Title: ***The impact of primary tumour location and molecular profile on recurrence patterns following liver resection for colorectal liver metastases (CRLM): a multicentre cohort study***

Rationale

Liver resection offers the best chance of long-term survival for patients with colorectal liver metastases (CRLMs), with 5-year overall survival (OS) rates of 40-60%[1, 2]. Despite these encouraging results, recurrence rates following liver resection are high at 50-60%, with the majority occurring within 2 years of resection[3]. Therefore, understanding the factors that influence recurrence patterns and timing is crucial to improve patient selection for surgery and systemic anticancer therapy. Tumour biology may be underlined by molecular characteristics and anatomical location of the primary tumour (right vs. left vs. rectal), however the effect of these factors on recurrence rates in patients with CRLMs is uncertain.

Molecular characteristics of the tumour, particularly mutations in KRAS/NRAS, BRAF and mismatch repair status (microsatellite instability, MSI) have emerged as important prognostic indicators in metastatic colorectal cancer (mCRC)[4-6]. Patients with KRAS and/or BRAF mutations are observed to have more advanced/invasive disease, with BRAF mutations associated with rapid, multisite recurrences following surgery. MSI-high status confers a better prognosis in patients with localised disease, but associated with worse outcomes in mCRC[6]. The poorer survival may relate to high-risk tumour biology as MSI-high metastatic tumours often harbour BRAF mutations and resistance to conventional systemic chemotherapy.

Emerging data suggests that the location of the primary tumour may also influence the characteristics of metastatic disease and patient outcomes. Right sided colon cancers have distinct embryological origin compared to left sided and rectal tumours, which may reflect differences in their genomic patterns. Retrospective studies have reported worse outcomes with right sided primary tumours after CRLM resection, and observational studies have reported more advanced disease with right sided primary tumours (e.g. T4 primary, bilobar metastases, etc)[7].

Given the multifactorial nature of recurrence risk, an integrated approach to patient assessment is required. Current risk-scoring rely either on traditional clinicopathological markers (e.g. Fong Clinical Risk Score), but lack integration with tumour biology. Currently, no studies have specifically examined how primary tumour location interacts with molecular profile to influence recurrence patterns, however characterising this relationship may improve prognostication and our understanding of the natural history of mCRC. For example, certain high-risk constellations (right sided primary + BRAF mutation) may predispose to a particularly aggressive phenotype (widespread recurrence) whereas a left-sided RAS wildtype tumour may represent the opposite end of the spectrum.

This multicentre retrospective cohort study is designed to capture a large real-world cohort to characterise these inter-relationships and their impact on outcome. By identifying how primary tumour sidedness and molecular profile affect recurrence patterns and survival following liver resection, we aim to refine risk stratification.

Study objectives and hypothesis

Hypothesis

Primary tumour location and molecular profile (e.g., KRAS/NRAS, BRAF mutations, MSI status) significantly influence the recurrence patterns (site and timing) and survival outcomes following curative-intent liver resection for colorectal liver metastases (CRLM).

Primary objective

To determine the impact of primary tumour location and tumour molecular profile on patterns of recurrence and survival following curative-intent liver resection for CRLMs. Specifically we will evaluate whether primary tumour location and key mutations are associated with differences in outcome:

- RFS following liver resection
- Site of first recurrence (intrahepatic vs. extrahepatic, lung vs. other)
- Overall survival

Secondary objectives

To determine how the use of systemic therapy (downstaging or post-resection palliative) varies between patients with different primary tumour site, and whether the benefit of chemotherapy is differs by molecular subtype. We also aim to identify predictors of isolated liver only recurrence vs. lung only recurrence versus disseminated disease.

Study design

Multicentre retrospective cohort study of all eligible patients across participating centres. The study is observational with no intervention, focussing on standard of care treatments. The study period is from January 2017- October 2023 to ensure adequate follow up time for recurrence and survival outcomes. The precise timeframe may vary between individual centres depending on when molecular testing became available. April 2025 will remain as the follow up cutoff to ensure each patient has at least 18 months follow up available.

Inclusion criteria

- Adult patients aged ≥18 years at the time of liver surgery with histologically confirmed colorectal adenocarcinoma and liver metastases.
- Patients who underwent curative-intent liver resection from January 2017 to October 2023.
- Known molecular profile for at least 1 of the following: KRAS/NRAS, BRAF or MSI. Ideally, KRAS/NRAS and BRAF status will be known for all patients after 2010.

Exclusion criteria

- Incomplete macroscopic (R2) resection
- Concomitant unresectable extrahepatic disease
- Insufficient data for key variables (unknown primary, lack of follow up data)
- Non-adenocarcinoma histology

Definitions

Right-sided colon is defined as a tumour originating at any site in the colon from the caecum up to, but not including, the splenic flexure. Left sided colon is from the splenic flexure down to the sigmoid colon. Rectal tumours are those originating below the sigmoid colon. For rectosigmoid tumours, we will rely on the surgical designation in the operation note. Primary tumour location will be grouped into 3 categories: Right, left or rectal. If necessitated by the analysis, rectal tumours may be grouped together with left sided tumours depending on group sizes.

Data collection

Key variables will be collected for each consecutive patient (appendix 1) using a standardised excel spreadsheet. If available, RedCap may be used instead. The Newcastle team will be contactable for any questions regarding data collection and categorisation of variables, if required.

Analysis plan

Descriptive statistics will be used to describe the cohort stratified by primary tumour location and molecular subtype.

Our primary outcomes are RFS and OS after liver resection. RFS is from liver resection to the death of first recurrence or death, censoring for patients alive without recurrence at last follow up. OS is from liver resection to death from any cause. Kaplan-Meier curves will be constructed for key subgroup comparisoms:

- Primary tumour location – right vs left vs rectum
- Molecular profile – RAS mutant vs WT, BRAF mutant vs WT, MSI vs MSS
- By combinations for exploratory insight – e.g. right sided + RAS + BRAF vs others, etc.

Separate Cox regression models will be built for OS and RFS to identify potential predictors. Factors will be identified and fitted a priori based on clinical knowledge and evidence in the literature. Criterion based fitting will not be used.

We will perform a competing risk analysis for first site of recurrence. We will consider death without recurrence as a competing event if it occurs. Cumulative incidence functions will be plotted for liver recurrence vs lung recurrence vs other, stratified by factors of interest (primary tumour site, KRAS status, etc).

We will categorise recurrent patients into early (<12 months) and late recurrence (≥12 months), and use logistic regression to identify predictors of early recurrence. We suspect factors like BRAF mutation and high tumour burden to strongly predict early relapse.

Using a landmark analysis or subset analysis, we will compare RFS in those who received perioperative systemic chemotherapy versus those who did not, after adjusting for baseline risk factors. This may replicate the findings of the EORTC trial in a real-world setting. Additionally, we will test the interaction between chemotherapy use and molecular status (e.g. is chemotherapy associated with a larger RFS benefit in left sided RAS WT tumours compared to others).

As an exploratory analysis, we may investigate post-recurrence survival by factors in a separate study.

Latent class analysis

Latent Class Analysis (LCA) will be used to identify hidden subgroups of patients with colorectal liver metastases (CRLM) based on a combination of clinical, molecular, and recurrence-related variables. By uncovering latent (unobserved) subgroups, LCA will provide insights into distinct patterns of disease progression, recurrence, and response to treatment, which can further guide patient management strategies.

Model Selection: LCA will be applied to the data using an unsupervised approach to identify latent classes based on the specified variables. The number of classes will be determined

based on model fit indices, such as Akaike Information Criterion (AIC), Bayesian Information Criterion , and likelihood ratio tests.

Model Fit Evaluation: Multiple models with varying numbers of latent classes will be tested to determine the best-fitting model. Class membership will be based on the maximum posterior probability of each patient belonging to a particular latent class.

Subgroup Analysis: After the latent classes are identified, we will analyse how each class differs with regard to clinical outcomes, such as recurrence patterns, survival rates, and response to treatment.

Class Interpretation: Each latent class will be interpreted based on the probabilities of the observed features within each class, which will help identify meaningful patterns (e.g., high-risk classes with early recurrence, or classes with aggressive disease based on molecular profiles).

Expected Outcomes

Identification of Patient Subgroups: LCA will uncover distinct latent subgroups of patients based on clinical and molecular characteristics that are associated with different recurrence patterns or survival outcomes.

Prognostic Stratification: These subgroups will provide insights into prognostic risk and inform follow-up and treatment decisions, allowing for personalized care strategies.

Clinical Relevance: The results from LCA may help identify specific characteristics of patients who are at high risk of recurrence or those who may benefit from tailored treatments or closer monitoring.

Integration with Other Analytical Methods

Depending on our findings, LCA results will be integrated with supervised machine learning techniques, such as random forests or logistic regression, to develop predictive models for recurrence and survival outcomes, utilizing the latent classes as features in these models.

Missing data

We anticipate some missing data (e.g. MSI status), and will perform multiple imputationfor missing covariates. If MSI is mostly missing,we may exclude it from multivariable models or perform a subgroup analysis of patients with MSI-status available.

References

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Appendix 1 – *List of variables*

Demographics

- Patient ID – coded as centre, and patient number (e.g. N1, denotes the 1st patient in Newcastle)
- Age at liver resection
- Sex
- Performance status (if recorded)
- ASA score
- Smoking status
- BMI

Primary tumour

- Location
    - Right
    - Left
    - Rectum
    - Multiple synchronous primaries – specify number and site of each tumour site
- Histopathology
    - Differentiation
    - Mucinous subtype
    - Signet ring cells
- T-stage
- Lymph node status
- Synchronous or metachronous liver disease (synchronous is defined as a liver lesion detected no later than 6 months from the diagnosis of the primary tumour)

Molecular profile

- KRAS/NRAS status
    - Wild type vs mutant
- BRAF status (V600E vs wild type vs other mutation if reported)
- MS/MMR status

  Note, these are typically tested on the primary tumour, but record both if metastasis is tested and differs from primary

Liver metastases

- Number of liver lesions resected
- Size of largest liver metastasis
- Bilobar disease (yes/no)
- Tumour burden score (see calculation appendix 2 below)

- Background liver status (normal vs steatotic)

Liver resection

- Date of surgery
- Approach (open vs laparoscopic vs robotic)
- Resection type (**definitions in appendix 3**)
- Magnitude of resection
    - Minor ≤2 segments
    - Major ≥3 segments
- Margin status (R1 if tumour <1mm from cut surface)
- Concurrent local ablation (yes/no)

Postoperative course

- Clavien-Dindo grade within 90 days
- Postoperative bile leak (ISGLS)
- Post-hepatectomy liver failure (ISGLS)
- Post-hepatectomy haemorrhage (ISGLS)
- Reoperation (for any cause)
- 90-day mortality (Yes/no)

Systemic therapy

- Neoadjuvant
    - Regimen
    - Duration (number of cycles)
    - Use of biologics
- Adjuvant chemotherapy
    - Regimen
    - Duration (number of cycles)
    - Use of biologics (type)
- Other treatment (e.g. SIRT, radiation, etc)

Outcomes

- Recurrence
    - Yes/No
    - Date of recurrence following liver resection
    - Site of recurrence (liver, lung, peritoneal, nodal, other, multisite)
    - Recurrence pattern
        - Intrahepatic vs extrahepatic vs mixed
        - Early (<12 months) vs late (≥12 months)
    - Treatment of recurrence (resection, ablation, systemic therapy, best supportive care)

Survival status

- Date of last follow up
- Alive or deceased at last follow up
- Cause of death (cancer-specific vs other cause)
- Disease status at last follow up (no evidence of disease, alive with disease, deceased)

Appendix 2 – *Calculation of tumour burden score*

$TBS^2 = (\text{Maximum tumour diameter})^2 + (\text{number of tumours})^2$

Appendix 3 – *Definitions of different resection types*

- Right hemihepatectomy
    - *Removal of the right lobe, including segments 5, 6, 7 and 8*
- Left hemihepatectomy
    - *Removal of the left lobe, including segments 2, 3 and 4 (a and b)*
- Extended right hepatectomy
    - *Removal of the right lobe, in addition to segment 4*
- Extended left hepatectomy
    - *Removal of the left lobe, in addition to segments 5 and 8*
- Central hepatectomy
    - *Removal of the central portion of the liver, including segments 4, segment 5 and/or segment 8*
- Right anterior sectionectomy
    - *Removal of the right anterior section (segments 5 and 8)*
- Right posterior sectionectomy
    - *Removal of the right posterior section (segments 6 and 7)*
- Left lateral sectionectomy
    - *Removal of left lateral section (segments 2 and 3)*
- Anatomical segmentectomy
    - *Removal of a single liver segment based on vascular and biliary anatomy and following anatomical segmental boundaries*
- Non-anatomical metastatectomy
    - *A wedge resection of the liver where the tumour is removed without adhering to segmental boundaries*