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Mutations of *RAS/RAF* Proto-oncogenes Impair Survival After Cytoreductive Surgery and HIPEC for Peritoneal Metastasis of Colorectal Origin

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Background: Adequate selection of patients with peritoneal metastasis (PM) for cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) remains critical for successful long-term outcomes. Factors reflecting tumor biology are currently poorly represented in the selection process. The prognostic relevance of *RAS/RAF* mutations in patients with PM remains unclear.

Methods: Survival data of patients with colorectal PM operated in 6 European tertiary centers were retrospectively collected and predictive factors for survival identified by Cox regression analyses. A simple point-based risk score was developed to allow patient selection and outcome prediction.

Results: Data of 524 patients with a median age of 59 years and a median peritoneal cancer index of 7 (interquartile range: 3–12) were collected. A complete resection was possible in 505 patients; overall morbidity and 90-day mortality were 50.9% and 2.1%, respectively. PCI [hazard ratio (HR): 1.08], N1 stage (HR: 2.15), N2 stage (HR: 2.57), G3 stage (HR: 1.80) as well as *KRAS* (HR: 1.46) and *BRAF* (HR: 3.97) mutations were found to significantly impair survival after CRS/HIPEC on multivariate analyses. Mutations of *RAS/RAF* impaired survival independently of targeted treatment against EGFR. Consequently, a simple point-based risk score termed BIOSCOPE (BIOlogical Score of COlorectal PEritoneal metastasis) based on PCI, N-, G-, and *RAS/RAF* status was developed, which showed good discrimination [development area under the curve (AUC) = 0.72, validation AUC = 0.70], calibration (*P* = 0.401) and allowed categorization of patients into 4 groups with strongly divergent survival outcomes.

Conclusion: *RAS/RAF* mutations impair survival after CRS/HIPEC. The novel BIOSCOPE score reflects tumor biology, adequately stratifies long-term outcomes, and improves patient assessment and selection.

Keywords: colorectal carcinoma, cytoreductive surgery (CRS), hyperthermic intraperitoneal chemotherapy (HIPEC), *KRAS/NRAS/BRAF*, peritoneal metastasis

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Peritoneal metastasis (PM) from colorectal carcinoma (CRC) carries a worse prognosis than other isolated distant metastases of CRC.[1] Over the last years, the combination of effective systemic therapy in combination with cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) improved cancer-specific survival (CSS) in patients with PM.[2] Although it has been shown that this invasive procedure can be performed with acceptable mortality and morbidity rates,[3] selection of patients is critical to avoid unnecessary procedures in patients, who do not benefit from CRS/HIPEC.[4] Multiple factors influence successful postoperative long-term oncological outcomes, for example, the completeness of resection[5] or the absence of major postoperative complications.[6,7]

However, the role of tumor biology and *RAS/RAF* mutations in the context of PM remains unclear. RAS/RAF proteins work as downstream secondary messengers of the epidermal growth factor receptor (EGFR) which is expressed on 85% of patients with metastatic CRC.[8] *RAS* mutations belong to the hallmark characteristics of CRC and affect between 30% to 50% (*KRAS*) and 3% to 5% (*NRAS*) of patients with metastatic CRC,[8,9] whereas mutations of *BRAF* are found in 3% to 10%[9–13] and are mutually exclusive with *RAS* mutations.[13,14] Antibodies blocking EGFR (Cetuximab and Panitumumab) and small-molecule inhibitors (Erlotinib and Gefitinib), preventing intracellular tyrosine kinase activation, were developed to counteract activation of RAS/RAF proteins and their downstream targets.[15] They modestly improve overall- and recurrence-free survival (RFS), when given alone or in combination with standard regimens.[16,17] In patients with PM of colorectal origin, the role of *RAS/RAF* mutations has not been examined and might be helpful to predict survival after CRS/HIPEC in combination with other prognostic factors. Available scores[18] and nomograms[19] are complex, and show a moderate ability to predict the outcome of patients after CRS/HIPEC.[20] Currently, there is no preoperative scoring system which incorporates tumor biology as a prognostic factor and accurately predicts oncological outcomes. The primary aim of our current analyses was therefore to investigate the role *RAS/RAF* on CSS and RFS in patients undergoing CRS/HIPEC.

Secondary goal was to develop a sore based on tumor biology-related factors to adequately predict oncological outcomes.

METHODS

Patients and Ethics

The study was approved by the lead ethics committee of the cantonal authorities in Zurich, Switzerland (KEK-ZH-Nr. 2017-01656) and the respective local authorities (Supp. Info. 1, http://links.lww.com/SLA/B449). Data of patients with PM from colorectal cancer from 6 European centers (Zurich, St. Gallen, and Lausanne, Switzerland; Lyon, France; Murcia, Spain; and Vienna, Austria) were collected and analyzed. Latest follow-up data including dates of death or recurrences and further oncological therapies were obtained from the hospital databases or by directly contacting patients or oncologists.

Treatment

Patients were presented at multidisciplinary tumor boards at the respective institutions and selected for CRS/HIPEC after clinical workup and exclusion of extra abdominal tumor manifestations by [18]FDG-PET/CT or thoracic-abdominal CT. Patients received standard of care pre- and postoperative chemotherapy according to international guidelines, based on leucovorin, 5-fluorouracil, irinotecan, and/or oxaliplatin, in combination with targeted therapies (monoclonal antibodies or small molecule inhibitors targeting VEGFα/angiogenesis or EGFR) where appropriate. CRS/HIPEC was performed by experienced and trained surgeons in all centers as specified elsewhere.[21,22] Mitomycin C sole [15–30 mg/m^2 Body Surface Area (BSA)] or in combination with doxorubicin (15 mg/m^2 BSA) at 42°C for 90 minutes or oxaliplatin (300–400 mg/m^2 BSA) as single agent at 43°C for 30 minutes were used for HIPEC. A procedure was defined as curative, if radical CRS (CC-Score 0,[23] no macroscopic residual tumor) followed by HIPEC could be performed.

Pathology

PCI was calculated after careful inspection following laparotomy at the beginning of CRS/HIPEC. Information on TNM-stage, G-stage, histology, and *RAS/RAF* status was retrieved from pathology reports of the operating hospital or referral letters and previous pathology reports.

Statistical Analyses and Risk Score Development

All statistical analyses were performed with R (Supp. Info. 2, http://links.lww.com/SLA/B449). Continuous data are reported as median (m) and interquartile range (IQR). Wilcoxon rank-sum test or Fisher exact test were used to compare medians, resp. frequencies between groups. Statistical significance was defined as $P < 0.05$. CSS and RFS were calculated with Kaplan–Meier estimates from the date of the operation until date of cancer-related death, disease

TABLE 1. Baseline Patient Demographic, Clinicopathological, and Treatment Characteristics of 524 Patients Undergoing CRS/HIPEC for PM of Colorectal Origin

Variable	n (%)
Sex	
Male	257 (49.1%)
Female	267 (50.9%)
Age, yr	59 (50–66)
T-stage of the primary tumor	
T3	119 (22.7%)
T4	213 (40.7%)
Tx/not available	192 (36.6%)
N-stage of the primary tumor	
N0	85 (16.2%)
N1	119 (22.7%)
N2	131 (25.0%)
Nx/not available	189 (36.1%)
G-stage/histological grading	
G1	90 (17.2%)
G2	211 (40.3%)
G3	88 (16.8%)
Gx/not available	135 (25.7%)
Histological subtype	
Intestinal adenocarcinoma	401 (76.5%)
Mucinous adenocacinoma	62 (11.8%)
Signet-ring cell adenocarcinoma	37 (7.1%)
Not available	24 (4.6%)
Tumor localization	
Caecum	57 (10.9%)
Colon ascendens	123 (23.5%)
Colon transversum	22 (4.2%)
Colon descendens	82 (15.6%)
Sigmoid colon	195 (37.2%)
Rectum	36 (6.9%)
Not available	9 (1.7%)
Appearance of PM	
Synchronous	283 (54.0%)
Metachronous	241 (46.0%)
RAS/RAF mutations	
Wild-type	202 (38.5%)
KRAS mutation	154 (29.5%)
NRAS mutation	24 (4.6%)
BRAF mutation	20 (3.8%)
Not available	154 (29.4%)
Center	
Lausanne	15 (2.7%)
Lyon	363 (69.3%)
Murica	25 (5.0%)
St. Gallen	33 (6.3%)
Vienna	17 (3.3%)
Zurich	70 (13.4%)
PCI	
PCI 0–5	212 (40.5%)
PCI 6–10	163 (31.1%)
PCI 11–15	55 (10.5%)
PCI 16–20	56 (10.6%)
PCI 21–25	29 (5.4%)
PCI 26–30	7 (1.3%)
PCI 31–35	1 (0.2%)
PCI 36–39	1 (0.2%)
Median PCI (+IQR)	7 (3–12)
HIPEC regimen	
Oxaliplatin	266 (50.8%)
Mitomycin C/Doxorubicin	44 (8.4%)
Mitomycin	171 (32.6%)
Other/not documented	43 (8.2%)

(*Continued*)

TABLE 1. (*Continued*)

Variable	n (%)
Operation time, min	308 (240–420)
Not available	179
Bloodloss, mL	300 (112.5–537.5)
Not available	458
Postoperative ICU stay, d	1 (1–2)
Not available	9
Postoperative hospital stay, d	16.5 (13–25)
Not available	6
OP potentially curative treatment	
Yes	505 (96.4%)
No	19 (3.6%)
Complications according to Clavien-Dindo classification	
None	257 (49.1%)
Grade 1	45 (8.6%)
Grade 2	133 (25.4%)
Grade 3a	32 (6.1%)
Grade 3b	24 (4.6%)
Grade 4a	17 (3.2%)
Grade 4b	5 (0.9%)
Grade 5	11 (2.1%)
Comprehensive complication index (*Slankamenac, Ann Surg, 2013*)	20.9 (20.9–34.4)
Pre-HIPEC chemotherapy	
No chemotherapy	112 (21.4%)
Conventional chemotherapy only	176 (33.6%)
No. of cycles	6 (4–9)
Chemotherapy + anti-VEFGα treatment	152 (29.0%)
No. of cycles	6 (4–9.5)
Chemotherapy + anti-EGFR treatment	74 (14.1%)
No. of cycles	5 (4–7)
Chemotherapy + multiple targeted therapies	10 (1.9%)
No. of cycles	12 (6.5–12)
Post-HIPEC chemotherapy	
No chemotherapy	205 (39.1%)
Conventional chemotherapy only	165 (31.5%)
No. of cycles	6 (5–8)
Chemotherapy + anti-VEFGα treatment	85 (16.2%)
No. of cycles	6 (5–8.5)
Chemotherapy + anti-EGFR treatment	50 (9.5%)
No. of cycles	7 (5–9)
Chemotherapy + multiple targeted therapies	19 (3.7%)
No. of cycles	6 (5–11.5)

CRS indicates cytoreductive surgery; HIPEC, hyperthermic intraperitoneal chemotherapy; PM, peritoneal metastasis.
Continuous variables are shown as median and interquartile range.

(n = 115), and patients with wild-type *RAS/RAF* treated with anti-EGFR treatment (n = 77) (Fig. 1C). Interestingly, patients with wild-type *RAS/RAF* had improved survival regardless of treatment with ($P = 0.047$) or without ($P = 0.028$) targeted anti-EGFR treatment compared with patients with RAS/RAF mutations, indicating a mutation-caused rather than treatment-related effect on survival.

Development of a New Risk Score for Patient Selection Based on Tumor Biology

Randomized splitting allocated 358 (72.4%) patients into the development cohort and 136 (27.6%) patients into the validation cohort (Fig. 2A). A Cox regression model with the four identified predictive factors PCI, N-, G-, and *RAS/RAF* mutation status was consequently fitted to the development cohort, which discriminated survival at 36 months with a AUC-value of 0.72 in the development cohort (Fig. 2B) and 0.70 in the validation cohort (Fig. 2C). The good discrimination of >0.7 was consistent over all time points tested

TABLE 2. Uni- and Multivariate Analysis of Factors Predicting Cancer-specific Survival (CSS) in Patients With PM of Colorectal Origin After Complete CRS/HIPEC (n = 494)

			n var ate Ana ys s				Mu t var ate Ana ys s			
Var ab e	n	Med an C I o CI	Hazard at o	CI	*P*	Hazard at o	CI	*P*
Sex									
Male	236 (47.8%)	50 (35–65)							
Female	258 (52.2%)	43 (35–53)	1.17 (0.87–1.57)		0.284				
Age (per additional year)						0.98 (0.97–1.00)		0.405	
Year of CRS/HIPEC									
2005–2012	193 (39.1%)	45 (40–50)							
2013–2017	301 (60.9%)	44 (34–53)	1.18 (0.86–1.63)		0.299				
T-stage									
T3	113 (36.4%)	45 (26–63)							
T4	197 (63.6%)	38 (28–48)	1.23 (0.85–1.79)		0.259				
N-stage									
N0	80 (25.6%)	57 (51–62)							
N1	111 (35.5%)	36 (26–45)							
N2	122 (39.0%)	36 (27–46)		≤			≤	
G-stage									
G1	86 (23.6%)	51 (38–65)							
G2	200 (55.0%)	45 (30–60)	0.92 (0.62–1.38)		0.714	1.09 (0.71–1.67)		0.672	
G3	78 (21.4%)	31 (22–40)							
Histology									
Adenocarcinoma	380 (80.5%)	47 (39–54)							
Mucinous adenocarcinoma	61 (12.9%)	49 (23–75)	1.21 (0.78–1.89)		0.385	1.19 (0.69–2.04)		0.526	
Singet ring cell	31 (6.6%)	34 (21–47)				1.07 (0.56–2.04)		0.835	
Localisation									
Coecum/ascendens	171 (35.2%)	36 (29–43)							
Transversum/descendens	99 (20.4%)	77 (40–104)				0.67 (0.42–1.04)		0.073	
Rectosigmoid	216 (44.4%)	51 (39–63)				0.78 (0.55–1.09)		0.155	
Temporal appearance of PM									
Synchronous	268 (54.3%)	41 (33–49)							
Metachronous	226 (45.7%)	48 (39–57)	0.85 (0.63–1.15)		0.302				
PCI per additional point					≤			≤	
***RAS/RAF* mutation**									
Wildtype	192 (50.8%)	52 (40–65)							
KRAS mutation	145 (38.4%)	38 (31–46)							
NRAS mutation	19 (5.0%)	49 (19–80)	1.22 (0.65–2.33)		0.533	0.88 (0.45–1.72)		0.711	
BRAF mutation	22 (5.8%)	18 (9–26)			≤			≤	
Major complication (≥Clavien Dindo classification 3A)									
No major complication	421 (85.2%)	49 (40–57)							
Major complication	73 (14.8%)	38 (27–49)				1.20 (0.78–1.84)		0.406	

CI indicates confidence Interval; CRS, cytoreductive surgery; CSS, cancer-specific survival; HIPEC, hyperthermic intraperitoneal chemotherapy; IQR, interquartile range; PCI, peritoneal cancer index; PM, peritoneal metastasis.

Significant values are in bold letters.

(Supp. Fig. 2A, http://links.lww.com/SLA/B449). Comparison of observed survival frequencies and predicted survival probabilities at 36 months (Fig. 2D) and additional time points (Supp. Fig. 2B and C, http://links.lww.com/SLA/B449) confirmed the good calibration of the model in the development (goodness-of-fit test: *P* = 0.401) as well as validation cohort (goodness-of-fit test: *P* = 0.483). Cross-validation yielded a global shrinkage coefficient of 0.9098. Using the shrinkage-adjusted β regression coefficients for allocation of points to the respective factors levels, the risk score with a range of 0 to 12 points was calculated (Fig. 3A). The resulting score was consequently applied on development and validation cohort and showed a consistent stepwise decrease of mCSS with increasing amount of points (Fig. 3B and C). In a last step, 4 risk groups were defined based on the allocated points. Patients with a risk score of 0 (risk group A) showed excellent long-term outcomes. Patients with minimal (1–3) risk points had a median survival over the average of 43 months of the whole cohort and were defined as risk group B. Patients with intermediate risk scores between 4 and 7 (risk group C) showed an acceptable survival after CRS/HIPEC, whereas patients with risk scores 8 and higher (risk group D) had a dismal long-term outcome (Fig. 3D and E); the score also allowed stratification of PFS in both cohorts to a certain extent (Supp. Fig. 3A and B, http://links.lww.com/SLA/B449).

DISCUSSION

The present study for the first time identifies mutations of *RAS/RAF* oncogenes as a risk factor for overall survival after CRS/HIPEC in patients with colorectal PM. In addition, we present a novel score (BIOSCOPE) to stratify patients with colorectal PM before CRS/HIPEC.

The role of *RAS/RAF* mutations in CRC is known as a surrogate marker for response rates to targeted chemotherapy[30] and overall survival in the setting of palliative chemotherapy.[31] In patients with CRLM, *RAS* mutations are a prognostic factor[32,33,34] and were included in a newly proposed modified version of the Clinical Risk Score for Prediction of Recurrence after resection of CRLM.[35] In contrast, its role as a predictive factor in patients
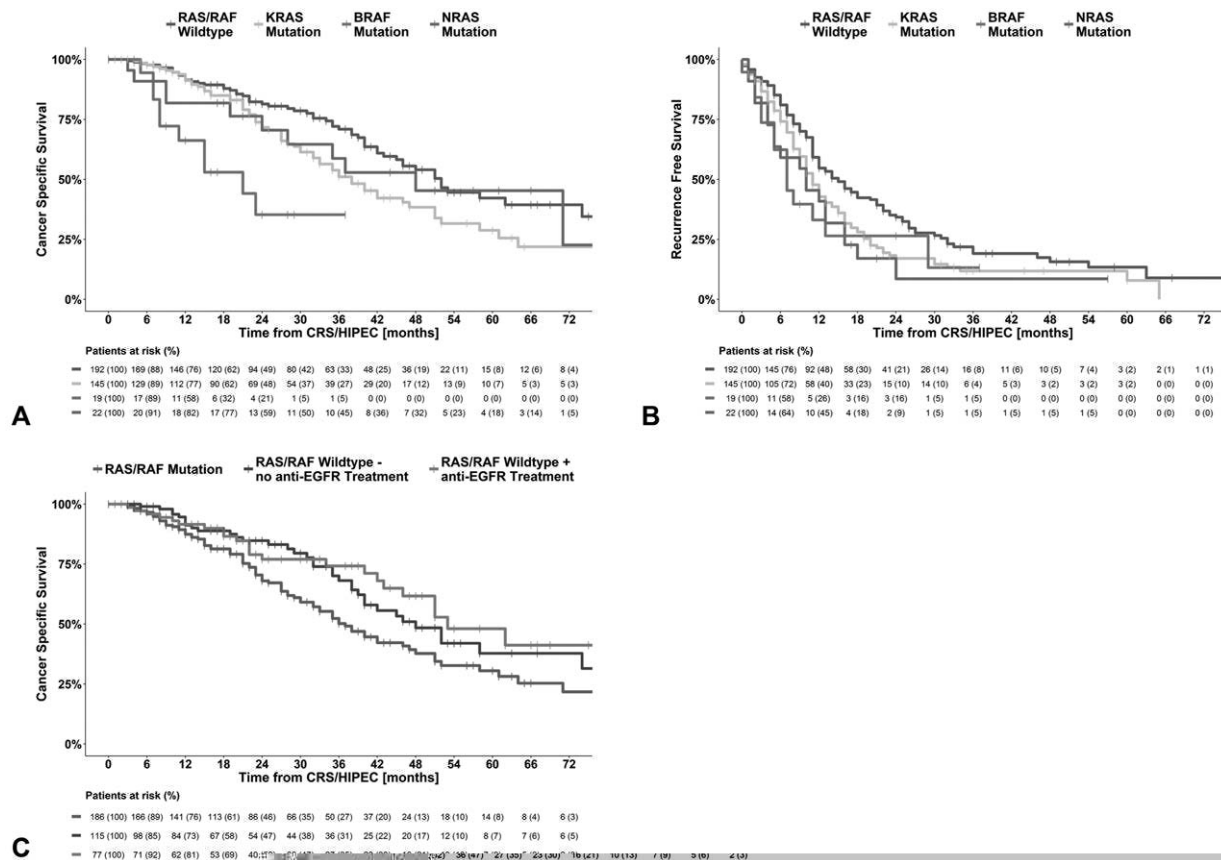
FIGURE 1. The influence of *RAS/RAF* mutations on outcomes after CRS/HIPEC. (A) While patients without *RAS/RAF* mutations show a median CSS of 52 months (CI: 40–65), outcomes for patients with *KRAS* mutations (mCSS 38 mo, CI: 31–46, *P* = 0.036) and *BRAF* mutations (mCSS of 21 mo, CI: 9–26, *P*≤0.001) were strongly impaired. No significant effect of *NRAS*mu3Tf1.89((mutatioTc/m76eTd()Tj/R328.9

undergoing surgery for PM, which are known to have a worse prognosis than liver or lung metastasis, is unclear.[1] In the present study, we are able to demonstrate the specific influence of *RAS/RAF* mutations on CSS and RFS in patients with PM from CRC undergoing CRS/HIPEC. We found impaired CSS and PFS after CRS/HIPEC for *KRAS* mutations, a heavy impact on CSS for *BRAF* mutations, whereas *NRAS* mutations did only impact on PFS in our cohort. Differences between *KRAS*, *NRAS*, and *BRAF* mutations in postsurgical outcomes have so far not been described.

Our analysis confirmed other predictive factors, for example, the amount of peritoneal disease, assessed by the PCI, positive lymph node status of the primary tumor, and tumor grading.[36,37] We consequently included PCI, N-, G-, and *RAS/RAF* mutational status in a Cox model, which showed good discrimination and calibration on the development as well as the validation cohort. We finally developed a score with a maximum of 12 points, which we termed **BIO COPE** (BIOlogical Score of COlorectal PEritoneal metastasis). When applied to both our cohorts, increasing score numbers strongly correlated with decreasing survival.

We then determined 4 groups based on points: BIOSCOPE A (0 risk points) represents patients with absent risk factors (PCI ≤10, N0, G1–2, *RAS/RAF* wt). These patients can expect an excellent long-term outcome, reflected by mCSS of 70 and 65 months in our cohorts. BIOSCOPE B (1–3 risk points) reflects patients with moderate risk factors; these patients are able to reach mCSS of 50 and 39 months respectively, which is equal and above the mCSS of 40 to 45 months reported in recent analyses of patients undergoing CRS/HIPEC.[6,7] BIOSCOPE C patients (4–7 points) profit from CRS/HIPEC with a mCSS of 33 and 25 months, which is clearly superior to the mCSS of 16.9 months in patients with PM treated systemically with modern targeted chemotherapy only.[1] In contrast, BIOSCOPE D patients (≥8 points) show a dismal survival of 13 (development) and 7 (validation) months only. CRS/HIPEC in these patients should be evaluated critically regarding possible complications and time for convalescence, and the decision for CRS/HIPEC should be made carefully on an individual basis.

We would like to acknowledge the limitations of our study. External validation is currently lacking and must be performed in an additional, independent patient cohort to confirm the value of the
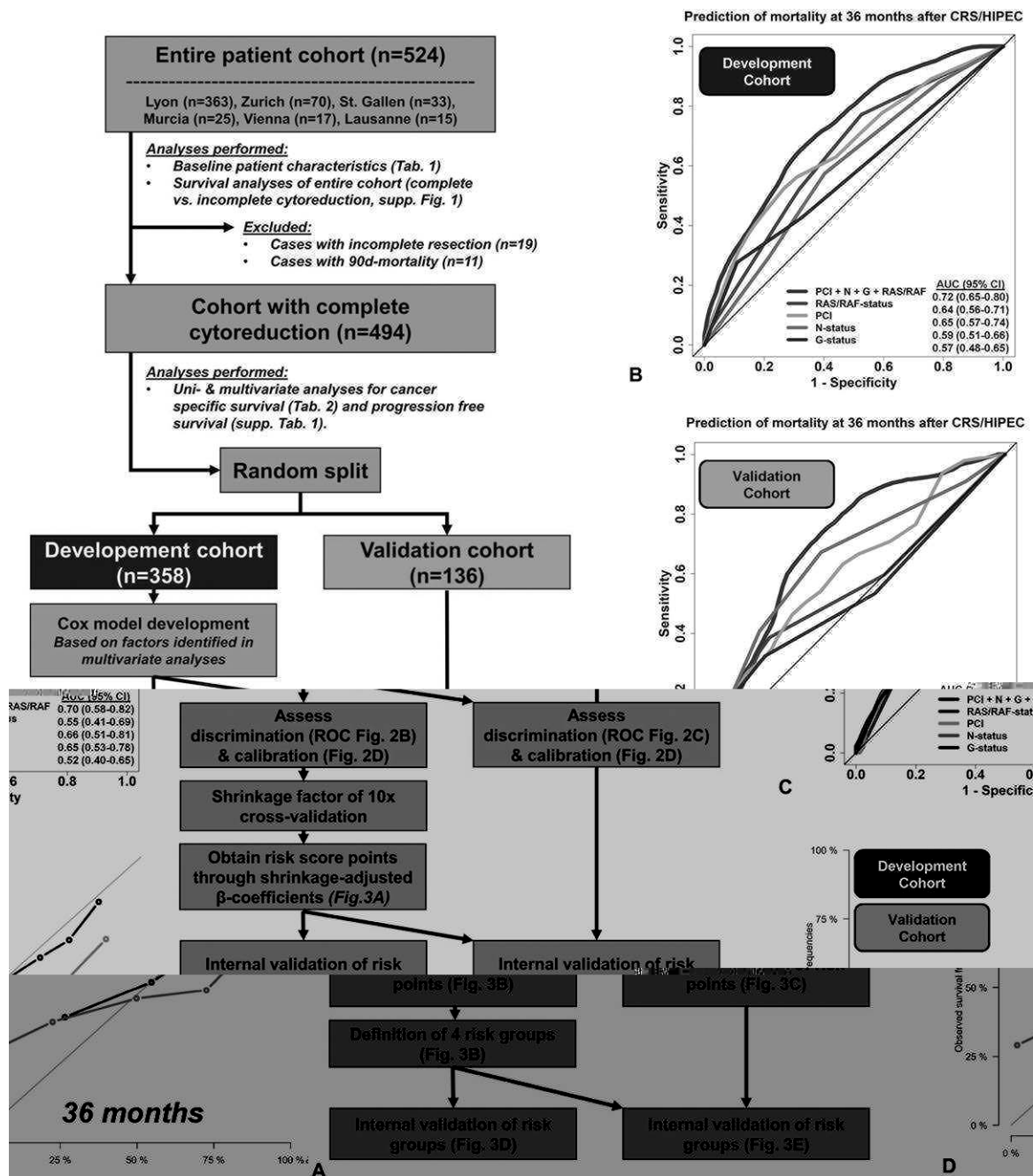
FIGURE 2. Discrimination and calibration of Cox regression model. (A) Methodological flow schema of patient cohort analyses and random splitting into development and validation cohort. (B) The Cox regression model with the 4 major predictive factors PCI, N-, G-, and *RAS/RAF* mutation status predicted survival at 3 years in the development cohort with a AUC value of 0.72 (CI: 0.65–0.80, R^2: 0.167) and showed superior discrimination compared to models consisting of the single predictive factors PCI (AUC: 0.65, R^2: 0.093), N-status (AUC: 0.59, R^2: 0.03), G-status (AUC: 0.57, R^2: 0.028), or *RAS/RAF* mutational status (AUC: 0.64, R^2: 0.031) only. (C) Consequent application of the model on the validation cohort confirmed the good discriminative ability with an AUC = 0.70. (D) Observed survival frequencies and predicted survival probabilities at 3 years after CRS/HIPEC for quintiles of survival groups confirmed the strong calibration of the model in both cohorts.
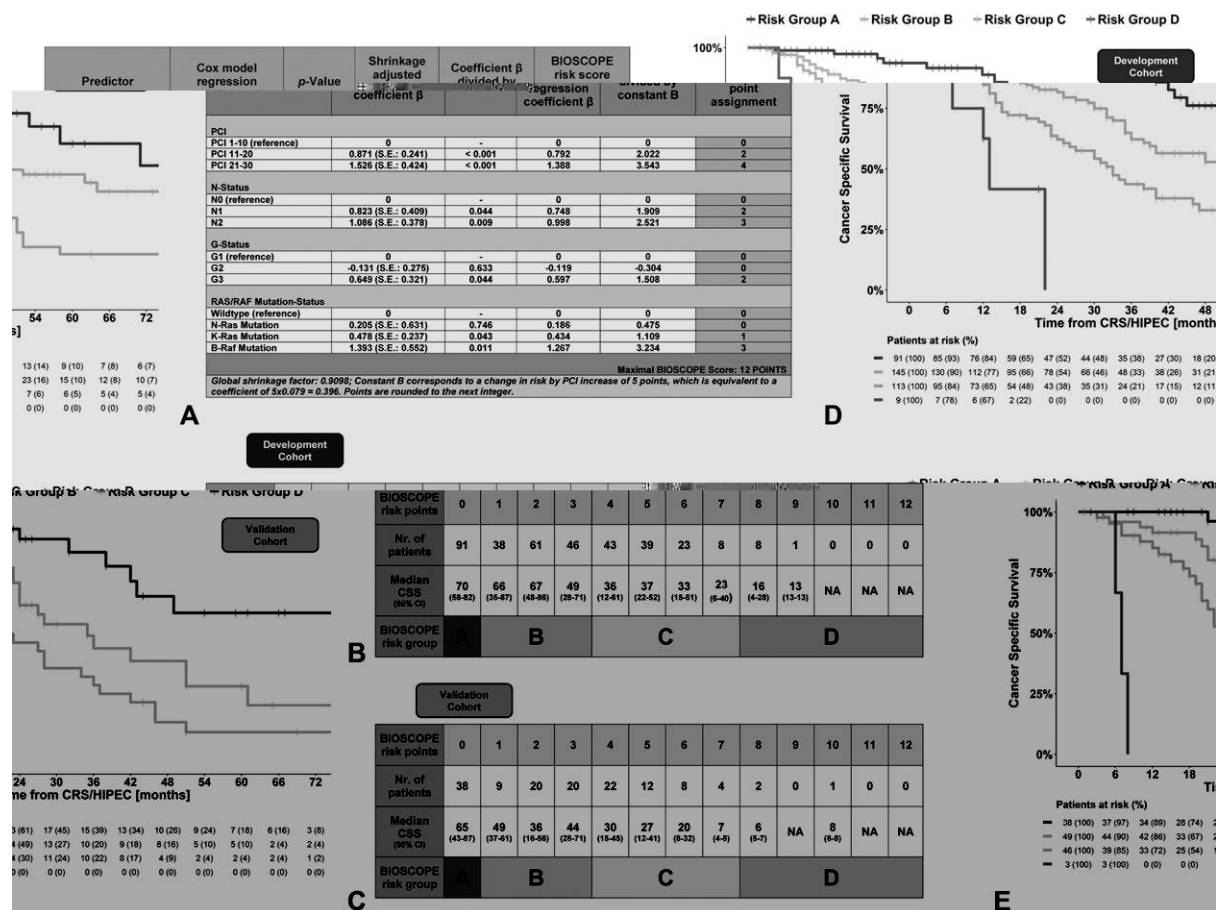
FIGURE 3. BIOSCOPE risk score development & internal validation. (A) A point-based risk score termed BIOSCOPE was developed by dividing shrinkage-adjusted β regression coefficients through constant B. Points were rounded to the next integer. (B) Application of the score on our development cohort showed a consistent decrease of mCSS with increasing risk points. Four risk groups were defined based on similar survival. Patients with 0 risk points showed excellent long-term outcome with a mCSS of 70 months; patients with risk score between 1 and 3 had mCSS above the median of the whole cohort (43 mo) and were defined as risk group B. Patients with risk scores between 4 and 7 (risk group C) showed an acceptable survival, whereas patients with risk scores 8 and higher (risk group D) had a dismal long-term outcome similar or below the current survival reached by systemic palliative chemotherapy. (C) Risk point allocation to the validation cohort confirmed decreasing survival with increasing risk points. (D) Application of the risk score with consequent group allocation divided patients in the development cohort into groups with strongly divergent outcomes of 70 months CSS (CI: 58–82) for group A, 55 months (CI: 42–68) for group B, 33 months (CI: 24–41) for group C, and 13 months (CI: 5–24) for group D ($P \leq 0.001$ for all comparisons between the 4 groups). (E) The score confirmed its value for stratification of patient outcomes after CRS/HIPEC by dividing patients in the validation cohort into 4 groups with mCSS of 65 months (CI: 43–87) for group A, 39 months (CI: 27–51) for group B, 25 (CI: 16–33) for group C, and 7 (CI: 5–9) for group D ($P \leq 0.01$ for all comparisons between the 4 groups).

BIOSCOPE score for patient selection and classification. Internal validation in this study was performed by splitting the cohort into a development and validation cohort.

Preoperative determination of the PCI remains challenging, despite modern imaging by MRI, CT, or PET. Therefore, most centers performing CRS/HIPEC for CRC advocate to perform laparoscopy to assess the PCI before CRS/HIPEC. The PCI documented in our study was evaluated on explorative laparotomy. For the development of our score, we decided to separate the PCI in relatively large categories of 10 points to facilitate preoperative classification of patients based on laparoscopy.

Information regarding *RAS* status of patients was based on the available retrospective data and no additional analysis of existing tumor tissue was performed. Most patients received testing of *KRAS*, but not *NRAS* or *BRAF*. Mutations of *KRAS* are the most frequent[9,11] and normally mutually exclusive toward mutation of *NRAS* or *BRAF*[38–40] and therefore render further testing unnecessary. However, in cases of absent *KRAS* mutation, it is nowadays recommended to consequently test for mutations in *NRAS*,[9] *BRAF*,[9,10] and most recently PI3KCA (mutated in 3.5% of CRC),[9,11] which all lead activation of EGFR downstream signals and render the patient resistant to anti-EGFR therapy. As additional retrospective testing of *NRAS* and *BRAF* was not possible, there might be the potential bias that patients without *KRAS* mutation were grouped in the wild-type group although being *NRAS* or *BRAF* mutated. Despite this, we consider this potential bias as negligible as rates of *RAS/RAF*

mutations in our patient sample compare well to the rates found in patients with CRC in systematic analyses. We therefore believe that our patient cohort represents an adequate sample of CRC patients.

CONCLUSION

Mutations of *KRAS*, and in particular *BRAF*, are negative prognostic factors in patients with PM of CRC undergoing CRS/HIPEC. The novel BIOSCOPE score, including *RAS/RAF* mutational status, PCI, and N- and G-status of the primary tumor, adequately predicts prognosis of patients, which can help to improve patient selection.

ACKNOWLEDGMENTS

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DISCUSSANTS

Beat P. Müller (Heidelberg, Germany):

I would like to thank and congratulate you for your innovative idea to develop the BIOSCOPE score, which should help us select patients for cytoreductive surgery and HIPEC for peritoneal metastasis originating from colorectal cancer in the future. Such a score is of great interest for both the surgical and oncological community. I very much appreciate the careful and thorough work you invested in this important idea.

First, I somewhat regret that you did not focus on this idea, and instead, spent most of the study introducing and discussing the importance of *RAS/RAF* proto-oncogenes as a predictive factor, which results in the fact that the BIOSCOPE score is not even mentioned in the title of the manuscript. From a surgical point of view – and *Annals of Surgery* is a surgical journal – a score, which helps us to better select surgical patients, would be of major interest. Could you please comment on this?

Second, unfortunately, external validation is missing, and therefore, the score cannot be generally recommended as a valid prognostic stratification tool for the time being. This should be stated in the conclusions, abstract, mini-abstract, and manuscript. The opportunity to address this flaw would have been to use a nonrandom split sample (TRIPOD 2B) – for example, the Lion population – for validation. Why did you decide against this? Unfortunately, according to the TRIPOD statement, using a random split-sample does not improve score development and validation compared with TRIPOD 1B, which you applied in the first version of your manuscript.

Third, how can we know that the BIOSCOPE score provides any improvement, in terms of the prediction of prognosis and better patient selection, when compared with existing selection criteria, such as the peritoneal carcinosis index or others?

Fourth, how did you deal with other potential confounders and risk factors, which have not been mentioned so far, such as different histologies, chemotherapy regimes, surgical expertise, changed management during the investigated period and so on? This should at least be mentioned in the supplemental material.

Response From Kuno Lehmann (Zurich, Switzerland):

Thank you, Professor Müller, for your interesting questions. There is a big discussion around the biology among different types of metastases, for example, lung, liver, or peritoneal metastases. Our main goal here was to explore the biology of peritoneal metastasis. This is why I personally think that the identification of novel risk factors, for example, *K-RAS* or *B-RAF*, is of major importance. BIOSCOPE is a useful tool that was developed afterward and will improve decision-making in the treatment of peritoneal metastasis.

Regarding your second question, the critical point here is external validation, and I acknowledge and agree with your point that this study needs independent external validations. I think that this is absolutely clear, and I'd be happy to welcome everyone, who would join us here and help us further develop BIOSCOPE.

Your third comment is very important. The peritoneal cancer index, PCI, is a known major predictor of survival in patients with peritoneal metastasis. The point here is that the surgeons operating these patients learned this already and tend to select patients in a quite narrow range of PCI. In our study, the median PCI ranged from 3 to 12, and 70% of patients were below 10. If the PCI range is narrowed in patients selected for surgery, then we need further parameters to predict outcomes, and I think our work can be of interest and help.

Regarding your last question, CRS/HIPEC was performed in all centers by qualified surgeons, and we did not observe any difference in survival among the institutions. We also analyzed factors, such as tumor histology or preoperative chemotherapy. For example, signet cell histology was significant in the univariate analysis, but not in the multivariate analysis. We did not find any significance for preoperative chemotherapy, which was applied in 80% of our patients.

Christiane Bruns (Cologne, Germany):

Basically, with the BIOSCOPE score, you tried to gain further information to select patients for cytoreductive surgery and HIPEC, with peritoneal metastasis originating from colorectal cancer beyond the PCI score. Did you integrate the location of the primary tumor as a confounder within the BIOSCOPE score, since the distribution of the mutations of the *RAS/RAF* proto-oncogenes is different? This automatically leads me to the next question: Did you integrate the impact of different chemotherapies and biologicals in the development of the BIOSCOPE score?

Response From Kuno Lehmann (Zurich, Switzerland):

Thank you, Professor Bruns, for your interesting question. We looked at the influence of the site of the primary tumor, but did not identify a predictive role. We also looked at different types of chemotherapies and the addition of VEGF antibodies, which did not have a significant impact. Importantly, the addition of EGFR antibodies did not improve the outcome, even in *K-RAS* wild-type patients.

Olivier Turrini (Marseille, France):

Congratulations on this very interesting work. I have one question. Did you look at the original location of the mutation? Was it on Codon 12 or 13 because it has been shown in liver metastases that recurrence rate was higher, when a *K-RAS* mutation occurred on Codon 13?

Response From Kuno Lehmann (Zurich, Switzerland):

Thank you, Professor Turrini, that is a very important question. Unfortunately, we do not have this data. However, this would be an interesting study for the future.