# Introduction

Primary tumor laterality (PTL) has been identified as a prognostic factor associated with mortality in patients with resectable colorectal cancer liver metastases (CRLM)[citation]. In 2016, Sasaki et al was the first to suggest that PTL may be associated with worse overall survival [citation]. Since then, several subsequent studies had showed unclear results [citations]. Wang et al. performed a meta-analysis showing that right sided (RS) tumors have worse overall survival than left sided (LS) tumors. However, they showed high heterogeneity implying that a subgroup effect may be present and acknowledging the lack of data on KRAS status was a major limitation.

In 2019 Margonis et al. suggested that the effect of PTL and thus its prognostic value may be dependent on KRAS status [citation]. Specifically, they showed that patients with RS tumors had worse overall survival than patients with LS tumors, but the effect was statistically significant only in patients with wild type KRAS status and not in patients with KRAS mutation. [εδώ να γράψουμε λίγα μπλα μπλα]

This finding is important for clinical practice since if the effect of PTL is truly contingent on KRAS status, PTL should only be used in patient with KRAS wild type status. Therefore, the goal of this study is to assess the prognostic role of tumor sidedness according to KRAS mutational status. To that scope, we performed a systematic literature research and meta-analysis. [λίγο μπλα μπλα και εδώ]

# Methods

## Objective

The present study aims to determine whether the effect of primary tumour location (left versus right side) is different between patients with stage IV KRAS mutated and patients with stage IV 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 [cit].

Data Sources and Search Strategies

We performed a comprehensive literature search in PubMed database for full-text articles published in print or online from inception until April 2020. An updated search was performed in April 2021. The detailed search strategy is as described in the Supplementary material. The search strategy was designed and conducted by an experienced librarian (A.T.) with input from the study investigators. Two of us (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 2 reviewers were resolved with consensus. We also included manually relevant studies using the similar articles function of pubmed.

## Inclusion Criteria

Consistent to our goal mentioned above we included studies that showed the effect of PTL stratified on KRAS mutational status either as a hazard ratio or any other relevant effect size or showed Kaplan Meier plots stratified per KRAS status We excluded studies not written in English, Dutch, Greek or German. In the case of more than one studies being published by the same authors or group, the newest or most informative single article was selected.

Data Extraction

From the eligible studies one of us (M.B.) extracted prespecified data elements including study specific information and the outcome of interest. Demographics included author, year of publication, country, number of patients and location of the primary tumour and KRAS mutational status (yes/no).

Outcomes of interest included HR or any other relevant effect size….

Analysis was performed in May 2021.

In case of an eligible study authored by the senior author (G.A.M) or his collaborators from the International Genetic Consortium for colorectal Liver Metastasis (IGCLM) we received individual participant data.

We transformed extracted effect sizes to HR.

In case of studies that reported survival curves we simulated data based on….

To infer the HRs for these studies, we extracted the survival probabilities at 5 years after surgery from each arm (patients with right vs left sided tumors) of the relevant KM plots. Censored subjects were identified either because the study used tick marks to indicate them (this was the case in one study-Chen et al) or

The methodology we used to infer HRs was tested by comparing the inferred HR of the Margonis’ study (the sole study using AD that reported on such HR) to that reported by the authors. Importantly, the inferred HR was almost identical to the reported one.

In regard to IPD, the HR of having a right vs left sided tumor separately in patients with KRAS mutated and wild type tumors was directly calculated using a survival analysis.

Statistical Analysis

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*Interaction analysis*: To avoid ecological bias we performed a 2-stage meta-analysis [citation]. In the first stage, for each study we applied a Cox PH model including KRAS, RTL and their interaction term (KRAS x RTL). Subsequently, we extracted the interaction terms along with their standard errors. In the second stage, we performed a random-effects meta-analysis with empirical Bayes τ2. By convention, an observed HR of <1 implied better survival for patients with left-sided cancers. To assess for across studies heterogeneity we used the I2 statistic. Two-sided P < .05 was deemed statistically significant. In order to inform clinicians what effect is to be expected in future studies, we also report pooled estimate’s 95% prediction intervals along with their 95% CI’s. 27406637

The results are presented in terms of forest plots. 31477023 28258124

*Sensitivity analysis:* Loree et al., showed that rectal tumors have a distinct molecular profile which differs substantially from that of other left sided tumors. 29180604 Kamphues et al showed that patients with non-metastatic CRC revealed a variable interplay of KRAS status and rectal vs other left sided tumors. 33368279 Therefore, with left side and rectum tumors Thus we repeated the meta-analysis as described above after excluded patients with rectal primaries.

*Influence analysis:* This analysis was based on the Leave-One-Out-method. Specifically, we first pooled the extracted (from AD) and calculated (from IPD) hazard ratios (HRs) from the included studies by using the fixed effects meta-analysis method with empirical Bayes τ2. Next, we re-run the analysis K – 1 times, excluding one study at a time as a measure of how sensitive the pooled effect is. “cite second”

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

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

Statistical packages

All analyses were performed in 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 survival package and for the meta-analysis the meta package.

# Results

## Study Selection

A total of 1169 titles and abstracts were identified by the screening electronic search strategy. After title and abstract screening following the aforementioned search strategy, 7 articles met the eligibility criteria (eFigure 1 in the Supplement). After full text inspection all 7 were considered relevant and included in the meta-analysis. IPD data were obtained for 3 studies but the study by Gagniere et al was binational and thus IPD were obtained and analysed separately. AD data were used for the other 4 studies. As such, 8 studies appear in this meta-analysis.

# Study Characteristics

The 7 studies comprised xxx patients ranging from xx to xx patients per study (median, xx). The major characteristics are shown in eTable xx and xx in the Supplement. Rates of RS ranged from xx% to xx% and LS from xx% to xx% of all included patients. Rates of KRAS mutation ranged from xx% to xx% and wild type from xx% to xx% of all included patients.

Meta-Analysis of Overall Survival stratified by the KRAS mutational status

A pooled HR of 1.03 (95% CI, 0.88-1.21) showed that tumor side was not associated with prognosis in patients with KRAS mutated tumors. In contrast, a pooled HR of 0.74 (95% CI, 0.62-0.87) showed that tumor side was associated with prognosis in patients with wild type tumors. Specifically, a left sided primary was associated with a 26% decrease in the risk of death in this patient group.

Sensitivity analysis after excluding patients with rectal tumors

The first sensitivity analysis confirmed the main finding of the meta-analysis as PTL was prognostic only in those with wild type tumors. Specifically, a pooled HR of 0.86 (95% CI, 0.58-1.28) showed that tumor side was not associated with prognosis in patients with KRAS mutated tumors. In contrast, a pooled HR of 0.68 (95% CI, 0.54-0.86) showed that tumor side was associated with prognosis in patients with wild type tumors. Specifically, a left sided primary was associated with a 32% decrease in the risk of death in this patient group.

Meta-Analysis of Overall Survival interaction terms (MA-IT)

The meta-analysis of interaction terms in both the entire cohort and the group pf patients without rectal tumors showed that there is a significant interaction between tumor side and KRAS mutational status. Specifically, for the former the pooled HR for interaction terms was 1.38 and the confidence intervals were 1.23 to 1.56 and for the latter the pooled HR for interaction terms was 1.28 and the confidence intervals were 1.01 to 1.62.

Influence analysis

The influence analysis confirmed the findings of the main analysis. The fixed pooled HR was calculated after excluding one study at a time. After excluding each of the 6 studies, the HR for left sided tumors among wild type patients was even lower than that in the main analysis (0.59 vs. 0.74, respectively). When the Goffredo study was excluded the pooled HR became 0.76, slightly higher that the HR reported in the main analysis (0.76 vs 0.74, respectively).

Similarly, the influence analysis confirmed the findings of the main analysis with regard to the interaction terms. Specifically, the fixed pooled HR for the interaction term ranged from 1.37 to 1.44 when in the main analysis it was 1.38.

Similar results were derived when we applied the influence analysis in the patient cohort after excluding those with rectal tumors. The pooled HR for left sided tumors among wild type patients ranged between 0.66 and 0.74 while a pooled HR of 0.68 was reported in the analysis that included all patients. The pooled HR for the interaction term ranged from 1.12 to 1.41 while a pooled HR of 1.28 was reported in the analysis that included all patients.