

Real-world evidence provides clinical insights into tissue-agnostic therapeutic approvals

Corresponding Author: Dr George W Sledge

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)

Sledge and colleagues present an important analysis of the efficacy across different tumor types of cancer therapies that have received tissue-agnostic approvals. While the trials that led to these tissue-agnostic approvals generally observed high response rates in cohorts that included a variety of tumor types, they did not have the very large sample sizes needed to adequately assess whether some tumor types are substantially more or less sensitive than others to these therapies. This article investigates some of these therapies by analysis of a real-world database of over a quarter million cancer patients, of whom a few thousand received treatments based on tissue-agnostic approvals. The article also assesses the prevalence of biomarker-positive patients for these indications within various tumor types; this too is important because for some of the rare indications, biomarker testing is not yet widely applied, and these data could help the community to at least prioritize biomarker testing in the cancer types with the highest likelihood of a positive test. For example, this study finds a ~5-fold higher prevalence of NTRK fusions in NSCLC compared with other cancer types.

The most in-depth analyses of the article apply to PD-1 inhibitors for MSS/TMB-high tumors, enabled by the large sample size available for this common indication. Some tumor types that were not included in the pivotal trials were found, in this study, to show similar median time-on-treatment from PD-1 inhibitors as those included in the pivotal trials. This is an important finding supporting the general validity of this particular tissue-agnostic approval. The real-world approach used here will be valuable in future to ask the same question of other approvals. The comparison of Hazard Ratios achieved by Nivolumab or Pembrolizumab, each comparing outcomes in MSS-high vs MSS-low cohorts in the database, was a clever way of using real-world data to compare the activity of competing drugs for the same indication. This finding is also quite important, applying to a frequent tissue-agnostic indication. Although, it is not clear to me that this could be used to extend tissue-agnostic approvals to other non-approved drugs, because if there had not been an approval in the first place, I can't see how the real-world database would come to contain enough patients treated with said drug.

In summary this study uses an exceptionally well-powered real world database to answer multiple important questions about cancer medicines with tissue-agnostic approvals. This study has the potential to usefully inform current practice in the area of biomarker testing and use of tissue-agnostic therapies. I have only one important question about the data, and a few minor comments.

Finally, if space permits, the authors and editors might include Supplementary Table S1 and Supplementary Figure S1 in the main text; in my view these are important and interesting enough to belong the main text.

Major question:

Is this Caris database specific to advanced/metastatic cancers, or does it encompass cancers of all stages? One might guess that biomarker testing is not often applied to stage 1 cancers, but it would be better to know how this might affect the interpretation of this dataset. A substantial prevalence of early-stage cancers in this data could be an alternative explanation for the seemingly low uptake of NTRK inhibitors: they would not often be needed for stage 1 cancers, for example. If data on stage is unavailable, the question for NTRK inhibitors might alternatively be addressed by examining OS in NTRK-positive tumors that did not receive NTRK inhibitors (with very high survival hinting at early stage diagnoses, and poor survival hinting at advanced disease that meaningfully missed the opportunity to benefit from NTRK inhibition).

Minor questions:

Line 337 (methods) writes "In cases with no insurance claims for a period exceeding 100 days, it was inferred that the patient had deceased."

Does any prior RWE study, or some piece of more granular data (perhaps where death can be definitively known), confirm that this is a valid assumption, at least most of the time?

Line 180 writes

"Together, these data suggest that the initial tissue-agnostic indication for TMB-High cancers granted by the FDA may plausibly extend beyond the tumor types included in the studies that formed the basis of that approval, which may hold true for other tissue-agnostic indications as well."

To me, this language is suggestive of the regulatory approval being extended, but I assume this analysis is about tumor types that were always part of the approval. The language could be more clear and specific, to note that the tissue-agnostic approval in effect extended beyond tumor types included in the studies, and this analysis provides the means to check whether this extension is valid.

Line 82 writes "real-word" instead of "real-world".

Reviewer #2

(Remarks to the Author)

The authors used the data that have clinically tested tissue-agnostic biomarkers and clinical outcomes to show in the real world, how these tissue-agnostic markers might contribute to the overall treatment outcomes across different cancers. The authors identified that 1/5 cancers harbored at least one marker and the most commonly occurred markers are TMB and MSI/MMRd which are indications for immune checkpoint inhibitors. The other markers including NTRK and RET fusion are relatively rare. They also showed the possibility of extending the benefit on clinical outcomes using the tissue agnostic biomarkers to the same class of drugs. The paper in general is well written and the real-world data is important to help assess the impact of these FDA-approved markers and associated treatments. However, given the limited information on these patient cohorts, the novelty is also limited. Most of these markers are well known, and the current clinical associations did not provide additional new information on these biomarkers.

Main Points shown as follows:

1. The cohort had the biomarker testing the potential treatment. However, the assumption would be that most of these patients were treated with multiple agents, so what information can be extracted or obtained from the claims or any other sources to test the biomarker effect on co-treatments or the impact of the co-treatment on the outcomes
2. There was a spectrum of response to, for example, immune checkpoint inhibitors across different cancers, any additional analysis to include factors such as pathology can be performed to provide insights into why some are more sensitive vs. others even though the tumors had the FDA-approved marker.
3. They mentioned the limitation of the study that they do not have access to demographic information, which is a big limitation in the analysis and potentially decreased innovation and new discovery.

Reviewer #3

(Remarks to the Author)

In this paper, Sledge et al. conducted a comprehensive evaluation of clinical outcome on patients treated based on tissue-agnostic indications. By both expanding potential therapeutic avenues for a given tissue-agnostic indication and identifying tumor types most likely to benefit, the authors illustrate the potential of using real-world data to inform the clinical implementation of tissue-agnostic drug approvals.

This is a comprehensive observational study. The significance is high. The findings of the study have the potential to influence clinical decision for oncologists. I do have a few questions. Most are minor.

I have some concerns about data sharing. For such a study that has potential impact on clinical oncology care, it is important to make sure the results are reproducible. I understand the data is sensitive. But with the current data sharing plan, it is simply not possible to reproduce or verify the finding. This needs to be addressed.

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Figure 4. suggest adding a title for the 3 rows so the differences among these plots are easier to tell.

Version 1:

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Reviewer #1

(Remarks to the Author)

The authors have provided thorough answers to each of my questions, with appropriate revisions to the article. The revised article is important, rigorous, and clearly explained, and I enthusiastically recommend it for publication.

Reviewer #2

(Remarks to the Author)

The authors have addressed the concerns. The information from the manuscript is valuable to the community.

Reviewer #3

(Remarks to the Author)

My concerns have been fully addressed in the revision. i have no further comment.

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REVIEWER COMMENTS and RESPONSES (in blue)

Reviewer #1 (Remarks to the Author):

Sledge and colleagues present an important analysis of the efficacy across different tumor types of cancer therapies that have received tissue-agnostic approvals. While the trials that led to these tissue-agnostic approvals generally observed high response rates in cohorts that included a variety of tumor types, they did not have the very large sample sizes needed to adequately assess whether some tumor types are substantially more or less sensitive than others to these therapies. This article investigates some of these therapies by analysis of a real-world database of over a quarter million cancer patients, of whom a few thousand received treatments based on tissue-agnostic approvals. The article also assesses the prevalence of biomarker-positive patients for these indications within various tumor types; this too is important because for some of the rare indications, biomarker testing is not yet widely applied, and these data could help the community to at least prioritize biomarker testing in the cancer types with the highest likelihood of a positive test. For example, this study finds a ~5-fold higher prevalence of NTRK fusions in NSCLC compared with other cancer types.

We appreciate the reviewer's support and recognition of our study's contribution. We fully agree that this work provides critical insights, helping to contextualize tissue-agnostic approvals within real-world evidence and guiding future research and clinical practice.

The most in-depth analyses of the article apply to PD-1 inhibitors for MSS/TMB-high tumors, enabled by the large sample size available for this common indication. Some tumor types that were not included in the pivotal trials were found, in this study, to show similar median time-on-treatment from PD-1 inhibitors as those included in the pivotal trials. This is an important finding supporting the general validity of this particular tissue-agnostic approval. The real-world approach used here will be valuable in future to ask the same question of other approvals. The comparison of Hazard Ratios achieved by Nivolumab or Pembrolizumab, each comparing outcomes in MSS-high vs MSS-low cohorts in the database, was a clever way of using real-world data to compare the activity of competing drugs for the same indication. This finding is also quite important, applying to a frequent tissue-agnostic indication. Although, it is not clear to me that this could be used to extend tissue-agnostic approvals to other non-approved drugs, because if there had not been an approval in the first place, I can't see how the real-world database would come to contain enough patients treated with said drug.

The reviewer raises an excellent point: Since RWE primarily reflects treatments that have been approved and widely adopted, data for non-approved therapies remain limited. While off-label use occasionally occurs, often driven by strong biological rationale or clinical need, such instances are rare and subject to biases, including unequal access to specialized care.

To address this limitation, we have revised the discussion (lines 276-280) to include the following statement: "Although this approach may not be feasible for many targeted therapies, which are rarely used outside of approved indications in clinical practice, immune checkpoint inhibitors such as nivolumab are frequently administered beyond TMB-H and MSI-H/MMRd indications. As TMB and MSI status are routinely assessed through comprehensive NGS testing, ample real-world data exist to investigate whether these tissue-agnostic markers can be extended to nivolumab."

In summary this study uses an exceptionally well-powered real world database to answer multiple important questions about cancer medicines with tissue-agnostic approvals. This study has the potential to usefully inform current practice in the area of biomarker testing and use of tissue-agnostic therapies. I have only one important question about the data, and a few minor comments.

Finally, if space permits, the authors and editors might include Supplementary Table S1 and Supplementary Figure S1 in the main text; in my view these are important and interesting enough to belong the main text.

We agree these are important and have moved these to the main text as suggested.

Major question:

Is this Caris database specific to advanced/metastatic cancers, or does it encompass cancers of all stages? One might guess that biomarker testing is not often applied to stage 1 cancers, but it would be better to know how this might affect the interpretation of this dataset. A substantial prevalence of early-stage cancers in this data could be an alternative explanation for the seemingly low uptake of NTRK inhibitors: they would not often be needed for stage 1 cancers, for example. If data on stage is unavailable, the question for NTRK inhibitors might alternatively be addressed by examining OS in NTRK-positive tumors that did not receive NTRK inhibitors (with very high survival hinting at early stage diagnoses, and poor survival hinting at advanced disease that meaningfully missed the opportunity to benefit from NTRK inhibition).

The Caris database predominantly represents advanced/metastatic diseases, as NGS testing is routinely requested by treating physicians to guide therapy selection in patients with advanced cancers.

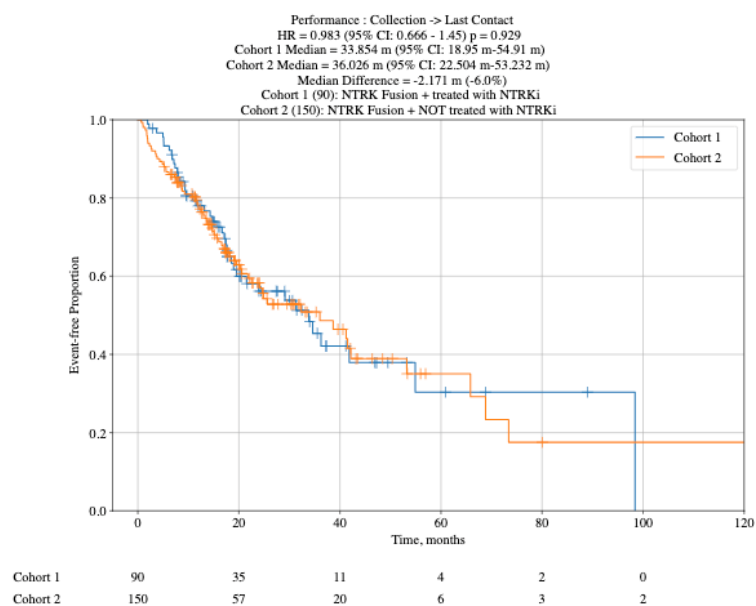
In order to investigate the reviewer's concern regarding earlier-stage cancer comprising a significant fraction of the data, first, we reviewed detailed records of 28 patients in our ROADMAP study (Caris Retrospective Real-world Outcomes-Associated Database, Multi-site Precision Oncology Alliance TM Protocol (ROADMAP). We noted that out of the 22 patients with non-CNS tumors, 18 were confirmed as stage IV at time of tumor profiling, for the remaining 4, although noted as unknown or earlier stage at time of profiling, one was a rare fibrosarcoma of the cervix with residual disease noted after surgery, one was a breast cancer with local recurrence to the axillary lymph node, one was a malignant GIST likely T4b with potential local invasion and the patient also had secondary primary of fibrosarcoma metastatic to C-Spine. The fourth patient had papillary thyroid cancer and the only information available was surgery. For the 6 CNS tumors where the staging information is irrelevant, patients either had grade IV disease at time of profiling, carrying aggressive molecular features like wild type IDH status, had residual disease after surgery or a rare pilocytic brain tumors seen in a mid-aged adult from a challenging location of brain stem. Overall, this review reveals that with extremely rare exceptions, the vast majority of cases included in the Caris database are metastatic or locally advanced diseases.

This new review of ROADMAP database also revealed updates of the data, and we edited the text from previous 8 of 23 NTRK fusion positive cases (data reviewed in end of 2023) treated with NTRK inhibitor to the current record of 14 of 28 treated (line 118).

Secondly, from the larger database, we had an honest broker review the clinical history and pathology reports of 245 cases that were positive for NTRK1/2/3 fusions. Based on limited documents and information available, we were able to confirm that overall, 75% (183) of reviewed cases were diseases of advanced stage while 25% of cases were unknown/unclear for their clinical stage.

In summary, this additional data review confirms that the Caris database largely includes patients with later-stage disease. In both datasets, we also confirmed whether patients were treated with NTRKi or not was independent of whether their disease was confirmed metastatic/advanced.

In addition, we also explored the reviewer’s suggestion to examine overall survival (OS) among NTRK fusion–positive patients who did or did not receive NTRK inhibitors. The Kaplan–Meier curve below indicates that the overall survival of patients not treated with an NTRK inhibitor is similar to the OS of patients who were treated, confirming that the non-treated patients are not predominantly earlier-stage cases. Together, these findings support that the limited uptake of NTRK inhibitors in our dataset is unlikely to be explained by a substantial proportion of early-stage cancers. We appreciate the reviewer’s input and have included this clarification in the revised manuscript (lines 86-87).



Minor questions:
Line 337 (methods) writes “In cases with no insurance claims for a period exceeding 100 days, it was inferred that the patient had deceased.”
Does any prior RWE study, or some piece of more granular data (perhaps where death can be definitively known), confirm that this is a valid assumption, at least most of the time?

We appreciate the reviewer’s thoughtful question. The use of a 100-day inference method to calculate patient survival has been previously described (Abraham et al 2021, CCR, PMID: 33293373) and is routinely used in publications generated from Caris’ database (e.g, Hsiehchen 2024, Cancer Cell, PMID: 39577419; Muquith 2024, Nat Cancer, PMID: 38528112). We have added the Abraham citation to the methods (line 346). A subgroup analysis showed that the assumption holds true for more than 95% of

patients with a recorded death in the National Death Index. In the subset of patients with recorded death in insurance claims database, the overall survival based on documented death differed by <1 month compared to the inferred method (internal data).

Line 180 writes

“Together, these data suggest that the initial tissue-agnostic indication for TMB-High cancers granted by the FDA may plausibly extend beyond the tumor types included in the studies that formed the basis of that approval, which may hold true for other tissue-agnostic indications as well.”

To me, this language is suggestive of the regulatory approval being extended, but I assume this analysis is about tumor types that were always part of the approval. The language could be more clear and specific, to note that the tissue-agnostic approval in effect extended beyond tumor types included in the studies, and this analysis provides the means to check whether this extension is valid.

We agree with the reviewer’s point of view and have reworded this statement (lines 184-185) accordingly: “Together, these data justify and strengthen the pembrolizumab tissue-agnostic indication for TMB-High cancers granted by the FDA based on data from limited cancer types.” As mentioned previously, we have further expanded this point in discussion (line 265-269).

Line 82 writes “real-word” instead of “real-world”.

We have corrected the typological mistake.

Reviewer #2 (Remarks to the Author):

The authors used the data that have clinically tested tissue-agnostic biomarkers and clinical outcomes to show in the real world, how these tissue-agnostic markers might contribute to the overall treatment outcomes across different cancers. The authors identified that 1/5 cancers harbored at least one marker and the most commonly occurred markers are TMB and MSI/MMRd which are indications for immune checkpoint inhibitors. The other markers including NTRK and RET fusion are relatively rare. They also showed the possibility of extending the benefit on clinical outcomes using the tissue agnostic biomarkers to the same class of drugs. The paper in general is well written and the real-world data is important to help assess the impact of these FDA-approved markers and associated treatments. However, given the limited information on these patient cohorts, the novelty is also limited. Most of these markers are well known, and the current clinical associations did not provide additional new information on these biomarkers.

Main Points shown as follows:

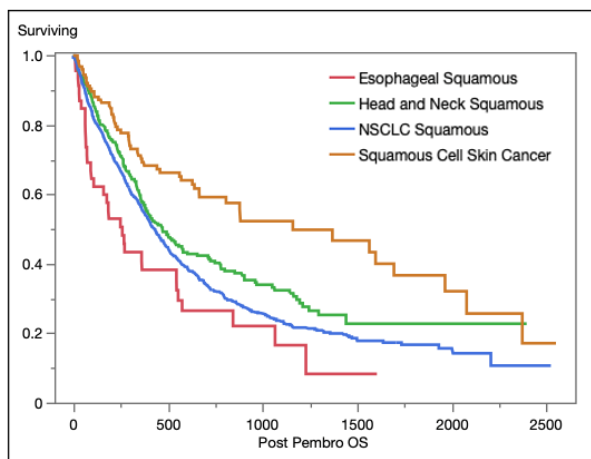
1. The cohort had the biomarker testing the potential treatment. However, the assumption would be that most of these patients were treated with multiple agents, so what information can be extracted or obtained from the claims or any other sources to test the biomarker effect on co-treatments or the impact of the co-treatment on the outcomes

The reviewer rightfully brought up various confounders of the current analysis; while the pathology and demographic information mentioned below was somewhat more straightforward to account for (please see the answers to the two comments below), as the insurance claims dataset captures the real-world clinical use of therapies, the effect of co-treatment is difficult to analyze due to the heterogeneity of treatment strategy at different cancer treatment sites and the complexity of the

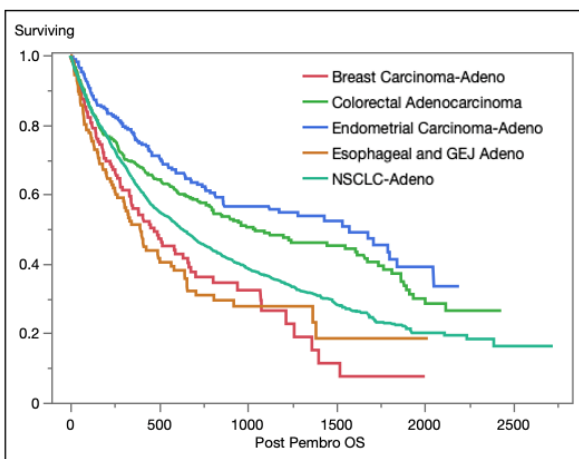
data. However, as the investigated cohort includes patients with metastatic and advanced diseases within each cancer type, it is not unreasonable to assume an overall balance between treatment arms.

2. There was a spectrum of response to, for example, immune checkpoint inhibitors across different cancers, any additional analysis to include factors such as pathology can be performed to provide insights into why some are more sensitive vs. others even though the tumors had the FDA-approved marker.

We recognize the importance of investigating the biological rationale underlying the differences in immune checkpoint inhibitor response and realize that our current data might not have sufficient details to address this point fully, however, when categorizing the 295,316 tumors into different cancer types, we took into account the pathological entities. For example, epithelial ovarian cancers and non-epithelial ovarian cancers are investigated separately (with the latter having too small of cohort size to be included in figure 3). In order to address the reviewer's concern on impact of factors like pathology, we compared IO-associated survival in TMB-H tumors with squamous cell carcinoma histology, including tumors of the esophagus, head and neck, lung, and skin, and noted a wide range of survival where squamous cell skin cancer showed a significantly longer survival than the other three cancer types. Similarly, within adenocarcinoma histology, we compared cancers of the breast, colon, endometrium, esophagus/GEJ and lung. Colorectal and endometrial carcinoma had significantly longest survival while esophagus GEJ tumors had the shortest OS. Our additional analysis shows that overall, TMB-H squamous cell tumors have shorter IO-therapy associated OS than TMB-H adenocarcinoma, and that there is substantial variability within each histological group, similar to the observation in the complete cohort. We have now added this figure to our supplemental materials and referenced it in the discussion (line 257-258).



Group	N	mOS (m)	Lower 95%	Upper 95%
Esophageal Squamous	46	4.3	1.6	9.1
NSCLC Squamous	1065	7.1	6.5	7.8
Head and Neck Squamous	366	7.9	6.4	9.6
Squamous Cell Skin Cancer	126	19.3	11.1	32.7
All Squamous cell carcinoma	1603	7.5	6.9	8.2



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Esophageal and GEJ Adeno	127	6.6	4.4	8.4
Breast Carcinoma-Adeno	139	7.9	5.7	11.0
NSCLC-Adeno	2882	10.5	9.8	11.4
Colorectal Adenocarcinoma	628	17.4	13.5	26.8
Endometrial Carcinoma-Adeno	278	26.2	14.5	30.7
All Adenocarcinoma	4054	11.3	10.6	12.0

3. They mentioned the limitation of the study that they do not have access to demographic information, which is a big limitation in the analysis and potentially decreased innovation and new discovery.

In order to address the reviewer's concern, we extracted key demographic information from the dataset and described the prevalence of "any positive" alterations in the context of patient age, sex and race, ethnicity. The data are now included in Supplementary Tables S2-3 and referenced in the results (lines 103-105).

Reviewer #3 (Remarks to the Author):

In this paper, Sledge et al. conducted a comprehensive evaluation of clinical outcome on patients treated based on tissue-agnostic indications. By both expanding potential therapeutic avenues for a given tissue-agnostic indication and identifying tumor types most likely to benefit, the authors illustrate the potential of using real-world data to inform the clinical implementation of tissue-agnostic drug approvals.

This is a comprehensive observational study. The significance is high. The findings of the study have the potential to influence clinical decision for oncologists. I do have a few questions. Most are minor.

I have some concerns about data sharing. For such a study that has potential impact on clinical oncology care, it is important to make sure the results are reproducible. I understand the data is sensitive. But with the current data sharing plan, it is simply not possible to reproduce or verify the finding. This needs to be addressed.

We understand and appreciate the reviewer's comment. In order to address the concern regarding data sharing, we have adopted the Nature Research data availability statements and data citations policy guidance for authors and edited our data policy statement accordingly. "Data available on request from the authors: The data that support the findings of this study are available from the corresponding author upon reasonable request." This statement is included in the updated manuscript.

While we fully recognize the importance of open data access, as the current study analyzed the entire database at Caris Life Sciences, the scope and sample size preclude us from making all the data publicly available. Further, from a contractual and legal constraint point of view, the data use agreements between Caris, the claims data provider as well as the contributing institutions also limit unrestricted sharing. However, we are fully committed to making the data available for researchers based on their requests and we are willing to providing analytical assistance as well. Please see example publications in which we used the same data sharing policy: (<https://pubmed.ncbi.nlm.nih.gov/36681091/>, <https://pubmed.ncbi.nlm.nih.gov/39577419/>; <https://pubmed.ncbi.nlm.nih.gov/38528112/>). There have been near 300 peer-reviewed publications based on the Caris database and we have been servicing readers' data requests through our letter-of-intent process: <https://www.carislifesciences.com/letter-of-intent/> . In addition, we want to highlight that we provide a total of 14 comprehensive supplementary tables that include a tremendous amount of key datasets and analyses, enabling the readers to reproduce analytical results and conclusions presented.

Table 1. What is the p-value shown in the table? How is it calculated?

In this table, Hazard ratios (HR) were computed utilizing the Cox proportional hazards model, and p values were assessed with the log-rank test. We have added this information to the Table legend.

Figure 1. The heatmap is really large and the fonts are too small to read. It is hard to tell what, if any message the authors are trying to convey in this figure.

We acknowledge that the original figure's size and font make it difficult to interpret and we appreciate the reviewer's feedback. This heatmap aims to provide a visual of variability or imbalance of distribution of the tissue-agnostic alteration across a broad range of cancer types. We used red boxes to mark cancer types where both tissue-specific and biomarker-specific drug approvals exist, and black boxes to indicate cancer types with only tissue-specific drug approvals. These annotations illustrate the current clinical approval landscape, while highlighting the added benefit of tissue-agnostic approvals for improving patient care. Due to the extensive data displayed, we provide a high level description of the data in the main text and a table indexing details in Supplemental Table S1. Based on the reviewer's input, we have increased the font size and adjusted the figure dimensions to improve readability and enhance its accessibility for interpretation.

Figure 2. "(A) Clinical update of NTRK-..." Should this be clinical uptake?
Yes, we have corrected this error in the revised version.

Figure 3. suggest adding sample for each cancer type to the figure legend. Because very small sample size will make the comparison difficult.

We completely agree with the reviewer's point. We have included the cohort size in parentheses along the Y axis. Figure 3 excludes patient cohorts with small sizes; included are cancer types with $N \geq 40$ for A-B (TMB-H patients treated with pembrolizumab), $N \geq 10$ for C-D (MSI-H/MMRd patients treated with pembrolizumab), and $N \geq 25$ for E-F (MSS/TMB-H patients treated with pembrolizumab).

Figure 4. suggest adding a title for the 3 rows so the differences among these plots are easier to tell. We agree with the reviewer's suggestion and have added the titles accordingly.

REVIEWER COMMENTS and RESPONSES (in blue)

Reviewer #1 (Remarks to the Author):

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To address this limitation, we have revised the discussion (lines 276-280) to include the following statement: "Although this approach may not be feasible for many targeted therapies, which are rarely used outside of approved indications in clinical practice, immune checkpoint inhibitors such as nivolumab are frequently administered beyond TMB-H and MSI-H/MMRd indications. As TMB and MSI status are routinely assessed through comprehensive NGS testing, ample real-world data exist to investigate whether these tissue-agnostic markers can be extended to nivolumab."

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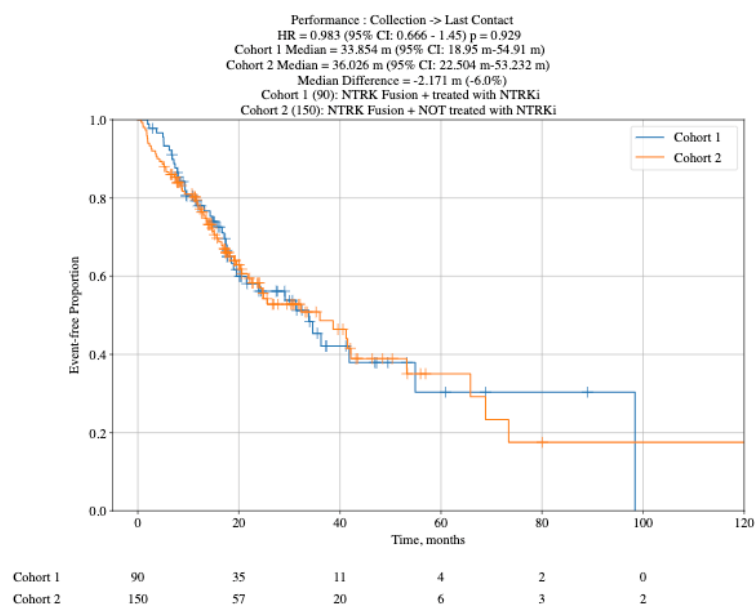
In order to investigate the reviewer's concern regarding earlier-stage cancer comprising a significant fraction of the data, first, we reviewed detailed records of 28 patients in our ROADMAP study (Caris Retrospective Real-world Outcomes-Associated Database, Multi-site Precision Oncology Alliance TM Protocol (ROADMAP). We noted that out of the 22 patients with non-CNS tumors, 18 were confirmed as stage IV at time of tumor profiling, for the remaining 4, although noted as unknown or earlier stage at time of profiling, one was a rare fibrosarcoma of the cervix with residual disease noted after surgery, one was a breast cancer with local recurrence to the axillary lymph node, one was a malignant GIST likely T4b with potential local invasion and the patient also had secondary primary of fibrosarcoma metastatic to C-Spine. The fourth patient had papillary thyroid cancer and the only information available was surgery. For the 6 CNS tumors where the staging information is irrelevant, patients either had grade IV disease at time of profiling, carrying aggressive molecular features like wild type IDH status, had residual disease after surgery or a rare pilocytic brain tumors seen in a mid-aged adult from a challenging location of brain stem. Overall, this review reveals that with extremely rare exceptions, the vast majority of cases included in the Caris database are metastatic or locally advanced diseases.

This new review of ROADMAP database also revealed updates of the data, and we edited the text from previous 8 of 23 NTRK fusion positive cases (data reviewed in end of 2023) treated with NTRK inhibitor to the current record of 14 of 28 treated (line 118).

Secondly, from the larger database, we had an honest broker review the clinical history and pathology reports of 245 cases that were positive for NTRK1/2/3 fusions. Based on limited documents and information available, we were able to confirm that overall, 75% (183) of reviewed cases were diseases of advanced stage while 25% of cases were unknown/unclear for their clinical stage.

In summary, this additional data review confirms that the Caris database largely includes patients with later-stage disease. In both datasets, we also confirmed whether patients were treated with NTRKi or not was independent of whether their disease was confirmed metastatic/advanced.

In addition, we also explored the reviewer’s suggestion to examine overall survival (OS) among NTRK fusion–positive patients who did or did not receive NTRK inhibitors. The Kaplan–Meier curve below indicates that the overall survival of patients not treated with an NTRK inhibitor is similar to the OS of patients who were treated, confirming that the non-treated patients are not predominantly earlier-stage cases. Together, these findings support that the limited uptake of NTRK inhibitors in our dataset is unlikely to be explained by a substantial proportion of early-stage cancers. We appreciate the reviewer’s input and have included this clarification in the revised manuscript (lines 86-87).



Minor questions:
Line 337 (methods) writes “In cases with no insurance claims for a period exceeding 100 days, it was inferred that the patient had deceased.”
Does any prior RWE study, or some piece of more granular data (perhaps where death can be definitively known), confirm that this is a valid assumption, at least most of the time?

We appreciate the reviewer’s thoughtful question. The use of a 100-day inference method to calculate patient survival has been previously described (Abraham et al 2021, CCR, PMID: 33293373) and is routinely used in publications generated from Caris’ database (e.g, Hsiehchen 2024, Cancer Cell, PMID: 39577419; Muquith 2024, Nat Cancer, PMID: 38528112). We have added the Abraham citation to the methods (line 346). A subgroup analysis showed that the assumption holds true for more than 95% of

patients with a recorded death in the National Death Index. In the subset of patients with recorded death in insurance claims database, the overall survival based on documented death differed by <1 month compared to the inferred method (internal data).

Line 180 writes

“Together, these data suggest that the initial tissue-agnostic indication for TMB-High cancers granted by the FDA may plausibly extend beyond the tumor types included in the studies that formed the basis of that approval, which may hold true for other tissue-agnostic indications as well.”

To me, this language is suggestive of the regulatory approval being extended, but I assume this analysis is about tumor types that were always part of the approval. The language could be more clear and specific, to note that the tissue-agnostic approval in effect extended beyond tumor types included in the studies, and this analysis provides the means to check whether this extension is valid.

We agree with the reviewer’s point of view and have reworded this statement (lines 184-185) accordingly: “Together, these data justify and strengthen the pembrolizumab tissue-agnostic indication for TMB-High cancers granted by the FDA based on data from limited cancer types.” As mentioned previously, we have further expanded this point in discussion (line 265-269).

Line 82 writes “real-word” instead of “real-world”.

We have corrected the typological mistake.

Reviewer #2 (Remarks to the Author):

The authors used the data that have clinically tested tissue-agnostic biomarkers and clinical outcomes to show in the real world, how these tissue-agnostic markers might contribute to the overall treatment outcomes across different cancers. The authors identified that 1/5 cancers harbored at least one marker and the most commonly occurred markers are TMB and MSI/MMRd which are indications for immune checkpoint inhibitors. The other markers including NTRK and RET fusion are relatively rare. They also showed the possibility of extending the benefit on clinical outcomes using the tissue agnostic biomarkers to the same class of drugs. The paper in general is well written and the real-world data is important to help assess the impact of these FDA-approved markers and associated treatments. However, given the limited information on these patient cohorts, the novelty is also limited. Most of these markers are well known, and the current clinical associations did not provide additional new information on these biomarkers.

Main Points shown as follows:

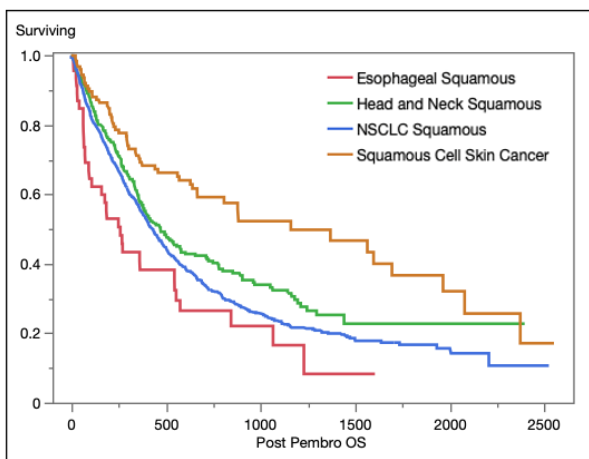
1. The cohort had the biomarker testing the potential treatment. However, the assumption would be that most of these patients were treated with multiple agents, so what information can be extracted or obtained from the claims or any other sources to test the biomarker effect on co-treatments or the impact of the co-treatment on the outcomes

The reviewer rightfully brought up various confounders of the current analysis; while the pathology and demographic information mentioned below was somewhat more straightforward to account for (please see the answers to the two comments below), as the insurance claims dataset captures the real-world clinical use of therapies, the effect of co-treatment is difficult to analyze due to the heterogeneity of treatment strategy at different cancer treatment sites and the complexity of the

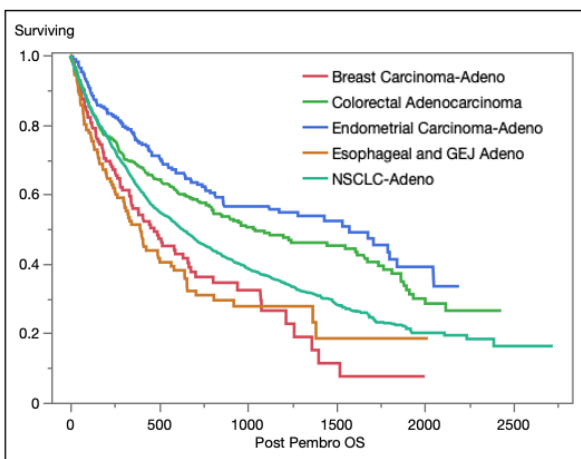
data. However, as the investigated cohort includes patients with metastatic and advanced diseases within each cancer type, it is not unreasonable to assume an overall balance between treatment arms.

2. There was a spectrum of response to, for example, immune checkpoint inhibitors across different cancers, any additional analysis to include factors such as pathology can be performed to provide insights into why some are more sensitive vs. others even though the tumors had the FDA-approved marker.

We recognize the importance of investigating the biological rationale underlying the differences in immune checkpoint inhibitor response and realize that our current data might not have sufficient details to address this point fully, however, when categorizing the 295,316 tumors into different cancer types, we took into account the pathological entities. For example, epithelial ovarian cancers and non-epithelial ovarian cancers are investigated separately (with the latter having too small of cohort size to be included in figure 3). In order to address the reviewer's concern on impact of factors like pathology, we compared IO-associated survival in TMB-H tumors with squamous cell carcinoma histology, including tumors of the esophagus, head and neck, lung, and skin, and noted a wide range of survival where squamous cell skin cancer showed a significantly longer survival than the other three cancer types. Similarly, within adenocarcinoma histology, we compared cancers of the breast, colon, endometrium, esophagus/GEJ and lung. Colorectal and endometrial carcinoma had significantly longest survival while esophagus GEJ tumors had the shortest OS. Our additional analysis shows that overall, TMB-H squamous cell tumors have shorter IO-therapy associated OS than TMB-H adenocarcinoma, and that there is substantial variability within each histological group, similar to the observation in the complete cohort. We have now added this figure to our supplemental materials and referenced it in the discussion (line 257-258).



Group	N	mOS (m)	Lower 95%	Upper 95%
Esophageal Squamous	46	4.3	1.6	9.1
NSCLC Squamous	1065	7.1	6.5	7.8
Head and Neck Squamous	366	7.9	6.4	9.6
Squamous Cell Skin Cancer	126	19.3	11.1	32.7
All Squamous cell carcinoma	1603	7.5	6.9	8.2



Group	N	mOS (m)	Lower 95%	Upper 95%
Esophageal and GEJ Adeno	127	6.6	4.4	8.4
Breast Carcinoma-Adeno	139	7.9	5.7	11.0
NSCLC-Adeno	2882	10.5	9.8	11.4
Colorectal Adenocarcinoma	628	17.4	13.5	26.8
Endometrial Carcinoma-Adeno	278	26.2	14.5	30.7
All Adenocarcinoma	4054	11.3	10.6	12.0

3. They mentioned the limitation of the study that they do not have access to demographic information, which is a big limitation in the analysis and potentially decreased innovation and new discovery.

In order to address the reviewer's concern, we extracted key demographic information from the dataset and described the prevalence of "any positive" alterations in the context of patient age, sex and race, ethnicity. The data are now included in Supplementary Tables S2-3 and referenced in the results (lines 103-105).

Reviewer #3 (Remarks to the Author):

In this paper, Sledge et al. conducted a comprehensive evaluation of clinical outcome on patients treated based on tissue-agnostic indications. By both expanding potential therapeutic avenues for a given tissue-agnostic indication and identifying tumor types most likely to benefit, the authors illustrate the potential of using real-world data to inform the clinical implementation of tissue-agnostic drug approvals.

This is a comprehensive observational study. The significance is high. The findings of the study have the potential to influence clinical decision for oncologists. I do have a few questions. Most are minor.

I have some concerns about data sharing. For such a study that has potential impact on clinical oncology care, it is important to make sure the results are reproducible. I understand the data is sensitive. But with the current data sharing plan, it is simply not possible to reproduce or verify the finding. This needs to be addressed.

We understand and appreciate the reviewer's comment. In order to address the concern regarding data sharing, we have adopted the Nature Research data availability statements and data citations policy guidance for authors and edited our data policy statement accordingly. "Data available on request from the authors: The data that support the findings of this study are available from the corresponding author upon reasonable request." This statement is included in the updated manuscript.

While we fully recognize the importance of open data access, as the current study analyzed the entire database at Caris Life Sciences, the scope and sample size preclude us from making all the data publicly available. Further, from a contractual and legal constraint point of view, the data use agreements between Caris, the claims data provider as well as the contributing institutions also limit unrestricted sharing. However, we are fully committed to making the data available for researchers based on their requests and we are willing to providing analytical assistance as well. Please see example publications in which we used the same data sharing policy: (<https://pubmed.ncbi.nlm.nih.gov/36681091/>, <https://pubmed.ncbi.nlm.nih.gov/39577419/>; <https://pubmed.ncbi.nlm.nih.gov/38528112/>). There have been near 300 peer-reviewed publications based on the Caris database and we have been servicing readers' data requests through our letter-of-intent process: <https://www.carislifesciences.com/letter-of-intent/> . In addition, we want to highlight that we provide a total of 14 comprehensive supplementary tables that include a tremendous amount of key datasets and analyses, enabling the readers to reproduce analytical results and conclusions presented.

Table 1. What is the p-value shown in the table? How is it calculated?

In this table, Hazard ratios (HR) were computed utilizing the Cox proportional hazards model, and p values were assessed with the log-rank test. We have added this information to the Table legend.

Figure 1. The heatmap is really large and the fonts are too small to read. It is hard to tell what, if any message the authors are trying to convey in this figure.

We acknowledge that the original figure's size and font make it difficult to interpret and we appreciate the reviewer's feedback. This heatmap aims to provide a visual of variability or imbalance of distribution of the tissue-agnostic alteration across a broad range of cancer types. We used red boxes to mark cancer types where both tissue-specific and biomarker-specific drug approvals exist, and black boxes to indicate cancer types with only tissue-specific drug approvals. These annotations illustrate the current clinical approval landscape, while highlighting the added benefit of tissue-agnostic approvals for improving patient care. Due to the extensive data displayed, we provide a high level description of the data in the main text and a table indexing details in Supplemental Table S1. Based on the reviewer's input, we have increased the font size and adjusted the figure dimensions to improve readability and enhance its accessibility for interpretation.

Figure 2. "(A) Clinical update of NTRK-...". Should this be clinical uptake?
Yes, we have corrected this error in the revised version.

Figure 3. suggest adding sample for each cancer type to the figure legend. Because very small sample size will make the comparison difficult.

We completely agree with the reviewer's point. We have included the cohort size in parentheses along the Y axis. Figure 3 excludes patient cohorts with small sizes; included are cancer types with $N \geq 40$ for A-B (TMB-H patients treated with pembrolizumab), $N \geq 10$ for C-D (MSI-H/MMRd patients treated with pembrolizumab), and $N \geq 25$ for E-F (MSS/TMB-H patients treated with pembrolizumab).

Figure 4. suggest adding a title for the 3 rows so the differences among these plots are easier to tell. We agree with the reviewer's suggestion and have added the titles accordingly.