomized single-center clinical trial	USA	All solid tumors	The IMPACT trial evaluated the clinical benefit of genomic biomarker-linked therapies in cases referred for phase I clinical trials. The program progressively expanded in scale, from an initial validation cohort to a retrospective 10-year follow-up study. This study retrospectively defined "matched therapies" and demonstrated that matched therapies improve response and survival compared with unmatched therapies.	In a retrospective analysis of 3,487 cases who underwent tumor molecular profiling, 711 received genomic biomarker-linked therapies (matched therapies), while 586 received unmatched therapies. The objective response rates were 16.4% and 5.4% for the matched and unmatched groups, respectively. The median PFS was 4.0 months versus 2.8 months, and OS was 9.3 months versus 7.3 months, which were both significantly different (Tsimberidou AM, et al. <i>J. Hematol. Oncol</i> . 2019.).	Tsimberidou AM, et al. <i>Clin Cancer Res</i> . 2014. Tsimberidou AM, et al. <i>JCO Precis Oncol</i> . 2017. Tsimberidou AM, et al. <i>J. Hematol. Oncol</i> . 2019.	
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	PROFILER: NCT01774409	A prospective, non-randomized multi-center clinical trial	France	All tumors (including both solid and hematological malignancies)	The PROFILER trial was designed to assess the feasibility and clinical impact of tumor molecular profiling. The study enrolled both adult and pediatric cases with solid or hematologic malignancies. In this study, sequencing results were reviewed by a multidisciplinary MTB, which recommended biomarker-linked therapies when appropriate. The study demonstrated a low initiation rate and response rate for genomic biomarker-linked therapies, underscoring the challenges in translating genomic findings into effective personalized treatments for most cases.	A total of 2,579 cases were enrolled, and the MTB reviewed the molecular profiles achieved for 1,980 cases (76.8%). Actionable alterations were identified in 1,032 cases (40%), and genomic biomarker-linked therapies were proposed in 699 (27%). However, only 163 cases (6%) received such therapies, with 23 partial responses (13%) observed, corresponding to just 0.9% of the total cohort.	Trédan O, et al. <i>Ann Oncol</i> . 2019.	
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	CoPPO: NCT02290522	A prospective, non-randomized, single-center clinical trial	Denmark	All solid tumors	The CoPPO trial evaluated the clinical benefit of tumor molecular profiling for treatment selection in a phase I setting. Cases underwent whole-genome or whole-exome sequencing, RNA sequencing, and SNP array analysis. Results were reviewed by an MTB, which recommended biomarker-linked therapies when appropriate. This study retrospectively classified targets according to the ESCAT framework and demonstrated that genomic profiling is efficient in identifying actionable targets and matching cases to targeted therapies.	A total of 2,147 cases were included, and genomic profiles were obtained in 1,868 cases. Actionable alterations were identified in 1,062 cases (57%), including 559 cases with high RNA CEACAM5 expression, and 256 received biomarker-linked therapies. ESCAT III was associated with significantly improved PFS and OS compared to ESCAT II/IV (PFS: 5.02 months versus 2.26 months, OS: 10.49 months versus 6.66 months) (Belcald, et al. <i>Ann Oncol</i> . 2025.).	Tuxen N, et al. <i>Clin Cancer Res</i> . 2019. Belcald, et al. <i>Ann Oncol</i> . 2025.	
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	i-PREDICT: NCT02534675	A prospective, non-randomized, multi-center clinical trial	USA	All tumors (mostly solid tumors)	i-PREDICT was a trial designed to evaluate whether combination genomic biomarker-linked therapies could improve outcomes in cases with refractory malignancies. The study retrospectively evaluated a "matching score," calculated by dividing the number of alterations targeted by the administered therapies by the total number of characterized alterations in each case's tumor, and demonstrated that a high matching score was associated with better prognosis, indicating the potential clinical benefit of combination therapies in precision oncology.	Among 149 enrolled cases, 83 were treated, of which 73 received genomic biomarker-linked therapies. The disease control rate was 50% in cases with a high matching score (>50%) compared to 22.4% in those with a low score (<=50%). PFS was longer in the high-score group (6.5 months versus 3.1 months), and OS was also significantly different (not reached versus 10.2 months).	Sicklick JK, et al. <i>Nat Med</i> . 2019.	
Randomized controlled trial of CGP and related genomic biomarker-linked therapies	SAFIR02-BREAST: NCT02999999	A phase II, prospective, randomized, multi-center clinical trial	France	HER2-negative breast cancer	The SAFIR02-BREAST trial evaluated whether genomic biomarker-linked therapy, guided by ESCAT, improves outcomes compared with standard maintenance therapy. Subsequent benefit from biomarker-linked therapy was observed only when treatments were matched to genomic alterations with proven drug sensitivity (ESCAT level IV), emphasizing the need for evidence-based precision oncology.	A total of 1,462 cases were included, and 238 cases were randomized to targeted therapy and maintenance chemotherapy. In cases with ESCAT III genomic alterations (n = 115), cases receiving genomic biomarker-linked therapies had significantly longer PFS than those receiving maintenance chemotherapy (9.1 months versus 2.8 months). No benefit was observed when alterations were unselected or of lower actionability.	Andre F, et al. <i>Nature</i> . 2022.	
Randomized controlled trial of CGP and related genomic biomarker-linked therapies	SHIVA: NCT01771458	A phase II, prospective, randomized, multi-center clinical trial	France	All solid tumors	The SHIVA trial was one of the earliest randomized controlled trials to evaluate the prognosis of biomarker-linked therapies compared to physician's choice treatment. Although this study found no significant improvement in PFS, it is a landmark study demonstrating the limitations of broad, unselected precision oncology approaches.	A total of 741 cases were analyzed, and 195 were randomly assigned to treatment. The median PFS in cases assigned to biomarker-linked therapy was 2.3 months, which was not significantly different from that of cases receiving physician's choice treatment (median, 2.0 months) (Le Tourneau C, et al. <i>Lancet Oncol</i> . 2015.). In the crossover analysis, the PFS ratio, defined as PFS on biomarker-linked therapy divided by PFS on physician's choice treatment, exceeded 1.3 in 37% of cases who crossed over from physician's choice treatment to biomarker-linked therapy (Belin L, et al. <i>Ann Oncol</i> . 2017.).	Le Tourneau C, et al. <i>Lancet Oncol</i> . 2015. Belin L, et al. <i>Ann Oncol</i> . 2017.	
Randomized controlled trial of CGP and related genomic biomarker-linked therapies	IMPACT2: NCT02152254	A phase II, prospective, randomized, single-center clinical trial	USA	All solid tumors	IMPACT2 was a randomized trial designed to evaluate whether genomic biomarker-matched therapies improve PFS compared with non-targeted therapies to address the limitations of the IMPACT trial. Due to challenges in randomization, the protocol was amended to include a patient-preference cohort. This study adopted an adaptive design to allow the inclusion of additional molecular alterations and treatments as they became available. The trial found no significant difference in response, PFS, or OS between the two arms, underscoring the challenges of randomizing cases in this setting.	Among the 829 cases enrolled, 109 were randomized or selected their treatment arms, while 297 were treated without consideration for randomization. The PFS for cases receiving genomic biomarker-linked therapies —whether randomized or not— was 4.41 months, which was not significantly different from that of those receiving a physician's choice treatment (3.75 months). Similarly, there was no significant difference in OS (9.44 months versus 9.21 months).	Vo HH, et al. <i>NPJ Precis Oncol</i> . 2022. Tsimberidou AM, et al. <i>Ann Oncol</i> . 2024. (abstract presented at the ESMO Congress 2024)	
Platform trial of genomic biomarker-linked therapies	NCI-MATCH: NCT02460560	A phase II, prospective, non-randomized, multi-center, platform clinical trial	USA	All solid tumors (lymphoma, and myeloma)	The NCI-MATCH trial was a large-scale, nationwide, signal-seeking precision medicine platform trial initiated by the NCI. The trial demonstrated the feasibility of large-scale genomic screening and treatment allocation based on actionable alterations. It provided important insights into the challenges of single-agent targeted therapies, which influenced the design of successor trials evaluating combination therapies, such as comboMATCH. One subprotocol (Arm H) contributed to the FDA approval of dabrafenib and trametinib for the treatment of unresectable or metastatic solid tumors harboring BRAF V600E mutations.	The trial screened 5,954 cases. Actionable alterations were identified in 37.6% of cases, and 17.8% were assigned to one of the 38 subprotocols evaluating genomic biomarker-linked therapy, whether FDA-approved or investigational (Faherty KT, et al. <i>J Clin Oncol</i> . 2020.). Among the initial 27 subprotocols, 7 were positive (25.9%) (O'Dwyer PJ, et al. <i>Nat Med</i> . 2023.). A list of NCI-MATCH publications is available on the ECOG-ACRIN Cancer Research Group website: https://ecog-acrin.org/clinical-trials/early131-nci-match-sequence-medicines/match-early131-findings/ .	Faherty KT, et al. <i>J Clin Oncol</i> . 2020. O'Dwyer PJ, et al. <i>Nat Med</i> . 2023. Salama AKS, et al. <i>J Clin Oncol</i> . 2020.	
Basket trial of genomic biomarker-linked therapies	TAPUR: NCT02693535	A phase II, prospective, non-randomized, multi-center, multi-basket clinical trial	USA	All solid tumors (lymphoma, and myeloma)	The TAPUR study was a large-scale, multiple basket trial initiated by ASCO, which evaluated signals of antitumor activity of FDA-approved genomic biomarker-linked therapies outside their approved indications in a pragmatic design. This study emphasized real-world implementation and broad access to genomic biomarker-linked therapies.	As of the 2018 report, more than 1,000 cases had been registered, and over 800 patients had received biomarker-linked therapies (Mangat PK, et al. <i>JCO Precis Oncol</i> . 2018.). According to the 2025 report, the number of registered cases has exceeded 3,400 (Mangat PK, et al. <i>NPJ Precis Oncol</i> . 2025.). The results of each subprotocol are summarized on the ASCO website: https://www.asco.org/research-data/tapur-study/study-results .	Mangat PK, et al. <i>JCO Precis Oncol</i> . 2018. Mangat PK, et al. <i>NPJ Precis Oncol</i> . 2025.	
Basket trial of genomic biomarker-linked therapies	MyPathway: NCT02091141	A phase II, prospective, non-randomized multi-center, multi-basket clinical trial	USA	All solid tumors	MyPathway was a multiple basket study designed to evaluate the efficacy and safety of FDA-approved genomic biomarker-linked therapies outside their approved indications, similar to the TAPUR study. This study also helped establish the feasibility and clinical value of basket trials in precision oncology.	A total of 251 cases with 35 different tumor types received study treatment, and 23% had objective responses. Tumor-pathway cohorts with notable objective response rates included HER2 amplified/overexpressing colorectal cancer and BRAF V600-mutated NSCLC.	Hainsworth JD, et al. <i>J Clin Oncol</i> . 2018.	
OS, overall survival; PFS, progression free survival; MTB, molecular tumor board; ESMO, European Society for Medical Oncology; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; NCI, National Cancer Institute; MSKCC, Memorial Sloan Kettering Cancer Center; GIST, gastrointestinal stromal tumor; AACR, American Association for Cancer Research; FDA, U.S. Food and Drug Administration; NSCLC, non-small cell lung cancer; ASCO, American Society of Clinical Oncology; CGP, comprehensive genomic profiling; TCGA, The Cancer Genome Atlas.								



Extended data Fig. 7: Response to TRK inhibitors and prognostic impact of *NTRK* fusions.
(Fig. 6 of the original manuscript)

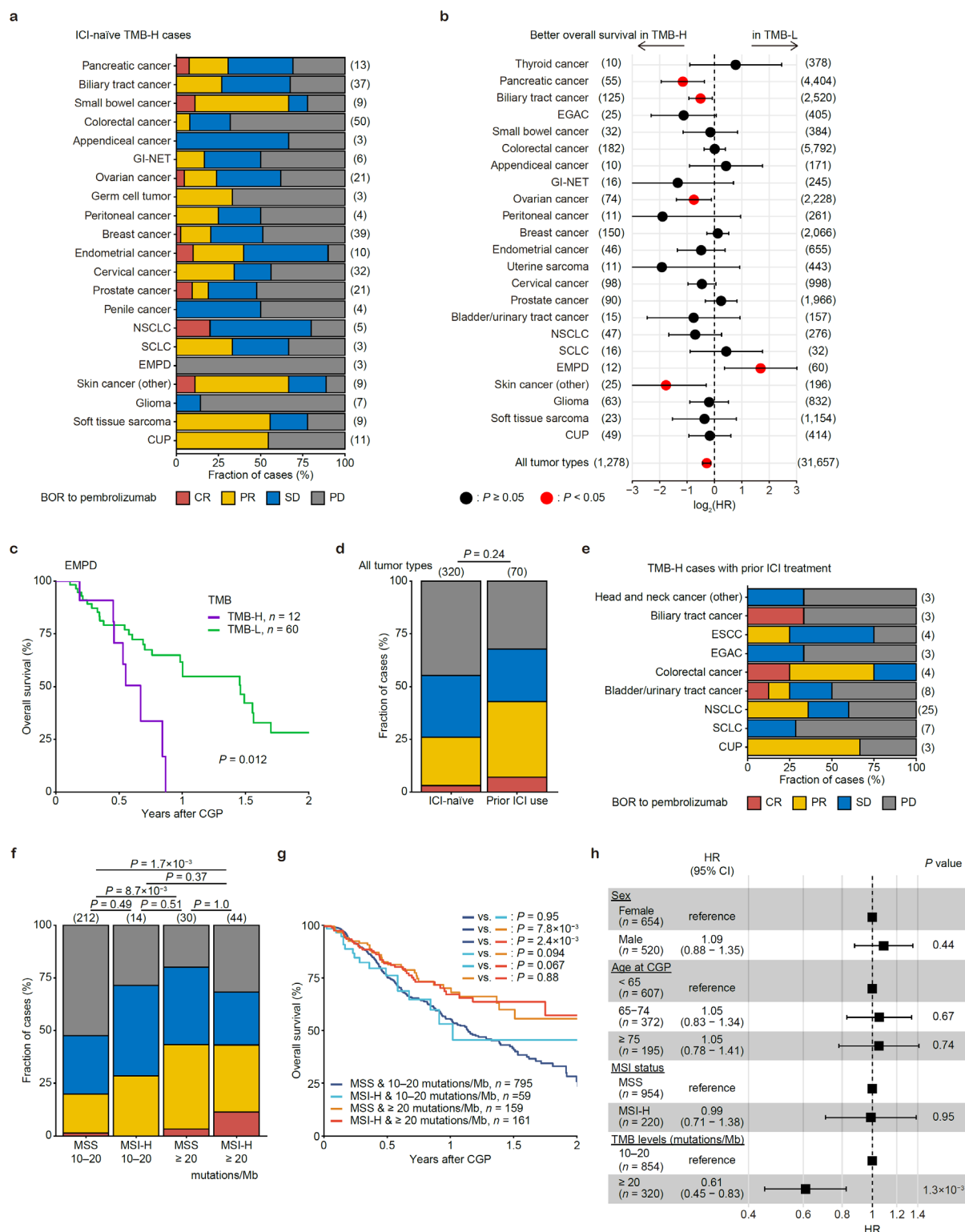


Fig. 4: Impact of tumor type and TMB levels on clinical outcome in TMB-H cases.

(Figs. 4 and 5 of the original manuscript)

#1-3

Is it possible to retrieve outcome data (either disease status or death) for the earlier cases. One of the key issues is whether the use - presumably sooner of NGS guided therapy really does have an impact on outcome in the real-world setting. An attempt to address this would be a major addition to this fine work.

As the reviewer suggested, we analyzed the survival data of patients who received CGP early in their clinical course. We found that patients who had received no or one treatment regimen before CGP showed a better prognosis than those who had received two or more regimens (**Extended Data Fig. 2b** in the revised manuscript), which may be due to lead time bias. However, it is important to note that the impact of level A (approved) alterations compared to level C–E alterations was comparable irrespective of the number of prior regimens (**Fig. 1c** in the revised manuscript). Similar results were obtained for level A (unapproved) alterations. These results clearly demonstrate the clinical utility of CGP regardless of the timing of CGP. Therefore, we have added these data and explanations in **Results, Discussion, Methods, Fig. 1**, and **Extended data Figs. 1 and 2** in the revised manuscript as follows.

(Page 9, line 104)

Prognostic impact of CGP-guided clinical evidence level classification.

Although patients who had received no or one treatment regimen before CGP showed a better prognosis than those who had received two or more regimens, the impact of level A alterations was comparable irrespective of the number of prior regimens (**Fig. 1c** and **Extended Data Fig. 2b**).

(Page 23, line 343)

Discussion

More importantly, through utilizing prospective annotation for genetic alterations, we demonstrate that CGP-guided clinical evidence level classification enables prognostic stratification in advanced solid tumors irrespective of the timing of CGP.

(Page 27, line 416)

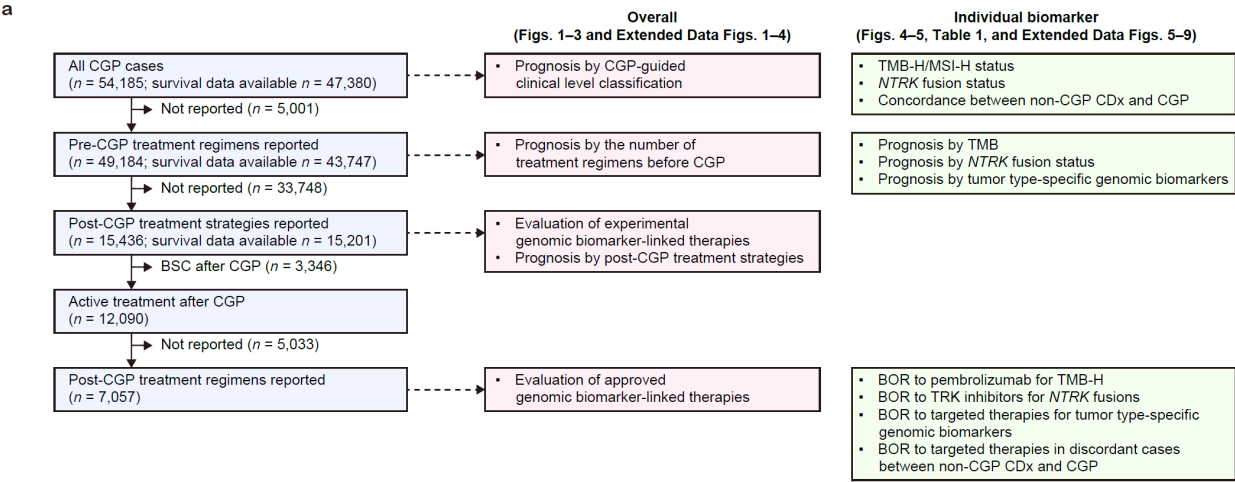
Evaluation of pre-CGP treatment regimens

The number of treatment regimens before CGP was counted unless they were used in the perioperative period.

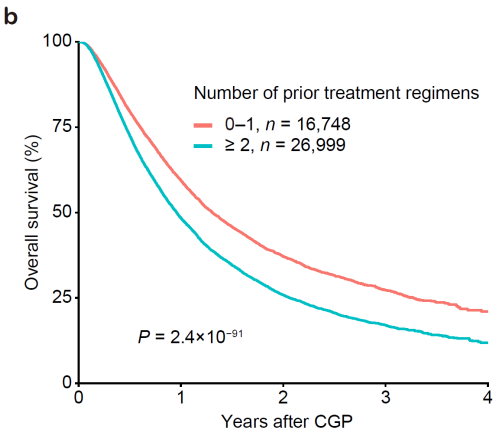


Fig. 1c: Log-transformed HRs with 95% confidence interval (CI) for overall survival, comparing cases

with level A (approved) (left) or level A (unapproved) (right) alterations to those with level C–E alterations, stratified by the number of treatment regimens before CGP. Numbers in parentheses indicate the number of evaluated cases with level A (approved) or level A (unapproved) (left) and level C–E (right) alterations, respectively.



Extended Data Fig. 1a: Flow chart of case selection (left) and corresponding subsets for overall (center; **Figs. 1–3** and **Extended Data Figs. 1–4**) and individual biomarker analyses (right; **Figs. 4–5**, **Table 1**, and **Extended Data Figs. 5–9**).



Extended Data Fig. 2b: Kaplan-Meier survival curves for overall survival in 43,747 cases with pre-CGP treatment regimen and survival data available, stratified by the number of treatment regimens before CGP.

#1-4

Minor issue: in the provided manuscript, the text from lines 274-277 was not clear and perhaps should be rephrased.

To address the reviewer's concern, we have added the details of non-CGP CDx assays for genetic alterations which have been available in Japan (**Supplementary Table 2**). In addition, to make it clearer, we have modified sentences in **Results** in the revised manuscript as follows:

(Page 20, line 296)

Effectiveness of genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP.

As non-CGP CDx assays are available for several genetic alterations (**Supplementary Table 2**), we evaluated the concordance between non-CGP CDx and CGP results. Among cases positive for either or both tests, 80.1% of cases showed concordant positive results, whereas only non-CGP CDx and only CGP were positive for 8.6% and 11.2% of cases, respectively (**Fig. 5a**), suggesting the presence of cases showing discordant results between them.

(Page 21, line 304)

Effectiveness of genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP.

Then, we investigated the efficacy of genomic biomarker-linked therapies in cases with discordant results.

Supplementary Table 2 (only added columns are shown).

Alteration (tumor type)	Tumor type	Non-CGP CDx assays approved in Japan [†]
TMB-H	All solid tumor	N/A
MSI-H	All solid tumor [‡]	PCR
<i>BRCA1/2</i> mutation/loss	Ovarian cancer	PCR (for germline variant), NGS
<i>ERBB2</i> amplification	Breast cancer	IHC, ISH
<i>ERBB2</i> amplification	EGAC	IHC, ISH
<i>BRAF</i> V600E mutation	Colorectal cancer	PCR
Mutations/loss in MMR genes [§]	All solid tumor	IHC
<i>BRCA1/2</i> mutation/loss	Prostate cancer	PCR (for germline variant)
<i>EGFR</i> mutation	NSCLC	PCR, NGS
<i>ERBB2</i> amplification	Colorectal cancer	IHC, ISH
<i>KIT</i> mutation	GIST	PCR
<i>BRAF</i> V600 mutation	Melanoma	PCR
<i>NTRK</i> fusion	All solid tumor	N/A
<i>FGFR2</i> fusion	Biliary tract cancer	N/A
<i>ERBB2</i> amplification	Salivary gland cancer	IHC, ISH
<i>MET</i> exon14 skipping mutation	NSCLC	PCR, NGS
<i>PIK3CA</i> mutation	Breast cancer	N/A
<i>ALK</i> fusion	NSCLC	IHC, ISH, PCR, NGS
MSI-H	Colorectal cancer	PCR
<i>EGFR</i> amplification [¶]	NSCLC	N/A
<i>ROS1</i> fusion	NSCLC	PCR, NGS
<i>ERBB2</i> amplification	NSCLC	N/A
<i>PTEN</i> mutation/loss	Breast cancer	N/A
<i>BRAF</i> V600E mutation	All solid tumor	PCR
<i>KRAS</i> G12C mutation	NSCLC	PCR, NGS
<i>BRAF</i> V600E mutation	Thyroid cancer	PCR, NGS
<i>RET</i> fusion	NSCLC	PCR, NGS
<i>ERBB2</i> mutation	NSCLC	NGS
<i>AKT1</i> E17K mutation	Breast cancer	N/A
<i>BRAF</i> V600E mutation	NSCLC	PCR, NGS
<i>KIT</i> amplification [¶]	Soft tissue sarcoma	N/A
<i>RET</i> fusion	Thyroid cancer	NGS
<i>MET</i> amplification	Renal cell carcinoma	N/A
Alterations with >= 10 cases are shown.		
TMB-H, high tumor mutation burden; MSI-H, high microsatellite instability; MMR, mismatch repair; EGAC, esophagogastric adenocarcinoma; NSCLC, non-small cell lung cancer; GIST, gastrointestinal stromal tumor; CGP, comprehensive genomic profiling; CDx, companion diagnostic; PCR, polymerase chain reaction; NGS, next-generation sequencing; IHC, immunohistochemistry; ISH, in-situ hybridization; N/A, not available.		
† Non-CGP CDx assays approved after the study period are not shown. NGS refers to NGS-based multiplex gene panels.		
‡ Colorectal cancer was excluded.		
§ MMR genes include <i>MSH2</i> , <i>MSH6</i> , <i>MLH1</i> , and <i>PMS2</i> .		
A PCR-based assay for <i>KIT</i> mutation detection in GIST is available in Japan; however, it is not strictly classified as a CDx, as mutation testing is not required for the administration of imatinib in GIST cases.		
¶ These alterations are currently not classified as evidence A (approved).		

Reviewer #1 (Remarks on code availability):

N/A

Reviewer #2 (Remarks to the Author):

In their manuscript, Saito et al describe the use and impact of comprehensive genomic profiling in patients with cancer using a large database.

We appreciate the reviewer for the time and expertise in reviewing the manuscript and for providing constructive comments. We have addressed each one, as detailed in the point-by-point response below.

#2-1

Overall, the results are quite disappointing because patients who present Level A alterations present only a modest improvement on their overall survival. The paper should better investigate the reasons for such a modest improvement.

We thank the reviewer for the valuable comment. We agree that cases with level A (approved) and level A (unapproved) alterations had significantly superior outcomes compared to cases with level C–E alterations in the entire cohort, but the effect sizes were moderate (hazard ratio [HR], 0.79; 95% confidence interval [CI], 0.75–0.83; $P = 2.6 \times 10^{-18}$ for level A [approved] and HR, 0.84; 95% CI, 0.79–0.90; $P = 1.1 \times 10^{-7}$ for level A [unapproved]) (**Fig. 1b**). The median overall survival (OS) was 16.4 (95%CI, 15.1–17.4), 14.5 (95%CI, 13.7–16.3), and 12.2 (95%CI, 11.9–12.4) months for cases with level A (approved), level A (unapproved), and level C–E alterations, respectively. Early non-randomized clinical trials, in which the definition and/or extent of matching were retrospectively determined, showed that patients receiving matched therapies (or CGP-guided genomic biomarker-linked therapies) achieve improved survival (refs. 15-18 of the revised manuscript) (**Supplementary Table 6**). On the other hand, several randomized controlled trials (RCT) in all solid tumors failed to reproduce these findings, while an RCT in HER2-negative metastatic breast cancer reported a positive result (refs. 19-21). Thus, there are differing views on whether CGP and related genomic biomarker-linked therapies improve the overall outcomes with advanced solid tumors. In addition, the observed differences in survival were modest, generally limited to a few months, even in studies showing the benefit of CGP (refs. 15-19). Thus, our findings are consistent with previous studies and are of value in that we demonstrated that CGP-guided clinical evidence level classification enables prognostic stratification.

One major reason for such a moderate improvement in OS should be attributed to substantial differences across tumor types. In fact, substantial improvements in OS were observed for cases with level A (approved) alterations in several cancer types, such as endometrial (HR, 0.47; 95%CI, 0.29–0.77) and biliary tract (HR, 0.67; 95%CI, 0.53–0.85) cancers as well as NSCLC (HR, 0.67; 95%CI, 0.55–0.81) (**Fig. 1d**). On the other hand, almost no improvement was observed in approximately one-third of the tumor types, which considerably attenuates the effect size in the entire cohort. We also investigated other contributing factors to such a moderate improvement in OS by performing subgroup analyses with respect to sex and age. However, we found that there were little differences in effect size among subgroups (**Reviewer-only Fig. 1**). Taken together, although these analyses were inherently limited by the lack of detailed clinical information, our findings suggest that inter-tumor type differences can account for a

moderate improvement in OS in the entire cohort. To clarify these points, we have added these explanations in **Introduction**, **Results** and **Discussion** and summarized the statistical metrics for each clinical evidence level across tumor types in **Supplementary Table 1** in the revised manuscript as follows:

(Page 5, line 42)

Introduction

Large-scale clinical sequencing projects have demonstrated the utility of CGP by showing that a large proportion of patients harbor actionable alterations^{1,2,8,9}. However, CGP has several drawbacks, such as long turn-around time, high cost, unavailability as CDx for many molecularly targeted therapies, and limitation of testing timing and hospitals capable of performing CGP^{1,5}. Thus, although several guidelines recommend CGP early in the management of advanced solid tumors in multiple tumor types^{10,11}, many patients undergo non-NGS-based CDx and related therapies before CGP^{12,13}, resulting in a smaller proportion of patients receiving CGP-guided genomic biomarker-linked therapies. In addition, although the efficacy of specific genomic biomarker-linked therapies against a subset of tumors with corresponding actionable alterations has been demonstrated in clinical trials, whether the availability of diverse genomic biomarker-linked therapies improves the clinical outcome of the overall patient population receiving CGP has remained controversial^{5,14-21}. Therefore, actual utility of NGS-based CGP and its difference among tumor types remains unknown in the real world.

(Page 9, line 97)

Prognostic impact of CGP-guided clinical evidence level classification.

Overall, cases with level A (approved) and level A (unapproved) alterations had significantly superior outcomes, albeit with a moderate effect size (hazard ratio [HR], 0.79 and 0.84), compared to cases with level C–E alterations (as a reference) (Fig. 1b). In addition, cases with level B alterations showed slightly better prognosis (HR, 0.93). On the other hand, cases with level C–E alterations showed almost the same and the worst clinical outcome. The median overall survival was 16.4, 14.5, and 12.2 months for cases with level A (approved), level A (unapproved), and level C–E alterations, respectively.

(Page 9, line 109)

Prognostic impact of CGP-guided clinical evidence level classification.

Cases with level A (approved) alterations had significantly better prognosis than cases with level C–E alterations in ten tumor types, with endometrial and biliary tract cancers as well as NSCLC being prominent (Fig. 1d and Supplementary Table 1).

(Page 22, line 320)

Discussion

Large-scale clinical sequencing projects have demonstrated the value of CGP by showing that many patients harbor actionable alterations (Supplementary Table 6)^{1,2,8,9}. Particularly, the initiatives at the Memorial Sloan Kettering Cancer Center laid the groundwork as a prospective clinical sequencing platform, clarifying the difference of clinical actionability across major tumor types and its temporal change

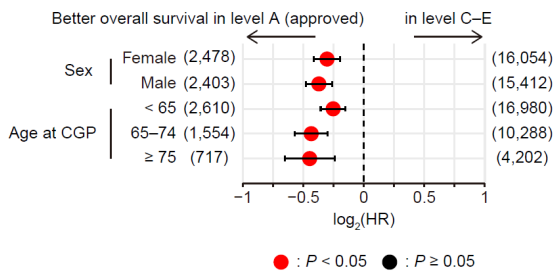
using the OncoKB knowledge base ^{2,8}. The registry databases (e.g., AACR Project GENIE) have collected such real-world sequencing data on a larger scale, facilitating the discovery of rare alterations across diverse tumors and the characterization of alterations in rare tumors ^{3,4,37}. Moreover, early non-randomized clinical trials, in which the definition and/or extent of matching were retrospectively determined, showed that patients receiving matched therapies (or CGP-guided genomic biomarker-linked therapies) achieve improved survival ¹⁵⁻¹⁸. On the other hand, several randomized controlled trials (RCT) in all solid tumors failed to reproduce these findings, while an RCT in HER2-negative metastatic breast cancer reported a positive result ¹⁹⁻²¹. Thus, there are differing views on whether CGP and related genomic biomarker-linked therapies improve the overall outcomes with advanced solid tumors. Meanwhile, multiple basket or platform trials (e.g., NCI-MATCH) recruiting hundreds to thousands of patients have yielded clinical evidence for the efficacy of biomarker-linked therapies within specific genomically defined subgroups, ultimately supporting the regulatory approval of tumor-agnostic indication ³⁸⁻⁴¹.

In this context, the C-CAT database can bridge the knowledge gap not covered by previous studies. The nationwide coverage allows investigating a patient population that better reflects real-world practice. The large sample size and detailed clinical information enables assessment of the efficacy of genomic biomarker-linked therapies targeting rare tumor types (such as ICIs for EMPD) and rare alterations (including *NTRK* fusions) as well as in contexts where evidence is limited, such as discordant results between non-CGP CDx and CGP. More importantly, through utilizing prospective annotation for genetic alterations, we demonstrate that CGP-guided clinical evidence level classification enables prognostic stratification in advanced solid tumors irrespective of the timing of CGP.

(Page 23, line 351)

Discussion

In addition, we leveraged adequate information on treatment details before and after CGP to evaluate whether to initiate CGP-guided approved/experimental genomic biomarker-linked therapies.



Reviewer-only Fig. 1: Log-transformed HRs with 95% CI for overall survival, comparing cases with level A (approved) alterations to those with level C–E alterations in subgroups stratified by sex or age category at CGP. Numbers in parentheses indicate the number of evaluated cases with level A (approved) (left) and level C–E (right) alterations, respectively.

Supplementary Table 1 (only added columns are shown).

Tumor type	OS of all cases (months)*			OS of cases with level A (approved) (months)*			OS of cases with level A (unapproved) (months)*			OS of cases with level B (months)*			OS of cases with level C-E (months)*			OS of cases with level A (approved) compared to those with level C-E			OS of cases with level A (unapproved) compared to those with level C-E			OS of cases with level B compared to those with level C-E						
	Median	95% CI (Lower)	95% CI (Upper)	Median	95% CI (Lower)	95% CI (Upper)	Median	95% CI (Lower)	95% CI (Upper)	Median	95% CI (Lower)	95% CI (Upper)	Median	95% CI (Lower)	95% CI (Upper)	HR	P value		HR	P value		HR	P value					
Thyroid tumor	32.8	23.4	N/A	N/A	8.8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	23.4	16.4	N/A	1.14	0.15	8.58	0.896	N/A	N/A	0.26	0.03	1.91	0.185			
Thyroid cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A			
Thyroid cancer	23.6	20.0	35.8	N/R	N/R	N/R	19.5	14.9	N/R	16.1	10.8	N/R	27.1	20.7	N/R	0.38	0.11	1.21	0.103	1.23	0.07	1.74	0.244	1.92	0.002			
Salivary gland cancer	33.7	30.2	N/R	26.7	21.0	N/R	N/R	N/R	N/R	N/R	15.4	N/R	33.7	21.6	N/R	0.70	0.38	1.31	0.266	0.00	0.00	0.94	0.994	0.52	0.37	3.77	0.518	
Parathyroid cancer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A		
Head and neck cancer (other)	15.9	13.7	17.0	9.0	7.4	N/R	4.7	3.8	N/R	7.4	N/R	13.9	12.8	15.8	0.94	0.55	1.61	0.811	2.20	0.98	4.95	0.055	1.05	0.47	2.35	0.914		
Pancreatic cancer	9.3	8.0	9.7	N/R	11.0	N/R	16.5	15.4	N/R	8.9	5.7	N/R	9.1	8.8	9.5	0.57	0.35	0.82	0.021	0.24	0.06	0.95	0.043	1.09	0.67	1.78	0.733	
Biliary tract cancer	10.0	9.6	10.6	13.6	11.0	N/R	17.1	11.8	19.6	13.5	8.8	N/R	9.4	9.0	10.0	0.67	0.53	0.85	0.001	0.65	0.51	0.83	0.001	0.74	0.53	1.05	0.093	
Undifferentiated embryonal sarcoma of the liver	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Malignant rhabdoid tumor of the liver	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Liver cancer	13.1	11.7	15.3	13.7	9.6	N/R	12.3	8.3	N/R	N/R	N/R	11.8	9.0	14.9	0.90	0.41	1.95	0.783	0.57	0.14	2.34	0.439	0.43	0.06	3.15	0.404		
ESCC	10.5	9.4	12.3	12.5	8.2	N/R	9.3	7.0	14.1	N/A	N/A	N/A	10.8	9.6	12.5	0.83	0.50	1.37	0.466	1.10	0.77	1.57	0.587	N/A	N/A	N/A	N/A	
EGAC	8.0	6.1	10.0	11.8	9.4	16.6	20.5	7.9	N/R	1.8	1.1	N/R	8.3	7.6	9.6	0.71	0.59	0.86	0.000	0.55	0.25	1.24	0.150	4.97	1.59	15.56	0.006	
Esophageal/stomach cancer (detail not reported/other)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Angiomyolipoma	11.0	8.9	13.1	N/R	5.9	N/R	2.5	2.5	N/R	9.8	4.6	N/R	11.2	8.9	13.2	0.77	0.11	5.57	0.798	5.14	0.68	39.06	0.114	1.67	0.52	5.37	0.387	
Small bowel cancer	15.5	13.0	18.2	12.6	10.1	N/R	N/R	6.0	20.1	8.7	7.1	N/R	15.5	13.0	18.2	1.10	0.57	2.12	0.774	0.61	0.15	2.50	0.463	1.16	0.54	2.49	0.713	
Colorectal cancer	13.4	12.9	14.0	12.1	11.0	14.7	11.5	6.3	20.1	13.5	12.2	14.7	13.6	13.0	14.3	1.19	1.02	1.39	0.029	1.23	0.89	1.72	0.215	1.04	0.94	1.16	0.465	
Appendiceal cancer	13.8	11.3	18.1	N/R	5.4	N/R	18.1	9.9	N/R	20.0	13.8	N/R	12.2	9.3	18.6	1.01	0.35	2.86	0.950	1.07	0.37	3.10	0.894	0.72	0.22	2.35	0.586	
Ana cancer	10.8	9.5	N/R	7.0	7.0	N/R	N/R	N/R	N/R	N/R	N/R	N/R	11.7	9.6	N/R	2.43	0.79	7.48	0.121	0.00	0.00	0.998	0.00	0.00	0.999	0.999		
GI-NET	17.2	12.8	25.9	N/R	6.7	N/R	3.3	N/R	11.5	3.9	N/R	12.6	9.9	17.5	0.57	0.14	2.34	0.431	0.44	0.14	1.30	0.160	1.02	0.25	4.17	0.978		
Bowel cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Ovarian cancer	11.4	10.7	12.4	15.4	11.6	18.0	13.9	10.9	N/R	9.5	6.7	N/R	10.9	10.2	11.8	0.81	0.67	0.97	0.025	0.70	0.33	1.48	0.347	1.00	0.68	1.47	0.999	
Germ cell tumor	N/A	11.4	N/R	N/A	4.5	N/R	N/A	N/A	N/A	N/A	N/A	N/A	11.7	10.0	N/R	0.68	0.14	3.29	0.633	N/A	N/A	N/A	N/A	N/A	0.00	0.00	N/A	
Sex cord stromal tumor	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Ovarian/fallopian tube cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Pancreatic cancer	13.7	11.6	18.0	18.5	N/R	N/R	16.9	11.9	N/R	3.2	N/R	N/R	13.2	10.3	17.0	0.55	0.14	2.25	0.409	0.87	0.46	1.56	0.637	10.33	1.39	76.86	0.023	
Breast cancer	12.8	12.0	13.9	16.8	14.2	21.7	12.0	10.5	13.9	10.5	8.5	12.8	11.8	10.4	13.5	0.77	0.65	0.91	0.002	0.98	0.85	1.12	0.780	1.17	1.00	1.37	0.050	
Breast sarcoma	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Endometrial cancer	14.3	12.9	16.1	N/R	N/R	N/R	2.2	0.9	N/R	N/A	N/A	14.2	N/R	13.5	11.9	15.0	0.47	0.29	0.77	0.003	17.04	5.38	53.96	0.001	0.68	0.43	1.00	0.088
Uterine sarcoma	16.0	12.6	18.5	N/R	5.9	N/R	N/R	8.6	8.1	N/R	N/A	14.3	11.9	18.3	0.91	0.37	2.23	0.843	0.00	0.00	0.00	0.993	1.05	0.33	3.30	0.936		
Uterine cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Gestational trophoblastic disease	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Cervical cancer	11.9	11.0	13.0	14.7	11.1	N/R	18.1	7.2	N/R	16.1	9.5	N/R	12.2	11.0	13.3	0.80	0.56	1.16	0.240	0.96	0.54	1.71	0.890	0.92	0.56	1.52	0.745	
Vaginal cancer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Vulvar carcinoma	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Vulvar/vaginal cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Prostate cancer	18.9	16.5	19.7	22.4	18.7	27.6	16.7	14.7	21.2	27.2	6.3	N/R	15.3	14.2	18.6	0.72	0.59	0.89	0.002	0.89	0.73	1.05	0.151	0.86	0.45	1.71	0.708	
Bladder/urinary tract cancer	14.5	11.8	16.6	8.0	7.7	N/R	16.4	12.3	27.8	N/R	N/R	N/R	14.6	11.5	17.9	1.15	0.76	1.74	0.519	0.79	0.57	1.10	0.162	0.73	0.30	1.76	0.479	
Renal cell carcinoma	15.3	12.8	N/R	N/R	N/R	N/A	N/A	N/A	N/A	15.3	12.8	N/R	11.2	7.7	N/R	0.43	0.13	1.42	0.158	N/A	N/A	N/A	N/A	N/A	0.69	0.43	1.10	0.122
Wilms tumor	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Rhabdoid cancer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Clear cell sarcoma of kidney	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Renal neuroendocrine tumor	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Kidney cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Testicular cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Penile cancer	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
NSCLC	13.1	11.9	14.5	18.7	15.4	24.3	13.0	10.4	26.3	13.7	11.4	20.0	10.0	9.9	11.5	0.67	0.55	0.81	0.000	0.74	0.58	0.96	0.023	0.80	0.65	0.99	0.041	
SCLC	8.1	6.8	11.9	6.8	5.3	N/R	14.0	N/R	4.4	N/R	N/A	8.5	7.1	13.8	1.47	0.84	2.58	0.191	0.83	0.26	2.89	0.761	1.73	0.23	13.12	0.586		
Lung cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Mesothelioma	14.8	11.0	19.3	N/R	N/R	N/R	N/A	N/A	N/R	N/R	N/R	N/R	12.5	8.2	17.4	0.00	0.00	0.00	0.997	N/A	N/A	N/A	N/A	N/A	0.00	0.00	0.998	
Pleural cancer (detail not reported)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Melanoma	14.6	13.3	18.6	15.6	11.1	N/R	22.5	13.8	N/R	9.2	6.5	N/R	14.3	11.3	19.1	0.83	0.55	1.24	0.364	0.55	0.25	1.18	0.126	1.45	0.59	2.14	0.059	
EMPD	17.5	12.0	20.4	8.0	5.5	N/R	10.4	N/R	N/R</																			

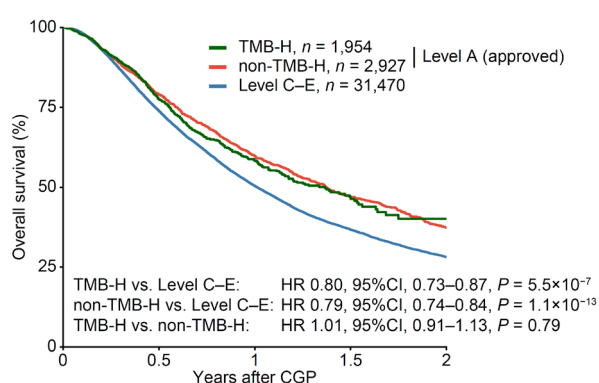
(Page 15, line 213)

Differences in the proportion of cases receiving CGP-guided genomic biomarker-linked therapies across tumor types.

Overall, 8.5% of cases were TMB-H (≥ 10 mutations/megabase [Mb]), most frequently observed in SCLC, NSCLC, and EMPD, accounting for 38.7% of cases with level A (approved) alterations (Extended Data Fig. 4a and 5a).

(Page 1, line 15 of Supplementary Discussion)

These observations suggest the potential complementarity of CGP-based TMB measurement and MSI testing in this setting.



Reviewer-only Fig. 2: Kaplan-Meier survival curves of overall survival for TMB-H cases with level A (approved) evidence, non-TMB-H cases with level A (approved) evidence, and for cases with level C-E evidence.

#2-3

A description of the outcome in other cohorts (ERBB2, BRCA, ALK etc) should be reported in the same way as patients with NTRK fusions

We appreciate the reviewer's comment. In the original manuscript, we performed prognostic analysis of TMB-H and *NTRK* fusions in cases without prior administration of the corresponding genomic biomarker-linked therapy (**Methods**). Unlike TMB-H and *NTRK* fusions, non-CGP CDx assays are routinely used before CGP for several biomarkers, such as *BRAF* mutation in bowel and skin cancers, *EGFR* mutation and *ALK* and *ROS1* fusions in lung cancer as well as HER2 expression and/or amplification in breast, head/neck, esophagus/stomach, and bowel cancers (**Supplementary Table 2**). For these biomarkers, most cases who were positive by CGP were also positive by non-CGP CDx and had already received the corresponding genomic biomarker-linked therapies (**Extended data Fig. 4a**). On the other hand, the majority of cases who were positive by CGP but had not received the corresponding genomic biomarker-linked therapies were negative by non-CGP CDx, thus considered to have discordant results between non-CGP CDx and CGP (**Reviewer-only Fig. 3**). Such cases with discordant results were described and discussed in detail in the "Effectiveness of genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP" section.

Therefore, here we focused on tumor type-specific level A genomic biomarkers, for which non-CGP CDx assays are not available, namely *BRCA1/2* mutation/loss in prostate cancer and *FGFR2* fusion in biliary tract cancer. We evaluated prognosis and response to the corresponding genomic biomarker-linked therapy for these biomarkers in a similar manner to *NTRK* fusion. We found that the targeted therapies for these alterations yielded a clinically meaningful ORR (**Extended data Fig. 8a**). In addition, cases harboring these alterations had better prognosis than those without (**Extended data Fig. 8b, c**). We have added these data and explanations in **Results, Methods, and Extended data Fig. 8** in the revised manuscript as follows:

(Page 20, line 287)

Differences in the response of TRK inhibitors to various *NTRK* fusions.

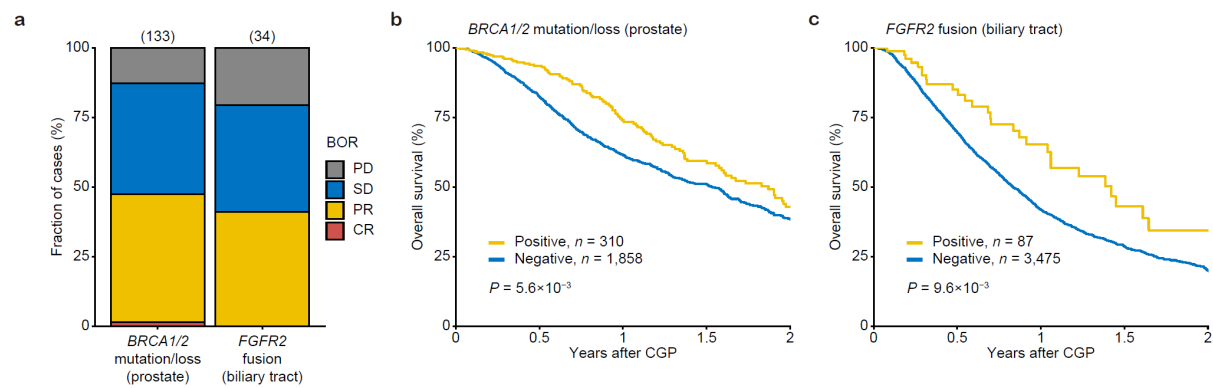
There are several tumor type-specific genomic biomarkers with level A evidence for which non-CGP CDx was not available, namely *BRCA1/2* mutation/loss in prostate cancer (targeted by PARP inhibitor [olaparib]) and *FGFR2* fusion in biliary tract cancer (by FGFR inhibitors [futibatinib or pemigatinib]). The targeted therapies for these alterations yielded a clinically meaningful ORR (**Extended data Fig. 8a**). Moreover, cases harboring these alterations had better prognosis than those without (**Extended data Fig. 8b, c**).

(Page 32, line 502)

Tumor type-specific genomic biomarkers with level A evidence

Response to targeted therapies and prognosis were assessed for tumor type-specific genomic biomarkers with level A evidence for which non-CGP CDx was not available. *BRCA1/2* mutation/loss in

prostate cancer was included, as non-CGP CDx detects only germline variants.

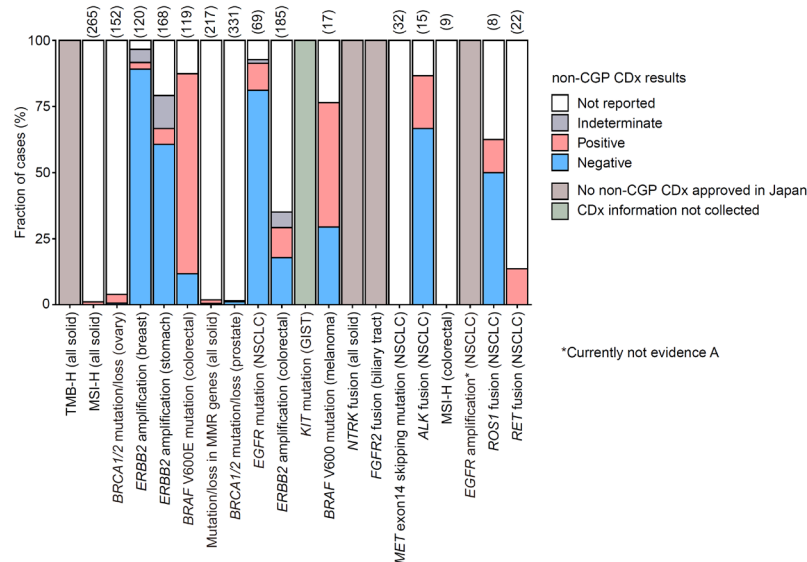


Extended Data Fig. 8: Response and prognostic impact of tumor type-specific genomic biomarkers with level A evidence.

a, BOR to PARP inhibitor (olaparib) in cases with *BRCA1/2* mutation/loss in prostate cancer and *FGFR2* inhibitors (futibatinib or pemigatinib) in cases with *FGFR2* fusion in biliary tract cancer.

b, Kaplan-Meier survival curves for overall survival with and without *BRCA1/2* mutation/loss in prostate cancer.

c, Kaplan-Meier survival curves for overall survival cases with and without *FGFR2* fusion in biliary tract cancer.



Reviewer-only Fig. 3: Results of non-CGP CDx in cases who were positive for the biomarker by CGP and had not received the corresponding genomic biomarker-linked therapies before CGP. Pink and blue colors indicate positive and negative (i.e., discordant) results by non-CGP CDx, respectively. HER2 low expression by IHC is categorized into negative for *ERBB2* amplification.

#2-4

There is a general criticism surrounding the use of CGP as "only approach" for precision medicine. The authors confirm this impression by reporting a high rate of false negative results for CGP as compared to standard testing (companion diagnostic). This observation would deserve a specific figure in the main document and a chapter of results.

We agree with the reviewer's comment. As the reviewer pointed out, non-CGP CDx and CGP are complementary to each other in cancer precision medicine. As suggested, we have moved **Extended Data Fig. 7a** (together with **Extended Data Fig. 7e, f**) of the original manuscript to **Fig. 5** of the revised manuscript. In fact, here we analyzed cases that were positive by either or both of non-CGP CDx and CGP and reported a positive concordance rate of 80.1% in the original manuscript. When including cases that are negative by both tests, an overall concordance rate is 96.3% (**Reviewer-only Fig. 4**). In addition, when cases positive for non-CGP CDx assays are considered as true positive, a false negative rate is 9.7%. Given the false negative rates of non-CGP CDx assays can reach 12-18% (PMID: 33272981, 34145315, 24878018, 38492039), this value is not considered to be high. In addition, as shown in **Table 1**, a considerable proportion of cases where genomic biomarkers are negative by non-CGP CDx but positive by CGP respond to molecularly targeted therapies, suggesting that such cases may have clinically relevant alterations. However, we accept that the manuscript could be improved by explaining these points more clearly. Therefore, although it is difficult to create a chapter for this observation in **Results** due to the limited space, we have added these explanations in **Results** and **Supplementary Discussion** in the revised manuscript as follows:

(Page 20, line 296)

Effectiveness of genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP.

As non-CGP CDx assays are available for several genetic alterations (**Supplementary Table 2**), we evaluated the concordance between non-CGP CDx and CGP results. Among cases positive for either or both tests, 80.1% of cases showed concordant positive results, whereas only non-CGP CDx and only CGP were positive for 8.6% and 11.2% of cases, respectively (**Fig. 5a**), suggesting the presence of cases showing discordant results between them.

(Page 21, line 304)

Effectiveness of genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP.

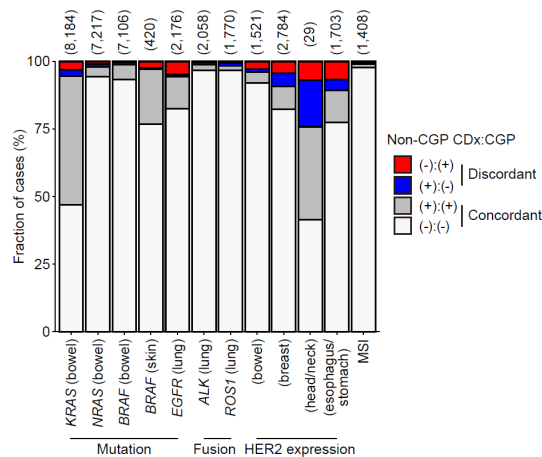
Then, we investigated the efficacy of genomic biomarker-linked therapies in cases with discordant results.

(Page 3, line 37 of Supplementary Discussion)

Genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP.

Although non-CGP CDx and CGP are complementary to each other in cancer precision medicine, a

clinically challenging and evidence-scarce situation is when **their** results are discordant.



Reviewer-only Fig. 4: Concordance rate between non-CGP CDx and CGP for each genetic alteration and primary organ/tissue. (+) and (-) indicate positive and negative results, respectively.

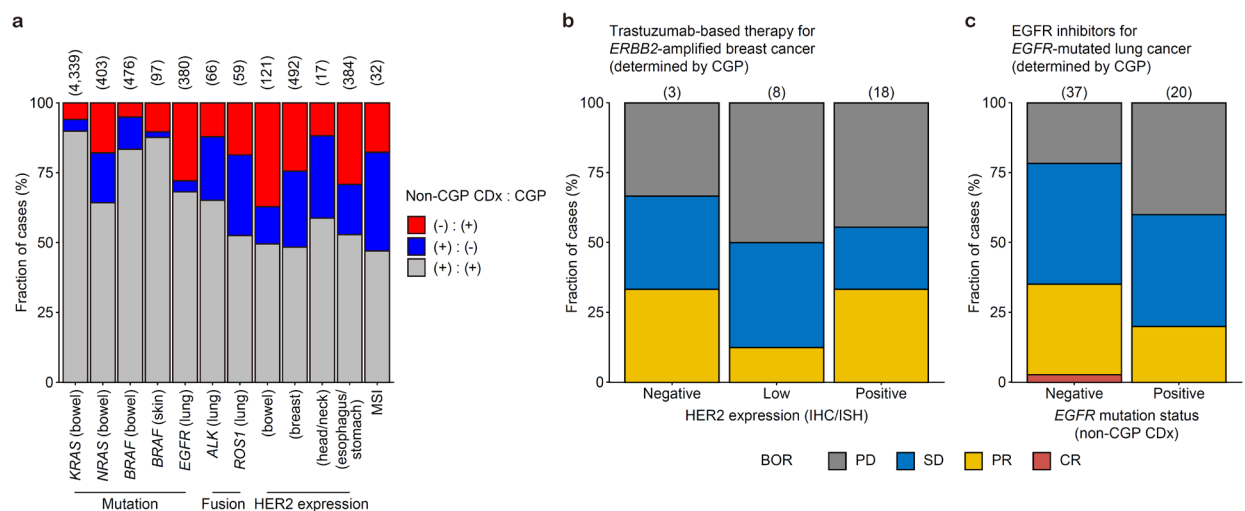
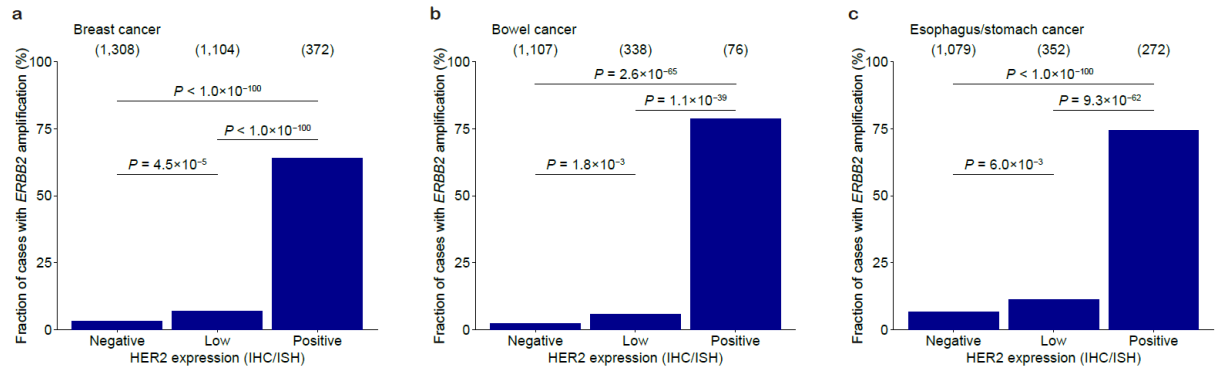


Fig. 5 (Extended data Fig. 7 in the original manuscript): Genomic biomarker-linked therapies in cases with discordant results between non-CGP CDx and CGP.



Extended data Fig. 9 (Extended data Fig. 7 in the original manuscript): Concordance between non-

CGP CDx (IHC/ISH) and CGP in cases with *ERBB2* amplification.

#2-5

Authors should better describe the use of off-label drugs since this is a major issue when CGP is used since it generates major costs without proven benefit.

In response to the reviewer's suggestion, we have provided a more detailed description of off-label drug use. Particularly, we clarified the proportion of cases who were enrolled in clinical trials and received off-label drugs across tumor types (**Extended Data Fig. 4b**). In addition, we found that clinical trial enrollment did not affect prognosis across tumor types, whereas off-label drug use was associated with significantly worse prognosis in several tumor types (**Extended Data Fig. 4c**). Therefore, off-label drug use warrants careful consideration, balancing scientific evidence with cost-effectiveness. We have added these explanations in **Results**, **Discussion**, and **Extended Data Fig. 4** in the revised manuscript as follows:

(Page 14, line 185)

Differences in the proportion of cases receiving CGP-guided genomic biomarker-linked therapies across tumor types.

The estimated proportion of cases with level A (unapproved) or B–D alterations who received unapproved or experimental genomic biomarker-linked therapies was high in thyroid cancer and melanoma (**Fig. 3b and Extended Data Fig. 4b**).

(Page 14, line 191)

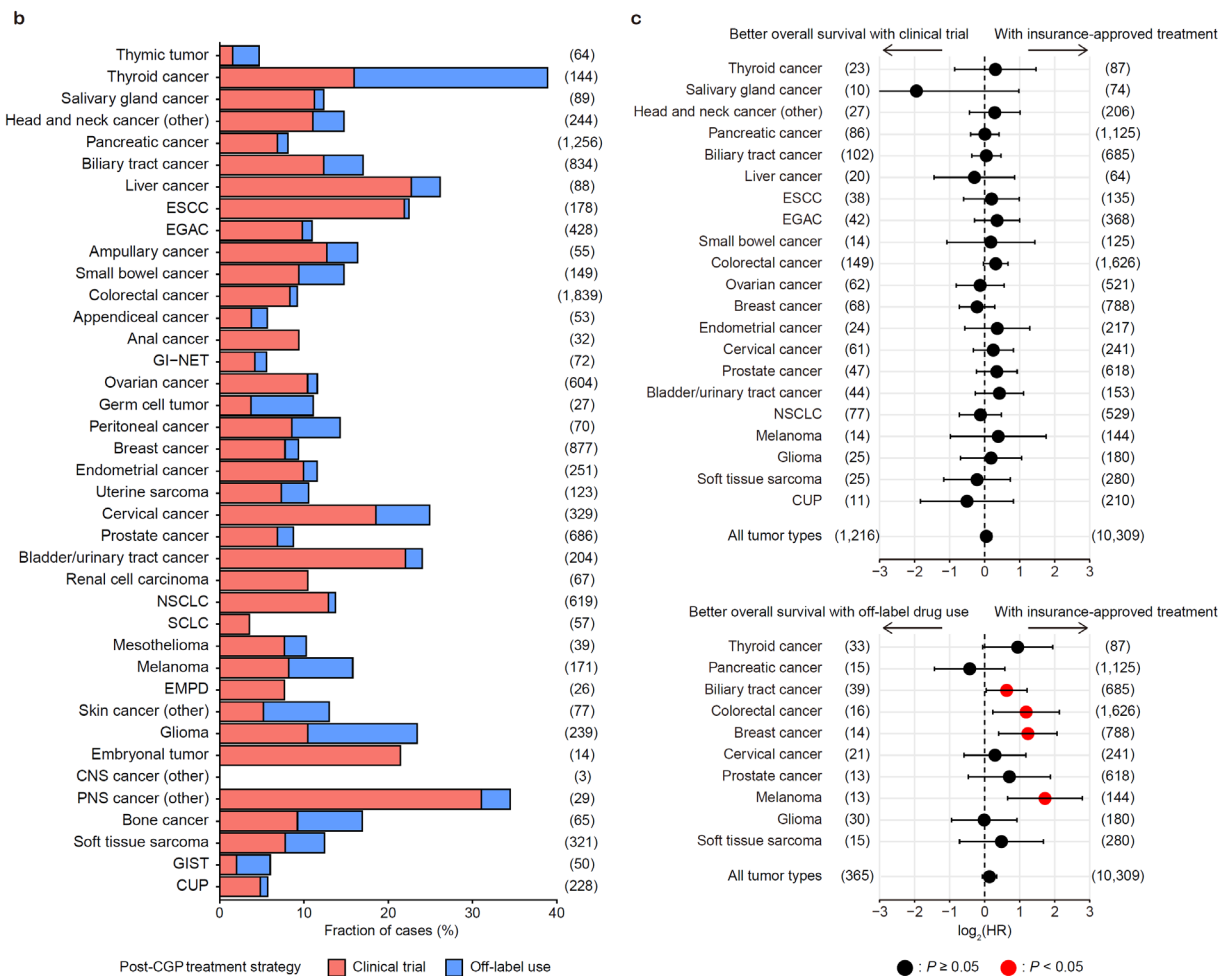
Differences in the proportion of cases receiving CGP-guided genomic biomarker-linked therapies across tumor types.

Of note, clinical trial enrollment did not affect prognosis in any tumor types, whereas off-label drug use was associated with worse prognosis in several tumor types (**Extended Data Fig. 4c**).

(Page 23, line 348)

Discussion

Therefore, it is reasonable to assume that CGP-guided **approved/experimental** administration of genomic biomarker-linked therapies (**potentially excluding off-label drug use**) can more consistently improve clinical outcomes, although several alterations themselves affect prognosis.



Extended Data Fig. 4.

b, Fraction of cases enrolled in clinical trials (red) and receiving off-label drugs (blue) in cases who received active treatment after CGP across 39 tumor types (with ≥ 100 cases).

c, Log-transformed HRs with 95% CI for overall survival, comparing cases enrolled in clinical trials (top) or receiving off-label drugs (bottom) to those receiving insurance-approved treatment across 21 tumor types (with ≥ 10 cases enrolled in clinical trials [top] or receiving off-label drugs [bottom]). Numbers in parentheses indicate the number of evaluated cases enrolled in clinical trials (top) or receiving off-label drugs (bottom) (left), and those receiving insurance-approved treatment (right).

Minor points:

#2-6

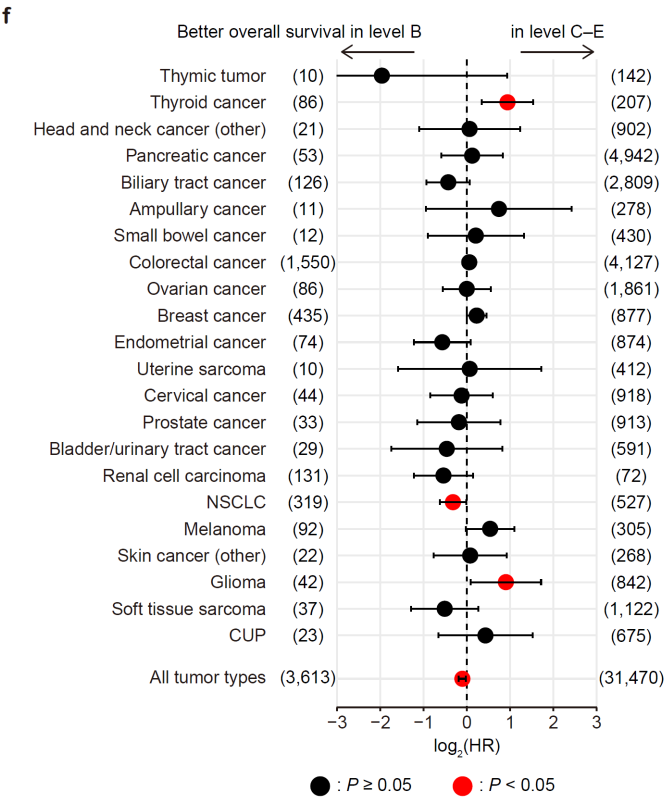
Why are level B not included in figure 1?

As the reviewer suggested, we investigated the differences in prognostic impact of level B alterations across tumor types and found that NSCLC cases with level B alterations also had better prognosis than those with level C–E alterations (**Extended Data Fig. 2f**). Therefore, we have added these explanations in **Results** and **Extended Data Fig. 2** in the revised manuscript as follows:

(Page 10, line 118)

Prognostic impact of CGP-guided clinical evidence level classification.

NSCLC cases with level B alterations also had better prognosis (**Extended Data Fig. 2f**).



Extended Data Fig. 2f: Log-transformed HRs with 95% CI for overall survival, comparing cases with level B alterations to those with level C–E alterations across 22 tumor types (with ≥ 10 cases having level B alterations). Numbers in parentheses indicate the number of evaluated cases with level B (left) and level C–E (right) alterations, respectively.

#2-7

How are responses assessed? It's written in the paper that authors use the data from hospital records. Nevertheless, not all hospitals and investigators assess RECIST in their daily practice. This point should be more precise in the method section.

We thank the reviewer for the valuable comment. To ensure data consistency, C-CAT has established a data submission manual for all participating institutions and physicians, which recommends using RECIST v1.1 when reporting best overall response. To clarify this point, we have added these explanations in **Methods** in the revised manuscript as follows:

(Page 28, line 435)

Evaluation of post-CGP treatment regimens and their responses

BOR to post-CGP treatment was reported by the treating physicians at each institution according to the response evaluation criteria in solid tumors (RECIST 1.1)⁴⁴, as recommended by the C-CAT data submission manual.

#2-8

HR should be multivariate

As the reviewer suggested, we performed multivariable survival analyses using Cox proportional hazards model adjusted for sex and age to calculate hazard ratios in **Fig. 1b–e, 4b, c, g, h, as well as Extended Data Fig. 2b–f, 3b, 4c, 5c, d, 6d, f–h, 7d, and 8b, c**. We found that almost all the statistically significant results observed in the original univariate analyses remained significant. Therefore, we have updated these Figs and Extended Data Figs and have modified sentences related to survival analysis in **Methods** in the revised manuscript as follows:

(Page 31, line 475)

Survival analysis

Overall survival was defined as the time from the date of CGP to the date of death or last follow-up. Survival analyses were performed using the R package survival (version 3.5-5). Multivariable analyses were performed using the Kaplan-Meier method with Cox proportional hazards model, adjusted for sex and age category (< 65, 65–74, and ≥ 75 years), unless otherwise specified. *P* values from multivariable Cox proportional hazards models were calculated using two-sided Wald test.

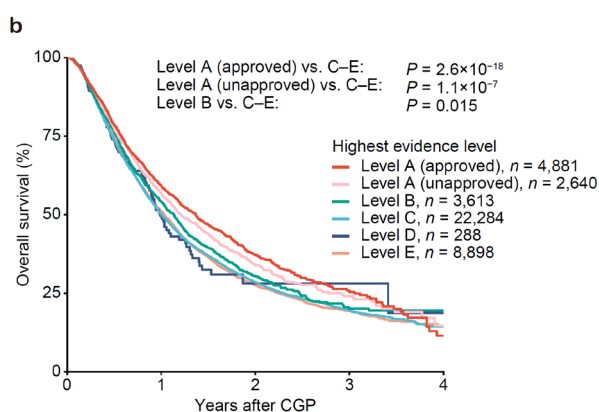


Fig. 1b

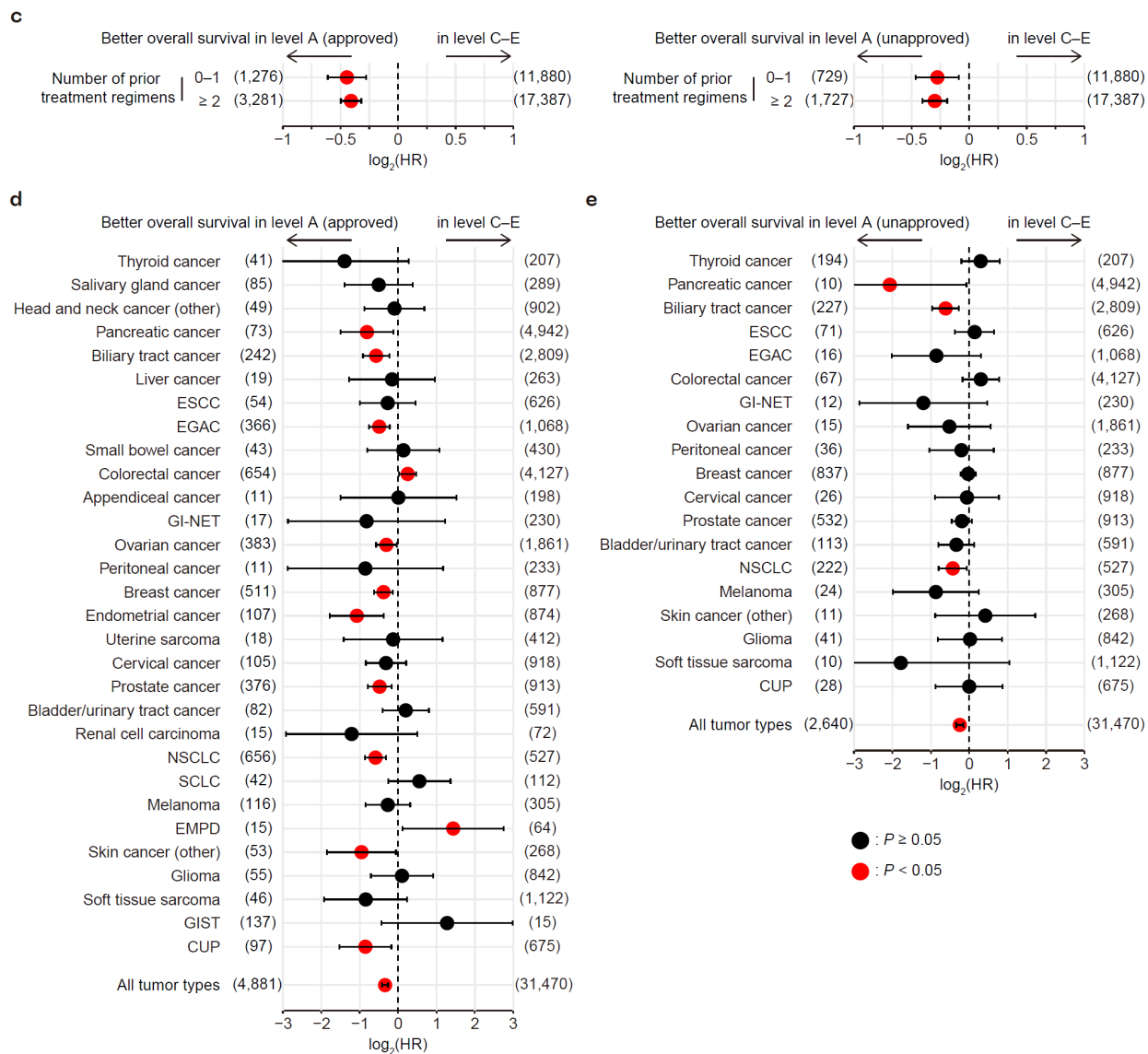


Fig. 1c-e

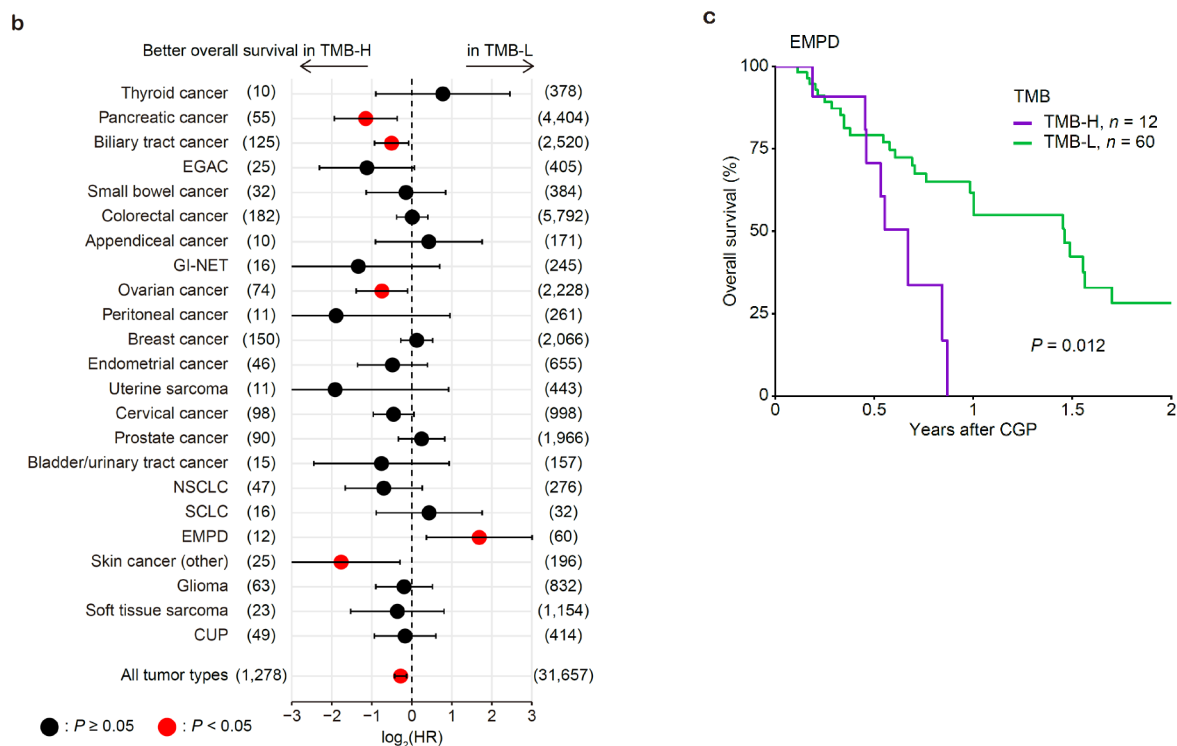


Fig. 4b, c

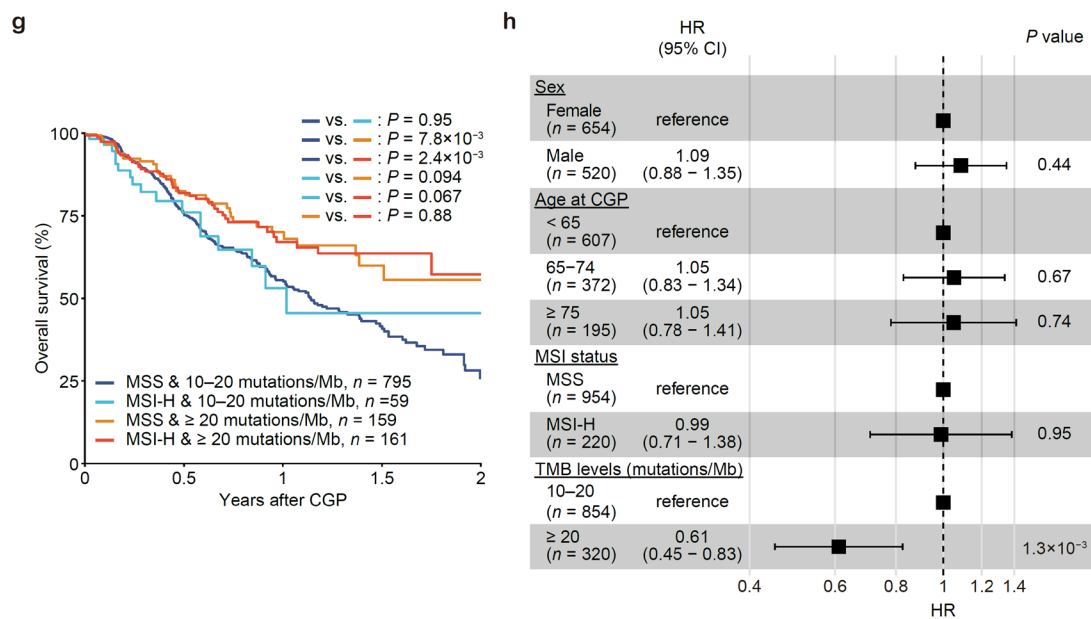
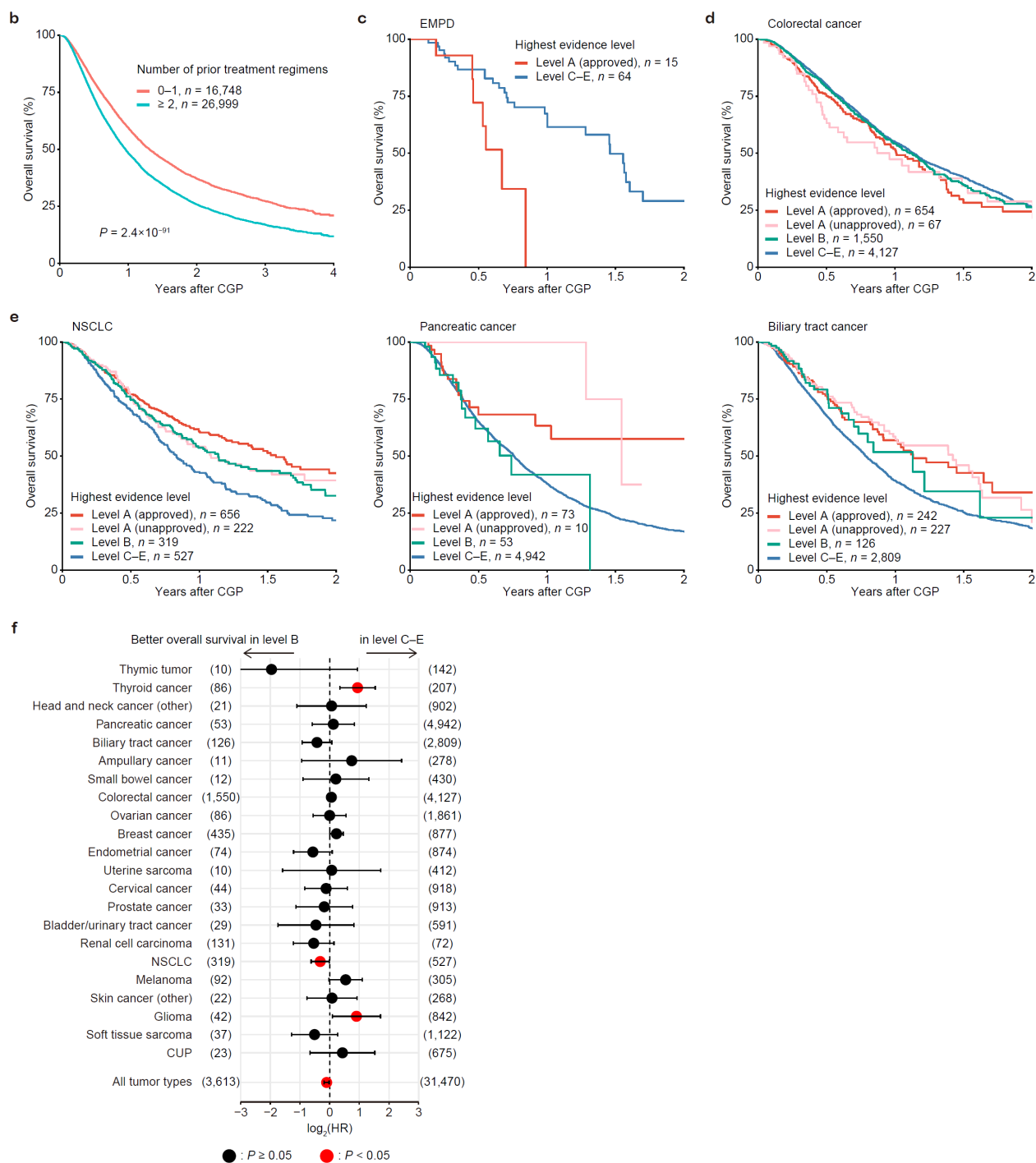
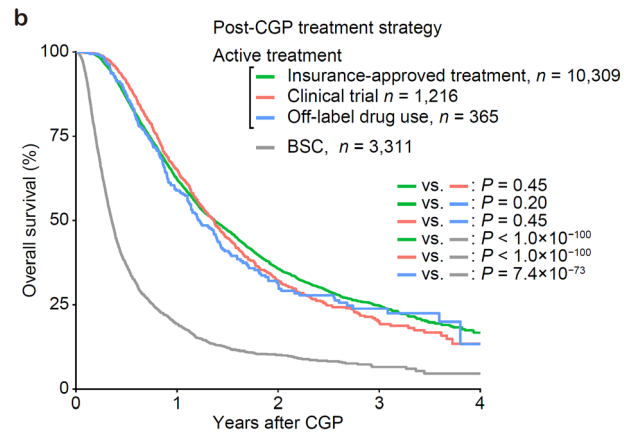


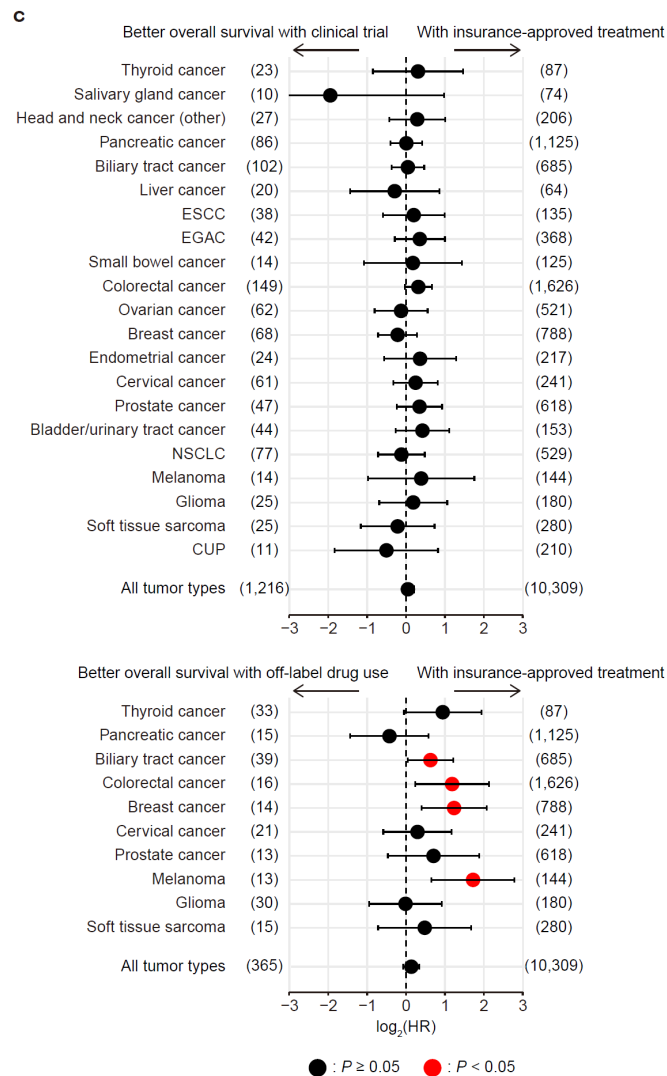
Fig. 4g, h



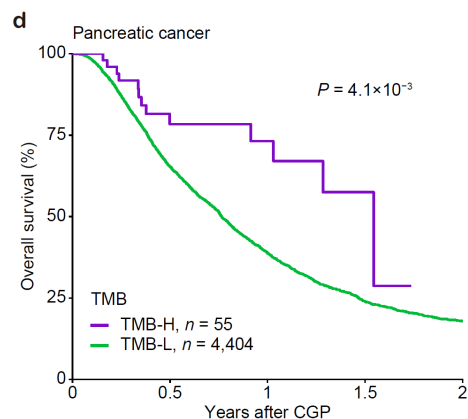
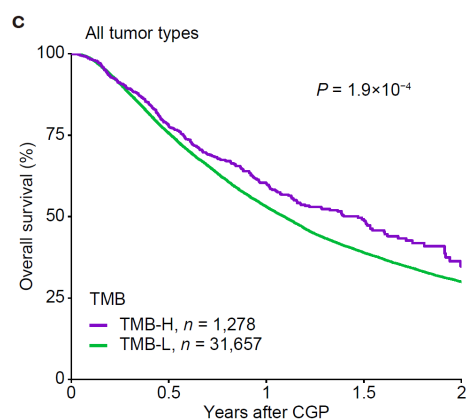
Extended Data Fig. 2b-f



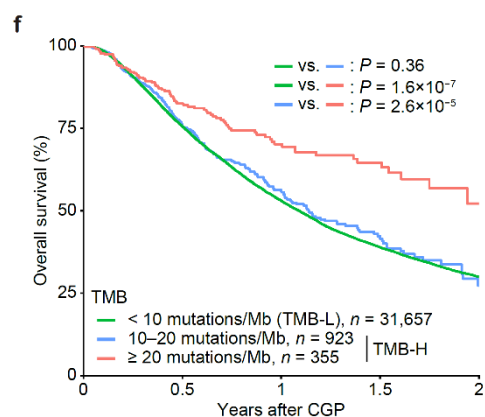
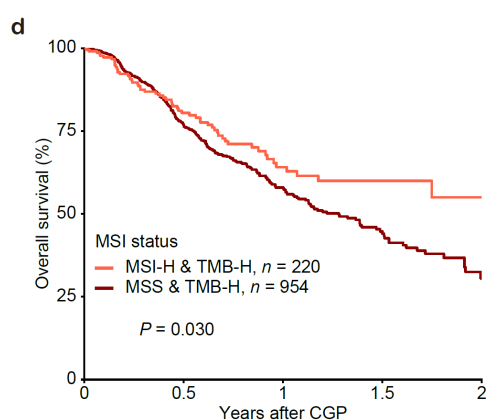
Extended Data Fig. 3b



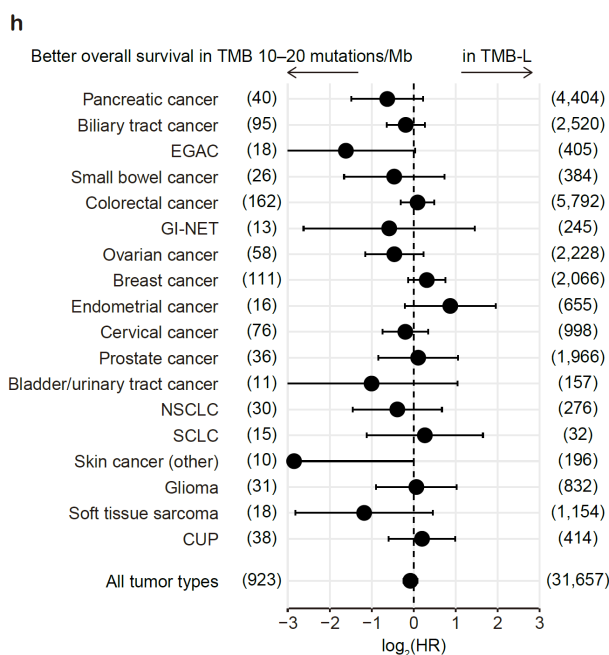
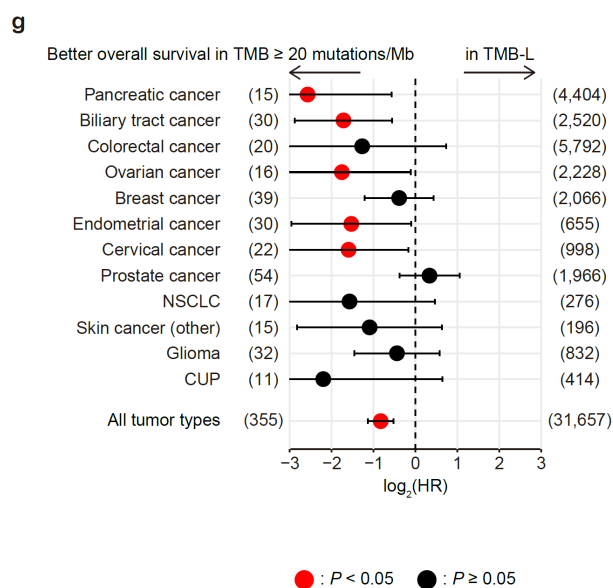
Extended Data Fig. 4c



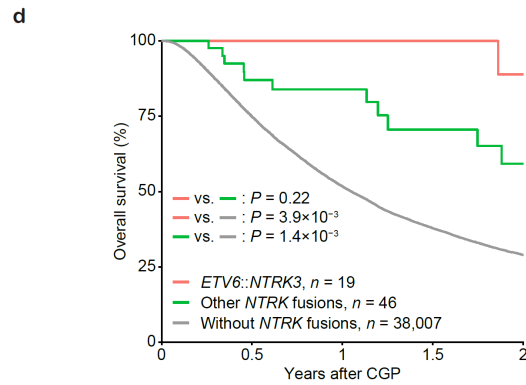
Extended Data Fig. 5c, d



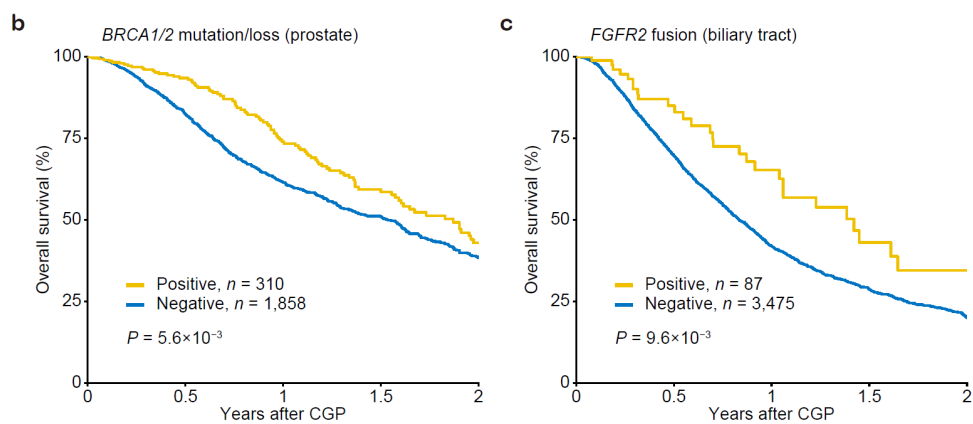
Extended Data Fig. 6d, f



Extended Data Fig. 6g, h



Extended Data Fig. 7d



Extended Data Fig. 8b, c

#2-9

Figure 2 is difficult to understand, authors should make it more clear.

We thank the reviewer for the valuable comment. We agree that **Fig. 2** was not sufficiently clear. In response, we have revised the figure to improve clarity by adding more description and providing a clearer legend. We hope the revised version enhances the reader's understanding.

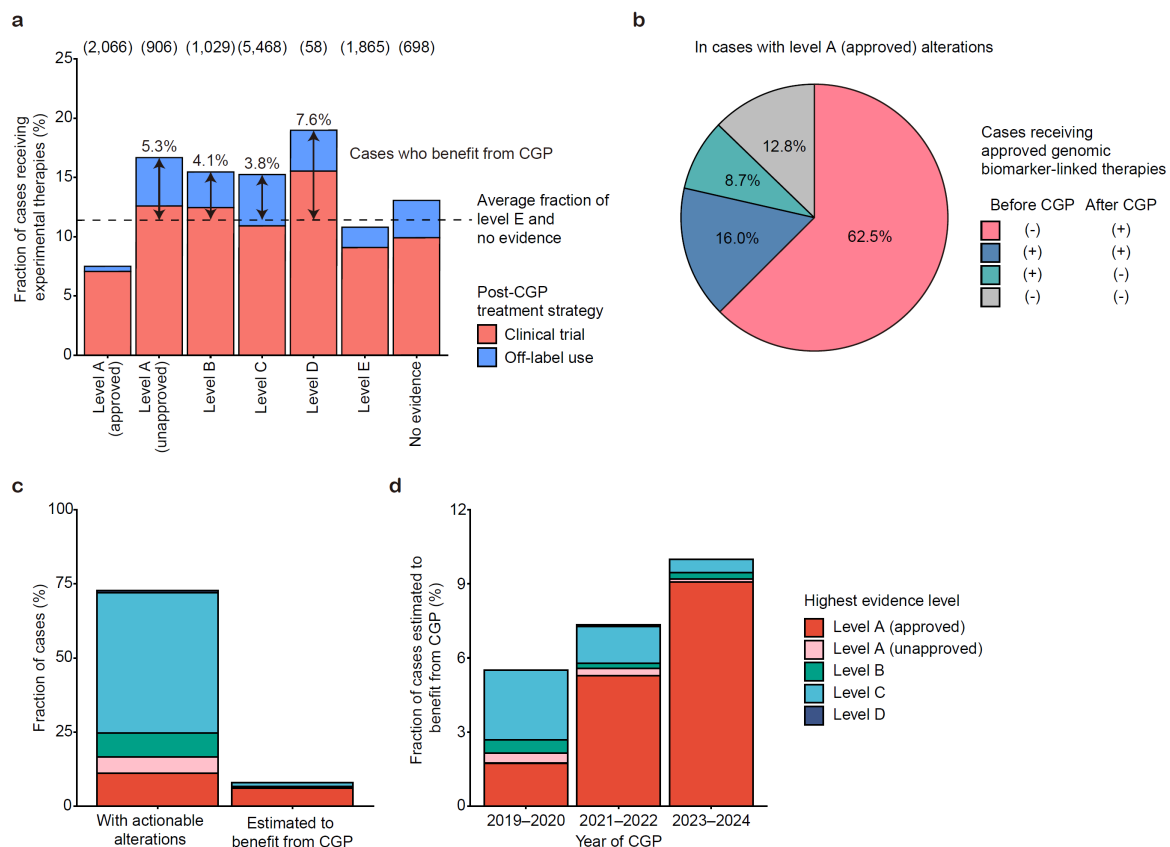


Fig. 2: Evaluation of cases receiving CGP-guided genomic biomarker-linked therapies.

a, Fraction of cases enrolled in clinical trials (red) and receiving off-label drugs (blue) according to the C-CAT evidence level in cases who received active treatment after CGP. Numbers in parentheses indicate the number of evaluated cases. The dotted line represents the **average** fraction of cases **harboring** alterations with level E or no evidence (= 11.4%). **Double-headed arrows with percentages indicate the increase in the fraction compared to the average fraction of cases harboring alterations with level E or no evidence, which is the proportion of cases who are considered to benefit from CGP.**

b, Fraction of cases receiving corresponding genomic biomarker-linked therapies before and/or after CGP in 1,558 cases with level A (approved) alterations and post-CGP treatment regimens available. **Cases receiving the corresponding genomic biomarker-linked therapy for the first time after CGP (pink) are considered to benefit from CGP.**

c, Fraction of cases with clinically actionable alterations (with evidence levels A–D) (left) and cases estimated to **benefit from CGP** (right) in the entire cohort.

d, Fraction of cases estimated to **benefit from CGP** according to year of CGP testing.

#2-10

Some diseases present a low rate of Level A. As example, breast and BTC present <10% level A while their frequency is usually more important (PIK3CA for breast cancer for example). Authors should comment it.

We thank the reviewer for the valuable comment. We agree that some tumor types showed a smaller proportion of cases with level A alterations than those expected from the literature (refs. 1, 2 of the revised manuscript). These results are partly because the clinical evidence level of each genetic alteration was annotated according to the C-CAT evidence-level classification at the time of CGP testing. They may also reflect the presence of unapproved indications in Japan, such as *PIK3CA* amplification in breast cancer (alpelisib and capivasertib), *FGFR3* fusion in bladder/urinary tract cancer (erdafitinib), *IDH1* mutation in biliary tract cancer (ivosidenib), and mutation/loss of genes involved in homologous recombination (other than *BRCA1/2*) in prostate cancer (olaparib and talazoparib), as already described in **Methods**. To clarify these points, we have added these explanations in **Results** in the revised manuscript as follows:

(Page 14, line 197)

Differences in the proportion of cases receiving CGP-guided genomic biomarker-linked therapies across tumor types.

In addition, several tumor types, such as breast and bladder/urinary tract cancers, showed a smaller proportion than those expected from the literature ^{1,2}, partly due to the presence of unapproved indications in Japan (**Methods**).

Reviewer #2 (Remarks on code availability):

I went to the github but there are many codes, I could not review all of them.

We acknowledge that the GitHub repository was not sufficiently clear. To improve readability and facilitate review, we have substantially revised the GitHub repository (https://github.com/nccmo/C-CAT_actionability_2025) as follows:

- (1) We have revised the README file to present the directory structure and provide explanations for individual R markdown scripts.
- (2) We have organized the R markdown scripts into directories according to analytical content. In addition, we have arranged the R markdown scripts within each directory to explicitly reflect their execution sequence.
- (3) We have added an explanation of the analysis to the top of each R markdown script.

Reviewer #3 (Remarks to the Author):

This manuscript analyzes comprehensive genomic profiling (CGP) in over 54,000 patients with advanced solid tumors in Japan, leveraging the national C-CAT registry. It provides a valuable real-world perspective on the implementation of precision oncology at scale, revealing important insights into the clinical uptake of CGP, treatment stratification based on biomarker evidence levels, and tumor-specific responses to targeted and immune therapies.

The study is especially impactful due to its size, nationwide scope, and outcomes analysis. It underscores the gap between identifying actionable alterations (present in 73% of cases) and actual delivery of CGP-guided therapies (only 8%) and explores the reasons behind such discrepancies across tumor types. Furthermore, the authors highlight important findings around tumor mutational burden (TMB), suggesting a revised threshold (≥ 20 mutations/Mb) may better predict the benefit of immune checkpoint inhibitors. These real-world findings have important implications for refining biomarker-driven therapy.

The dataset, derived from the Center for Cancer Genomics and Advanced Therapeutics (C-CAT), significantly contributes to the global research community. It complements previously published experiences, such as the MSK-IMPACT cohort (PMID: 28481359), which used paired tumor-normal sequencing in a single-institution setting. The comparison between a national registry and institutional datasets offers a unique opportunity to assess generalizability, capture rare cancers, and evaluate systemic implementation challenges. The figures are well done and provide a good summary of the data.

We appreciate the reviewer for their time and expert opinion, and greatly appreciate the enthusiasm for the importance of our manuscript. We have addressed each comment, as detailed in the point-by-point response below.

#3-1

Pre-CGP testing context: Many patients had received treatment before CGP. Can the authors explain what diagnostic or molecular testing was used before CGP? For example, were tumor-type specific panels (e.g., EGFR for lung, HER2 for breast) routinely used?

We agree that it is important to explain what kind of non-CGP CDx assays are routinely used before CGP in Japan. Thus, we have added this information to **Supplementary Table 2** and added explanations in the **Methods** of the revised manuscript as follows:

(Page 33, line 507)

Concordance of CGP testing results and non-CGP companion diagnostics (CDx)

The results of all non-CGP CDx for level A (approved) biomarkers, together with *KRAS* and *NRAS* mutations in bowel cancer, were reported by the treating physicians at each institution (**Supplementary**

Table 2). Biomarkers with at least 10 positive cases by non-CGP CDx in each primary organ/tissue were evaluated for concordance between CGP and non-CGP CDx, excluding CDx assays that evaluate only germline *BRCA1/2* variants.

Supplementary Table 2 (only added columns are shown).

Alteration (tumor type)	Tumor type	Non-CGP CDx assays approved in Japan [†]
TMB-H	All solid tumor	N/A
MSI-H	All solid tumor [‡]	PCR
<i>BRCA1/2</i> mutation/loss	Ovarian cancer	PCR (for germline variant), NGS
<i>ERBB2</i> amplification	Breast cancer	IHC, ISH
<i>ERBB2</i> amplification	EGAC	IHC, ISH
<i>BRAF</i> V600E mutation	Colorectal cancer	PCR
Mutations/loss in MMR genes [§]	All solid tumor	IHC
<i>BRCA1/2</i> mutation/loss	Prostate cancer	PCR (for germline variant)
<i>EGFR</i> mutation	NSCLC	PCR, NGS
<i>ERBB2</i> amplification	Colorectal cancer	IHC, ISH
<i>KIT</i> mutation	GIST	PCR
<i>BRAF</i> V600 mutation	Melanoma	PCR
<i>NTRK</i> fusion	All solid tumor	N/A
<i>FGFR2</i> fusion	Biliary tract cancer	N/A
<i>ERBB2</i> amplification	Salivary gland cancer	IHC, ISH
<i>MET</i> exon14 skipping mutation	NSCLC	PCR, NGS
<i>PIK3CA</i> mutation	Breast cancer	N/A
<i>ALK</i> fusion	NSCLC	IHC, ISH, PCR, NGS
MSI-H	Colorectal cancer	PCR
<i>EGFR</i> amplification [¶]	NSCLC	N/A
<i>ROS1</i> fusion	NSCLC	PCR, NGS
<i>ERBB2</i> amplification	NSCLC	N/A
<i>PTEN</i> mutation/loss	Breast cancer	N/A
<i>BRAF</i> V600E mutation	All solid tumor	PCR
<i>KRAS</i> G12C mutation	NSCLC	PCR, NGS
<i>BRAF</i> V600E mutation	Thyroid cancer	PCR, NGS
<i>RET</i> fusion	NSCLC	PCR, NGS
<i>ERBB2</i> mutation	NSCLC	NGS
<i>AKT1</i> E17K mutation	Breast cancer	N/A
<i>BRAF</i> V600E mutation	NSCLC	PCR, NGS
<i>KIT</i> amplification [¶]	Soft tissue sarcoma	N/A
<i>RET</i> fusion	Thyroid cancer	NGS
<i>MET</i> amplification	Renal cell carcinoma	N/A
Alterations with >= 10 cases are shown.		
TMB-H, high tumor mutation burden; MSI-H, high microsatellite instability; MMR, mismatch repair; EGAC, esophagogastric adenocarcinoma; NSCLC, non-small cell lung cancer; GIST, gastrointestinal stromal tumor; CGP, comprehensive genomic profiling; CDx, companion diagnostic; PCR, polymerase chain reaction; NGS, next-generation sequencing; IHC, immunohistochemistry; ISH, in-situ hybridization; N/A, not available.		
† Non-CGP CDx assays approved after the study period are not shown. NGS refers to NGS-based multiplex gene panels.		
‡ Colorectal cancer was excluded.		
§ MMR genes include <i>MSH2</i> , <i>MSH6</i> , <i>MLH1</i> , and <i>PMS2</i> .		
A PCR-based assay for <i>KIT</i> mutation detection in GIST is available in Japan; however, it is not strictly classified as a CDx, as mutation testing is not required for the administration of imatinib in GIST cases.		
¶ These alterations are currently not classified as evidence A (approved).		

#3-2

Data access and sharing: Given the high value of this dataset, it would be important to clarify the process for outside researchers to access C-CAT data. Are there plans to make a version of the dataset publicly accessible via platforms like cBioPortal or a comparable repository, as done with other large-scale datasets (e.g., MSK-IMPACT)?

We agree with the reviewer that the process to access the C-CAT data should be clearly stated. In addition to its primary utilization for improving medical practice and insurance medical care, the C-CAT data can

be used for academic research and drug development as secondary utilization. Although we do not belong to C-CAT, we conducted this study by using the C-CAT data, with the approval of the C-CAT data utilization review board (C-CAT proposal control number: CDU2022-003), under controlled access due to ethical and privacy restrictions.

Researchers can use the C-CAT data through “Research-Use Portal site”. To apply for data use, researchers need to submit the C-CAT data use application form with a research protocol and a copy of the approval from their research ethics committee and the head of their institution to C-CAT (E-mail: c_cat_use@ml.res.ncc.go.jp), which is expected to respond within approximately one week. Please refer to “Steps from application to data utilization” (URL: https://www.ncc.go.jp/en/c_cat/use/flow/index.html) for details.

At present, access to the C-CAT data is limited to hospitals for cancer genomic medicine, industries, and research institutes in Japan. This restriction reflects the fact that C-CAT is a national initiative linked to the national health insurance system and is based on decisions made by the government. However, data sharing for researchers outside Japan is planned for the near future. Currently, researchers outside Japan may access the C-CAT data through a collaborative study with researchers based in Japan, upon approval from the C-CAT data utilization review board. Instead, the “Number-of-Records Search site” (URL: <https://simplesearch-use.c-cat.ncc.go.jp/en/>), which provides the number of the registered records of your interest in the C-CAT cohort, is publicly available. To clarify these points, we have revised sentences in **Methods** and **Data availability** in the revised manuscript as follows:

(Page 25, line 376)

C-CAT dataset preparation

This study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by the institutional review board at the National Cancer Center (approval number: 2021-249). The secondary utilization of the C-CAT data for academic research purposes was approved by the C-CAT data utilization review board (C-CAT proposal control number: CDU2022-003).

(Page 34, line 530)

Data availability

The data that support the findings of this study are available from C-CAT (URL: https://www.ncc.go.jp/en/c_cat/about) under controlled access due to ethical and privacy restrictions. Researchers can use the C-CAT data through “Research-Use Portal site”. To apply for data use, researchers need to submit the C-CAT data use application form with a research protocol and a copy of the approval from their research ethics committee and the head of their institution to C-CAT (E-mail: c_cat_use@ml.res.ncc.go.jp), which is expected to respond within approximately one week. Please refer to “Steps from application to data utilization” (URL: https://www.ncc.go.jp/en/c_cat/use/flow/index.html) for details. The data access is currently restricted to hospitals for cancer genomic medicine, industries, and research institutes in Japan, but is expected to be extended to institutions outside Japan in the near future. Currently, researchers outside Japan may access the C-CAT data through a collaborative study with researchers based in Japan, upon approval from the C-CAT data utilization review board. The “Number-

of-Records Search site" (URL: <https://simplesearch-use.c-cat.ncc.go.jp/en/>), which provides the number of the registered records of your interest in the C-CAT cohort, is publicly available.

#3-3

Contextualization with prior CGP efforts: A brief comparison with earlier institutional CGP efforts—such as MSKCC's work—could further highlight the strengths of the current national approach and inform how a centralized registry enables broader insights into clinical implementation, biomarker variability, and real-world treatment patterns.

We appreciate the reviewer's comment. Large-scale clinical sequencing projects have demonstrated the value of CGP by showing that many patients harbor actionable alterations (summarized in **Supplementary Table 6**) (refs. 1, 2, 8, 9 of the revised manuscript). Particularly, the initiatives at the Memorial Sloan Kettering Cancer Center laid the groundwork as a prospective clinical sequencing platform, clarifying the difference of clinical actionability across major tumor types and its temporal change using the OncoKB knowledge base (refs. 2, 8). The registry databases (e.g., AACR Project GENIE) have collected such real-world sequencing data on a larger scale, facilitating the discovery of rare alterations across diverse tumors and the characterization of alterations in rare tumors (refs. 3, 4, 37). Moreover, early non-randomized clinical trials, in which the definition and/or extent of matching were retrospectively determined, showed that patients receiving matched therapies (or CGP-guided genomic biomarker-linked therapies) achieve improved survival (refs. 15-18). On the other hand, several randomized controlled trials (RCT) in all solid tumors failed to reproduce these findings, while an RCT in HER2-negative metastatic breast cancer reported a positive result (refs. 19-21). Thus, there are differing views on whether CGP and related genomic biomarker-linked therapies improve the overall outcomes with advanced solid tumors. Meanwhile, multiple basket or platform trials (e.g., NCI-MATCH) recruiting hundreds to thousands of patients have yielded clinical evidence for the efficacy of biomarker-linked therapies within specific genomically defined subgroups, ultimately supporting the regulatory approval of tumor-agnostic indication (refs. 38-41).

In this context, the C-CAT database can bridge the knowledge gap not covered by previous studies. The nationwide coverage allows investigating a patient population that better reflects real-world practice. The large sample size and detailed clinical information enables assessment of the efficacy of genomic biomarker-linked therapies targeting rare tumor types (such as ICIs for EMPD) and rare alterations (including *NTRK* fusions) as well as in contexts where evidence is limited, such as discordant results between non-CGP CDx and CGP. More importantly, through utilizing prospective annotation for genetic alterations, we demonstrate that CGP-guided clinical evidence level classification enables prognostic stratification in advanced solid tumors irrespective of the timing of CGP. Particularly, level A and B alterations can predict better outcomes, which are more prominent in NSCLC and pancreatobiliary cancers. Therefore, it is reasonable to assume that CGP-guided approved/experimental administration of genomic biomarker-linked therapies (potentially excluding off-label drug use) can more consistently

improve clinical outcomes, although several alterations themselves affect prognosis. In addition, we leveraged adequate information on treatment details before and after CGP to evaluate whether to initiate CGP-guided approved/experimental genomic biomarker-linked therapies. We estimate that only a small proportion (8.0%) of cases benefit from CGP at present, despite clinically actionable alterations (with evidence levels A–D) being identified in 72.7% of cases. The large gap between the two proportions was compatible with those reported from prospective cohort studies conducted at selected institutions (refs. 8, 14, 17). Remarkably, our pan-cancer analysis identifies a significant difference in the proportion of cases benefitting from CGP testing across tumor types, which is greater than the difference in the proportion of cases harboring actionable alterations. Therefore, these findings provide more reliable estimates for determining where CGP provides the most clinical benefit at present.

To clarify these points, we have summarized clinical trials, large-scale clinical sequencing projects, and registry databases in precision oncology in **Supplementary Table 6** and added these explanations in **Introduction** and **Discussion** in the revised manuscript as follows:

(Page 5, line 42)

Introduction

Large-scale clinical sequencing projects have demonstrated the utility of CGP by showing that a large proportion of patients harbor actionable alterations ^{1,2,8,9}. However, CGP has several drawbacks, such as long turn-around time, high cost, unavailability as CDx for many molecularly targeted therapies, and limitation of testing timing and hospitals capable of performing CGP ^{1,5}. Thus, although several guidelines recommend CGP early in the management of advanced solid tumors in multiple tumor types ^{10,11}, many patients undergo non-NGS-based CDx and related therapies before CGP ^{12,13}, resulting in a smaller proportion of patients receiving CGP-guided genomic biomarker-linked therapies. In addition, although the efficacy of specific genomic biomarker-linked therapies against a subset of tumors with corresponding actionable alterations has been demonstrated in clinical trials, whether the availability of diverse genomic biomarker-linked therapies improves the clinical outcome of the overall patient population receiving CGP has remained controversial ^{5,14-21}. Therefore, actual utility of NGS-based CGP and its difference among tumor types remains unknown in the real world.

(Page 22, line 320)

Discussion

Large-scale clinical sequencing projects have demonstrated the value of CGP by showing that many patients harbor actionable alterations (**Supplementary Table 6**) ^{1,2,8,9}. Particularly, the initiatives at the Memorial Sloan Kettering Cancer Center laid the groundwork as a prospective clinical sequencing platform, clarifying the difference of clinical actionability across major tumor types and its temporal change using the OncoKB knowledge base ^{2,8}. The registry databases (e.g., AACR Project GENIE) have collected such real-world sequencing data on a larger scale, facilitating the discovery of rare alterations across diverse tumors and the characterization of alterations in rare tumors ^{3,4,37}. Moreover, early non-randomized clinical trials, in which the definition and/or extent of matching were retrospectively determined, showed that patients receiving matched therapies (or CGP-guided genomic biomarker-linked

therapies) achieve improved survival ¹⁵⁻¹⁸. On the other hand, several randomized controlled trials (RCT) in all solid tumors failed to reproduce these findings, while an RCT in HER2-negative metastatic breast cancer reported a positive result ¹⁹⁻²¹. Thus, there are differing views on whether CGP and related genomic biomarker-linked therapies improve the overall outcomes with advanced solid tumors. Meanwhile, multiple basket or platform trials (e.g., NCI-MATCH) recruiting hundreds to thousands of patients have yielded clinical evidence for the efficacy of biomarker-linked therapies within specific genomically defined subgroups, ultimately supporting the regulatory approval of tumor-agnostic indication ³⁸⁻⁴¹.

In this context, the C-CAT database can bridge the knowledge gap not covered by previous studies. The nationwide coverage allows investigating a patient population that better reflects real-world practice. The large sample size and detailed clinical information enables assessment of the efficacy of genomic biomarker-linked therapies targeting rare tumor types (such as ICIs for EMPD) and rare alterations (including *NTRK* fusions) as well as in contexts where evidence is limited, such as discordant results between non-CGP CDx and CGP. More importantly, through utilizing prospective annotation for genetic alterations, we demonstrate that CGP-guided clinical evidence level classification enables prognostic stratification in advanced solid tumors irrespective of the timing of CGP.

(Page 23, line 351)

Discussion

In addition, we leveraged adequate information on treatment details before and after CGP to evaluate whether to initiate CGP-guided approved/experimental genomic biomarker-linked therapies.

Supplementary Table 6: Summary of clinical trials, large-scale clinical sequencing projects, and registry databases in precision oncology.

Supplementary Table 6. Summary of clinical trials, large-scale clinical sequencing projects, and registry databases in precision oncology.						
Study type	Study name: registration number	Study design	Country	Tumor type	Study description and significance	Study results
Clinical sequencing project	MSK-IMPACT: NCT01775072	A prospective, single-center clinical sequencing project	USA	All solid tumors	The initiatives using MSK-IMPACT, the first CGP test authorized by FDA, laid the groundwork as a prospective clinical sequencing platform, clarifying the difference of clinical actionability across major tumor types and its temporal change using the OncoKB knowledge base. These initiatives have also contributed to cancer research, such as cancer genomics, metastatic biology, germline pathogenicity, and clonal hematopoiesis. In addition, they have facilitated the application of artificial intelligence, such as natural language processing-based clinicogenomic data integration (Jee J, et al. <i>Nature</i> , 2024.).	Initially, data from 11,369 cases sequenced using MSK-IMPACT were reported. Of these, 11% of cases were enrolled in genomically matched clinical trials (Zehir A, et al. <i>Nat Med</i> , 2017.). Subsequent reports have included data on 47,271 samples (42,154 cases) detailing their clinical actionability over time. This study showed an increase from 8.9% to 31.6% in the fraction of tumors harboring an OncoKB level 1 or 2 biomarker between 2017 and 2022. Additionally, the fraction of tumors with a clinically actionable biomarker varied by tumor type, being highest in GIST and lowest in mesothelioma (Suehnholz SP, et al. <i>Cancer Discov</i> , 2024.). Key papers are summarized on the MSKCC website: https://www.mskcc.org/mskcc-impact .
Clinical sequencing project	K-MASTER	A prospective, multi-center clinical sequencing project	South Korea	All solid tumors	K-MASTER, conducted in South Korea, performed CGP to identify targetable alterations and match them to appropriate clinical trials. The study provided a genomic overview of Korean patients, highlighting differences in molecular features and clinical actionability compared with Western populations.	A total of 4,028 cases were sequenced. Druggable alterations were identified in 28.7% of cases, with 440 cases enrolled into 20 different clinical trials. Comparison with TCGA revealed the differences of clinically actionable alterations based on ethnicity.
Clinical sequencing project	SCRUM-MONSTAR	A prospective, multi-center clinical sequencing project	Japan	All tumors (excluding lung cancer)	The SCRUM-Japan MONSTAR-SCREEN consortium is a large-scale molecular profiling project in Japan, employing artificial intelligence-driven multimodal analyses for cases with advanced malignancies. It is intricately linked to multiple clinical trials and observational studies, enabling enrollment in matched clinical trials.	More than 24,000 cases were enrolled, and 5.0% participated in matched clinical trials. Cases who received biomarker-linked therapies demonstrated significantly prolonged OS compared with those who did not (19.1 months versus 15.3 months).
Registry database of clinical sequencing data	C-CAT	A registry database to collect clinical sequencing data	Japan	All solid tumors	C-CAT is a nationwide registry database in Japan, where almost all CGP tests conducted under the national insurance system have been registered. C-CAT includes not only genomic but also clinical information, including prognosis and treatment details. The large-scale database allows evaluating inter-tumor type differences and investigating evidence-scarce situations.	Data from 48,627 cases in C-CAT were analyzed to define the pan-cancer landscape of driver alterations and their clinical actionability in Japanese cases. Comparison with White populations revealed higher TP53 mutation frequencies in Asian cases across multiple tumor types (Horie S, et al. <i>Cancer Discov</i> , 2024.). Our subsequent analysis of 54,185 cases further demonstrates the prognostic value of CGP-guided evidence-level classification and identifies where CGP provides the greatest clinical benefit in real-world practice (this paper).
Registry database of clinical sequencing data	GENIE	A registry database to collect clinical sequencing data	USA and other countries	All tumors (including both solid and hematological malignancies)	AACR Project GENIE is an international, multi-institutional registry of clinical sequencing data from participating institutions. This is the largest genomic registry to date, enabling the assessment of prevalence of clinically actionable alterations across tumor types, including rare ones.	The latest release (version 17.0-public) includes more than 220,000 sequenced samples from over 100,000 cases. A list of AACR Project GENIE publications is available on the AACR website: https://www.aacr.org/professionals/research/aacr-project-genie/aacr-project-genie-publications/ .
Registry database of clinical sequencing data	GENIE BPC	A registry database to collect clinical sequencing data	USA and Canada	Bladder, breast, colorectal, lung, pancreatic, and prostate cancers	GENIE Biopharma Collaborative (BPC) is an ongoing project that manually curates and annotates in-depth clinical data, including treatment information, to link with clinical sequencing data for a subset of cases from the GENIE project.	In phase 1, GENIE BPC integrated clinical data, including cancer treatments, with genomic data from nearly 8,000 cases across four institutions. Currently, datasets comprising 1,846 NSCLC cases and 1,485 colorectal cancer cases have been released.
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	MOSCATO-01: NCT01566019	A prospective, non-randomized single-center clinical trial	France	All solid tumors	The MOSCATO-01 trial evaluated whether clinical sequencing could identify actionable alterations and improve clinical outcomes when genomic biomarker-linked therapies were administered. This study was one of the earliest studies demonstrating that genomic biomarker-linked therapy could improve outcomes for a subset of cases using the "PFS2PFS1 ratio".	A total of 1,035 cases were included, and 199 cases were treated with genomic biomarker-linked therapies. The PFS2PFS1 ratio, defined as the PFS on biomarker-linked therapy divided by the PFS on the immediately preceding therapy, was greater than 1.3 in 33% of cases.
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	IMPACT: NCT00851032	A prospective, non-randomized single-center clinical trial	USA	All solid tumors	The IMPACT trial evaluated the clinical benefit of genomic biomarker-linked therapies in cases referred for phase I clinical trials. The program progressively expanded in scale, from an initial validation cohort to a retrospective 10-year follow-up study. This study retrospectively defined "matched therapies" and demonstrated that matched therapies improve response and survival compared with unmatched therapies.	In a retrospective analysis of 3,487 cases who underwent tumor molecular profiling, 711 received genomic biomarker-linked therapies (matched therapies), while 566 received unmatched therapies. The objective response rates were 16.4% and 5.4% for the matched and unmatched groups, respectively. The median PFS was 4.0 months versus 2.8 months, and OS was 9.3 months versus 7.3 months, which were both significantly different (Tsimberidou AM, et al. <i>J. Hematol. Oncol</i> , 2019.).
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	ProFILER: NCT01774409	A prospective, non-randomized multi-center clinical trial	France	All tumors (including both solid and hematological malignancies)	The ProFILER trial was designed to assess the feasibility and clinical impact of tumor molecular profiling. The study enrolled both adult and pediatric cases with solid or hematologic malignancies. In this study, sequencing results were reviewed by a multidisciplinary MTD, which recommended genomic biomarker-linked therapies when appropriate. The study demonstrated a low initiation rate and response rate for genomic biomarker-linked therapies, underscoring the challenges in translating genomic findings into effective personalized treatments for most cases.	A total of 2,579 cases were enrolled, and the MTB reviewed the molecular profiles achieved for 1,980 cases (76.8%). Actionable alterations were identified in 1,032 cases (40%), and genomic biomarker-linked therapies were proposed for 699 (27%). However, only 163 cases (8%) received such therapies, with 23 partial responses (13%) observed, corresponding to just 0.9% of the total cohort.
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	CoPro: NCT02205022	A prospective, non-randomized, single-center clinical trial	Denmark	All solid tumors	The CoPro trial evaluated the clinical benefit of tumor molecular profiling for treatment selection in a phase I setting. Cases underwent whole-genome or whole-exome sequencing, RNA sequencing, and SNP array analysis. Results were reviewed by an MTD, which recommended biomarker-linked therapies when appropriate. This study retrospectively classified targets according to the ESCAT framework and demonstrated that genomic profiling is efficient in identifying actionable targets and matching cases to targeted therapies.	A total of 2,147 cases were included, and genomic profiles were obtained in 1,888 cases. Actionable alterations were identified in 1,062 cases (57%), including 559 cases with high RNA CEACAM5 expression, and 252 received biomarker-linked therapies. ESCAT III was associated with significantly improved PFS and OS compared to ESCAT II/IV (PFS: 5.02 months versus 2.26 months, OS: 10.49 months versus 6.66 months) (Belcald, et al. <i>Ann Oncol</i> , 2025.).
Non-randomized clinical trial of CGP and related genomic biomarker-linked therapies	I-PREDICT: NCT02534675	A prospective, non-randomized, single-center clinical trial	USA	All tumors (mostly solid tumors)	I-PREDICT was a trial designed to evaluate whether combination genomic biomarker-linked therapies could improve outcomes in cases with refractory malignancies. The study retrospectively evaluated a "matching score," calculated by dividing the number of alterations targeted by the administered therapies by the total number of characterized alterations in each case's tumor, and demonstrated that a high matching score was associated with better prognosis, indicating the potential clinical benefit of combination therapies in precision oncology.	Among 149 enrolled cases, 83 were treated, of which 73 received genomic biomarker-linked therapies. The disease control rate was 50% in cases with a high matching score (>50%) compared to 22.4% in those with a low score (<=50%). PFS was longer in the high-score group (6.5 months versus 3.1 months), and OS was also significantly different (not reached versus 10.2 months).
Randomized controlled trial of CGP and related genomic biomarker-linked therapies	SAFIR02-BREAST: NCT02299999	A phase II, prospective, randomized, multi-center clinical trial	France	HER2-negative breast cancer	The SAFIR02-BREAST trial evaluated whether genomic biomarker-linked therapy, guided by ESCAT, improves outcomes compared with standard maintenance therapy. Subsequent benefit from biomarker-linked therapy was observed only when treatments were matched to genomic alterations with proven drug sensitivity (ESCAT level III), emphasizing the need for evidence-based precision oncology.	A total of 1,462 cases were included, and 238 cases were randomized to targeted therapy and maintenance chemotherapy. In cases with ESCAT III genomic alterations (n = 115), cases receiving genomic biomarker-linked therapies had significantly longer PFS than those receiving maintenance chemotherapy (9.1 months versus 2.8 months). No benefit was observed when alterations were unselected or of lower actionability.
Randomized controlled trial of CGP and related genomic biomarker-linked therapies	SHIVA: NCT01771458	A phase II, prospective, randomized multi-center clinical trial	France	All solid tumors	The SHIVA trial was one of the earliest randomized controlled trials to evaluate the prognosis of biomarker-linked therapies compared to physician's choice treatment. Although this study found no significant improvement in PFS, it is a landmark study demonstrating the limitations of broad, unselected precision oncology approaches.	A total of 741 cases were analyzed, and 195 were randomly assigned to treatment. The median PFS in cases assigned to biomarker-linked therapy was 2.3 months, which was not significantly different from that of cases receiving physician's choice treatment (median, 2.0 months) (Le Tourneau C, et al. <i>Lancet Oncol</i> , 2015.). In the crossover analysis, the PFS ratio, defined as PFS on biomarker-linked therapy divided by PFS on physician's choice treatment, exceeded 1.3 in 37% of cases who crossed over from physician's choice treatment to biomarker-linked therapy (Belin L, et al. <i>Ann Oncol</i> , 2017.).
Randomized controlled trial of CGP and related genomic biomarker-linked therapies	IMPACT2: NCT02152254	A phase II, prospective, randomized, single-center clinical trial	USA	All solid tumors	IMPACT2 was a randomized trial designed to evaluate whether genomic biomarker-matched therapies improve PFS compared with non-targeted therapies to address the limitations of the IMPACT trial. Due to challenges in randomization, the protocol was amended to include a patient-preference cohort. This study adopted an adaptive design to allow the inclusion of additional molecular alterations and treatments as they became available. The trial found no significant difference in response, PFS, or OS between the two arms, underscoring the challenges of randomizing cases in this setting.	Among the 829 cases enrolled, 109 were randomized or selected their treatment arms, while 297 were treated without consideration for randomization. The PFS for cases receiving genomic biomarker-linked therapies —whether randomized or not— was 4.41 months, which was not significantly different from that of those receiving a physician's choice treatment (3.75 months). Similarly, there was no significant difference in OS (9.44 months versus 9.21 months).
Platform trial of genomic biomarker-linked therapies	NCI-MATCH: NCT02465060	A phase II, prospective, non-randomized, multi-center, platform clinical trial	USA	All solid tumors: lymphoma, and myeloma	The NCI-MATCH trial was a large-scale, nationwide, signal-seeking precision medicine platform trial initiated by the NCI. The trial demonstrated the feasibility of large-scale genomic screening and treatment allocation based on actionable alterations. It provided important insights into the challenges of single-agent targeted therapies, which influenced the design of successor trials evaluating combination therapies, such as comboMATCH. One subprotocol (Arm H) contributed to the FDA approval of dabrafenib and trametinib for the treatment of unresectable or metastatic solid tumors harboring BRAF V600E mutations.	The trial screened 5,954 cases. Actionable alterations were identified in 37.6% of cases, and 17.8% were assigned to one of the 38 subprotocols evaluating genomic biomarker-linked therapy, whether FDA-approved or investigational (Fiaherly KT, et al. <i>J Clin Oncol</i> , 2020.). Among the initial 27 substudies, 7 were positive (25.9%) (O'Dwyer PJ, et al. <i>Nat. Med.</i> , 2023.). A list of NCI-MATCH publications is available on the ECOG-ACRIN Cancer Research Group website: https://ecog-acrin.org/clinical-trials/eay131-nci-match-precision-medicine-nci-match-eay131-findings/ .
Basket trial of genomic biomarker-linked therapies	TAPUR: NCT02693535	A phase II, prospective, non-randomized multi-center, multi-basket clinical trial	USA	All solid tumors: lymphoma, and myeloma	The TAPUR study was a large-scale, multiple basket trial initiated by ASCO, which evaluated signals of antitumor activity of FDA-approved genomic biomarker-linked therapies outside their approved indications in a pragmatic design. This study emphasized real-world implementation and broad access to genomic biomarker-linked therapies.	As of the 2018 report, more than 1,000 cases had been registered, and over 800 patients had received biomarker-linked therapies (Mangat PK, et al. <i>JCO Precis Oncol</i> , 2018.). According to the 2025 report, the number of registered cases has exceeded 3,400 (Mangat PK, et al. <i>NPJ Precis Oncol</i> , 2025.). The results of each subprotocol are summarized on the ASCO website: https://www.asco.org/research-data/tapur-study/study-results .
Basket trial of genomic biomarker-linked therapies	MyPathway: NCT02091141	A phase II, prospective, non-randomized multi-center, multi-basket clinical trial	USA	All solid tumors	MyPathway was a multiple basket study designed to evaluate the efficacy and safety of FDA-approved genomic biomarker-linked therapies outside their approved indications, similar to the TAPUR study. This study also helped establish the feasibility and clinical value of basket trials in precision oncology.	A total of 251 cases with 35 different tumor types received study treatment, and 23% had objective responses. Tumor-pathway cohorts with notable objective response rates included HER2 amplified/overexpressing colorectal cancer and BRAF V600-mutated NSCLC.
OS, overall survival; PFS, progression free survival; MTB, molecular tumor board; ESMO, European Society for Medical Oncology; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; NCI, National Cancer Institute; MSKCC, Memorial Sloan Kettering Cancer Center; GIST, gastrointestinal stromal tumor; AACR, American Association for Cancer Research; FDA, U.S. Food and Drug Administration; NSCLC, non-small cell lung cancer; ASCO, American Society of Clinical Oncology; CGP, comprehensive genomic profiling; TCGA, The Cancer Genome Atlas.						

#3-4

Could they address if the data, in part, could be included in C-Biportal? This would provide a subset of the data to the research community. There may be restrictions preventing this, which would be

understandable.

As described in #3-1, currently, access to the C-CAT data is limited to hospitals for cancer genomic medicine, industries, and research institutes in Japan. This restriction reflects the fact that C-CAT is a national initiative linked to the national health insurance system and is based on decisions made by the government. Although we do not belong to C-CAT, we conducted this study by using the C-CAT data, with the approval of the C-CAT data utilization review board (C-CAT proposal control number: CDU2022-003), under controlled access due to ethical and privacy restrictions. C-CAT prohibits data licensees from offering, selling, or reselling relevant data to any third parties. Thus, we are not able to determine whether these data can be included in cBioPortal.

The registration of C-CAT data to cBioPortal is not planned at this time. However, the “Number-of-Records Search site” (URL: <https://simplesearch-use.c-cat.ncc.go.jp/en/>), which provides the number of the registered records of your interest in the C-CAT cohort, is publicly available. To clarify these points, we have revised the sentences in **Data availability** in the revised manuscript as follows:

(Page 35, line 527)

Data availability

The data that support the findings of this study are available from C-CAT (URL: https://www.ncc.go.jp/en/c_cat/about) under controlled access due to ethical and privacy restrictions. Researchers can use the C-CAT data through “Research-Use Portal site”. To apply for data use, researchers need to submit the C-CAT data use application form with a research protocol and a copy of the approval from their research ethics committee and the head of their institution to C-CAT (E-mail: c_cat_use@ml.res.ncc.go.jp), which is expected to respond within approximately one week. Please refer to “Steps from application to data utilization” (URL: https://www.ncc.go.jp/en/c_cat/use/flow/index.html) for details. The data access is currently restricted to hospitals for cancer genomic medicine, industries, and research institutes in Japan, but is expected to be extended to institutions outside Japan in the near future. Currently, researchers outside Japan may access the C-CAT data through a collaborative study with researchers based in Japan, upon approval from the C-CAT data utilization review board. The “Number-of-Records Search site” (URL: <https://simplesearch-use.c-cat.ncc.go.jp/en/>), which provides the number of the registered records of your interest in the C-CAT cohort, is publicly available.

Overall, this is a well-written, highly valuable study representing a significant step forward in the real-world application of precision oncology. Minor additions and clarifications will significantly benefit clinicians, researchers, and health policymakers.

We again appreciate the reviewer for the time and expertise and are pleased that the reviewer considers our study as highly valuable study and significant step forward in the real-world application of precision oncology.

Response to referees and editors

Editor:

The editorial team requests that more information is provided in the Data Availability Statement on the steps that researchers (both in Japan and abroad) need to take to gain access to the data. As you mention that there are plans to expand direct access to researchers outside of Japan, please provide more details on the timeframe. You can find an example here: <https://www.nature.com/articles/s41591-023-02682-0#data-availability>.

We appreciate the editorial team for the concrete comments. Referring to the article you kindly provided, we have now included all the steps for data access for both researchers in Japan and abroad. With respect to direct access for researchers outside of Japan, as C-CAT is a national initiative, the timeframe depends on government consultation and decision-making. Unfortunately, we are unable to provide an estimated schedule at this time. We have updated the Data Availability Statement as follows:

Data Availability

The data that support the findings of this study are available from C-CAT (URL: https://www.ncc.go.jp/en/c_cat/about) under controlled access due to ethical and privacy restrictions. The application process for data access is described on the C-CAT website under “Steps from application to data utilization” (URL: https://www.ncc.go.jp/en/c_cat/use/flow/index.html) and consists of the following steps: (1) a principal investigator submits the C-CAT data use application form with a research protocol and a copy of the approval from their research ethics committee and the head of their institution to C-CAT (E-mail: c-cat_use@ml.res.ncc.go.jp), which is expected to respond within approximately one week; (2) the application is reviewed by the C-CAT data utilization review board; (3) once approved, an “Agreement on Utilization of C-CAT Data” is concluded between National Cancer Center Japan (governing C-CAT) and the institution of the principal investigator; (4) after the agreement is signed and the access fee is paid (with academic institutions generally exempt), the access license to the “C-CAT Research-Use Portal site” is granted; and (5) upon obtaining access, registered data licensees can connect to the portal using their registered computers over Transport Layer Security (TLS). The granted access is valid for one year, after which renewal of both the agreement and the access license is required. The data available to registered data licensees include: (1) de-identified data from individual patients, including patient background, treatment details (before and after CGP), and clinical outcomes; and (2) somatic alteration calls with clinical evidence levels, drug approval status, and clinical trial availability in Japan. C-CAT prohibits data licensees from offering, selling, or reselling relevant data to any third parties.

At present, principal investigators are restricted to individuals who belong to hospitals for cancer genomic medicine, industries, and research institutes in Japan. Therefore, researchers outside Japan may access the C-CAT data by collaborating with investigators based in Japan. In such cases, the

same procedure, including the approval from the C-CAT data utilization review board, is required. Although the timeframe is yet to be determined, direct data access is expected to become available to researchers/applicants outside Japan in the near future. The “Number-of-Records Search site” (URL: <https://simplesearch-use.c-cat.ncc.go.jp/en/>), which provides the number of the registered records of interest in the C-CAT cohort, is publicly available.

Please also note that no discussion text can be included in the supplement. Please incorporate the supplementary discussion text into the main discussion section. We also feel that the discussion can be condensed, to avoid the repetition of results. Please ensure that a paragraph on the limitations of the study is retained in the discussion.

Thank you for your comments. As instructed, we have incorporated the Supplementary Discussion into the Discussion section. We have also shortened the manuscript to comply with the word limit, primarily by condensing the Discussion section to reduce repetition of results. Please see the manuscript with tracked changes for details. Please note that the section copied from the Supplementary Discussion was inserted without tracked changes and shown in red font for readability. Subsequent modifications to this section have been made with tracked changes.

We also suggest to revise the manuscript abstract to provide more clarity on what "level A and level B" mean in this context.

We appreciate and agree with the editorial team's suggestions. We have added explanations of levels A and B in the Abstract as follows:

Abstract

Comprehensive genomic profiling (CGP) is crucial in precision oncology, yet its real-world utility remains unclear. Through analyzing 54,185 advanced solid tumor cases receiving CGP with FoundationOne CDx from the Japanese C-CAT database, we demonstrate the prognostic value of CGP-guided clinical evidence level classification. Level A (16.6%) and B (8.1%) alterations, which predict response to PMDA- or FDA-approved therapies and therapies supported by well-powered studies with expert consensus, respectively, are associated with better prognosis.

Reviewer #1 (Remarks to the Author):

Thank you for providing a markedly improved and clearer manuscript. It now focuses on the key issues and leaves some of the distracting anecdotes to the supplementals. The new figures are better and the authors address the outcome as well as subset analyses adeptly. Well done.

We appreciate the reviewer for the time and expertise in reviewing the manuscript and for providing positive feedback.

Reviewer #2 (Remarks to the Author):

The authors have addressed my comments

We appreciate the reviewer for the time and expertise in reviewing the manuscript and for providing positive feedback.

Reviewer #3 (Remarks to the Author):

The authors have fully addressed all of my queries, including clarification of pre-CGP testing practices, description of data access and sharing through C-CAT, and additional methodological details regarding concordance with non-CGP assays. They have also strengthened the manuscript by refining figures, expanding comparisons with other large datasets, and improving clarity around the prognostic and therapeutic impact of comprehensive genomic profiling.

Overall, I believe the revised manuscript is much improved and will provide an important data resource for the research community.

We appreciate the reviewer for the time and expertise in reviewing the manuscript and for providing positive feedback.