**Meta-analysis of the effect of primary tumor location in patients with KRAS mutated vs wild type colorectal liver metastases: Is laterality still prognostic?**

Background: Primary tumor location (PTL) is an important prognostic factor likely related to tumor biology. However, it is unclear whether PTL is prognostic in all colorectal liver metastases (CRLM) patients or only those with wild type KRAS status.

Objective: To determine the effect of PTL on overall survival in KRAS mutated vs wild type CRLM.

Data: We searched PubMed for studies reporting data on 5-year overall survival for CRLM originating from left-sided (LS) versus right-sided (RS) colon cancer stratified by KRAS status. Individual participant data (IPD) were used if available, if not IPD were simulated from the KM curves. To estimate the effect of PTL by KRAS mutation status, we performed two meta-analyses using the two definitions of PTL. To assess the difference between the effects of PTL across the KRAS subgroups, we similarly performed two meta-analyses of interaction terms.

Results: This meta-analysis included 8 studies and 6976 patients. PTL had prognostic value only in patients with wild type tumours (HR for LS: 0.71 [95% CI, 0.62-0.82]), but not in those with KRAS mutated tumors (HR: 0.99 [95% CI, 0.85-1.15]). The meta-analysis of interaction terms showed a significant interaction between tumor side and KRAS mutation status (HR: 1.38 (95% CI 1.24-1.53). Similar results were obtained when another definition of PTL was used.

Conclusions: PTL has a different effect in patients with wild type versus KRAS mutated tumors, with RS tumors translating to worse OS only in the former. Given the wide application of KRAS as a marker of tumor biology and PTL as a prognostic factor, our findings suggest a major change in how we can utilize these two variables.

# Introduction

Primary tumor laterality (PTL) is the most recently identified prognostic factor associated with mortality in patients with resectable colorectal cancer liver metastases (CRLM). In 2016, Sasaki et al was the first to suggest that PTL may be associated with worse overall survival. 27792291 Since then, most studies confirmed that right sided (RS) primaries may show worse overall survival, although others could not show a relationship between PTL and mortality. 29181680 29580735 32011815 Wang et al. synthesized relevant studies published until the end of 2018 and performed the first meta-analysis which showed that RS tumors have worse overall survival than left sided (LS) tumors. 31386192 However, their meta-analysis showed high heterogeneity implying that a subgroup effect may be present.

In 2019, Margonis et al. suggested that this subgrouping variable may be the KRAS mutational status. 31389831 Specifically, they showed that patients with RS tumors had worse overall survival than those with LS tumors, but only in patients with wild type KRAS status and not in those with KRAS mutations. However, this finding contradicted a study by Yamashita et al who showed that RS tumors had worse overall survival in both patients with KRAS wild type and KRAS mutated tumors. 28002060 This debated topic has clinical relevance, as PTL should only be used as a prognostic factor in patients with KRAS wild type status if a subgroup effect is present.

To resolve this debate, we aimed to perform a systematic literature search and meta-analysis using all relevant studies. Given that left sided disease can be defined in two possible ways (excluding or including rectal tumors) we performed two separate meta-analyses using each definition.

# Methods

## Objective

The present study aims to determine whether the effect of primary tumor location (left versus right side according to two different definitions) on overall survival is different between patients with KRAS mutated and KRAS wild-type colorectal cancer liver metastases who underwent metastasectomy. The reporting of this systematic review follows the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement. 33782057

Data Sources and Search Strategies

We performed a comprehensive literature search in the PubMed database for full-text articles published in print or online from inception until May 2021. The detailed search strategy is described in the eFile 1 in the supplement. The search strategy was designed and conducted by an experienced librarian (A.T.) with input from the study investigators. Two authors (M.B and G.A.M.) identified and reviewed full-text articles that were deemed relevant by screening their titles and abstracts. Disagreements between the two reviewers were resolved with consensus. We also manually included relevant studies using the similar articles function of Pubmed.

## Inclusion Criteria

We included original studies that either reported the effect of PTL stratified by KRAS status as a hazard ratio (or any other relevant effect size) or showed Kaplan Meier plots stratified by KRAS status. The outcome of interest was 5-year overall survival measured from the date of CRLM surgery. We excluded studies not written in English, Dutch, Greek, or German. When we encountered more than one study published by the same authors, we selected the newest or most informative article.

Data Extraction

For eligible studies authored by the senior author (G.A.M) or his collaborators from the International Genetic Consortium for Colorectal Liver Metastasis (IGCLM), we received and used individual participant data (IPD). For the remaining studies, we used aggregate data (AD) or we simulated IPD based on their Kaplan Meier plots.

One author (M.B.) extracted prespecified data elements from the eligible studies, including study specific information and the outcome of interest. Study specific information included author name, year of publication, country, study interval, number of patients, definition of right vs left side (and whether rectum was included in the LS), location of the primary tumor, and KRAS mutational status.

The outcome of interest was HR. If other relevant effect size indices were used, we transformed them to HR. If the survival information was only presented in Kaplan Meier survival curves, we simulated their IPD based on the method developed by Guyot et al. 22297116

Statistical Analysis

To estimate the effect of PTL per KRAS mutational status we performed 2 separate meta-analyses according to the two different definitions of PTL. For each study where IPD were available we first applied an univariable Cox PH model per KRAS mutational status group. We extracted their HR along with their standard errors. Subsequently, we combined them along with the corresponding AD estimates using both a fixed and random effect meta-analysis with empirical Bayes τ2.

To assess the difference between the effects of PTL across the KRAS subgroups we performed a meta-analysis of interaction terms. For each study we applied a Cox PH model including KRAS, PTL, and their interaction term (KRAS x PTL).

Subsequently, we pooled the extracted estimates from IPD with the corresponding AD using both a fixed and random-effects meta-analysis with empirical Bayes τ2. 30634920To assess for study heterogeneity, we used the I2 statistic. In case of high heterogeneity, we report the random effects meta-analysis pooled estimate and show both fixed and random effects pooled estimates in their forest plots. By convention, an observed HR of <1 implied better survival for patients with left-sided cancers. Two-sided P < .05 was deemed statistically significant. To inform clinicians what effect to expect in future studies, we also report the 95% prediction intervals of the pooled estimates along with the 95% CIs. 27406637

*Bias assessment:* We did not perform a risk of bias assessment as the quality of the studies was expected to be similar (all were retrospective studies of observational data).

*Publication bias*: To assess whether publication bias was present, we performed both a rank correlation and linear regression test for funnel asymmetry. 21784880

Statistical packages

All analyses were performed using the statistical software R version 3.6.0 (2019-04-26). For data manipulation we used the *tidyverse* package; for the Cox PH we used the *survival* package; and for the meta-analysis we used the meta package.

# Results

## Study Selection

A total of 1169 titles and abstracts were identified by the aforementioned search strategy. After title and abstract screening, ten articles met the eligibility criteria (eFigure 1 in the Supplement). After full text inspection, eight had extractable data and were included in the meta-analysis. IPD data were obtained for three studies, although the study by Gagniere et al was binational and thus IPD were obtained and analysed separately. AD data were used for the other four studies.

# Study Characteristics

The eight studies comprised 6976 patients ranging from 227 to 2655 patients per study (median: 645, IQR: 587.5). The major characteristics are shown in eTables 1 and 2 in the Supplement. With regard to the variables of interest, 1189 patients had KRAS mutated RS tumors, 1273 had KRAS mutated LS tumors, 1248 patients had wild-type RS and 3040 had wild-type LS tumors.

Meta-Analysis of Overall Survival stratified by KRAS mutational status using the first definition of PTL

All our meta-analyses showed high heterogeneity; therefore, we report the random effects pooled HR and include the fixed effects pooled estimate only in our forest plots. For the KRAS mutated tumors the pooled HR was 0.99 (95% CI, 0.85-1.15)(Figure 1A) while for the KRAS wild type tumors the pooled HR was 0.71 (95% CI, 0.62-0.82)(Figure 1B), indicating that PTL has a prognostic value only in patients with wild type tumours.

Meta-Analysis of Overall Survival stratified by KRAS mutational status using the second definition of PTL

When an alternate definition of PTL was used (patients with rectal tumors were not included in the left sided group), the analysis showed similar results confirming that PTL has prognostic value only in patients with wild type tumors. Specifically, the pooled HRs were 0.86 (95% CI, 0.58-1.28) (Figure 2A) and 0.68 (95% CI, 0.54-0.86)(Figure 2B) for KRAS mutated and wild type tumors, respectively.

Meta-Analysis of Overall Survival interaction terms (MA-IT) using the first definition of PTL

The meta-analysis of interaction terms showed that there is a significant interaction between tumor side and KRAS mutational status. Specifically, the pooled HR for interaction terms was 1.38 (95% CI 1.24-1.53)(Figure 3).

Meta-Analysis of Overall Survival interaction terms (MA-IT) using the second definition of PTL

When an alternate definition of PTL was used (patients with rectal tumors were not included in the left sided group), a similarly significant interaction between tumor side and KRAS mutational status was observed. Specifically, the pooled HR for interaction terms was 1.28 (95% CI 1.01-1.62)(Figure 4).

Publication bias: The publication bias tests could not been performed because a minimum of 10 studies is required.

**Discussion**

This meta-analysis included a large number of patients (n=7,165) with data on primary tumor side and KRAS mutational status, and the robustness of the study was further increased by the inclusion of IPD. To our knowledge, it is the first meta-analysis that explicitly investigated whether the effect of PTL is independent of or contingent on KRAS status. The study ultimately showed that PTL and KRAS mutational status have a statistically significant interaction. Specifically, PTL has a different effect in patients with wild type versus KRAS mutated tumors, with RS tumors translating to worse OS only in the former. The variable effect of KRAS status on PTL persisted regardless of whether patients with rectal tumors were included in the LS group. This is important because there is evidence that grouping patients with left sided and rectum rectal tumors may not be methodologically and biologically sound. 29180604 33368279 Importantly, the clinical significance of this meta-analysis is that PTL should only be used to predict survival in patients with KRAS wild type status.

Furthermore, our findings may explain why previous studies on PTL generated conflicting results. For example, a recent meta-analysis on PTL reported that although RS was overall associated with worse OS, about half of the included studies (22/43) did not show significant associations between RS tumors and worse OS. 31386192 Given that the frequency of KRAS mutations varies widely (15-38% according to a recent meta-analysis), it is possible that in smaller studies, a relatively high frequency of KRAS mutations can tip the scale in favour of no survival difference between RS and LS tumors. 26206254

Although this is the first meta-analysis to show a variable effect of PTL based on KRAS mutational status, a previous meta-analysis presented findings that implied such a relationship existed. 31386192. Specifically, Wang et al found that RAS/RAF mutations were one of the three variables that accounted for 99% of that heterogeneity implying that the relationship of PTL and OS may be different across wild-type and KRAS mutated patients. However, due to its different purpose, that meta-analysis did not use patient level KRAS mutational data but only pooled study level frequencies of KRAS mutations. In turn, the lack of patient level information on KRAS status precluded a dedicated analysis on the relationship between PTL and RFS.

The findings of the present meta-analysis are also consistent with a prior study from our group which was the first to investigate the possible interplay of KRAS mutation status and PTL. 27352204 Specifically, in 2016, Sasaki et al found that among patients with wild type KRAS tumors, the overall survival of patients with LS tumors was numerically superior to those with RS tumors (median OS: 65.8 vs 56.4 months, respectively). In contrast, among patients with KRAS mutated tumors, OS was similar (median OS: 46.8 vs 44.0 months for those with RS vs LS tumors, respectively).

These results are in line with those reported by Cavallaro et al, who investigated the relationship between PTL and KRAS status in a mixed National Cancer Data Base (NCDB) cohort of resectable and unresectable patients with CRC and synchronous metastases to the liver. 31899147 Specifically, they found that among those with wild-type tumors, the overall survival of patients with LS tumors was numerically superior to those with RS disease (median OS: 31.5 vs 16.7 months, respectively) while in patients with KRAS mutated tumors OS was comparable (median OS was 21.1 months for those with RS tumors and 23.7 for those with LS tumors). Unfortunately, the NCDB does not provide data on whether curative intent surgery was performed and thus this study could not be included in the present meta-analysis. Importantly, our findings may apply even to patients with non-metastatic CRC. Specifically, a study by Kamphues et al evaluated the interplay between KRAS status and PTL in a cohort of patients with non-metastatic CRC treated at six academic centers in Europe and Japan. In this cohort, KRAS mutation status was only found to be prognostic among patients with LS disease, which is consistent with the present study.

Given the findings of this meta-analysis, it is tempting to speculate on the molecular profiles that make KRAS mutated tumors largely indifferent to PTL. Some studies have suggested that KRAS mutation is only prognostic when there is a coexisting TP53 or SMAD4 mutation. 31221662 31719050 Interestingly, the relatively equal distribution of these two “activating” mutations between RS and LS disease may at least partially account for the similar prognosis of KRAS mutated tumors regardless of PTL. Among patients with wild type tumors, PTL likely impacts outcomes through other activating mutations such as BRAF V600E, which is not only largely mutually exclusive of KRAS and associated with poor prognosis but is also encountered far more frequently in RS disease.

This meta-analysis has some inherent weaknesses that must be acknowledged. All studies included in the meta-analysis were retrospective and thus have inherent limitations including the notable heterogeneity that we observed. To address the high heterogeneity, we employed a random effects model analysis. The strengths of this meta-analysis include its novelty, the large number of patients included, and the incorporation of individual patient data.

Collectively, our findings suggest that patients with RS or LS KRASmut have similarly poor prognoses, while those with RS wild type tumors have a 26% increased risk of death compared to their LS counterparts. This finding may help resolve the current debate on the effect of PTL on survival, as it suggests that the relative frequency of KRAS mutated tumors may determine if PTL will be prognostic in a given cohort. A recent editorial suggested that an open question is if such results stem from true interactions between PTL and KRAS status or by a simple superimposition of distinct effects. 32959141 This meta-analysis was able to answer this question for the first time by detecting significant interaction between PTL and KRAS status. Taken together with existing evidence on nonmetastatic and unresectable metastatic CRC as discussed in this manuscript, our findings suggest that the interactions between KRAS and PTL exist across multiple stages of disease, ranging from non-metastatic CRC to resectable and unresectable metastatic liver disease. Ultimately, given the wide application of KRAS status as a marker of tumor biology and PTL as a predictive and prognostic factor, our findings suggest a major change in how we can utilize these two variables. Specifically, they imply that the current practice of assigning the same points for a right sided tumor regardless of KRAS status, as is the case with the most recent nomogram to predict survival in CRLM, may not be appropriate. 34023948