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The Emerging Role of Genetic Data in the Surgical Treatment of Patients with Colorectal Cancer Liver Metastasis: Clinical Implications and Open Questions

zur Erlangung der Lehrbefähigung

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Table of Contents

Abbreviations

ACHBT	(French) Association de Chirurgie Hépato-Bilio-Pancréatique et Transplantation
AGEO	(French) Association des Gastro-Enterologues Oncologues
AI	Artificial Intelligence
AIC	Akaike Information Criterion
ALPPS	Associating Liver Partition and Portal vein ligation for Staged Hepatectomy
AR	Anatomical Resection
AUC	Area Under the Curve
BRAF	V-Raf Murine Sarcoma Viral Oncogene Homolog B1
CEA	Carcinoembryonic Antigen
CRS	Clinical Risk Score
CRLM	Colorectal Cancer Liver Metastases
CS	Conditional Survival
e-CS	extended Clinical Score
EGFR	Epidermal Growth Factor Receptor
ESMO	European Society for Medical Oncology
FDA	Food and Drug Administration
FRENCH	(French) Fédération de Recherche EN Chirurgie
GAME score	Genetic And Morphological Evaluation score
GDP	Guanosine Diphosphate
GTP	Guanosine Triphosphate
HR	Hazard Ratio
IGCLM	International Genetic Consortium for colorectal Liver Metastasis
JHH	Johns Hopkins Hospital
OS	Overall Survival
KRAS	Kirsten RAt Sarcoma
LS	Left Sided
m-CS	modified Clinical Score
mCRC	metastatic Colorectal Cancer
MSKCC	Memorial Sloan Kettering Cancer Center

mutBRAF	BRAF Mutation
mutKRAS	KRAS Mutation
NAR	Non-Anatomical Resection
RFS	Recurrence-Free Survival
RS	Right Sided
PFS	Progression-Free Survival
PTL	Primary Tumor Laterality
PVE	Portal Vein Embolization
RH	Repeat Hepatectomy
STS	Soft Tissue Sarcoma
TBS	Tumor Burden Score
TSH	Two-Stage Hepatectomy
V600E	Valine-to-glutamic acid substitution at codon 600
wtBRAF	wild-type BRAF
wtKRAS	wild-type KRAS

The emerging role of genetic data in the surgical treatment of patients with colorectal cancer liver metastasis: clinical implications and open questions

1. Introduction

1.1 Epidemiology, treatment, and prognosis

Colorectal cancer is one of the most common malignancies worldwide, accounting for at least 1.8 million new cases in 2018.[1] In around one quarter to half of these patients, tumor cells will spread hematogenously to the liver during the course of their disease.[2-4] Metastatic involvement of the liver is a chief contributor of cancer-related deaths in this patient group and largely drives prognosis.[5, 6] Metastasectomy of colorectal cancer liver metastases (CRLM) can provide a significant survival benefit in selected cases, with operative indications continuously expanding in recent years; previous absolute contraindications such as bilobar disease, > 3 liver lesions and the anticipation of an R1 resection have been abandoned.[7-9] In fact, even the presence of limited extrahepatic disease is no longer an absolute contraindication. Instead, the inability to preserve an adequate volume of future liver remnant, as well as sustainable vascular inflow, outflow, and biliary drainage are, in contemporary practice, the only factors that can preclude an operation.[8]

Nonetheless, only around 20% of those diagnosed with CRLM will be deemed resectable at the time of initial diagnosis.[10] Metastasectomy will allow at least 50% of patients to survive for at least 5 years following surgery, with almost half of these patients eventually achieving 10-year survival without evidence of disease, an endpoint commonly considered synonymous with "cure."[11] It should be noted that these survival rates are the result of progress made in this field during the last three decades; 5-year overall survival (OS) was only around 30% in the 1980s.[12] Ultimately, cure remains impossible without surgery, but with advances in systemic therapy, the 2-year survival of

medically treated patients has risen to roughly 40%.[3] However, because surgery is still associated with significantly better survival, there have been aggressive efforts to expand the pool of patients who are candidates for liver resection, with the aid of conversion chemotherapy and specific surgical techniques (e.g., portal vein embolization [PVE], two-stage hepatectomy [TSH], and associating liver partition and portal vein ligation for staged hepatectomy [ALPPS]).[13-16] While combining operative and non-operative approaches is part of the current standard of care, many questions remain regarding the optimal utilization of perioperative chemotherapy (especially among patients with upfront resectable disease), and contemporary practice continues to be guided by expert opinion.[17]

1.2 Areas that need improvement

The prognosis of patients with CRLM is not uniformly favorable. Unfortunately, prior attempts to classify these patients into groups with distinctly different prognosis have largely failed as reflected by the conclusion of a recent comprehensive systematic review. Specifically, after examining all 26 prognostic models published up to 2015, Mahar et al concluded that "In the stage IV population with liver metastases, variation in the prognostic factors used across tools may reflect a gap in understanding prognosis for that population."[18] As such, improvement is warranted in patient prognostication, both at the time of surgery and during subsequent follow-up. Aside from facilitating physician-patient discussions, improved prognostication is important because groups with distinctly different prognosis may have intrinsically different disease. In addition to improving our understanding of disease biology, the first practical implication of discovering prognostically distinct subgroups is to identify individuals at such high risk of recurrence and progression that non-invasive approaches may be a reasonable alternative to surgery thus improving patient selection. An additional concrete application would be the ability to tailor

surgical techniques to distinct patient groups by anticipating the pattern of intra-parenchymal disease spread, which could reduce recurrence rates. Lastly, a third practical implication would be to inform post-hepatectomy surveillance according to the patterns of recurrence observed among different patient groups.

1.3 Short note

To achieve these aims, clinicopathologic factors have long been employed, but their usefulness has reached a plateau. In 2013, Vauthey et al demonstrated that a marker of tumor biology (Kirsten RAt Sarcoma virus [KRAS] mutation status) is a potent prognosticator for patients with CRLM.[19] This observation was subsequently confirmed by the author and others.[20] Given that KRAS status is now routinely determined in the clinical setting so as to assess eligibility for anti- epidermal growth factor receptor (EGFR) treatments, the last decade has witnessed a revolution in the availability of clinically useful genetic data. The studies included in this habilitation assess the contribution of KRAS mutation status, as well as a closely related biomarker, v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation status, to advancing the aims stated above. The *clinical problems* we attempted to solve, relevant background knowledge, and the thought process we followed to arrive at specific hypotheses are described below.

1.4 First clinical problem: Patient selection for surgery

Clinical problem: It has long been argued that some patients have such poor prognosis (e.g., by developing early, extensive extrahepatic recurrence) that liver resection may not be associated with a meaningful survival benefit. If these patients could be identified preoperatively, they could be spared unnecessary morbidity and mortality. To this end, many prognostic models that include

clinicopathologic variables have been developed, but have failed to consistently identify a subgroup of patients with extremely poor prognosis that would be ill-suited for surgical treatment.

Background knowledge: In the majority of published clinical risk scores, even the highest risk groups had relatively favorable prognosis. For example, the high-risk group of the Nordlinger score had a 2-year OS rate of around 50% and minimum survival rates were even higher for other clinical risk scores.[21] Only the grade C group of the Nagashima score reportedly had a 5-year survival rate of 0%.[22] However, this finding was derived from a single-center study of only 81 patients.

The failure of risk scores that consist solely of clinicopathologic factors to identify patients with extremely poor prognosis demonstrates that variables such as bilobar disease or high tumor burden, while originally considered contraindications to surgery, are, in fact, not reliable markers of aggressive tumor biology. The introduction of novel, genetic biomarkers is therefore necessary before significant improvements in prognostication can be realized. To this end, the presence of BRAF mutation (mutBRAF) has recently been proposed as an indicator of extremely aggressive tumor biology and may serve as a robust predictor of poor outcomes.[23] In fact, these early data led some groups to postulate that the presence of BRAF mutation may serve as a biological (as opposed to technical) contraindication to CRLM resection.[24]

Thought process: While the concept of "biological contraindications" is innovative, it must be recognized that the aforementioned data regarding the unfavorable prognostic role of mutBRAF status were derived from patients with unresectable, metastatic colorectal cancer (mCRC), rather than CRLM cohorts.[25-27] As such, it would be premature and likely hazardous to extrapolate from these results for the purpose of potentially excluding patients from metastasectomy, unless similar findings could be replicated in patients with CRLM. Studies in patients with resectable, mutBRAF

CRLM have been sparse because of the inherently low incidence of this somatic mutation, which has been estimated at 8-10% in all-comers with metastatic disease.[28] In fact, due to the propensity of these tumors to metastasize widely, BRAF mutations are rarer still among technically resectable patients with CRLM; all relevant surgical series collectively published in a 2016 meta-analysis cumulatively included only 22 patients with BRAF mutations.[29] In 2018 we assembled a large multi-institutional, international cohort of patients with surgically treated, mutBRAF CRLM in order to accurately characterize their outcomes.[30] At the time, it was the largest study of its kind.

1.5 Second clinical problem: Informing prognosis at the time of surgery

Clinical problem: As stated above, a total of 26 prognostic models for patients with CRLM were reported between 1996 and 2015, but all had relatively low prognostic abilities, especially when validated in external cohorts.[18] As a result, their clinical utility remains quite limited.

Background knowledge: This conclusion is further supported by studies that aimed to externally validate these prognostic models. For example, in 2007, Zakaria and colleagues externally validated the risk scores proposed by Fong et al, Nordlinger et al, and Iwatsuki et al and demonstrated their limited prognostic accuracy (AUCs ranged between 0.53 and 0.56) and clinical value.[31]

Thought process: The common denominator of all previous prognostic models is that they only include clinicopathologic variables, as genetic biomarkers had not been identified when they were originally developed. Of those, while the BRAF mutation is far too rare to add much additional prognostic power to an all-comer prognostic model, the KRAS mutation is sufficiently common (27-40% of patients with CRLM) and well-established as a prognostic factor to have possible value as a component of a revised prognostic system.[19, 20, 32] To this end, in 2018 we devised a novel,

composite prognostic model (GAME score) that was the first to incorporate KRAS mutation status and traditional clinicopathologic factors for the purpose of predicting outcomes among patients with CRLM.[33] Published around the same time as a similar, independent work by investigators from MD Anderson, the GAME score has since served as a frequently cited, benchmark prognostic model in the CRLM literature.[34]

1.6 Third clinical problem: Determining prognosis over time

Clinical problem: Some CRLM patients with grim prognoses at the time of surgery survive for a long time, refuting initial predictions. Providing updated prognostication of these patients is challenging, as existing models are designed to provide static survival estimates at the time of surgery under the assumption that the risk of cancer-related death is uniformly distributed over time.[35] Novel approaches using conditional survival are needed to remedy this shortcoming.

Background knowledge: While this concept had not been investigated in CRLM, prior studies in soft tissue sarcoma (STS) have demonstrated that baseline prognostic factors do not have a consistent impact over time. Pisters et al found that the impact of high tumor grade on the risk of developing metastasis from extremity STS was important in the first 3 years following surgery, but continuously diminished over time.[36] Stojadinovic et al similarly demonstrated that the impact of tumor size and tumor grade on sarcoma-related death among patients with extremity/truncal STS was mitigated significantly over time.[37]

Thought process: Evidence indicates that the risk of cancer-related death decreases rapidly over time following surgery for CRLM.[38] If this is the case, the impact of prognostic factors may change over time as well, and the initial prognostic factors are unlikely to influence long-term survival. In 2018, we performed the first study in CRLM to test this hypothesis by estimating the

conditional survival of patients at a number of time points following surgery in relation to various established prognostic factors, including KRAS and BRAF mutation status.[39]

1.7 Fourth clinical problem: Precision surgery

Clinical problem: Among all variables that influence prognosis, the only ones that are under the direct control of the surgeon are surgical margin width (to an extent) and choice of surgical technique (anatomical versus non-anatomical hepatectomy). As expected, both have been investigated for decades to define the optimal resection strategy in patients with CRLM. Nonetheless, the findings reported in the literature have often been contradictory and little consensus exists on the possible impact of different surgical approaches among patients with CRLM. Whether certain patient subgroups may benefit from resection strategies "tailored" to their disease biology has not been thoroughly investigated.

Background knowledge: In the late 80's, Blake Cady and Ekberg and colleagues suggested that surgeons should ideally strive for a resection margin width of at least 1 cm.[7, 40] Although no longer considered a criterion of resectability, the ''1 cm margin rule'' has underwent a limited revival, as some studies suggest that it may be associated with superior prognosis compared to narrower margins.[41] Some studies, however, have demonstrated that no additional prognostic benefit is derived from extending the margin width beyond 1 mm, while others have supported that even an R1 resection does not appreciably impact long-term survival, thus further fueling the ongoing controversy.[42] In 2016, we hypothesized that a single optimal margin width may not exist and may instead differ for patients with distinct underlying disease biology, such as KRAS mutation status.[43] A similar view was expressed by investigators from MD Anderson in a relevant study published at approximately the same time.[44] Our preliminary findings suggested that the aggressive tumor biology implied by the presence of KRAS mutation (mutKRAS) could not be

counterbalanced by extensive resection and thus even an R1 margin may not seriously impact outcomes in these patients. In comparison, the aforementioned MD Anderson investigators suggested that mutKRAS CRLM should be treated with “aggressive” margins of 10 or even 15 mm to overcome the aggressive tumor biology. It should be noted that this study did not perform a dedicated sub-analysis to assess this hypothesis. Interestingly, both studies could be proven correct depending on the pattern of intrahepatic spread of mutKRAS lesions (wider spread would support more extensive margins) and their propensity for distant intrahepatic and extrahepatic recurrence (which if sufficiently high would tend to nullify the results of effective hepatic resection irrespective of margin). As resection margin width can be affected by other factors outside the surgeon’s control, further exploration of this hypothesis could be performed by assessing the relative efficacy of anatomical and non-anatomical resection among patients with mutKRAS lesions. Existing data in all-comers had by that time generally favored non-anatomical resection as equally efficacious, less morbid, and more conducive to repeat resection than anatomical hepatectomy; however, the apparent advantage of the latter in some large series had not been convincingly explained.[45]

Thought process: In light of these preliminary findings, we sought to assess for the first time whether anatomical hepatectomy would lead to distinct outcomes among patients with mutKRAS CRLM in a single institution cohort study published in 2017.[46] Superiority of anatomical resection in this patient group would imply the presence of micrometastatic spread in the involved Couinaud segments and potentially change the clinical landscape of CRLM surgery from a "one-size-fits-all" approach to precision surgery.

1.8 Fifth clinical problem: KRAS status as a modifier of other prognostic factors, towards resolving the tumor laterality debate

Clinical problem: Following the identification of the prognostic role of KRAS mutations in CRLM in 2013, arguably the next most significant prognostic factor to emerge in the literature was primary tumor laterality. A proxy of underlying disease biology, tumor laterality has been shown to be both prognostic (patients with right-sided tumors fare worse than those with left-sided tumors) and predictive (patients with wild-type (wt) KRAS and wtBRAF left-sided tumors are more likely to benefit from anti-EGFR first line combination therapy) in patients with mCRC.[47, 48] However, until 2018 it was unclear whether this was also the case for resectable CRLM. The association of tumor laterality with other genetic biomarkers in this patient group also remained to be determined.

Background knowledge: The aforementioned uncertainty stemmed from the conflicting results of several initial studies that assessed the impact of tumor laterality on prognosis among patients with CRLM. An initial study in 2016 co-authored by Sasaki and Andreatos at Johns Hopkins Hospital (with the author's participation) was the first to report that right-sided primaries are associated with worse survival among patients with CRLM; this was later corroborated by Yamashita et al in a cohort of patients from MD Anderson.[49, 50] Nonetheless, others such as Creasy et al who reported on a series from the Memorial Sloan Kettering Cancer Center (MSKCC) questioned the prognostic relevance of primary tumor laterality in the long-term, as did Wang et al and Scherman et al in a separate cohorts.[51-53]

Thought process: Given that tumor laterality has been consistently prognostic in unresectable mCRC while results are mixed in resectable CRLM, we investigated differences in methodologies between these two groups of studies. We found that studies in unresectable mCRC were generally conducted in patients with wtKRAS tumors, while those in resectable CRLM included mixed cohorts of both wtKRAS and mutKRAS tumors. As a result, we hypothesized that the prognostic

impact of tumor laterality may vary according to KRAS mutation status. We were the first to explore this possibility in a multi-institutional cohort study published in 2019.[54]

1.9 Methodology

KRAS and BRAF oncogenes

Among the many constitutively active oncogenes identified in human cancers, RAS was among the first to be discovered and remains one of the most studied. RAS mutations are found in ~25% of all human cancers, rendering RAS pathway activation one of the most frequently encountered genetic aberrations in carcinogenesis.[55] Among the different RAS oncogene isoforms, KRAS mutations are mostly prevalent in CRC (52%), pancreatic ductal adenocarcinoma (98%), and lung cancer (32%).[56-58] KRAS encodes a small GTPase that functions as a signal switch (by pivoting between active GTP-bound and inactive GDP-bound conformations) that controls signal transduction from activated cell surface receptors to the nucleus.[59] In turn, these signals regulate downstream effector pathways that regulate cellular proliferation, differentiation, and survival. The BRAF protein belongs to the same signaling pathway and lies downstream of KRAS; BRAF mutation results in persistent RAS-independent activation of the pathway which drives increased cellular proliferation and survival. A more detailed description of the KRAS and BRAF signaling pathways is found in the figure below.

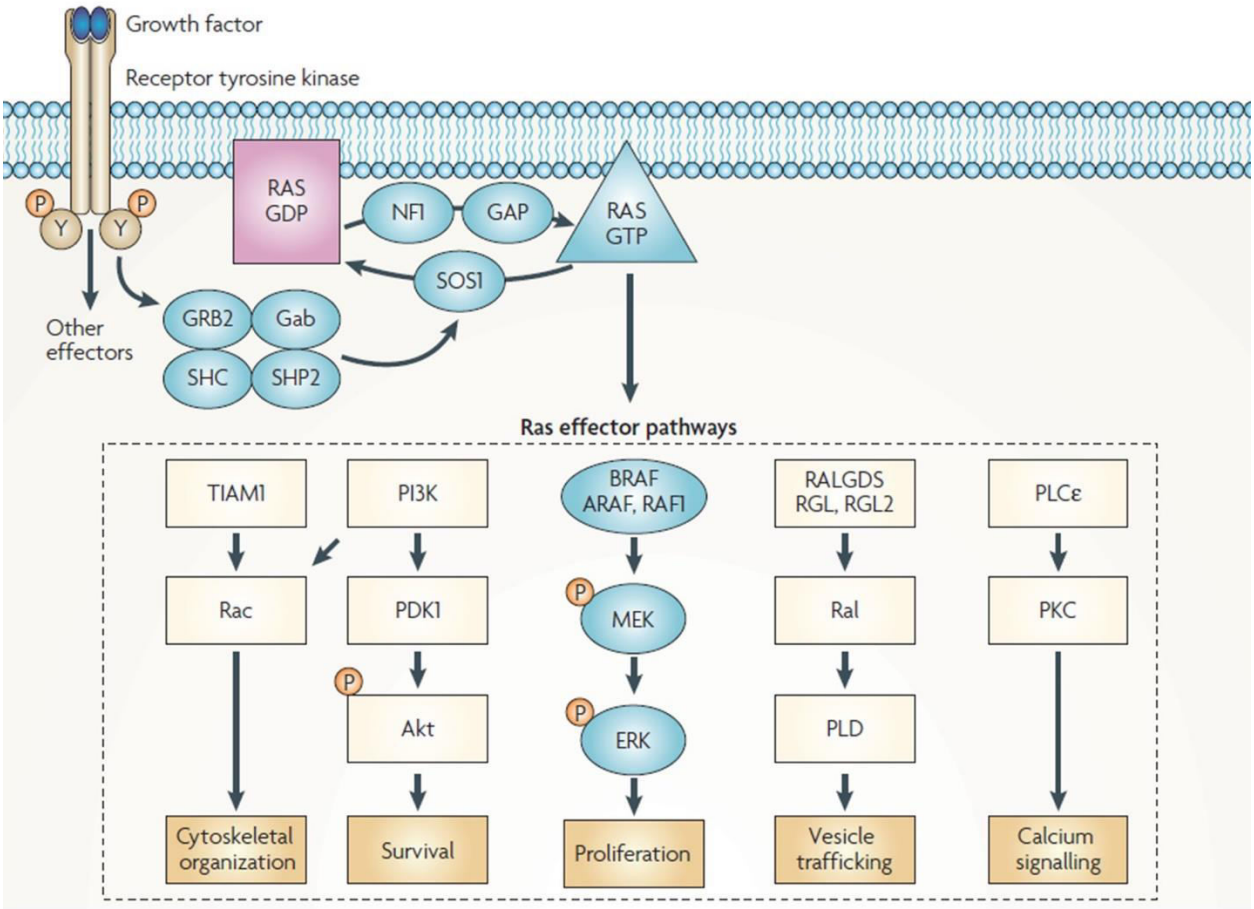

Figure. The Ras signalling pathway. This illustration of the Ras signalling pathway highlights proteins affected by mutations in developmental disorders and cancer. Growth factor binding to cell-surface receptors results in activated receptor complexes, which contain adaptors such as SHC (SH2-containing protein), GRB2 (growth-factor-receptor bound protein 2) and Gab (GRB2-associated binding) proteins. These proteins recruit SHP2 and SOS1, the latter increasing Ras–guanosine triphosphate (Ras–GTP) levels by catalysing nucleotide exchange on Ras. The GTPase-activating protein (GAP) neurofibromin (NF1) binds to Ras–GTP and accelerates the conversion of Ras–GTP to Ras–GDP (guanosine diphosphate), which terminates signalling. Several Ras–GTP effector pathways have been described, and some of the key effectors are depicted here. The BRAF–mitogen-activated and extracellular-signal regulated kinase kinase (MEK)– extracellular signal-regulated kinase (ERK) cascade often determines proliferation and becomes deregulated in certain cancers and in developmental disorders such as cardio-facio-cutaneous syndrome. Ras also activates the phosphatidylinositol 3-kinase (PI3K)– 3-phosphoinositide-dependent protein kinase 1 (PDK1)–Akt pathway that frequently determines cellular survival. RALGDS, RALGDS-like gene (RGL), RGL2 and TIAM1 are exchange factors of Ral and Rac, respectively. Among the effectors of Ral is phospholipase D (PLD) an enzyme that regulates vesicle trafficking. Rac regulates actin dynamics and, therefore, the cytoskeleton. Ras also binds and activates the enzyme phospholipase Cε (PLCε), the hydrolytic products of which regulate calcium signalling and the protein kinase C (PKC) family. P, phosphate; Y, receptor tyrosine.
(Used from Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer. 2007 Apr;7(4):295-308 through a request to the publisher, license number 4972470573469)

Although long considered "undruggable", recent efforts to directly inhibit RAS have been partially successful with the groundbreaking discovery of pharmacological agents that specifically target the KRAS(G12C) mutant. While this genotype is rare in CRC, the fact that sotorasib, the first agent to target KRAS(G12C), was recently granted breakthrough status by the Food and Drug

Administration (FDA) for non-small cell lung cancer raises hope for future advances.[60, 61] Similarly, after initially underwhelming results, patients with valine-to-glutamic acid substitution at codon 600 (V600E) mCRC now have access to genotype-directed combination regimens following the results of the BEACON trial.[62]

Multi-institutional studies

The use of genetic data to solve these clinical problems requires several stratifications, which in turn severely decrease statistical power. To mitigate this problem, a large patient population needs to be analyzed. To achieve this, the author played a leading role in establishing the International Genetic Consortium for colorectal Liver Metastasis (IGCLM) which is a collaboration of Johns Hopkins University (Baltimore, USA), Stanford University School of Medicine (Stanford, USA), Cleveland Clinic (Cleveland, USA), Medical University of Vienna (Vienna, Austria), Medical University of Graz (Graz, Austria), Charité - University of Berlin (Berlin, Germany), Haukeland University Hospital (Bergen, Norway), Yokohama City University Graduate School of Medicine (Yokohama, Japan), and Kumamoto University (Kumamoto, Japan). Furthermore, an independent collaboration with MSKCC was eventually established via the author's own initiative.

1.10 Short note

The author has sought to avoid merely recapping the discussion section of each paper included in this habilitation thesis. Instead, the focus has been on examining the findings of the selected studies in the context of subsequent work by the author, his collaborators, and other independent investigators. The aim was to examine if these topics proved to be of interest to the scientific community and whether the results stood the test of time in their original form or need to be re-

considered in light of subsequent findings. A critical appraisal of our work and a brief review of open questions and ongoing avenues of investigation complete the thesis.

2. Presentation of the author's work

2.1 Patient selection for surgery

BRAF mutations are reportedly associated with aggressive tumor biology.[23, 63] However, a meta-analysis published in 2016 that included multiple surgical studies on BRAF-mutated CRLM cumulatively included only 22 patients with BRAF mutations.[29] This limits our ability to reliably conclude whether BRAF mutation should serve as a contraindication to surgery, and ultimately triggered the study that is presented below.

Publication 1: Association of BRAF mutations with survival and recurrence in surgically treated patients with metastatic colorectal liver cancer.

Margonis GA, Buettner S, Andreatos N, Kim Y, Wagner D, Sasaki K, Beer A, Schwarz C, Løes IM, Smolle M, Kamphues C, He J, Pawlik TM, Kaczirek K, Poultsides G, Lønning PE, Cameron JL, Burkhart RA, Gerger A, Aucejo FN, Kreis ME, Wolfgang CL, Weiss MJ. *JAMA Surg*. 2018 Jul 18;153(7):e180996. doi: https://doi.org/10.1001/jamasurg.2018.0996

The following text is reproduced in full from the abstract of the publication "Association of BRAF Mutations With Survival and Recurrence in Surgically Treated Patients With Metastatic Colorectal Liver Cancer."

> *"Importance: BRAF mutations are reportedly associated with aggressive tumor biology. However, in contrast with primary colorectal cancer, the association of V600E and non-V600E BRAF mutations with survival and recurrence after resection of colorectal liver metastases (CRLM) has not been well studied.*
>
> *Objective: To investigate the prognostic association of BRAF mutations with survival and recurrence independently and compared with other prognostic determinants, such as KRAS mutations.*

Design, setting, and participants: In this cohort study, all patients who underwent resection for CRLM with curative intent from January 1, 2000, through December 31, 2016, at the institutions participating in the International Genetic Consortium for Colorectal Liver Metastasis and had data on BRAF and KRAS mutational status were retrospectively identified. Multivariate Cox proportional hazards regression models were used to assess long-term outcomes.

Interventions: Hepatectomy in patients with CRLM.

Main outcomes and measures: The association of V600E and non-V600E BRAF mutations with disease-free survival (DFS) and overall survival (OS).

Results: Of 853 patients who met inclusion criteria (510 men [59.8%] and 343 women [40.2%]; mean [SD] age, 60.2 [12.4] years), 849 were included in the study analyses. Forty-three (5.1%) had a mutated (mut) BRAF/wild-type (wt) KRAS (V600E and non-V600E) genotype; 480 (56.5%), a wtBRAF/wtKRAS genotype; and 326 (38.4%), a wtBRAF/mutKRAS genotype. Compared with the wtBRAF/wtKRAS genotype group, patients with a mutBRAF/wtKRAS genotype more frequently were female (27 [62.8%] vs 169 [35.2%]) and 65 years or older (22 [51.2%] vs 176 [36.9%]), had right-sided primary tumors (27 [62.8%] vs 83 [17.4%]), and presented with a metachronous liver metastasis (28 [64.3%] vs 229 [46.8%]). On multivariable analysis, V600E but not non-V600E BRAF mutation was associated with worse OS (hazard ratio [HR], 2.76; 95% CI, 1.74-4.37; P < .001) and DFS (HR, 2.04; 95% CI, 1.30-3.20; P = .002). The V600E BRAF mutation had a stronger association with OS and DFS than the KRAS mutations (β for OS, 10.15 vs 2.94; β for DFS, 7.14 vs 2.27).

Conclusions and relevance: The presence of the V600E BRAF mutation was associated with worse prognosis and increased risk of recurrence. The V600E mutation was not only a stronger prognostic factor than KRAS but also was the strongest prognostic determinant in the overall cohort."[30]

2.2 Informing prognosis at the time of surgery

Although the outcomes of patients with BRAF mutated tumors on average were not so poor as to suggest that surgery may be futile, the presence of various genotypes with distinct prognoses (V600E vs non-V600E) suggests that subgroups of these patients may fare quite differently.[30] In regards to those who will undergo curative intent surgery, the importance of prognostication is evidenced by the large number of models that have attempted to predict their long-term outcomes following surgery.[18] Unfortunately, most of these models have limited predictive abilities, which suggests that there exists a conceptual weakness in including clinicopathologic features alone.[18] In the study that is presented below, we hypothesized that a hybrid model that includes both clinicopathologic features and a biomarker of tumor biology that is widely available (i.e., KRAS status) may overcome this deficiency.

Publication 2: Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases.

Margonis GA, Sasaki K, Gholami S, Kim Y, Andreatos N, Rezaee N, Deshwar A, Buettner S, Allen PJ, Kingham TP, Pawlik TM, He J, Cameron JL, Jarnagin WR, Wolfgang CL, D'Angelica MI, Weiss MJ. *Br J Surg*. 2018 Aug;105(9):1210-1220. doi: https://doi.org/10.1002/bjs.10838

The following text is reproduced in full from the abstract of the publication "Genetic And Morphological Evaluation (GAME) score for patients with colorectal liver metastases."

> *"Background: This study sought to develop a clinical risk score for resectable colorectal liver metastasis (CRLM) by combining clinicopathological and clinically available biological indicators, including KRAS.*
>
> *Methods: A cohort of patients who underwent resection for CRLM at the Johns Hopkins Hospital (JHH) was analysed to identify independent predictors of overall survival (OS) that can be assessed before operation; these factors were combined into the Genetic And Morphological Evaluation (GAME) score. The score was*

compared with the current standard (Fong score) and validated in an external cohort of patients from the Memorial Sloan Kettering Cancer Center (MSKCC).

Results: Six preoperative predictors of worse OS were identified on multivariable Cox regression analysis in the JHH cohort (502 patients). The GAME score was calculated by allocating points to each patient according to the presence of these predictive factors: KRAS-mutated tumours (1 point); carcinoembryonic antigen level 20 ng/ml or more (1 point), primary tumour lymph node metastasis (1 point); Tumour Burden Score between 3 and 8 (1 point) or 9 and over (2 points); and extrahepatic disease (2 points). The high-risk group in the JHH cohort (GAME score at least 4 points) had a 5-year OS rate of 11 per cent, compared with 73·4 per cent for those in the low-risk group (score 0-1 point). Importantly, in cohorts from both the JHH and MSKCC (747 patients), the discriminatory capacity of the GAME score was superior to that of the Fong score, as demonstrated by the C-index and the Akaike information criterion.

Conclusion: The GAME score is a preoperative prognostic tool that can be used to inform treatment selection."[33]

2.3 Determining prognosis over time

The GAME model demonstrated the added value of capturing tumor biology while retaining well validated clinicopathologic factors and offered a promising approach to CRLM prognostication.[33] Nonetheless, one should keep in mind that these conventional prognostic models are designed to be accurate at the time of surgery, while evidence indicates that the risk of cancer-related death may decrease rapidly over time following surgery for colorectal liver metastases.[38] If this is the case, the impact of prognostic factors may change over time as well, and the initial prognostic factors are unlikely to influence long-term survival. The following publication aimed to address this challenge.

Publication 3: Prognostic factors change over time after hepatectomy for Colorectal Liver Metastases: A multi-institutional, international analysis of 1099 patients.

Margonis GA, Buettner S, Andreatos N, Wagner D, Sasaki K, Barbon C, Beer A, Kamphues C, Løes IM, He J, Pawlik TM, Kaczirek K, Poultsides G, Lønning PE, Cameron JL, Mischinger HJ, Aucejo FN, Kreis ME, Wolfgang CL, Weiss MJ. *Ann Surg*. 2019 Jun;269(6):1129-1137. doi: https://doi.org/10.1097/SLA.0000000000002664

The following text is reproduced in full from the abstract of the publication "Prognostic Factors Change Over Time After Hepatectomy for Colorectal Liver Metastases."

> *"Objective: To evaluate the changing impact of genetic and clinicopathologic factors on conditional overall survival (CS) over time in patients with resectable colorectal liver metastasis.*
>
> *Background: CS estimates account for the changing likelihood of survival over time and may reveal the changing impact of prognostic factors as time accrues from the date of surgery.*
>
> *Methods: CS analysis was performed in 1099 patients of an international, multi-institutional cohort. Three-year CS (CS3) estimates at the "xth" year after surgery*

were calculated as follows: CS3 = CS (x + 3)/CS (x). The standardized difference (d) between CS3 rates was used to estimate the changing prognostic power of selected variables over time. A $d < 0.1$ indicated very small differences between groups, $0.1 \leq d < 0.3$ indicated small differences, $0.3 \leq d < 0.5$ indicated moderate differences, and $d \geq 0.5$ indicated strong differences.

Results: According to OS estimates calculated at the time of surgery, the presence of BRAF and KRAS mutations, R1 margin status, resected extrahepatic disease, patient age, primary tumor lymph node metastasis, tumor number, and carcinoembryonic antigen levels independently predicted worse survival. However, when temporal changes in the prognostic impact of these variables were considered using CS3 estimates, BRAF mutation dominated prognosis during the first year ($d = 0.48$), whereas surgeon-related variables (ie, surgical margin and resected extrahepatic disease) determined prognosis thereafter ($d \geq 0.5$). Traditional clinicopathologic factors affected survival constantly, but only to a moderate degree ($0.3 \leq d < 0.5$).

Conclusions: The impact of genetic, surgery-related, and clinicopathologic factors on OS and CS3 changed dramatically over time. Specifically, BRAF mutation status dominated prognosis in the first year, whereas positive surgical margins and resected extrahepatic disease determined prognosis thereafter."[39]

2.4 Precision surgery

Aside from our efforts to improve patient selection and prognostication, we performed a study with direct implications for the surgical management of patients with CRLM. The study, which was published in 2017, compared the long-term outcomes of two different surgical techniques (anatomical and non-anatomical hepatectomy) under the lens of tumor biology (i.e., KRAS mutation status).[46] It was prompted by the contradictory results of prior studies, which did not account for KRAS mutation status, and the need for biomarkers that can tailor surgical technique.[64-67] Although our group and others had extensively studied biomarkers in metastatic colorectal cancer, before this study their use was limited to only informing prognosis.

Publication 4: Anatomical resections improve Disease-free Survival in patients with KRAS-mutated Colorectal Liver Metastases.

Margonis GA, Buettner S, Andreatos N, Sasaki K, Ijzermans JNM, van Vugt JLA, Pawlik TM, Choti MA, Cameron JL, He J, Wolfgang CL, Weiss MJ. *Ann Surg*. 2017 Oct;266(4):641-649. doi: https://doi.org/10.1097/SLA.0000000000002367

The following text is reproduced in full from the abstract of the publication "Anatomical Resections Improve Disease-free Survival in Patients With KRAS-mutated Colorectal Liver Metastases."

> *"Objective: To investigate the potential clinical advantage of anatomical resection versus nonanatomical resection for colorectal liver metastases, according to KRAS mutational status.*
>
> *Background: KRAS-mutated colorectal liver metastases (CRLM) are known to be more aggressive than KRAS wild-type tumors. Although nonanatomical liver resections have been demonstrated as a viable approach for CRLM patients with similar oncologic outcomes to anatomical resections, this may not be the case for the subset of KRAS-mutated CRLM.*

Methods: 389 patients who underwent hepatic resection of CRLM with known KRAS mutational status were identified. Survival estimates were calculated using the Kaplan-Meier method, and multivariable analysis was conducted using the Cox proportional hazards regression model.

Results: In this study, 165 patients (42.4%) underwent nonanatomical resections and 140 (36.0%) presented with KRAS-mutated CRLM. Median disease-free survival (DFS) in the entire cohort was 21.3 months, whereas 1-, 3-, and 5-year DFS was 67.3%, 34.9%, and 31.5% respectively. Although there was no difference in DFS between anatomical and nonanatomical resections in patients with KRAS wild-type tumors (P = 0.142), a significant difference in favor of anatomical resection was observed in patients with a KRAS mutation (10.5 vs. 33.8 months; P < 0.001). Five-year DFS was only 14.4% in the nonanatomically resected group, versus 46.4% in the anatomically resected group. This observation persisted in multivariable analysis (hazard ratio: 0.45; 95% confidence interval: 0.27-0.74; P = 0.002), when corrected for number of tumors, bilobar disease, and intraoperative ablations.

Conclusions: Nonanatomical tissue-sparing hepatectomies are associated with worse DFS in patients with KRAS-mutated tumors. Because of the aggressive nature of KRAS-mutated CRLM, more extensive anatomical hepatectomies may be warranted."[46]

2.5 KRAS status as a modifier of other prognostic factors, towards resolving the tumor laterality debate

Although it is well established that right sided tumors are associated with worse prognoses than left sided tumors in patients with unresectable mCRC, data from studies in patients with resectable colorectal liver metastases have been conflicting.[49-52, 68, 69] Our most recent study aimed to clarify this relationship. After observing that almost all studies in unresectable mCRC were conducted in patients with wild-type KRAS tumors, while studies in resectable CRLM were conducted in cohorts with mixed KRAS statuses, we came to hypothesize that KRAS status may determine whether primary tumor side is prognostic.

Publication 5: The prognostic impact of primary tumor site differs according to the KRAS mutational status: A study by the International Genetic Consortium for Colorectal Liver Metastasis.

Margonis GA, Amini N, Buettner S, Kim Y, Wang J, Andreatos N, Wagner D, Sasaki K, Beer A, Kamphues C, Morioka D, Løes IM, Imai K, He J, Pawlik TM, Kaczirek K, Poultsides G, Lønning PE, Burkhart R, Endo I, Baba H, Mischinger HJ, Aucejo FN, Kreis ME, Wolfgang CL, Weiss MJ. *Ann Surg*. 2019 Aug 5. doi: https://doi.org/10.1097/SLA.0000000000003504

The following text is reproduced in full from the abstract of the publication "The Prognostic Impact of Primary Tumor Site Differs According to the KRAS Mutational Status."

> *"Objective: To examine the prognostic impact of tumor laterality in colon cancer liver metastases (CLM) after stratifying by Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) mutational status.*
>
> *Background: Although some studies have demonstrated that patients with CLM from a right sided (RS) primary cancer fare worse, others have found equivocal outcomes of patients with CLM with RS versus left-sided (LS) primary tumors.*

Importantly, recent evidence from unresectable metastatic CRC suggests that tumor laterality impacts prognosis only in those with wild-type tumors.

Methods: Patients with rectal or transverse colon tumors and those with unknown KRAS mutational status were excluded from analysis. The prognostic impact of RS versus LS primary CRC was determined after stratifying by KRAS mutational status.

Results: 277 patients had a RS (38.6%) and 441 (61.4%) had a LS tumor. Approximately one-third of tumors (28.1%) harbored KRAS mutations. In the entire cohort, RS was associated with worse 5-year overall survival (OS) compared with LS (39.4% vs 50.8%, P = 0.03) and remained significantly associated with worse OS in the multivariable analysis (hazard ratio 1.45, P = 0.04). In wild-type patients, a worse 5-year OS associated with a RS tumor was evident in univariable analysis (43.7% vs 55.5%, P = 0.02) and persisted in multivariable analysis (hazard ratio 1.49, P = 0.01). In contrast, among patients with KRAS mutated tumors, tumor laterality had no impact on 5-year OS, even in the univariable analysis (32.8% vs 34.0%, P = 0.38).

Conclusions: This study demonstrated, for the first time, that the prognostic impact of primary tumor side differs according to KRAS mutational status. RS tumors were associated with worse survival only in patients with wild-type tumors."[54]

3. Discussion

Until 2016, only 22 cases of patients who underwent resection for mutBRAF CRLM were eligible to be included in a relevant meta-analysis, thus precluding a thorough survival analysis.[29] Then, in 2018 and 2019, the three largest relevant studies to date were published. Our own analysis, which is presented in this habilitation thesis, was the first of those and included 43 mutBRAF patients derived from the largest cohort of surgically treated patients with available BRAF mutational status reported up to that date.[30] The aggregate results confirmed the powerful, negative prognostic role of mutBRAF status on outcomes; however, we noted that this effect was not as strong as previously considered (HR in our study was 2.76 versus 3.90 in the 2016 meta-analysis that is mentioned above) and was mostly driven by patients with BRAF V600E mutations, while those with non-V600E mutations seemed to fare much better. Indeed, a favorable prognosis for the BRAF non-V600E genotype had been previously reported in patients with unresectable mCRC.[70, 71] While we were unable to conclusively demonstrate the improved outcomes of patients with BRAF non-V600E mutations via formal analysis secondary to low statistical power, our study was, to our knowledge, the first to report this trend in a surgical cohort. The prognostic differences of the BRAF variants that we observed may be mediated by known differences in their in vivo/vitro oncogenic activities.[72]

Our main findings with respect to the prognostic power of mutBRAF status were corroborated by two subsequent reports, one from a group of investigators from MSKCC (n=35 mutBRAF cases) and another from a French multi-institutional collaborative group (n=66 mutBRAF cases).[73, 74] Collectively these 3 reports demonstrated that BRAF mutations are rare in surgical cohorts (around 2-5% of CRLMs) and that median OS is shorter for patients with mutBRAF (26-53 months) compared to wtBRAF tumors (60-81 months). Where the 3 reports diverged (with regard to OS)

was in their treatment of patients with V600E and non-V600E mutations; while we reported a trend suggesting a distinct prognosis for patients with non-V600E mutations, Gagniere et al from MSKCC suggested that the two groups fared similarly, while Bachet et al refrained from analysis given the low number of patients with this genotype in their cohort. To further assess this preliminary finding, we recently assembled a significantly larger cohort of 240 patients with mutBRAF CRLM (codon-specific data were available for 229) by collaborating with Johns Hopkins, Memorial Sloan Kettering, IGCLM, the French Fédération de Recherche en Chirurgie (FRENCH), the French Association de Chirurgie Hépato-Bilio-Pancréatique et Transplantation (ACHBT) and the French Association des Gastro-Enterologues Oncologues (AGEO). Elements of this work have been presented and the respective manuscript is currently in the final stages of preparation.[75] In summary, 182 patients had BRAF V600E mutations, while 47 harbored non-V600E mutations. The presence of a BRAF V600E mutation was associated with far shorter OS compared to non-V600E mutations (30.6 vs 144 months, P = 0.004). In fact, the median survival of 144 months noted among patients with BRAF non-V600E mutations was longer than what has been historically reported for patients with wtBRAF tumors.[30, 73]

As discussed above, mutBRAF status was originally proposed as a relative contraindication to metastasectomy. However, these results suggest that even patients with BRAF V600E genotypes have acceptable long-term outcomes thus making *prima facie* challenges to the utility of surgery difficult to support. Certainly, the benefit associated with a therapeutic strategy (e.g., surgery) can only be accurately assessed via direct comparison with alternative approaches (e.g., medical or interventional treatment) between well-matched patient groups, or, ideally, in the context of a randomized trial. While indirect comparison of outcomes between medically and surgically treated patients underlies much of the CRLM literature, “good” outcomes after a specific strategy do not

necessarily support its superiority and "poor" outcomes do not necessarily argue in favor of its abandonment; rather, it is possible that the characteristics of a patient group allocated to a specific strategy are responsible for the outcomes rather than the strategy itself. To further clarify the value of surgery in the mutBRAF group, we performed a new, matched comparison of patients treated with resection vs systemic therapy alone (an Italian consortium of medical oncologists collaborated with the author's group at JHH, IGCLM and MSKCC for this study); this work has been accepted as an oral presentation and the respective manuscript is nearing completion.[76] The cohort included 119 surgically treated and 51 medically treated patients with mutBRAF V600E, liver-limited disease. Importantly, the surgical cohort fared substantially better (median OS of 35 vs 20 months, $P < 0.001$) and was the only group with 10-year survivors, a benchmark associated with "cure" as discussed above (17% vs 0%, $P < 0.05$). The surgical cohort continued to fare better even after analysis was limited to its highest risk patients; specifically, high-risk surgical candidates with baseline disease similar to the medical cohort (A), a Clinical Risk Score (CRS) $\geq$ 3 (B) or a Tumor Burden Score (TBS) ≥ 7 (C) continued to have better outcomes than medically treated patients (A: 25 vs 20, B: 28 vs 15 and C: 24 vs 16 months, respectively, all $P < 0.05$). Furthermore, while recurrence after surgery was frequent (80%), it was limited to the liver in 49% of patients and was frequently amenable to repeat hepatectomy (RH); indeed, median OS after recurrence/progression was longer for surgically treated patients (17 vs 7 months, $P < 0.001$), especially if RH was performed.

In summary, this series of publications helped to better define the prognostic impact of BRAF mutations on patients with resectable CRLM. Contrary to what was originally postulated, our findings demonstrated that the negative prognostic impact of BRAF status has previously been overestimated. Moreover, we were able to demonstrate that even among patients with the high

risk mutBRAF V600E genotype, surgery does not appear to be oncologically futile as was previously feared. On the contrary, it can lead to favorable outcomes even among those with the V600E genotype and concurrent high-risk characteristics or following intrahepatic recurrence and RH. As such, technical limitations, performance status, and patient preference should be the only factors affecting the decision to offer metastasectomy in this group. It should be noted that these conclusions are limited to patients without concurrent extrahepatic disease, who formed the vast majority of the aforementioned study populations.

While there is currently no strong data that would justify the exclusion of a patient from operative consideration on prognostic grounds alone, accurate prognostication remains clinically relevant for the purpose of informing patient-physician discussions, tailoring follow-up protocols, and, provided the model in question can achieve adequate and consistent discriminatory ability, guiding patient allocation in clinical trials. In an effort to improve on prior models, we developed the GAME score in 2017, which combined the prognostic impact of KRAS mutation status and traditional clinicopathologic factors in a "hybrid" approach for the first time.[33] The validity of this concept is supported by the fact that similar models were developed independently by two independent groups of investigators, specifically Brudvik et al (modified clinical score [m-CS]) and Lang et al (extended clinical score [e-CS], which replaced RAS with RAS-RAF pathway and incorporates SMAD alterations as well), and published in close temporal proximity to our own.[34, 77] While all of these scores performed better than the then current standard-of-care, it is important to consider their external validity, which has traditionally been one of weakest elements of prior prognostication efforts. In the original publication, the GAME score was externally validated in a cohort from MSKCC and performed well, while the m-CS was successfully validated in an international cohort; the model by Lang and colleagues is the most recent and has not been

externally validated yet. Of all these approaches, external validation in an international cohort is certainly the most robust, as it assesses the stability of the model in patient populations that are likely to differ fundamentally from the one in which the model was developed. While the GAME score was not subjected to this challenge in the index publication, it has been successfully validated in diverse international cohorts ever since.

A group of independent investigators from the Hospital de la Santa Creu in Barcelona (Martin-Cullell et al) reported on such a validation study during the 2019 European Society for Medical Oncology (ESMO) congress, concluding that "The GAME score was validated as a useful preoperative prognostic tool in our cohort of patients."[78] Specifically, Martin-Cullell et al demonstrated that the GAME score was able to stratify patients into groups with distinct prognoses. The high-risk group (GAME score ≥ 4 points) had a median progression-free survival (PFS) of 15.8 months compared with 24.2 months for the low-risk group (GAME score 0–1 points; P = 0.019). Similarly, the high-risk group had a median OS of 38.6 months compared with 68.8 months for the low-risk group (P = 0.05). A second, considerably more extensive (n = 2,376 patients with known KRAS mutation status), external validation study was performed by the author and his collaborators in a dataset of patients from North America (Stanford University School of Medicine and Cleveland Clinic), Europe (University Hospital of Clermont-Ferrand and Inserm, Medical University of Vienna, Medical University of Graz, University of Berlin- Charité and Haukeland University Hospital), South America (Hospital Italiano de Buenos Aires), and Asia (Yokohama City University Graduate School of Medicine and Graduate School of Medical Sciences of Kumamoto University).[79] In the entire dataset, the c-index of the GAME score (0.61) exceeded that of the Fong score (0.57) and the m-CS (0.54). Interestingly, the Fong score, which includes only clinicopathologic variables, had a higher c-index than the m-CS, which also

incorporates RAS mutation status. A comparison of the models across different geographical regions demonstrated that the GAME score performed significantly better ($P < 0.01$) in terms of c-index than the m-CS in North America, Europe, and South America, while also maintaining a numerical advantage in Asia; the c-index of the GAME score was also consistently higher than that of the Fong score, except in the case of Asia where they were equal. The maximum difference between GAME and the other two scores was noted in the cohort derived from South America; the c-index of the GAME score was 0.65, outperforming by far both the m-CS (0.54) and the Fong score (0.57).[79] Collectively, these results render the GAME score the most widely validated of the new "hybrid" prognostic models, further solidifying its value.

Despite the encouraging generalizability of the GAME score, the aforementioned results also clearly demonstrate that the incorporation of KRAS mutation status led to only modest gains in discriminatory ability, failing to consistently exceed a c-index of 0.7 which has proven to be the "high-water mark" of almost all prognostic models (when externally validated) reported to date.[18] Understanding these shortcomings is as important as the incremental prognostic gains achieved via the GAME score. First of all, KRAS mutation status is likely a more modest predictor than originally thought. Indeed, the updated versions of several studies indicate that previously reported hazard ratios overinflated the true association of RAS mutation status with survival, likely due to small sample sizes and incomplete adjustment for confounders.[30, 49, 80] In fact, the author's group has performed a meta-analysis and found an HR of 1.5, which is considerably lower than what was previously reported by Brudvik et al in their 2015 meta-analysis (HR: 2.24).[32, 81] Interestingly, KRAS status is proving to be increasingly similar to older clinicopathologic predictors in exerting a consistent but modest prognostic effect across patient groups. This also applies to patients with extrahepatic disease, as we reported in a presentation to the American College of Surgeons in

2017.[82] In turn, the combination of such modest effects into risk scores has, perhaps predictably, failed to yield a high aggregate discriminatory ability. This may imply that the most potent prognostic factors remain to be discovered, or that, unlike other cancers in which prognostic nomograms have proven successful, CRC is highly complex and its prognosis cannot be captured by a "user friendly" linear model that incorporates a limited number of variables.

Regardless of which of these alternatives proves to be correct, further improvements in prognostication among patients with CRLM can only be achieved by: a) improving the modeling process, b) incorporating new prognostic factors or c) optimizing existing predictors. The gradual introduction of Artificial-Intelligence (AI)-based approaches promises to improve prognostic modeling by more effectively identifying distinct patient subgroups and variable nonlinear interactions. Discovery of novel biomarkers is eagerly anticipated, while introduction of genetic aberrations with potential prognostic significance (e.g., TP53, SMAD4 etc.) into risk models (as was the case with the e-CS by Lang and colleagues) will gradually gain ground as next-generation sequencing renders such data more widely available.[83] Optimization of existing predictors is likely to prove the most immediately practical approach and is equally applicable to KRAS mutation status and traditional clinicopathologic factors. In the former case, it has been previously demonstrated by the author's group and subsequently confirmed by others that different KRAS codon mutations have distinct clinical implications; the prognostic spectrum becomes even more varied once KRAS point mutations are considered.[84-87] Such data can help us reformulate KRAS mutation status as a non-binary variable, with resulting improvements in prognostic power. The optimization of clinicopathologic factors via the selection of appropriate cut-off values is even more important, given their ubiquity in clinical risk scores. For example, a recent systematic review identified three clinicopathologic variables used in the GAME score (size of the largest

liver lesion, number of liver metastases, and Carcinoembryonic Antigen [CEA] levels) as the three most commonly used predictors of survival across all clinical prediction models published until 2015.[18] Similarly, m-CS and e-CS use tumor size as one of their components. Nonetheless, formal statistical approaches to identify cut-offs for these variables have been inconsistently applied in prior reports, suggesting potential for improvement. In response, Kamphues and Andreatos recently applied recursive partitioning analysis to the problem of cut-off determination under the direction of the author, and succeeded in incrementally improving the prognostic impact of the aforementioned three variables.[88] While the *per variable* improvement attributable to each new cut-off was modest, their combination into a risk score can yield appreciable advances in prognostic performance and should be applied in future studies.

Improvements in prognostic modeling that started with the publication of the GAME score and similar "hybrid" models have the potential to improve forecasting of outcomes and ultimately directly influence clinical decision-making. Nonetheless, one should keep in mind that conventional prognostic models are designed to be accurate at the time of surgery, and thus may lose relevance at later time-points if the impact of variables on prognosis changes over time. Data from other malignancies suggest that this is the case, and in response, we explored this possibility for the first time in patients with CRLM in a 2018 study which employed conditional survival analysis.[39] Our findings confirmed this hypothesis, demonstrating that mutBRAF status, an established marker of poor disease biology, dominated prognosis during the first year, with surgery-related factors (i.e., positive surgical margins and resected concurrent extrahepatic disease) subsequently taking the lead in prognostic importance. Interestingly, KRAS mutation status exerted a continuous but moderate prognostic effect over time, in line with its apparent behavior as a component of the GAME score above.

From a clinical standpoint, our findings can be used to provide updated prognostic forecasts and following validation by well-designed clinical trials, can guide individualized postoperative surveillance for patients who underwent resection of CRLM. For example, while it is reasonable to be guarded in prognostic discussions when addressing patients with BRAF mutations at the outset (without precluding them from surgery if they are otherwise good candidates as discussed above), if these patients continue to survive beyond one year, their prognosis no longer appears to be dominated by their mutBRAF status and re-assurance is in order. While this temporal pattern may imply the need for more intensive surveillance and/or distinct adjuvant therapy strategies for mutBRAF patients during the first postoperative year, this remains an open question to be addressed by clinical trials.

The interpretation of these findings is as interesting as their possible clinical significance and may help elucidate the natural history of mutBRAF CRLM. The identified temporal pattern likely reflects the fact that patients with mutBRAF status and truly adverse disease biology (i.e., mutBRAF V600E +/- other unknown modifiers of biologic aggressiveness) largely die of disease within the first postoperative year, while the survivors represent a far more favorable risk sub-cohort or sub-cohorts (e.g., patients with BRAF non-V600E mutations). This complex association is far easier to conceptualize now in light of our recent findings regarding the prognosis of different BRAF mutation profiles (as discussed above) than it was originally, and further reinforces the value of conditional survival analysis. Indeed, this approach successfully identified a pattern that, while initially uninterpretable, ultimately proved biologically significant and would have been missed by a traditional survival model.

Importantly, our results also suggest that patients with microscopically positive margins remain at high risk of adverse outcome for many years after resection. This finding not only emphasizes the

importance of surgical technique in preventing adverse outcomes in the first place, but also suggests that these patients should perhaps be followed more closely, even if they appear to be doing well for a considerable interval after resection.

Although the concept of conditional survival is well-established and has been applied to many malignancies, the current study was original both in terms of applying the method to patients with CRLM and in shifting the focus of the analysis from the risk of death to the changing impact of prognostic factors over time. Thus far, our study has been followed by a single similar analysis reported in 2019 by Kawaguchi et al from MD Anderson which focused on conditional recurrence rather than OS. The authors concluded that the presence of RAS/TP53 co-mutation had a persistent, highly adverse impact on recurrence risk over time. The persistent effect is in line with our own findings on the impact of KRAS mutation status, although our results suggest a more moderate effect on outcomes than that report by Kawaguchi et al.[89] This discrepancy can be readily explained, however, as KRAS-TP53 co-mutation is associated with much worse prognosis compared to KRAS mutation alone; moreover, the two studies assess different outcomes, which prevents direct comparison.[90, 91] Indeed, within the relatively brief follow-up period of most studies, it is not difficult to conceptualize a scenario in which an adverse prognostic factor would be associated with more recurrence events than deaths; this is an additional possible explanation for the observed discrepancy.

Aside from our efforts to improve patient selection and prognostication, we performed a study with direct implications for the surgical management of patients with CRLM. This study was published in 2017 and initially aimed to indirectly assess whether mutKRAS lesions demonstrate a diffuse pattern of intrahepatic spread necessitating wider resection as postulated by some authors.[44, 46] Importantly, the findings exceeded this initial goal, and suggested for the first time

that surgical technique (i.e., anatomical hepatectomy) may be tailored to a specific mutation profile. Not only was this finding original in demonstrating the potential of "precision surgery" based on better understanding of disease biology, but it was also controversial as it questioned the standard of practice at that time, which favored more limited resections.

This work was received positively by the scientific community, summarized by an invited commentary to Nature Reviews that stated that "In conclusion, we congratulate Margonis et al. for bringing to the forefront surgery and tumour biology in patients with CLM."[92] Of note, the commentary did question our decision not to exclude patients who received concurrent intraoperative ablation. However, these criticisms were then addressed in a sub-analysis that excluded these patients; importantly, our main findings remained essentially unchanged.[93] Subsequently, Vigano et al expressed some additional concerns in a letter published in Annals of Surgery that were fully addressed in our respective response letter. Of note, a principal counter-argument centered around the increased theoretical potential for RH following recurrence among patients treated with more "conservative", non-anatomical resections, as well as the theoretical loss of OS benefit that such repeat resections would confer if the anatomical approach was followed. This argument being somewhat speculative is perhaps the hardest to rebut with data; its validity would have needed no further justification if the non-anatomical approach could be shown to result in improved survival both after recurrence and in aggregate, but this was not the case in our dataset. Indeed, it is possible that non-anatomical approaches may result in such suboptimal outcomes after the index procedure in certain patient subgroups (e.g., those with mutKRAS lesions) that any theoretical post-recurrence benefit is nullified. In addition, both feasibility and success of RH is especially unlikely among patients with mutRAS CRLM as was demonstrated in a series of studies by a group of investigators from MD Anderson in 2017.[94, 95] Clearly the findings

of a single retrospective study are subject to both predictable and unforeseeable sources of bias and cannot be used in isolation to support a radical change in therapeutic approaches. In the absence of clinical trial data, it is helpful to compare our findings with other similar reports in the literature.

While not directly analogous to our study, a report from a group of French investigators provided indirect evidence of the existence of a "KRAS-specific" pattern of tumor spread, which may be most effectively treated with anatomical resection. Specifically, Renaud et al demonstrated that in a cohort of patients undergoing resection of lung metastases from CRC anatomical and non-anatomical approaches yielded similar outcomes among patients with wtKRAS tumors.[96] However, patients with mutKRAS tumors had a significantly prolonged time to pulmonary recurrence (defined as the interval between a thoracic metastasectomy and lung recurrence) following an anatomical resection compared to a non-anatomical approach. While the underlying cause of these results remains speculative and the microscopic growth pattern of mutKRAS lesions has yet to be elucidated, this evident similarity in the findings of two geographically remote, independent surgical series investigating two distinct metastatic sites is unlikely to be coincidental and deserves further study.

Following a prolonged dearth of relevant publications, a group of investigators from MD Anderson very recently published a study that aims to re-assess the potential impact of anatomical and non-anatomical resections on patients with mutKRAS CRLM.[86] While the authors concluded that anatomical resections were not associated with a survival benefit in patients with mutKRAS tumors, a number of methodologic short-comings are worth mentioning. Careful examination of the respective Kaplan-Meier curves not only demonstrates a clear visual trend in favor of anatomical resection for both overall RFS and liver-specific RFS among mutKRAS patients, but

also questions the use of the log rank test in the setting of likely violation of the proportional hazards assumption (as evidenced by the crossing Kaplan-Meier curves). Indeed this methodologic approach likely further increased the risk of Type II error, which in combination with limited baseline statistical power, likely drove the study's negative findings.[97]

In summary, the aforementioned findings point to a link between KRAS mutation status and optimal surgical technique, which could theoretically be mediated by distinct growth patterns of mutKRAS and wtKRAS tumors. While these remain hypotheses to be proven and no definitive treatment recommendations can be made until these findings are validated by a well-designed clinical trial, our study played a significant role in initiating a lively debate on the role of precision surgery and individualized therapy in patients with CRLM. As our understanding of disease biology grows over the next few years, this debate is likely to prove fruitful.

The final study (from a chronological standpoint) included in this habilitation thesis was performed in 2019 with the aim of clarifying the interplay of KRAS mutation status with another proxy of tumor biology and outcomes, namely primary tumor laterality.[54] Specifically, we hypothesized that the inconsistent association of primary tumor laterality with survival among patients with CRLM was a result of as yet undetermined interplay with KRAS mutation status as described in the introduction above. Indeed, we demonstrated that while right-sided (RS) disease was associated with worse outcomes in patients with wtKRAS status, tumor laterality was not prognostic in those with mutKRAS genotypes. This implies that the relative frequency of patients with mutKRAS tumors in a given cohort can impact the ability to detect the prognostic effect of primary tumor laterality. This is consistent with a prior study by Sasaki et al conducted in a cohort of patients treated at the Johns Hopkins Hospital. Specifically, among patients with wtKRAS tumors, the overall survival of patients with LS tumors was numerically superior to those with RS

disease (median OS: 65.8 vs 56.4 months, respectively). In contrast, among those with mutKRAS tumors, OS was comparable (median OS was 46.8 months for those with RS tumors and 44.0 for those with LS tumors).[98, 99]

Given these findings, it is tempting to speculate on the differences in molecular profiles that provide mutKRAS lesions with a relatively uniform prognosis irrespective of tumor laterality, while outcomes among wtKRAS patients differ so significantly for RS vs LS disease. Some reports have suggested that KRAS mutation is not prognostic unless there is a coexisting TP53 or SMAD4 mutation.[90, 91] While this is likely a gross oversimplification, the relatively equal distribution of these two "activating" mutations between RS and LS disease may, to some extent, account for the similar prognosis of RS/mutKRAS and LS/mutKRAS tumors.[99] Among wtKRAS patients, tumor laterality drives outcomes likely as a result of other activating mutations that may be (largely) mutually exclusive with KRAS such as BRAF V600E, which is associated with poor prognosis and encountered far more frequently in RS disease.

The rough schema that emerges from these findings is that of a LS/wtKRAS group with excellent prognosis, a RS/wtKRAS group with guarded prognosis, and RS/LS/mutKRAS groups with the worst outcomes. Despite corroboration of these results in patients with CRLM, we were unsure if these findings could be extrapolated to patients with non-metastatic CRC, as stated in the limitations section of our work. A subsequent study that the author had a major role in designing was aimed precisely at assessing the interplay of KRAS status and tumor laterality in a cohort of patients with non-metastatic CRC treated at six academic centers in Europe and Japan.[100] In this cohort, KRAS mutation status was only found to be prognostic among patients with LS disease, a finding consistent with the general schema above (i.e., as the LS/wtKRAS group has the best prognosis and LS/mutKRAS is similar to RS/mutKRAS, it follows that LS/wtKRAS-

LS/mutKRAS>RS/wtKRAS-RS/mutKRAS).[100] A recent study from the Massachusetts General Hospital (Cavallaro et al, 2020) further confirmed these findings among patients with CRLM.[101] Whether these results stem from true interactions between tumor laterality and KRAS status or a simple superimposition of distinct prognostic effects remains to be determined, as discussed by the author in a recent relevant commentary.[99] In either case, a more thorough investigation of the molecular profile of RS and LS disease is warranted, as is the exploration of a possible predictive role of tumor laterality in patients with CRLM, as in the case of mCRC.

In conclusion, the common message of the five studies included in this habilitation thesis is that tumor biology is not only an important cause of, but also a possible solution to many clinical problems. The relationship between tumor biology and surgical practice is gradually being transformed by discoveries that allow us to comprehend the former and adapt to the challenges it imposes. This is indeed a novel development; until recently, few could deny the relevance of Blake Cady's famous dictum according to which "Biology is King; selection of cases is Queen, and the technical details of surgical procedures are princes and princesses of the realm who frequently try to overthrow the powerful forces of the King and Queen … usually to no long-term avail, although with some temporary apparent victories."[102] While the King's overthrow is far from imminent, we have at last began to comprehend the nature of his power, which may allow us more frequent and less temporary victories. Despite the persistence of several critical gaps in our understanding of tumor biology, recent developments in the field herald the beginning of a new era, removed from complete reliance on clinicopathologic factors and conducive to more personalized and effective treatment approaches. The studies discussed in the present thesis may not present a complete picture of what will surely remain a rapidly evolving biologic puzzle, but, in our view, have successfully contributed to the development of novel concepts and the re-appraisal of traditional

ones in a systematic and coherent fashion over the last five years. We look forward to continuing these investigations and hope that ongoing developments in genomics, molecular therapeutics, and Artificial Intelligence will help to completely transform the field in the near future.

4. Summary

The research work summarized in this thesis is largely focused on the role of KRAS and BRAF mutation status in determining prognosis and guiding management among patients with resectable CRLM. The decision to focus our investigation on these two biomarkers reflects both their central importance in colorectal carcinogenesis and the availability of relevant data which are collected as part of routine practice in most institutions. The publications presented in this work follow a well-defined continuum that traces the evolution of a number of interconnected ideas on the impact and possible role of available genetic data in the clinical management of patients with CRLM. This intellectual journey begun in 2015 with our first report confirming the prognostic role of KRAS mutation status in CRLM. Subsequently, we proceeded to demystify the impact of BRAF mutations on outcomes which at the time were considered synonymous with poor survival. While we confirmed that BRAF mutation status was indeed a potent predictor of poor prognosis, we were also able to identify distinct subgroups with favorable outcomes (i.e., patients with non-V600E mutations) and ultimately dispel the idea that these patients do not benefit from operative management. Despite these discoveries, when we sought to improve prognostication among patients with CRLM by introducing genetic biomarkers into contemporary risk scores, we found ourselves obligated to employ KRAS, rather than BRAF, due to the much higher incidence of KRAS mutation in CRLM cohorts. The result of this effort was the GAME score, which has gradually emerged as the most robust and accurate of a series of “hybrid” prognostic models that

combine genetic and clinicopathologic variables. More so than its successes, the limitations of the GAME score and its predecessors proved of vital importance to future research endeavors by identifying persistent deficits in prognostication among patients with CRLM. One such example was the realization that contemporary survival models offered a static picture of projected outcomes at the time of surgery, failing to reflect clinical developments in a dynamic fashion. Conditional survival analysis helped address this limitation, confirming the prognostic discrepancies between patients with different BRAF codon mutations and highlighting the long-term importance of traditional variables such as surgical margin. The idea that tumor biology could impact tumor growth patterns and thus dictate surgical technique most likely to result in disease control led us to identify the possible utility of anatomical hepatectomy among patients with KRAS mutations. While definitive proof of efficacy will need to await dedicated clinical trials, this work initiated a debate on the potential of "precision surgery," which remains active to this date. Lastly, having largely explored the prognostic implications of KRAS mutation status among patients with CRLM, we employed this variable to help reconcile disparate reports on the effect of tumor laterality on outcomes, paving the way for the utilization of this powerful prognostic factor in future risk scores.

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7. Disclosure

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