Genomic Predictors of Recurrence Patterns After Complete Resection of Colorectal Liver Metastases and Adjuvant Hepatic Artery Infusion Chemotherapy

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Abstract

Background: Despite curative hepatectomy, most colorectal liver metastasis (CRLM) patients relapse locally within two years. Genomic predictors for hepatic recurrence are poorly understood. This study aims to identify genomic signatures for recurrence in resected CRLM patients treated with adjuvant hepatic artery infusion (HAI) and/or systemic (SYS) chemotherapy.

Methods: Patients undergoing curative hepatectomy and adjuvant HAI+SYS or SYS between January 2000-October 2017 with next-generation sequencing data were catalogued. Gene and signaling-level alterations were checked for association with time to any (AR), liver (LR), and extrahepatic recurrence (ER) using Kaplan-Meier analysis.

Results: Of 172 receiving HAI+SYS, 100 patients recurred, with 69 LR and 83 ER. Five and ten-year LR rates were 57%(95%CI=48-65%) and 51%(95%CI=41-60%), respectively. Five and 10-year ER rates were 51%(95%CI=43-58%) and 45%(95%CI=36-54%), respectively. More ER was observed with tumors harboring

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altered *KRAS*(38%[95%CI=25-50%] vs 63%[95%CI=53-71%], p-adj=0.003) and RAS/ RAF(36%[95%CI=25-48%] vs 66%[95%CI=56-74%], p-adj<0.001) than wild-type. Co-altered RAS/RAF-*TP53* was associated with worse AR(26%[95%CI=14-40%] vs 48%[95%CI=39-57%], p-unadj<0.001), ER(30%[95%CI=17-45%] vs 62%[95%CI=53-70%], p-unadj<0.001), and LR rate(40%[95%CI=24-57%] vs 70%[95%CI=60-77%], p-unadj=0.002). On multivariable analysis, controlling for clinical risk score, ablation, margin status, and primary T-stage, co-altered RAS/RAF-*TP53* was associated with increased risk for AR(HR=2.14, 95%CI=1.38-3.31, p-unadj<0.001), LR(HR=1.79, 95%CI=1.06-3.02, p-unadj=0.029), and ER(HR=2.81, 95%CI=1.78-4.44, p-unadj<0.001).

Conclusions: Altered *KRAS*, RAS/RAF, and RAS/RAF-*TP53* associated with earlier local and distant recurrence in resected CRLM patients receiving adjuvant HAI+SYS. Co-altered RAS/ RAF-*TP53* was a novel predictor of LR warranting investigation of whether genomic cooperativity is associated with this relapsing phenotype. Systemic therapies tailored to high-risk tumor biology are needed to reduce distant relapse after hepatectomy.

INTRODUCTION

Colorectal cancer is the second most common cause of cancer death in the United States.[1] Most patients with resectable colorectal liver metastases (CRLM) relapse after hepatectomy and these recurrences frequently involve the liver remnant.[2,3] Among patients with completely resected CRLM, it is unclear which populations are at greater risk for hepatic or extrahepatic recurrence making it difficult to tailor appropriate adjuvant treatments and surveillance strategies.

Since liver recurrence is common after CRLM resection, adjuvant hepatic artery infusion (HAI) chemotherapy has been utilized to reduce the risk of hepatic relapse. Adjuvant HAI chemotherapy has been shown in randomized trials to significantly reduce intrahepatic recurrence and prolong overall recurrence-free survival.[4,5] The local impact of adjuvant HAI chemotherapy on CRLM patients harboring different genomic profiles, however, remains poorly understood. Prior reports suggest that treatment of CRLM with some systemic agents may induce selective expansion of tumor cells with more aggressive genomic profiles,[6] however, these studies have not identified specific profiles associated with hepatic recurrence after resection. Alterations in the RAS (e.g. *KRAS*, *NRAS*, *BRAF)* pathway, have been shown to be associated with a greater risk for lung, peritoneal, and brain recurrence after hepatectomy.[7-9]

Prior reports have investigated clinical and pathologic predictors of recurrence patterns in resected CRLM cohorts treated with adjuvant systemic chemotherapy, however, genomic predictors of recurrence patterns in resected cohorts treated with combined adjuvant HAI and systemic chemotherapy have not been studied. Investigation of cohorts that recur despite complete resection and combined HAI and systemic chemotherapy may unmask unique genomic signals associated with overall recurrence and/or specific recurrence patterns. In this study, we sought to identify genomic correlates of recurrence patterns in resected CRLM patients treated with adjuvant systemic and HAI chemotherapy.

METHODS

Patient and Tissue Selection

All sequenced CRLM patients undergoing complete resection followed by adjuvant HAI and systemic chemotherapy (HAI-CRLM) between January 1, 2000 and October 31, 2017 were identified from a prospectively maintained hepatectomy database. Race and ethnicity data were not recorded into this database during the study period. Patients with preoperatively identified extrahepatic disease, mismatch repair mutations, no HAI chemotherapy administration (i.e., floxuridine), ablation without resection, or with insufficient follow up (<3 months) were excluded. Patients with recurrent disease within 3 months of hepatectomy were presumed to have occult synchronous disease and were also excluded. For comparison of clinicopathologic and genomic profiles to HAI-CRLM patients, a sequenced cohort of completely resected CRLM patients treated with adjuvant systemic chemotherapy alone (SYS-CRLM) between January 1, 2000 and October 31, 2017 without extrahepatic disease, mismatch repair mutations, ablation without resection, or insufficient follow up (<3 months) were identified.

Clinicopathologic characteristics, surgical history, perioperative outcomes, survival, and recurrence patterns were collected. The clinical risk score (CRS), a composite metric of 5 parameters predicting higher risk of disease recurrence following curative-intent hepatectomy, was calculated as defined previously.[10] High CRS patients were defined as having a score of 3 or higher. Right colon tumors were defined as arising from the cecum to the distal transverse colon and left colon tumors originating from the splenic flexure to the distal sigmoid colon. Rectal tumors were defined as originating at or below the rectosigmoid junction. Preoperative chemotherapy was considered treatment with any systemic agent in anticipation of curative-intent hepatectomy. Adjuvant systemic chemotherapy was considered any therapy given after hepatectomy without evidence of active disease. Postoperatively, patients were monitored every 3-6 months for five years and then typically annually thereafter. Follow up included tumor marker serology and contrast-enhanced computed tomography and/or magnetic resonance imaging. Recurrent disease was established by identifying a new lesion on cross-sectional imaging and/or biopsy. This study was approved by our Institutional Review Board.

Genomic Analysis

Paired DNA from tumor specimens and matched normal tissue underwent targeted next generation sequencing using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay–a platform that detects point mutations, copy number alterations, and gene fusions from 341 (early iteration) to 468 (modern iteration) cancer-associated genes.[11] Sequenced genomic data was stored for analysis on a secure server for large-scale cancer genomics data (cBioPortal for Cancer Genomics).[12,13] Actionable gene targets were defined by the OncoKb database as somatic alterations reported to confer some heightened response or resistance to therapy relative to the wild-type (wt) configuration. Driver gene alterations resulted in some selective advantage for tumorigenesis such as gain-of-function for proto-oncogenes or loss-of-function for tumor suppression genes.[14] When both primary colorectal and CRLM tumor sequencing

data were available, the latter was used given its proximity to the date of hepatectomy. Genomic profiles of primary colorectal tumors were considered eligible for inclusion based on reports noting high genomic concordance between matched primary and metastatic liver lesions.[15,16] Select mutations from genes and signaling pathways were examined for associations with recurrence patterns if at least 10 patients had a mutation (mt) present.

Statistical Analysis

Time to any recurrence (AR), any liver recurrence (LR), and any extrahepatic recurrence (ER) were assessed from hepatectomy until detection of that specific recurrence. Regardless of whether recurrent disease occurred at an extrahepatic site or in the liver previously, detection of any LR or any ER were documented, respectively. Kaplan-Meier methods and the log-rank test assessed relationships between outcomes and genomic variables. Patients who died or were alive without recurrence at last follow up were censored. On univariable analyses, false discovery rate corrections were applied within each outcome for with possible genomic correlates.

Given the potential prognostic relevance of co-altered *TP53* and RAS/RAF pathway mutation (*KRAS*, *ARAF*, *BRAF*, *NRAS*, *HRAS*),[17,18] the relationship between co-altered *TP53* and RAS/RAF with recurrence was assessed. First, a dependent or multiplicative effect between RAS/RAF and *TP53* was checked using an interaction term in a logistic or Cox model, where appropriate. Next, an additive component to RAS/RAF-*TP53* co-occurrence was assessed with bivariable (*TP53* and RAS/RAF) Cox or logistic models as positive for both *TP53* and RAS/RAF (mt-mt) versus all other options in univariable analyses. Classification and regression tree (CART) analyses explored subgroups that could be masked through standard modeling procedures. For CART analyses, an exponential distribution, ten-fold cross validation, and cross-complexity pruning were used. Effect sizes were represented by the lambda (λ) parameter estimate (PE). P-values for co-occurrence analyses were not adjusted (p-unadj).

Cox regression methods assessed the relationship between clinicopathologic characteristics and outcomes. The composite CRS was utilized herein rather than its individual components. Multivariable models were built for significant clinicopathologic factors with altered RAS/RAF, *TP53*, or co-altered RAS/RAF-*TP53* for the investigated outcomes. Clinical characteristics and mutation rates were compared between the HAI-CRLM and SYS-CRLM cohorts with Fisher's Exact test and Wilcoxon Rank Sum test where appropriate. Univariable KM analyses were repeated in the SYS-CRLM patients.

Unless otherwise stated, unadjusted two-sided p-values less than 0.05 were considered statistically significant. Recursive partitioning was performed with the rpart package (version 4.1-13, 2018) in Cran R (R Core Team, Vienna Austria) and all other analyses were performed with SAS 9.4 TS1M5 (The SAS Institute, Cary, NC).

RESULTS

Clinical Characteristics and Recurrence Patterns of Resected HAI and SYS CRLM Patients

Of 230 sequenced CRLM patients operated upon during the study period, 172 (75%) underwent adjuvant HAI/SYS and 58 (25%) underwent only adjuvant SYS. Of the 172 HAI-CRLM patients, 56% (n=96/172) were male and the median age at hepatectomy was 54 years (range=28-77). At resection, 19% (n=32/172) underwent ablation combined with resection and 6% (n=10/172) had positive margins. Compared to 58 SYS-CRLM patients, the HAI-CRLM cohort was younger, had more liver metastases, more commonly received preoperative chemotherapy, underwent major hepatectomy more frequently, and had fewer positive margins (Table 1).

Median follow up among HAI-CRLM survivors (n=152) was 41 months (range=11-197) and median time to AR was 20 months (95%CI=15-37). Five and ten-year rates free of AR were 40% (95%CI=32-48%) and 39% (95%CI=31-46%), respectively (Figure 1A). Accounting for all recurrences, 69 patients developed LR and 83 ER. By last follow up, 17 recurred only in the liver, 31 recurred at an extrahepatic site only, 52 recurred in both the liver and an extrahepatic site, and 72 had no recurrences detected. Five and ten-year rates free of LR were 57% (95%CI=48-65%) and 51% (95%CI=41-60%), respectively (Figure 1B), and 5 and 10-year rates free of ER were 51% (95%CI=43-58%) and 45% (95%CI=36-54%), respectively (Figure 1C).

Median follow up among SYS-CRLM survivors (n=43) was 32 months (range=10-131) and median time to AR was 14 months (95%CI=11-22). Accounting for all recurrences, 37 patients developed LR and 37 ER. By last follow up, only 7 recurred in the liver, only 7 recurred at an extrahepatic site, 30 recurred in both the liver and an extrahepatic site, and 14 had no recurrences detected.

Genomic Profiles Associated with Recurrence

The most commonly altered genes among the HAI-CRLM patients were *APC* (85%, n=146/172), *TP53* (80%, n=137/172), and *KRAS* (33%, n=57/172, Figure 2). The most commonly altered signaling pathways were Wnt (92%, n=159/172), p53 (84%, n=144/172), RTK-RAS (55%, n=94/172), PI3K (31%, n=54/172), Notch (23%, n=40/172), TGF-beta (14%, n=24/172), HIPPO (12%, n=21/172), and Cell Cycle (6%, n=10/172).

No specific gene or signaling pathway alterations were significantly associated with worse AR and LR rates in HAI-CRLM patients. Compared to the wild-type, HAI-CRLM patients had worse ER rates with tumors altered at *FLT3* (3yr. 12% [95%CI=1-41%] vs 57% [95%CI=49-64%], p-adj=0.037), *KRAS* (3yr. 38% [95%CI=25-50%] vs 63% [95%CI=53-71%], p-adj=0.003), and *SMAD4* (3yr. 31% [95%CI=11-54%] vs 57% [95%CI=49-64%], p-adj=0.029, Supplemental Figure 1). At the signaling pathway level, alterations in the RTK-RAS (3yr. 41% [95%CI=31-51%] vs 71% [95%CI=59-80%], p-adj<0.001), RAS/RAF (3yr. 36% [95%CI=25-48%] vs 66% [95%CI=56-74%], p-adj<0.001), and Cell cycle pathways (3yr. 13% [95%CI=1-43%] vs 38% [95%CI=19-56%], p-adj=0.010) were significantly associated with worse ER rates (Supplemental Figure 2).

The SYS-CRLM cohort had a higher proportion of *KRAS* (55% vs 33%, p-unadj=0.005), *BRAF* (10% vs 2%, p-unadj=0.018), and *SMAD4* alterations (21% vs 9%, p-unadj=0.034) compared to the HAI-CRLM cohort. Additionally, SYS-CRLM patients had more RTK-RAS (76% vs 55%, p-unadj=0.005), RAS/RAF (69% vs 38%, p-unadj<0.001), and TGF-Beta pathway alterations (26% vs 14%, p-unadj=0.044). For this cohort, no genes or signaling pathways were significantly associated with AR, LR, or ER.

Additive and Interactive Analyses of TP53 and RAS

Co-alteration of RAS/RAF-*TP53* in HAI-CRLM patients was associated with worse AR (3yr. 26% [95%CI=14-40%] vs 48% [95%CI=39-57%], p-unadj<0.001), LR (3yr. 40% [95%CI=24-57%] vs 70% [95%CI=60-77%], p-unadj=0.002), and ER (3yr. 30% [95%CI=17-45%] vs 62% [95%CI=53-70%], p-unadj<0.001). In bivariable analyses, RAS/RAF remained significantly associated with worse AR (HR=1.96, 95%CI=1.29-2.96, p-unadj=0.002), LR (HR=1.80, 95%CI=1.09-2.97, p-unadj=0.021), and ER (HR=2.63, 95%CI=1.67-4.13, p-unadj<0.001). Altered *TP53* in bivariable analyses, however, was not found to have a significant association with AR (p-unadj=0.07), LR (p-unadj=0.08), or ER (p-unadj=0.23). No significant interactions were found between RAS/RAF and *TP53* for AR (p-unadj=0.68), LR (p-unadj=0.74), or ER (p-unadj=0.39).

When RAS/RAF-*TP53* co-alteration was tested among SYS-CRLM patients, no significant association was found with AR (3yr. 19% vs 26%, p-unadj=0.51), LR (3yr. 38% vs 43%, p-unadj=0.60), or ER (3yr. 31% vs 38%, p-unadj=0.26).

Classification and Regression Tree Analysis of Genomic Correlates for Recurrence

In the model for AR, an initial split occurred on RAS/RAF alterations with PE=0.82 for wt-RAS/RAF and PE=1.39 for mt-RAS/RAF indicating greater risk of recurrence (Figure 3). A subsequent split occurred on mt-RAS/RAF with *TP53* alterations where PE=0.97 for wt-*TP53* and PE=1.67 for mt-*TP53*. The three terminal nodes had the following 5-year rates free of AR: wt-RAS/RAF (48%, 95%CI=38-58%), mt-RAS/RAF+wt-*TP53* (47%, 95%CI=26-66%), and mt-RAS/RAF+mt-*TP53* (26%, 95%CI=14-40%). Attempts to build trees for ER and LR failed, as no splits remained after pruning.

Clinicopathologic and Genomic Factors Associated with Recurrence

On univariable analysis, advanced primary T-stage, high CRS, positive margins, and ablation with hepatectomy were associated with worse AR. Younger age at hepatectomy, high CRS, minor hepatectomy, positive margins, and ablation were associated with worse LR. Younger age at hepatectomy, advanced primary T-stage, and high CRS were associated with worse ER (Table 2).

Multivariable analyses were used to investigate the association of co-altered RAS/RAF-*TP53* with recurrence patterns while controlling for significant clinical covariates. Co-altered RAS/RAF-*TP53* (HR=2.14, 95%CI=1.38-3.31, p-unadj<0.001), advanced primary T-stage (HR=2.01, 95%CI=1.06-3.82, p-unadj=0.032), and positive margins (HR=2.32, 95%CI=1.13-4.74, p-unadj=0.022) remained significantly associated with worse AR. Additionally, co-altered RAS/RAF-*TP53* (HR=1.79, 95%CI=1.06-3.01, p-unadj=0.029),

age at hepatectomy (HR=0.97, 95%CI=0.95-0.99, p-unadj=0.005), ablation (HR=2.04, 95%CI=1.17-3.53, p-unadj=0.012), minor hepatectomy (HR=0.51, 95%CI=0.30-0.87, p-unadj=0.014), and positive margins (HR=2.38, 95%CI=1.03-5.49, p-unadj=0.042) remained significantly associated with LR. Co-altered RAS/RAF-*TP53* (HR=2.81, 95%CI=1.78-4.44, p-unadj<0.001), advanced primary T-stage (HR=2.06, 95%CI=1.02-4.16, p-unadj=0.044), and high CRS (HR=1.70, 95%CI=1.08-2.67, p-unadj=0.021) remained significantly associated with worse ER.

DISCUSSION

Although hepatectomy is the only curative option,[19,20] most CRLM patients relapse within two years of resection.[21] The liver is the most common site of recurrence and, as a result, hepatic metastases are the most common cause of death.[21] Novel therapies are necessary to reduce the risk for liver relapse after hepatectomy. Decreased liver recurrence has been reported with adjuvant HAI and systemic chemotherapy after complete resection of CRLM across four randomized trials.[4,22-24] Consequently, a unique opportunity arises to study predictors of recurrence patterns after curative hepatectomy, adjuvant HAI, and systemic chemotherapy. In this report, altered RAS/RAF and co-altered RAS/RAF-*TP53* were associated with worse AR, LR, and to a greater extent, ER for CRLM patients after resection with adjuvant HAI and systemic chemotherapy, but not systemic chemotherapy alone.

Predictably, advanced primary T-stage and high CRS were associated with worse ER reflecting more advanced initial tumors in HAI-CRLM patients. Independent risk factors for worse LR represented variables associated with patients at risk for having residual microscopic disease including minor hepatectomy, ablation with resection, and positive margins. Younger age at hepatectomy was also associated with worse LR, however, this likely reflects that younger patients have more advanced disease at presentation as noted in prior work and this cohort.[25,26]

The genomic profile of the HAI-CRLM cohort resembles multiple other reports on resected CRLM patients; for example, *BRAF* and *KRAS* were altered in less than 5% and a third of patients, respectively.[27-29] Altered *KRAS* was associated with worse ER, recapitulating findings from earlier work.[30,31] Although *KRAS* alteration has been linked to worse outcomes,[32-35] HAI chemotherapy after hepatectomy has been associated with prolonged survival regardless of *KRAS* status.[36] Interestingly, the SYS-CRLM cohort in this study had significantly more *KRAS* and RAS/RAF-altered patients; however, no significant associations with AR, LR, or ER were found. The lack of an identifiable association could be due to limited sampling. Alternatively, this may suggest that an additional trigger for relapse may be attributable to patient characteristics, the tumor, or selection of HAI in the first place. Additionally, HAI-CRLM patients can receive less systemic doses due to the added regional regimen, which may impact the risk for recurrence, however, this study was not designed to investigate this association. Given the increased risk for ER with RAS/RAF and RAS/RAF-*TP53* altered tumors, improved systemic chemotherapy options are necessary to improve distant control.

Tumors with altered RAS/RAF are notably resistant to anti-epidermal growth factor receptor chemotherapy[37,38] and are also associated with more aggressive features like poor pathologic response to chemotherapy,[7,39,40] diaphragm invasion,[41] and positive margins.[42,43] As a result, some have argued that hepatectomy in the context of RAS alteration may not be justified[44] due to its association with worse overall survival,[7-9,39-45] recurrence-free survival,[7,39,40,43,46] and post-recurrence survival.[47,48] A report from Passot et al found no RAS-altered survivors four years out from resection,[44] however, other reports have indicated that RAS alteration does not lead to a substantial decline in survival until co-altered with *TP53*.[49] Moreover, 5-year survival for the RAS/RAF-altered HAI-CRLM cohort herein was 70%, potentially from achieving regional hepatic control, as local recurrence after hepatectomy is a poor predictor of survival.[3] Given the possibility of long-term survival, RAS alteration should not preclude hepatectomy. Other reports have described associations between RAS alteration with lung and peritoneal recurrence after resection of CRLM,[7-9] however, this study is the first to show an association with aggregate ER. Notably, RAS alteration was not associated with LR in prior reports[7-9], however on bivariable analysis, an association was detected. Altered *TP53* was not associated with LR on univariable or bivariable analyses. Interestingly, co-altered RAS/RAF-*TP53* was associated with worse AR, LR, and ER suggesting that *TP53* alteration could play a collaborative role in promoting relapse. Preclinical evidence for the interplay between loss-of-function p53 alteration and RAS activation has been reported.[50,51] Emerging data also suggests that *TP53* alteration negatively correlates with cytolytic immune cell activity suggesting that liver recurrence after hepatectomy may leverage immune evasion to flourish.

Limitations

Interpretation of these findings should be done cautiously due to the limited number of recurrence events in patients altered for *FLT3*, *SMAD4*, and Cell Cycle pathway genes. Additionally, inclusion of patients with available sequencing data or those treated with adjuvant local and systemic chemotherapy created a selection bias. Patients with available sequencing data likely represent those with higher risk biology, which may have prompted sequencing in the first place. This is reflected by the relatively fewer patients in the SYS-CRLM cohort during the same study period. However, this patient sample was a unique cohort to study drivers of recurrence that emerge following immense selective pressure. Although the genomic profile of the HAI-CRLM cohort differed from the SYS-CRLM patients, tumor profiles from published work were more consistent with our HAI cohort. Regardless, studying a larger SYS-CRLM cohort for comparison would be beneficial. Finally, it is unclear if sequencing a single tumor sample is the best strategy to profile the cancer genome given that CRLM patients have, by definition, systemic disease. Although high concordance between the genomic profiles of primary and metastatic liver tumors has been noted,[52] recent reports call into question whether newer, non-invasive biomarkers such as circulating tumor cells or DNA would make for more reliable tumor markers.[53] Nonetheless, high concordance has been reported between CRLM and the primary colorectal tumor[16,52,54] but not with extrahepatic metastases[9] supporting the use of the primary and liver tumors and exclusion of extrahepatic samples.

CONCLUSIONS

Altered RAS/RAF and RAS/RAF-*TP53* CRLM tumors were associated with worse AR, LR, and ER for resected patients after adjuvant HAI and systemic chemotherapy, but not systemic chemotherapy alone. Future investigations into genomic profiles of recurrence should incorporate RAS/RAF-*TP53* co-alteration and seek other unexplored drivers of local and distant recurrence arising in locally treated patients. Furthermore, better systemic chemotherapy options are necessary to improve distant control after complete hepatectomy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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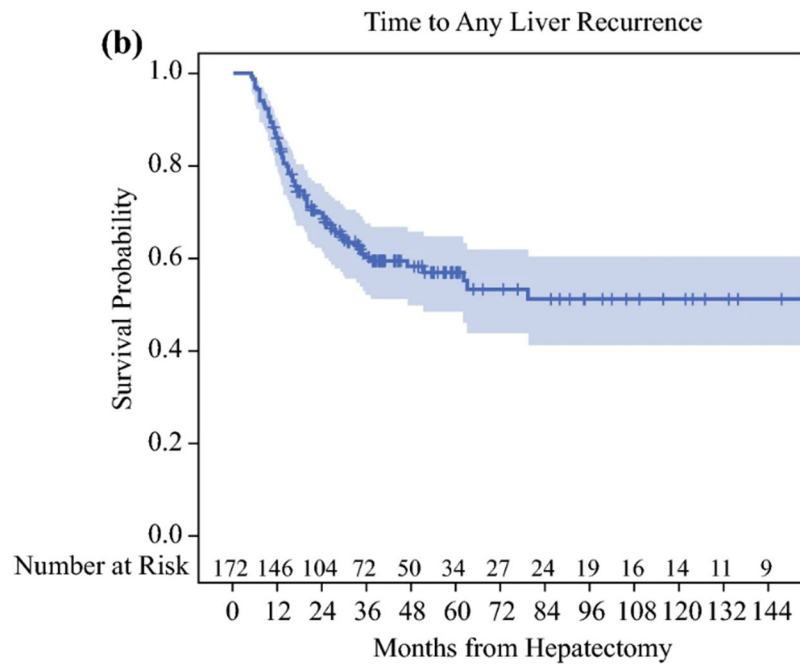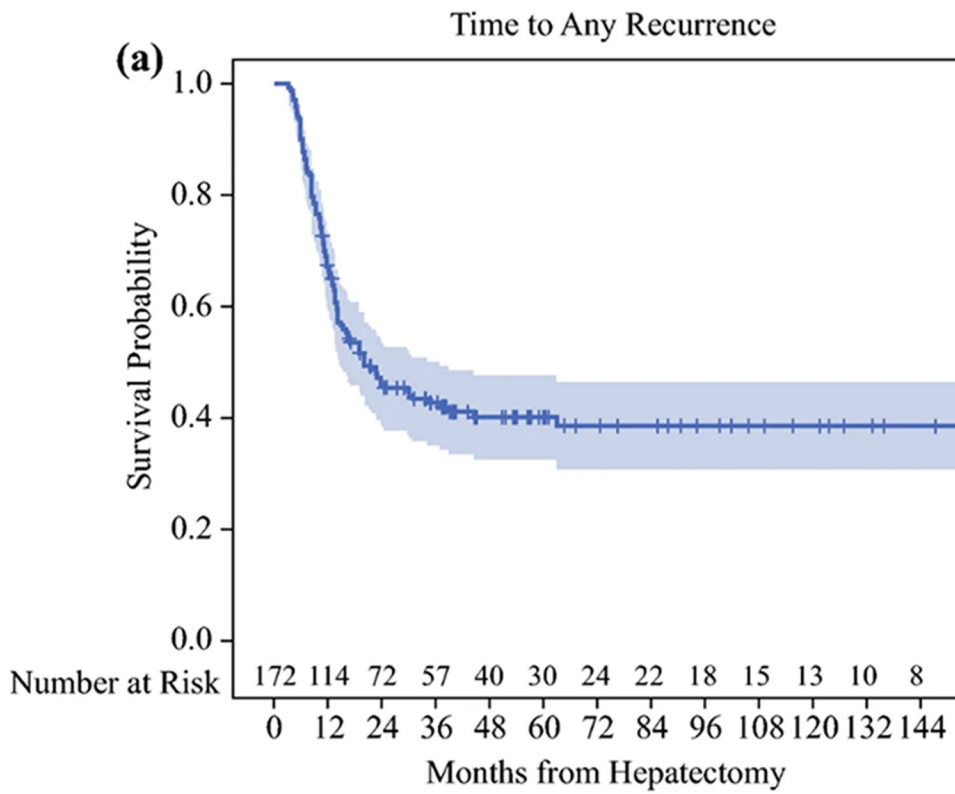
SYNOPSIS

Despite curative hepatectomy, most colorectal liver metastasis patients recur locally within two years of resection. Genomic predictors for hepatic recurrence are poorly understood but may hold implications for therapeutic devision making and are thus explored herein.

Time to Any Recurrence

(a)

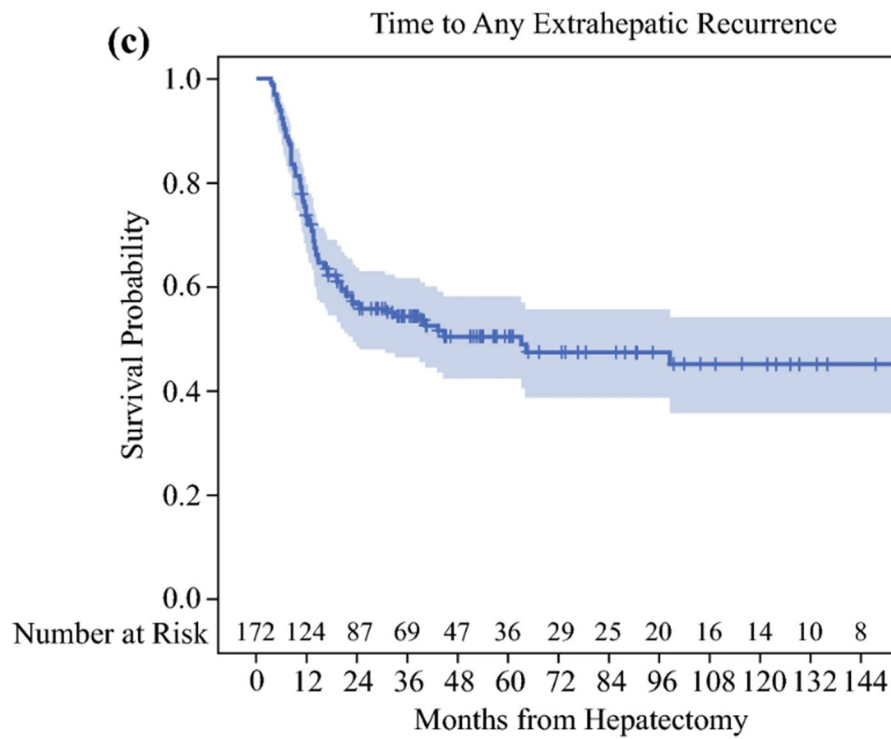**Time to Any Liver Recurrence**

(b)

Figure 1.
A: Time to Any Recurrence, **B:** Time to Any Liver Recurrence, **C:** Time to Any Extrahepatic Recurrence in colorectal liver metastasis patients after complete resection with adjuvant local and systemic chemotherapy (HAI-CRLM).
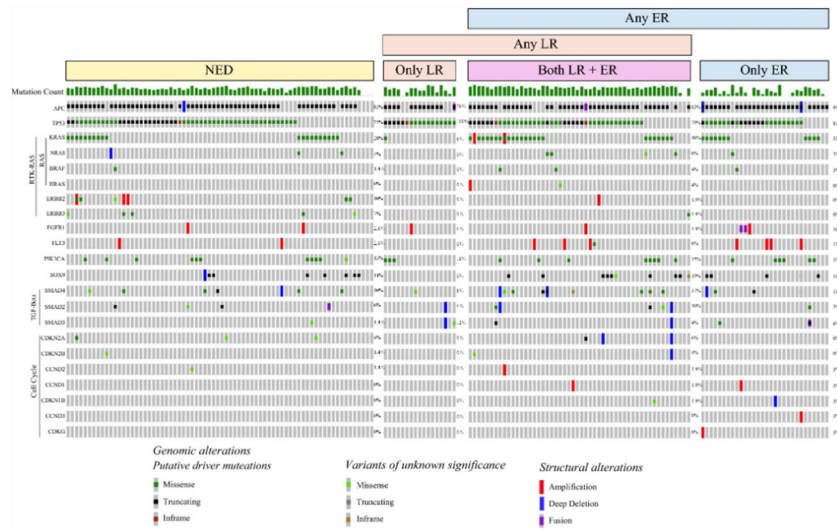
Figure 2:
Oncoprint of Genomic Alterations Stratified by Recurrence Patterns; NED: No evidence of disease, LR: Liver recurrence, ER: Extrahepatic recurrence. Timing of and censor events are not reflected, so inference about difference in recurrence patterns cannot be derived from this figure.
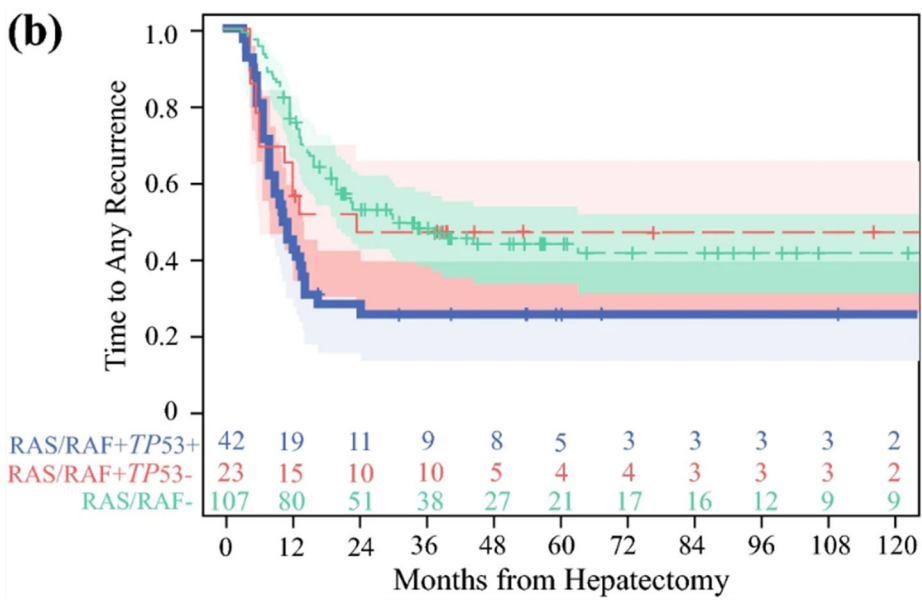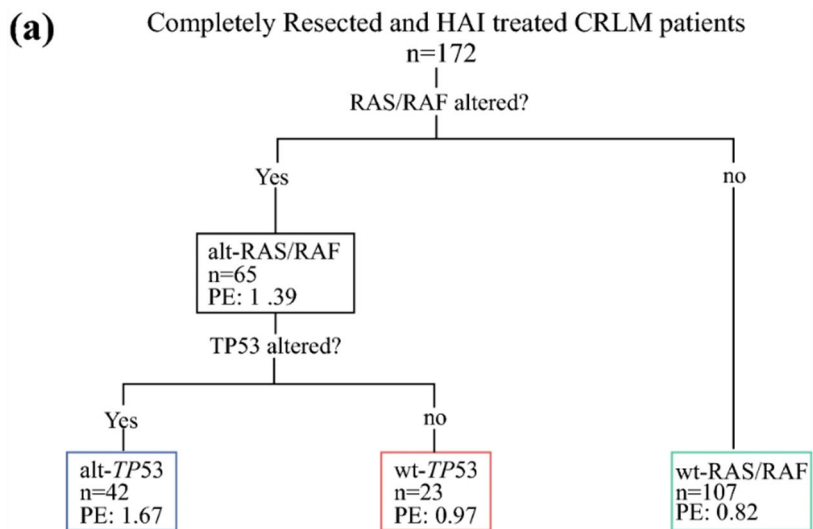
Figure 3.
A: Classification and regression tree analysis of patients with completely resected colorectal liver metastases and hepatic artery infusion chemotherapy demonstrating three terminal nodes of genomic risk for recurrence (n=172); PE: parameter estimate; **B:** Stratification of recurrence-free survival by the three genomic risk groups; alt-RAS-alt-*TP53*, alt-RAS-wt-*TP53*, wt-RAS. Number at risk over time indicated in the adjoining risk table.

Table 1:

Clinicopathologic characteristics of all colorectal liver metastasis (CRLM) patients completely resected with hepatic artery infusion (HAI) and systemic chemotherapy and systemic chemotherapy alone (SYS).

		All patients	HAI-CRLM	SYS-CRLM	p-value
Number of patients		230	172	58	
Gender	Male	129 (56)	96 (56)	33 (57)	>0.95
	Female	101 (44)	76 (44)	25 (43)	
Age at Surgery, years	Median (range)	55 (28-84)	54 (28-77)	59 (28-84)	**0.004**
Primary Tumor Location	Right Colon	59 (26)	39 (23)	20 (34)	0.26
	Left Colon	108 (47)	86 (50)	22 (38)	
	Rectum	59 (26)	44 (26)	15 (26)	
	Colon, NOS	4 (2)	3 (2)	1 (2)	
Primary Pathologic T-classification	T0-T2	36 (16)	31 (18)	5 (9)	0.14
	T3-T4	193 (84)	141 (82)	52 (90)	
Primary Pathologic Nodal Status	N0	71 (31)	57 (33)	14 (24)	0.25
	N+	159 (69)	115 (67)	44 (76)	
Disease Free Interval, months	Median (range)	0.6 (0.0-118.9)	1.0 (0-118.9)	0.4 (0.0-90.5)	0.22
Preoperative CEA, ng/mL	Median (range)	7.9 (0.5-5285.7)	7.7 (0.5-5285.7)	8.0 (1.6-983.8)	0.78
	N Missing	(6)	(4)	(2)	
Largest CRLM Size, cm	Median (range)	2.5 (0.3-16.1)	2.5 (0.3-14.5)	2.4 (0.5-16.1)	>0.95
Number of CRLM	Median (range)	3 (1-15)	3 (1-15)	2 (1-15)	**<0.001**
	Solitary	71 (31)	42 (24)	29 (50)	**<0.001**
	Multifocal	159 (69)	130 (76)	29 (50)	
Clinical Risk Score (CRS)	Low Risk (0-2)	117 (51)	85 (49)	32 (55)	0.54
	High Risk (3-5)	113 (49)	87 (51)	26 (45)	
Preoperative Chemotherapy	Yes	177 (77)	142 (83)	35 (60)	**0.001**
	No	53 (23)	30 (17)	23 (4)	
Extent of Hepatectomy	Minor	152 (66)	107 (62)	45 (78)	**0.037**
	Major	78 (34)	65 (38)	13 (22)	
Liver Margin	R0	210 (91)	162 (94)	48 (83)	**0.013**
	R1	20 (9)	10 (6)	10 (17)	
Ablation at Hepatectomy	Yes	37 (16)	32 (19)	5 (9)	0.10
	No	193 (84)	140 (81)	53 (91)	

Table 2:

Cox proportional hazards regression model for unadjusted recurrence, liver recurrence, and extrahepatic recurrence-free survival in patients following complete resection of colorectal liver metastases with adjuvant hepatic artery infusion and systemic chemotherapy.

Variable		Any Recurrence				Any Liver Recurrence				Any Extrahepatic Recurrence			
		Univariable Analysis		Multivariable Analysis		Univariable Analysis		Multivariable Analysis		Univariable Analysis		Multivariable Analysis	
		HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age at resection		0.98 (0.97-1.00)	0.12			0.97 (0.95-0.99)	0.007	0.97 (0.95-0.99)	0.005	0.98 (0.96-1.00)	0.043	0.98 (0.96-1.00)	0.07
Primary T-Stage	T3-T4	2.29 (1.22-4.30)	0.010	2.01 (1.06-3.82)	0.032	1.97 (0.94-4.13)	0.07			2.22 (1.11-4.43)	0.024	2.06 (1.02-4.16)	0.044
	T0-T2	1.00		1.00		1.00				1.00		1.00	
Clinical Risk Score	High (3-5)	1.66 (1.11-2.46)	0.013	1.45 (0.96-2.19)	0.08	1.73 (1.07-2.80)	0.026	1.39 (0.83-2.31)	0.21	1.79 (1.15-2.77)	0.010	1.70 (1.08-2.67)	0.021
	Low (0-2)	1.00		1.00		1.00		1.00		1.00		1.00	
Preoperative Chemotherapy	Yes	1.51 (0.84-2.71)	0.16			1.48 (0.74-2.99)	0.20			1.23 (0.67-2.28)	0.50		
	No	1.00				1.00				1.00			
Extent of Hepatectomy	Major	0.80 (0.53-1.20)	0.28			0.58 (0.34-0.97)	0.038	0.51 (0.30-0.87)	0.014	0.93 (0.60-1.46)	0.76		
	Minor	1.00				1.00		1.00		1.00			
Liver Margin	R1	2.62 (1.31-5.24)	0.006	2.32 (1.13-4.74)	0.022	3.25 (1.47-7.19)	0.004	2.38 (1.03-5.49)	0.042	1.27 (0.51-3.13)	0.61		
	R0	1.00		1.00		1.00		1.00		1.00			
Ablation at Hepatectomy	Yes	1.80 (1.13-2.85)	0.013	1.44 (0.90-2.31)	0.13	2.28 (1.35-3.84)	0.002	2.04 (1.17-3.53)	0.012	1.39 (0.82-2.34)	0.22		
	No	1.00		1.00		1.00		1.00		1.00			
RAS-TP53	Co-Altered	2.05 (1.34-3.13)	<0.001	2.14 (1.38-3.31)	<0.001	1.95 (1.17-3.24)	0.010	1.79 (1.06-3.01)	0.029	2.57 (1.64-4.04)	<0.001	2.81 (1.78-4.44)	<0.001
	All others	1.00		1.00		1.00		1.00		1.00		1.00	