Surgical Risk Is Not Linear: Derivation and Validation of a Novel, User-friendly, and Machine-learning-based Predictive OpTimal Trees in Emergency Surgery Risk (POTTER) Calculator

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Introduction: Most risk assessment tools assume that the impact of risk factors is linear and cumulative. Using novel machine-learning techniques, we sought to design an interactive, nonlinear risk calculator for Emergency Surgery (ES).

Methods: All ES patients in the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) 2007 to 2013 database were included (derivation cohort). Optimal Classification Trees (OCT) were leveraged to train machine-learning algorithms to predict postoperative mortality, morbidity, and 18 specific complications (eg, sepsis, surgical site infection). Unlike classic heuristics (eg, logistic regression), OCT is adaptive and reboots itself with each variable, thus accounting for nonlinear interactions among variables. An application [Predictive OpTimal Trees in Emergency Surgery Risk (POTTER)] was then designed as the algorithms' interactive and user-friendly interface. POTTER performance was measured (c-statistic) using the 2014 ACS-NSQIP database (validation cohort) and compared with the American Society of Anesthesiologists (ASA), Emergency Surgery Score (ESS), and ACS-NSQIP calculators' performance.

Results: Based on 382,960 ES patients, comprehensive decision-making algorithms were derived, and POTTER was created where the provider's answer to a question interactively dictates the subsequent question. For any specific patient, the number of questions needed to predict mortality ranged from 4 to 11. The mortality c-statistic was 0.9162, higher than ASA (0.8743), ESS (0.8910), and ACS (0.8975). The morbidity c-statistics was similarly the highest (0.8414).

Conclusion: POTTER is a highly accurate and user-friendly ES risk calculator with the potential to continuously improve accuracy with ongoing machine-learning. POTTER might prove useful as a tool for bedside preoperative counseling of ES patients and families.

Keywords: artificial intelligence, complication, Emergency General Surgery, Emergency Surgery, machine-learning, morbidity, mortality, Optimal Classification Trees, POTTER, risk calculator, risk prediction

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The burden of emergency surgical disease has continuously increased over the last 2 decades. Between 2001 and 2010, the United States alone reported >27 million Emergency Surgery (ES) admissions accounting for 7.1% of all hospitalizations.[1] The correlation between ES and adverse outcome has been studied extensively: when compared with similar elective surgery, ES carries a much higher risk of postoperative morbidity and mortality.[2–4] The ability to reliably predict postoperative risk is critical for surgical decision-making, counseling of patients and families, resource allocation, and quality benchmarking. The existent risk stratification models range from the simple and subjective, like the American Society of Anesthesiologists (ASA) classification,[5] to the comprehensive, like the Elixhauser[6] and Charlson[7] Comorbidity Indices. The American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP) has also produced its own Surgical Risk Calculator (ACS-SRC).[8] Given that most of these models have been created with the elective surgical patient in mind, many studies have questioned their performance in ES.[9–10] Because of that concern, the Emergency Surgery Score (ESS) was recently suggested as a better predictive model of mortality and morbidity after ES.[11–13] All these aforementioned risk calculators (including ESS), although useful, assume that the variables in their models interact in a linear and additive fashion. The mathematical and medical realities, however, suggest that the interaction of comorbidities and markers of disease acuity are far from linear, and that some variables gain or lose significance due to the absence or presence of other variables.[14] Take, for example, 3 variables which have been repeatedly found to be independent predictors of postoperative mortality: age >70 years, cirrhosis, and use of steroids. In existing, linear, and predictive models, each of these variables is treated as ''present'' or ''absent,'' and often assigned the same weight irrespective of the presence or absence of the other 2 risk factors. However, it is theoretically possible that, for patients >70 years, cirrhosis plays a role but the use of steroids does not; whereas in patients <70 years, cirrhosis does not play a role but use of steroids does. Therefore, in a nonlinear risk model, the age of the patient would determine whether cirrhosis or steroid use would be included in the prediction of outcomes. The inclusion of 1 of these 2 would then determine the next variable to be included, and this variable could be different for each of the 2 choices. For example, if cirrhosis was chosen, then temperature >100.4 could be the next variable added; if steroid use was chosen, then heart failure could be added. Therefore, in a linear model the surgical risk of these 2 ES patients would be established based on the presence or absence of the same set of variables. In a nonlinear model, the risk could be determined by 2 very different sets of variables. The latter arguably better represents the complexity, interactivity, and nonlinearity of real life.

In this paper, we sought to combine big data from a well-validated, national, surgical database with artificial intelligence (AI) to design and test a novel, interactive, and nonlinear risk calculator for ES. These machine-learning methods, namely, Optimal Classification Trees (OCT) and Optimal Imputation, promise a higher degree of accuracy, interpretability, and automatic integration into electronic health records (EHRs). If translated to user-friendly applications, they may be of real-time assistance to surgeons by the bedside.

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METHODS

Patient Population: Derivation and Validation Cohorts

We used the entire ACS-NSQIP 2007 to 2013 dataset for model derivation and algorithm training. The 2014 ACS-NSQIP dataset was used for model testing and validation. The dataset includes >150 preoperative, intraoperative, and postoperative variables.[15–16]

Data Variables

The preoperative variables were used to design our models, whereas the postoperative variables were used as the dependent variables or outcomes to predict. We restricted the dataset to those patients who underwent ES, indicated by the ''Emergency'' variable. We excluded variables that were not consistently collected between 2007 and 2014. We removed the ICD-9 and CPT codes because they are often unknown preoperatively and their complexity compromises use by the surgeon at the bedside. Also, their initial inclusion did not improve the model performance in preliminary testing. We left numerical variables, such as laboratory results, in their raw numeric form. When possible, we converted categorical variables and scales into numeric or ordinal ones (such as functional status and wound classification) to enhance model building. The ASA classification as a preoperative variable requires physician evaluation and arguably subjective judgment, and thus we opted to design 2 decision models, one without (OCT1) and one with (OCT2) the ASA variable. Our primary outcome, mortality, was formed from the ''Days from Operation to Death'' variable, with null values interpreted as survival and all others as death. Similarly, we formed 18 separate OCT algorithms and models to predict the 30-day postoperative ACS-NSQIP complications (eg, surgical site infection, postoperative pulmonary embolism, postoperative acute renal failure).

Optimal Imputation

A significant number of values in the ACS-NSQIP dataset are missing. We imputed missing values using a recently developed and novel machine-learning method called Optimal Impute,[17] which formulates the imputation task as a family of optimization problems. Imputing the missing values in this way before building predictive models has been shown in multiple real-world datasets to lead to significant improvements in prediction accuracy compared with classical missing values imputation methods.

Machine-learning OCT

To create our AI-based decision-trees, we used a recently developed innovative machine-learning method called OCT.[18] Through OCT, we produced a set of predictive models for 30-day postoperative mortality, morbidity, and each one of the 18 individual postoperative complications of the ACS-NSQIP. We trained a separate decision-tree for each of the above postoperative outcomes. The OCT method is adaptive and reboots itself with each variable, accounting for nonlinear interactions among variables. Beyond its promise for higher accuracy, OCT also increases interpretability due to its tree structure which allows predictions through a few decision splits on a small number of high-importance variables, a characteristic not shared by other machine-learning methods such as neural networks or gradient boosted decision-trees, which are more opaque ''black box" methods. Classical decision-tree methods typically cannot achieve the same level of accuracy as machine-learning methods. However, the early AI machine-learning trees often suffered from limited interpretability. Our novel OCT methodology is a recent advance in AI and machine-learning that trains a single-decision-tree, permitting high-accuracy predictions without sacrificing interpretability.[18] This high level of accuracy is achieved by leveraging modern optimization techniques to train decision-trees from the perspective of global optimality rather than using greedy heuristics like the classical methods.

To better understand OCT, an example of a decision-tree that estimates the risk of any complication (including mortality) after ES is displayed in Figure 1. The actual decision-tree is far more comprehensive but has been limited in this example to a maximum depth of 4 nodes for display purposes. The root node of the tree shows that there are approximately 313,000 patients in the dataset, and the overall risk of mortality or other complication is around 25%. The next decision-tree split refers to transfusion in the 72 hours before surgery. If none occurred, the algorithm leads to the left branch of the tree. There are 302,000 patients following this path, with an updated risk of 23%. If a transfusion occurred, the algorithm leads to the right branch of the tree, which analyzes 10,000 patients with transfusion and estimates the updated risk to 76%. The tree proceeds to further split both sides of the initial branch, and after each new split, the risk is recalculated. Importantly, the preoperative variables used by the tree are not the same at each level; the questions asked change based on the responses at the prior node. In this way, decision-trees can capture nonlinear interactions between variables rather than mandate that the variables interact in a linear and additive fashion, as classical logistic regression does.

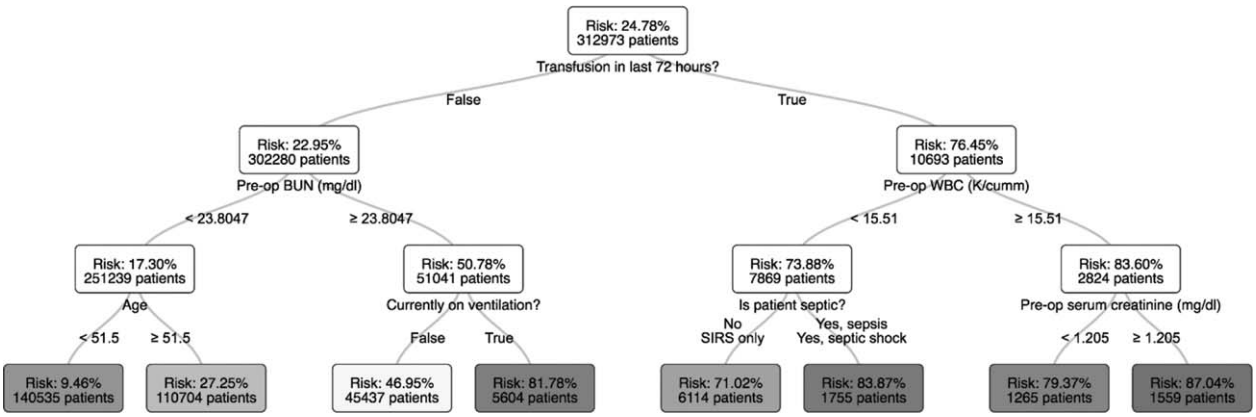

FIGURE 1. An illustrative example of a segment of a decision-tree to predict any complication (including mortality).

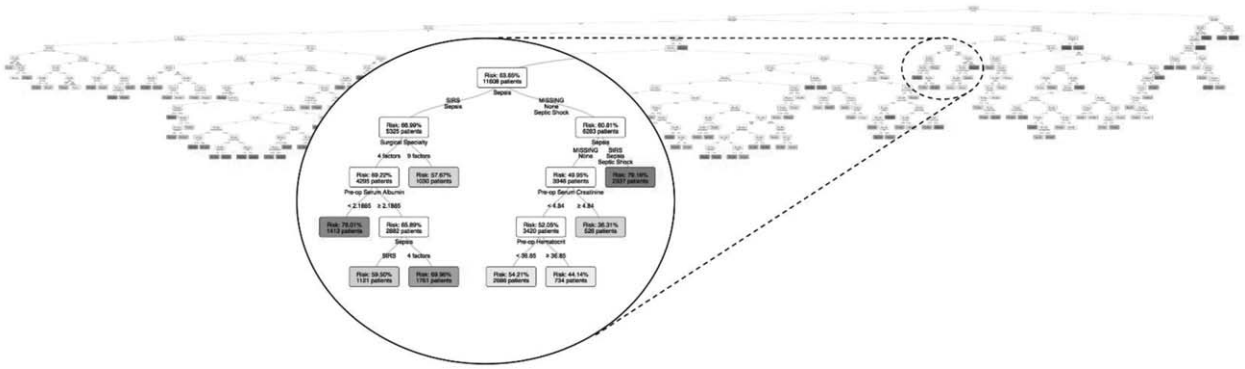

FIGURE 2. The comprehensive decision-tree to predict postoperative 30-day mortality with a random zoom in of one of its trees.

Measurement of Model Performance

The OCT algorithm performance and its ability to predict 30-day postoperative mortality, morbidity, and each of 18 postoperative complications was measured using the c-statistic, also known as the area under the curve (AUC). The c-statistic measures the ability of a model to discriminate between the outcomes of interest and has been used as a measure of model success in multiple prior risk-scoring development efforts.[8,19–21] The performance of our OCT models in predicting mortality was measured in the derivation, validation, and the entire ACS-NSQIP dataset against the performance of the ASA, ACS-SRC, and ESS. For the ACS-SRC comparison, we used the predictions of morbidity and mortality included in the ACS-NSQIP data under the MORBPROB and MORTPROB fields, respectively.

The User-friendly Interface

Using our trained decision-trees for each outcome, we built a dynamic online and phone application as the user-friendly interface of the algorithms for direct use by providers. The application presents the results in the form of an interactive questionnaire. The surgeon user, dealing with a specific ES patient in mind, is initially prompted to select the outcome for which the risk needs to be estimated. The user is then asked a series of simple questions about the presence or absence of certain preoperative variables. Like the machine-learning OCT algorithms, the questions are adaptive; the subject of each new question depends on the specific answer to the prior question. When all the questions are answered, the end-user receives the final risk estimate of the selected outcome for the particular patient. The application design allows easy integration into any EHR environment, so that it can potentially pull most available variables directly from the EHR database in an automated fashion. Once integrated into the EHR, the user would only be required to answer questions that cannot be pulled in automatically. If there is full EHR automation, the risk would be calculated at once.

Ethical Oversight

Institutional Review Board approval for the study was obtained.

RESULTS

Out of a total of 382,960 ES patients in the derivation cohort, comprehensive decision-making algorithms were derived, and a user-friendly application, the Predictive OpTimal Trees in Emergency Surgery Risk (POTTER), was created.

OCT and 30-day Postoperative Mortality

The OCT decision algorithms for 30-day mortality are depicted in Figure 2. Table 1 compares the performance of our OCT models on the derivation, validation, and entire datasets, compared with that of ASA, ESS, and the ACS-SRC. The OCT1 model does not use the ASA classification, whereas the OCT2 does. The ACS-SRC was not included in the derivation/validation cohorts' comparison; only the final predictions of the model were available and there was no ability to calibrate the model to the derivation set and evaluate out-of-sample on the validation set. In summary, the mortality c-statistic for the OCT2 algorithms was the highest at 0.9199, outperforming the ASA (0.8743), ESS (0.8910), and ACS-SRC (0.8979). In the validation dataset, the accuracy of the OCT1 and OCT2 models in detecting a risk of mortality >50% was 0.9533 and 0.9535, respectively.

OCT and 30-day Postoperative Morbidity

The OCT decision algorithms for 30-day postoperative morbidity (ie, the occurrence of any complication) are depicted in Figure 3. Table 2 compares the performance of our OCT models in predicting 30-day morbidity on the derivation and validation datasets, compared to that of ASA, ESS and ACS-SRC. In summary, the c-statistic of the OCT2 algorithms was the highest at 0.8414, outperforming the ASA (0.7842), ESS (0.7768), and the ACS-SRC (0.8063).

OCT and the 30-day Individual Postoperative Complications

Except for superficial surgical site infection, the OCT1 and OCT2 algorithms predicted the occurrence of individual 30-day postoperative complications with a moderate to extremely high accuracy (c-statistic range from 0.7358 to 0.9338) (Table 3). Their best performances were in predicting postoperative septic shock (c-statistic 0.9338), postoperative ventilator dependence for

TABLE 1. The Performance of Optimal Classification Trees (OCT) in the Predicting 30-day Postoperative Mortality, as Compared With Other Known Risk-Prediction Models

Model	Derivation Cohort	Validation Cohort	Entire Cohort
OCT1	0.9125	0.9064	0.8979
OCT2	0.9233	0.9199	0.9162
ASA	0.8743	0.8740	0.8743
ESS	0.8922	0.8838	0.8910
ACS-SRC	N/A	N/A	0.8975

ASA indicates American Society of Anesthesia; ASC-SRC, American College of Surgeons Surgical Risk Calculator; ESS, Emergency Surgery Score; OCT1, Optimal Classification Trees, excluding ASA; OCT2, Optimal Classification Trees, including ASA.

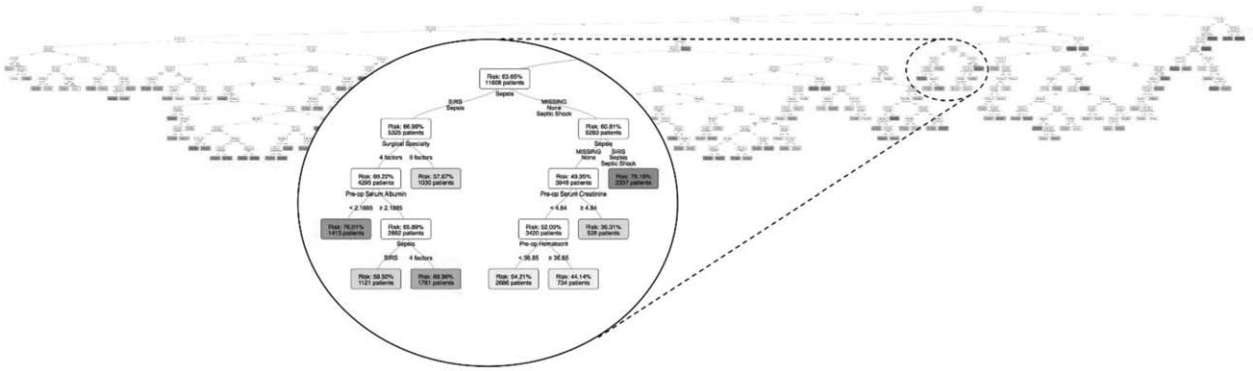

FIGURE 3. The comprehensive decision-tree to predict postoperative 30-day morbidity.

longer than 48 hours (0.9254), and postoperative renal failure (0.9126).

The Interface: POTTER

Using OCT1 and OCT2, a user-friendly, interactive, and comprehensive online and phone application was designed as the algorithms' end-user interface. With the POTTER calculator application, now available for free download in both android and iphone online stores, the provider's answer to a question interactively dictates the subsequent question. For any specific patient, the provider can predict the risk of 30-day postoperative mortality, overall postoperative morbidity or each of 18 individual postoperative complications, such as renal failure, respiratory failure, myocardial infarction, or deep vein thrombosis. For any specific patient, the number of questions needed to predict mortality ranged from 4 to 11, and a specific complication from 3 to 20. These numbers corresponded to the same number of ''clicks'' and typically consumed <1 minute. Figure 3 illustrates the simplicity and power of POTTER, where 2 screenshots depict how the change of answer to one question takes the decision-tree in a totally different direction with different questions and different variables required to predict the final risk of postoperative mortality. Figure 4 shows the almost completely different questions, variables, and decision-trees needed to predict different outcomes, in this case postoperative renal failure versus postoperative myocardial infarction Figure 5.

DISCUSSION

Combining the power of big data and the innovative logic of AI, we have designed POTTER, a novel calculator for the ES patient. As the interface of the OCT algorithms, POTTER offers the advantages of being (1) evidence-based, (2) accurate, (3) nonlinear/machine-learning-based, (4) user-friendly/interactive, (5) amenable to integration into existing EHR, and (6) potentially actionable.

Evidence-based: POTTER is evidence-based because its OCT algorithms do not rely on any modeling assumptions, but are completely derived from patient level data including actual patient outcomes. In this case, the data used for both the derivation and the validation are from the national ACS-NSQIP database, arguably the largest, most reliable, and best validated database in surgery.[22–24] Several studies have suggested the superior accuracy of the ACS-NSQIP database to administrative databases such as the Nationwide Inpatient Sample or insurance claims databases.[25–29]

Accurate: Despite the significantly fewer number of questions needed to estimate the postoperative risk of a specific patient, its accuracy in predicting 30-day postoperative outcome, as measured

TABLE 2. The Performance of Optimal Classification Trees (OCT) in the Predicting 30-day Postoperative Morbidity, as Compared With Other Known Risk-Prediction Models

Model	Derivation Cohort (2007–2013)	Validation Cohort (2014)	Entire Cohort
OCT1	0.8366	0.8397	0.8187
OCT2	0.8471	0.8511	0.8414
ASA	0.7884	0.7673	0.7842
ESS	0.7906	0.7715	0.7768
ACS-SRC	N/A	N/A	0.8063

ASA indicates American Society of Anesthesia; ASC-SRC, American College of Surgeons Surgical Risk Calculator; ESS, Emergency Surgery Score; OCT1, Optimal Classification Trees, excluding ASA; OCT2, Optimal Classification Trees, including ASA.

TABLE 3. The Performance of Optimal Classification Trees (OCT) in the Predicting Individual 30-day Postoperative Complications

	Derivation Cohort		Validation Cohort	
Complication	**OCT1**	**OCT2**	**OCT1**	**OCT2**
Superficial SSI	0.6804	0.6859	0.6762	0.6808
Deep incisional SSI	0.7358	0.7446	0.7405	0.7540
Pulmonary embolism	0.7470	0.7595	0.7196	0.7333
Organ space SSI	0.7723	0.7789	0.7828	0.7860
Sepsis	0.7744	0.7860	0.8444	0.8448
Wound disruption	0.7749	0.7891	0.7689	0.7790
Urinary tract infection	0.7766	0.7778	0.7378	0.7396
DVT/thrombophlebitis	0.7995	0.8129	0.7787	0.7886
Progressive renal insufficiency	0.8315	0.8353	0.8210	0.8188
Myocardial infarction	0.8343	0.8467	0.8151	0.8240
Pneumonia	0.8365	0.8432	0.8364	0.8470
Unplanned intubation	0.8381	0.8462	0.8402	0.8493
Stroke/CVA	0.8536	0.8590	0.8300	0.8343
Cardiac arrest requiring CPR	0.8661	0.8838	0.8722	0.8882
Septic shock	0.8808	0.8888	0.9204	0.9338
Bleeding requiring transfusions	0.8969	0.8984	0.8974	0.9028
Acute renal failure	0.9002	0.9107	0.9025	0.9126
On ventilator >48 h	0.9094	0.9210	0.9107	0.9254

CPR indicates cardiopulmonary resuscitation; CVA, cerebrovascular accident; DVT, deep vein thrombosis; SSI, surgical site infection.

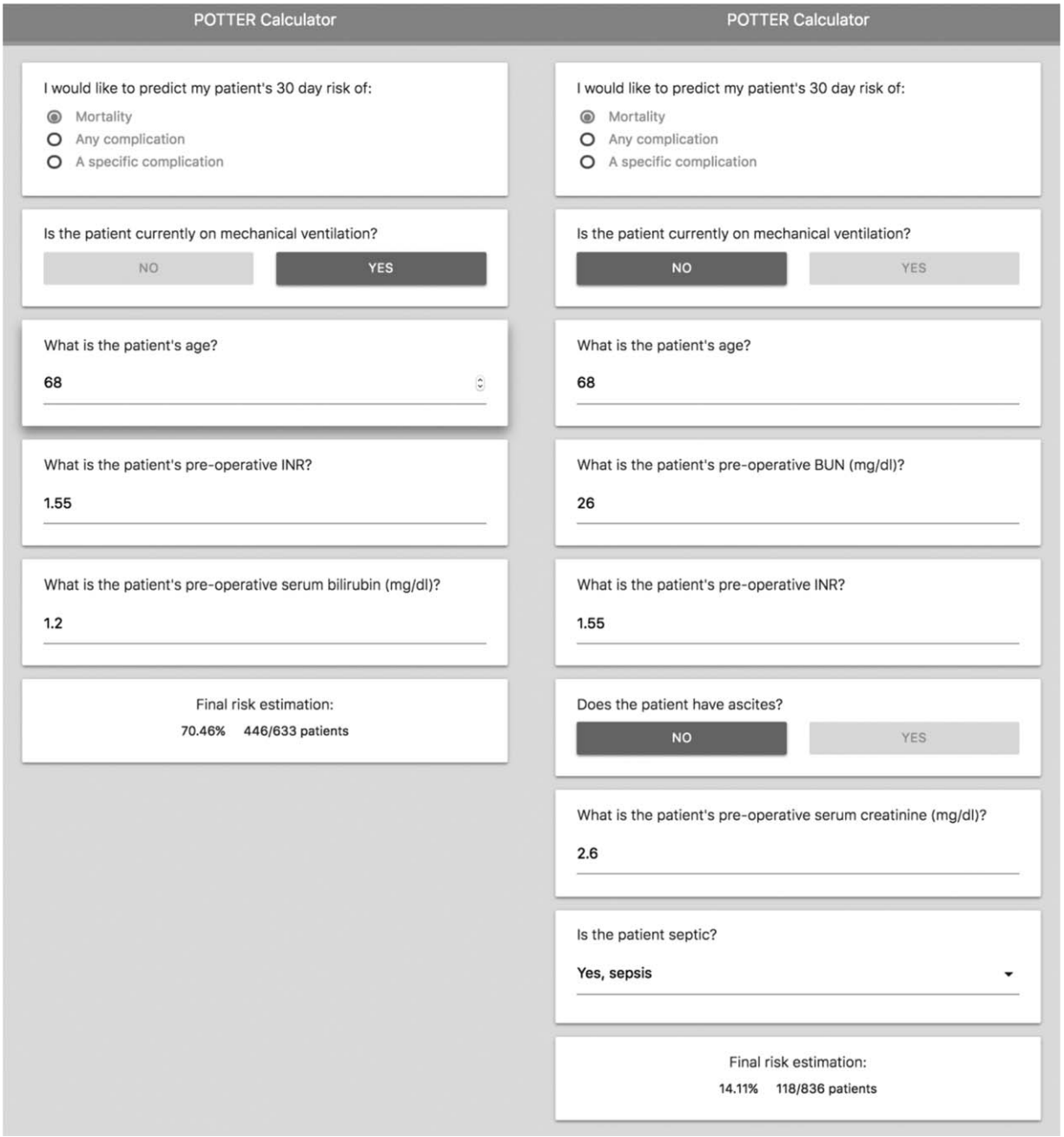

FIGURE 4. An example illustrating how POTTER is interactive, and the answer to a question dictates the next question. In this specific example, whether the provider answers yes to no to the question regarding mechanical ventilation takes the algorithm and questions in a different direction.

using the AUC and c-statistics, was significantly higher than the currently existing methods such as the ASA, ESS, and the ACS-SRC.

Nonlinear and machine-learning-based: Machine-learning is an application of AI where machines are enabled to recognize patterns and learn from their own experiences without being explicitly programmed to do so.[30–32] It is particularly useful to detect subtle intervariable complex relationships that are typically imperceivable to the human eye or mind. The current literature suggests that machine-learning algorithms in general and Classification and Regression Trees (CARTs) in particular can significantly improve the accuracy of classical risk prediction models based on multivariable analyses.[33–35] This is due to the fact that surgical risk is simply not linear, and the impact of a certain variable is dependent on the absence or presence of another variable upstream along the decision-tree. However, CART takes a top-down approach to determining the partitions: starting from the root node, a split is determined by solving an optimization problem before proceeding to repeat the efforts at the level of the 2 resulting new nodes. Such a top-down

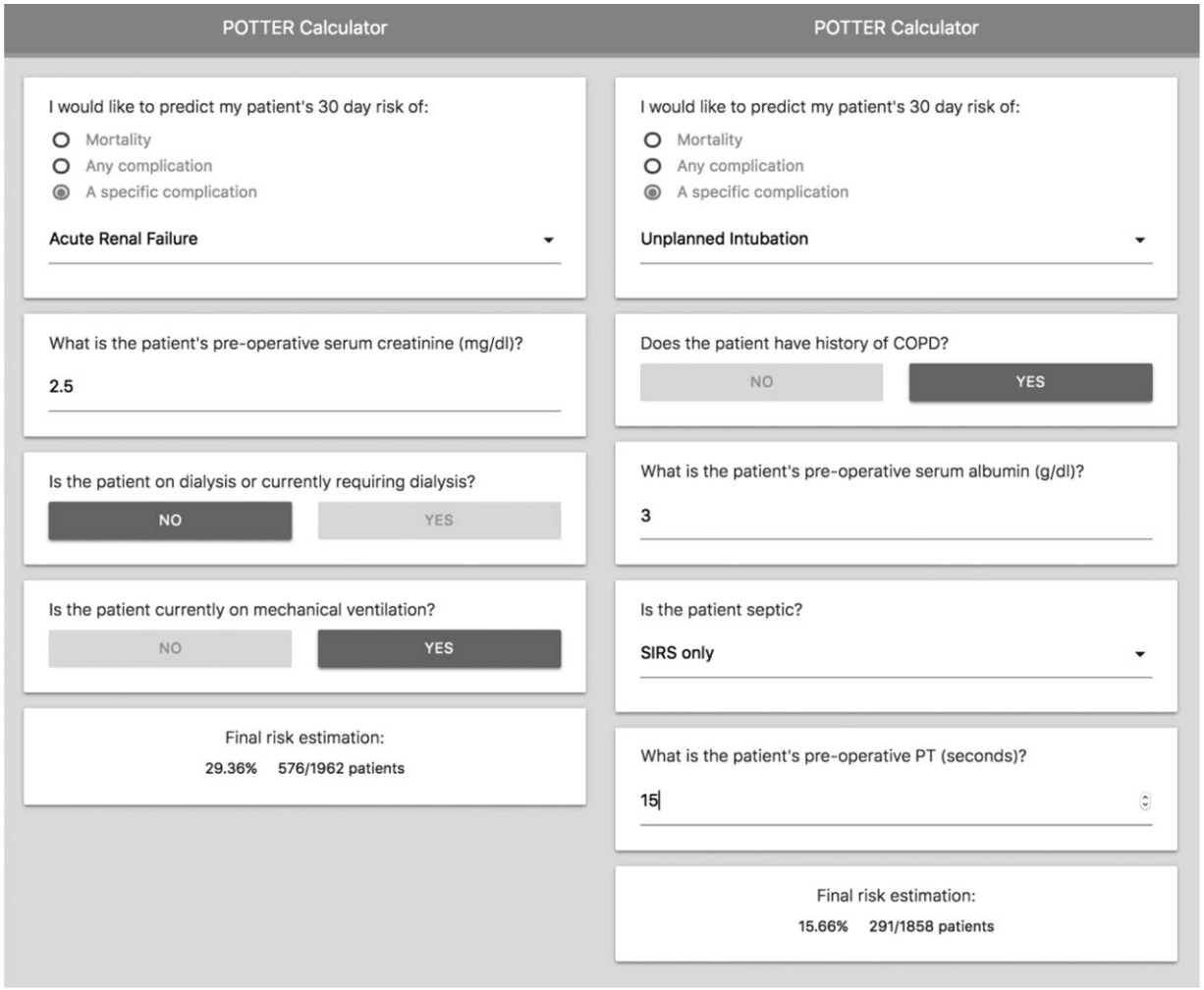

FIGURE 5. An example illustrating how POTTER interactively uses completely different algorithms, and thus different questions to predict different postoperative complications. In this specific example, we see different questions needed to predict the risk of developing postoperative acute renal failure versus requiring an unplanned intubation.

approach has been criticized because each tree split is determined in isolation without reconsidering the possible impact of future splits in the tree, and typically in practice leads to decision-trees having worse performance than alternative methods. In contrast, the OCT methodology used here and recently developed and validated by our team, constructs the entire decision-tree in a single step, yielding the single best decision-tree for the training data.[18] OCT has been suggested to outperform the accuracy of CART or Random Forest techniques by up to 7%.[18]

Interactive and user-friendly: Because of the complexity of the OCT decision-trees, we have created an interactive interface that starts by asking the providers what outcome they would like to predict on a specific patient. Through a series of short, specific questions where one chooses a yes/no answer or an answer from a drop-down menu, or simply enters an actual laboratory value, the provider quickly receives a specific percentage of risk, sometimes with as little as 3 questions. As a result of the machine-learning methodology used, the risk model is interactive in real time. A provider's answer to the first question will dictate what the next one will be, the answer to the second question will dictate the third, and so on. Each interaction with the application corresponds to a unique decision-tree node and is based on the specific patient characteristics and selected outcome (mortality, morbidity, or a specific complication).

Amenable to integration with HER: We have designed POTTER to be easily amenable to integration into an EHR environment, so that many of the answers can be pulled automatically from the EHR. In an advanced EHR, one can envision the immediate and automated generation of multiple risk estimates for mortality, morbidity, and the specific complications. Prior studies have shown promise in the ability of EHR-integrated, machine-learning algorithms to aid bedside decision-making.

Actionable: POTTER can equip surgeons with personalized and highly accurate risk estimates that will allow them to counsel ES patients and families before surgery. Such information might give the objective data needed to forgo surgery in the patient with little risk for meaningful survival instead of the surgeon having to rely on gestalt or preference.[36–37] Even if surgery will be pursued, using specific risk estimates of mortality and morbidity helps set the right expectations for recovery and what its journey entails. For example, the surgeon might choose to estimate the risk of respiratory failure in the COPD patient

while stressing the risk of myocardial infarction in the patient with severe heart disease. In addition, only a small subset of the variables are used in the trees, reducing the need of the physician to know or plug all of a patient's information before receiving a risk estimate. As importantly, it is plausible that POTTER is able to identify ''break points'' in the early perioperative patient care where a specific clinical care intervention can favorably alter the eventual outcome of a specific patient. Our team has also recently developed another mathematical machine-learning methodology, the *OPT*.[38] OPT is a promising tool that will learn from existing data to recommend/prescribe the best personalized care intervention for each patient that can effectively reduce the risk of postoperative complications or mortality. The challenge remains, of course, in identifying the specific actionable variables that are not mere indicators of the severity of illness or the acuteness of disease, but essential ''break point'' factors that directly impact patient outcome, if modified in a timely fashion.

Central to the limitations of our study lies the fact that the power of machine-learning prediction depends on the accuracy and comprehensiveness of the data it uses, in this case the ACS-NSQIP database.[14,39] As such, systematic biases resulting from the ACS-NSQIP data collection methodology and its changes over the the multiple years of data might exist. A second issue of our study is the exclusion of ICD and CPT codes from the model. Although it is difficult to accept that the risk of postoperative mortality in ES is not dependent on the diagnosis or the type of surgery performed, our analyses showed otherwise. The inclusion of these codes did not enhance the accuracy of the model, possibly because the type of surgery needed is theoretically reflected in the preoperative derangements that are included in the algorithms. For example, an ES who needs a simple incarcerated inguinal hernia repair might not be showing the same chemical, hematological, and coagulopathic derangements as the patient with perforated viscus or bleeding spleen requiring ES. A third limitation refers to causality between the variables and the outcomes, which is still not proven despite the high degree of connectivity between the 2. Therefore, interpretability and actionability on the relevant variables are controversial. For example, if the mortality decision-tree of a specific patient included a low sodium level, correcting it might not necessarily improve mortality. The decision-tree might simply change in a different direction, and ultimately estimate the same mortality risk.

CONCLUSION

We have developed POTTER, a highly accurate ES risk calculator that outperforms, in accuracy and user-friendliness, all the current existing risk prediction tools. POTTER might prove useful as an evidence-based, adaptive, and interactive tool for bedside preoperative counseling of ES patients and families. Further studies are needed to explore the ability of POTTER to identify actionable ''break points'' in preoperative patient care that can effectively favorably alter their postoperative outcome.

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DISCUSSANTS

Dr Paul C. Kuo (Tampa, FL):

I would like to thank the Association for the privilege of commenting on this paper, and also my thanks to the authors for sending their manuscript well ahead of time.

In this paper, the authors apply a big data technique to create a predictive model for outcomes after emergency surgery using the ACS NSQIP dataset from 2007 to 2013. They examine a variety of outcomes, including mortality. This dataset includes 382,000 patients and >150 perioperative variables. They use a classification tree methodology (OCT) and an imputation technique (Optimal Impute) for missing variables.

They compare this model with established models such as ASA and ESS. The AUC of their OCT models is better than that of the comparison models. They also present a user application, POTTER, that implements OCT and may be used at the bedside.

I love your title. I agree with the authors that not only is surgical risk not linear, but also life is not linear. As a result of newer techniques, larger datasets, and more powerful computing capacity, we no longer need to limit ourselves to linear approaches to analysis.

In the same way that protein chemistry started with just a linear consideration of amino acid sequences and advanced to considerations of tertiary and quaternary structures, data science and analysis have similarly evolved.

As a big data nerd wannabe, I will confine my comments to methodology. Big data predictive modeling is essentially hypothesis generating. The equivalent of prospective clinical trials is required to determine applicability. In addition, these models, although potentially powerful, suffer from lack of consideration of local environmental factors, such as hospital resources, that might either further enhance performance or, alternatively, indicate overfitting of your model.

I ask, have the authors begun to implement OCT in their own institution to determine performance prospectively? And, certainly, beyond AUC, although that is the common statistic used to compare these kinds of models, it would be nice to see accuracy, sensitivity, and specificity for each of the models.

The methodology used for imputing missing variables is novel, but, unfortunately, the reference is under review. As a simple test of proof of principle, I ask if the authors created a data subset in which known values were deleted, applied their imputation technique, and determined accuracy of Optimal Impute. How does it compare with alternative approaches for missing variables, which are replete throughout the big data literature?

As we understand and implement machine-learning techniques, the approach overall is empiric. It is a tool. Performance trumps elegance. So I ask if the authors have compared OCT with other techniques such as traditional regression, random forest, gradient boosting, neural networks, and so on. Perhaps it is the imputation methodology rather than a classification approach.

This has to do with the paper. I was a bit confused by the description of POTTER. Has it been used yet? The paper refers to POTTER performance. If POTTER has been used in the context of this modeling approach, a clarification in this paper would have been nice. Otherwise, I think OCT performance has been measured and not POTTER.

I am going to throw in a question I did not send to you. That is, if I enter my patient's data into POTTER, do you retain that data?

It would be of interest to see the relevant independent variables and weights that comprise the trees for mortality and the various complications.

Lastly, as a simple rhetorical academic flourish, when a biologist publishes a paper, there is an agreement that requires that the substrate and/or biologics become available to other researchers. For the purposes of a purely academic discussion, do you think publishing your paper would require that you make your code available to your readership?

In closing the paper, I think the paper emphasizes the need to include contemporary methodologies as we address ongoing clinical problems. I congratulate the authors on your work. Very good. Thank you.

Dr Haytham M. Kaafarani:

Thank you, Dr Kuo, for your kind remarks. These are extremely insightful questions. We agree that our data generate as many hypotheses as it answers questions. The advantage of machine-learning techniques resides in the fact that they can continue to improve performance as we add more data, and as the variables become more comprehensive. Although we have recently started at MGH using the POTTER calculator in our daily AM sign out rounds when discussing ES patients, and in the ED when consulting on such patients, we have not yet started a prospective study evaluating its performance. Our team is currently discussing with the hospital leadership adopting it in our EHR and automatically evaluating its performance. A multi-institutional prospective study is also on our agenda.

Regarding sensitivity and specificity: This is not only statistically possible, but also relatively easy to do. The only caveat is that we would need to choose a threshold (eg, if the probability of mortality >5% or >10%). We would be happy to provide that in the manuscript.

Regarding the Optimal Impute methodology: We have indeed tested it across 95 real-world datasets by removing some known values and comparing their imputation technique against other methods. We observed an overall improvement of 10% to 15% in imputation accuracy for Optimal Impute compared to the best of the other methods.

Regarding the OCT methodology itself, this is another great question with a lot of insight into AI in general and machine-learning methodologies in specific. The key advantage of OCT in this case is the interpretability of the method. We sought to develop a risk calculator that was easy for physicians to both use and understand. The decision-tree structure means that few variables (typically 5 to 10) are needed to make a prediction for a patient, whereas for these alternative approaches such as random forests or gradient boosting,

we would need to enter values for each of the >150 variables into a black-box methodology that does not make the logic clear in attaining the risk percentage. With OCT, you can follow the logic of the AI as it goes because as it takes you from one question to the other, there is a numerator and a denominator that tells you why this risk is evolving in that specific direction.

The other question you had was about the terminology. POTTER is just simply the interface that we use so people do not have to look at the algorithms and guess the risks of POTTER, and the optimal classification algorithms are one and the same. There is no difference between the 2. I apologize if that was not clear in the manuscript, and we will correct that.

Regarding the weights for each variable, each decision-tree has different variables with different weights because of the OCT methodology. As such, the nonlinear nature of the trees make it hard to come up with meaningful averages because the importance of each variable is so dependent on the previous answers. For example, the weight of diabetes is really dependent on the specific patient and might be different from one patient to the other.

The question regarding publishing the codes: In principle, we totally agree. It would aid in reproducing results or applying approaches to new data sources. The realities are slightly more complicated, introducing concerns such as the quality of the code being published and how usable it is required to be, whether the code is required to be supported or kept up to date by the author as new versions of software come out.

You asked me whether we have the ability to retain the data in our application. As of now, we have not done that. Again, the application was just approved by iPhone and Android 2 weeks ago for Android and last week for iPhone. That is something to look into, so that we can continue to improve the algorithms as multiple people around the country use it.

Dr Henry Pitt (Philadelphia, PA):

I would like to congratulate the authors on applying machine-learning to NSQIP data. I have 2 questions.

A year ago we published a paper in JACS evaluating the ACS NSQIP risk calculator in elective and emergent colorectal patients at Temple. The risk calculator was more accurate in the elective than in the emergent patients, and one of the areas of inaccuracy was the ability of the risk calculator to predict whether a patient would be discharged to a skilled nursing facility (SNF). I did not see that outcome in your data. Thus, my question is, can POTTER predict the ability to be discharged to a SNF? Which actually is very important for some of these end-of-life patients.

Also, at Temple University Hospital we do 100% mortality review. The majority of the patients who have an emergent operation and do not survive have a cardiac or a vascular operation. As you know, the procedures captured in NSQIP are not preferred by cardiac surgeons and vascular surgeons because of the existence of STS and SVS databases. Thus, my second question is whether your POTTER NSQIP data can really be applied to emergent cardiac and vascular surgery patients. Thank you.

Dr Haytham M. Kaafarani:

Thank you for your questions. Addressing the first one: Functional outcome is probably the most important outcome besides mortality. A patient may survive like you could survive, but with a predicted risk of complications as high as 95% or 97%. That suggests that, for those who survive, they might survive with a very poor functional outcome and be discharged to a nursing facility. We have not done the predictive models for discharge venues or functional outcomes, but that is a really good suggestion, and we probably should. Thank you.

To answer your second question, yes, the OCT algorithms can be applied to other databases. They need a lot of patients to distill some of the noise in the data. But if you get us the STS database or other the cardiothoracic, vascular, or transplant databases, we can run the OCT algorithms similarly to what we did. The bigger the data and the more accurate the data and the more comprehensive the data, the better the algorithms will be.

Dr Adil Haider (Boston, MA):

Dr Kaafarani, fantastic presentation and congratulations on getting this through the Apple iPhone app store. I know how difficult it is to get a medical app through the app store, so congratulations.

My question is regarding the area under the curve or the c-statistic that you have used to determine the discriminatory ability of the POTTER score. I noticed that when you look at any complication, your discriminatory ability is actually quite good. But when you look at the individual complications, it's not as good as even the previous scores that you have determined. Can you talk about the ability of this machine-learning algorithm to discriminate between one complication versus multiple complications?

Also, does the POTTER score have anything to do with a certain very famous book, the POTTER piece of that?

Dr Haytham M. Kaafarani:

Let me answer the first question first. You are absolutely correct. The machine-learning methodology really depends on the data that we plug in. If there is a certain baseline variable we are not measuring that affects outcome, then that undermines the c-statistics.

What we found in POTTER, which is amazing, is that for life threatening complications, the performance was really above everything that we know. The c-statistic is impressively high.

However, the worst c-statistic was for superficial surgical site infection. I think it is because the NSQIP itself does not collect enough data to accurately predict surgical site infection. In general, if you look at most of the literature in predicting surgical site infection, you can see the c-statistics tend to be much lower because the data we collect tend to be the data that predicts how patients do overall—whether they live or do not live, and whether they get major complications or do not.

Your second question: I have a 9-year-old who is obsessed and reading Harry Potter over and over and over again. When I was playing with the perioperative optimal classification trees, the letters were matching to include P, O, T, E, and R, and that is the mnemonic that naturally came to my head.

Dr Ari Leppaniemi (Helsinki, Finland):

The concept of failure to rescue has been used, as you know, to compare the outcomes of patients' after complications. Now, the problem with that is sometimes how do you define the denominator? How do you include patients in the analysis?

My question is, how do you use this risk assessment as a tool to determine, for example, that everybody whose risk of dying is >50% would be included in the analysis? And then you actually look at what happened, and, therefore, have a case-mix adjusted calculator of your outcomes.

Second, briefly, I checked the application. Some of the units that are used in the United States, is it available for units to be used in Europe? Like millimoles and stuff like that.

Dr Haytham M. Kaafarani:

Let me start with the second question. That is a good suggestion by our friends from Finland: We should be able to automatically do the conversion to every unit that every country wants to use. It should be a pretty easy I.T. problem to fix.

For your first question, which is the failure to rescue question, can we identify a high-risk population and then we can look at those specific risks? Yes, in the algorithms, we can probably draw certain threshold lines, say, mortality >50%, to identify the final tree nodes that lead to such mortality. Then, we can go back and see those patients, who they are, and we can do the analysis only on this subset of patients. I think it is technically doable.

But I actually think the more fascinating suggestion you had is that regarding looking at the subset of patients who had a complication and analyzing how they die. We are experimenting with another AI tool called optimal prescriptive trees, which can help us identify tree nodes at which interventions can alter the outcome following complications to prevent the patient's clinical situation from spiraling to death.

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

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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.

Keywords: KRAS, primary tumor location, prognosis

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In the last several years, laterality of the primary colon tumor has emerged as a new predictive and prognostic factor of colorectal cancer (CRC), particularly in the metastatic setting [metastatic colorectal cancer (mCRC)]. In fact, a recent meta-analysis of 66 relevant studies assembled a cohort of over 1 million patients with unresectable mCRC, and demonstrated that a left-sided (LS) primary tumor location (PTL) was associated with a significantly reduced risk of death.[1] A smaller but more homogenous meta-analysis of prospective clinical trials of patients with unresectable mCRC corroborated these findings and confirmed that PTL is prognostic in unresectable mCRC.[2] Therefore, there is little doubt regarding the predictive and prognostic role of PTL in patients with unresectable mCRC.

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However, the prognostic significance of primary tumor sidedness may differ when it comes to resectable colon cancer liver metastases (CLM). In contrast to studies regarding unresectable mCRC, those in resectable CLM have produced much more contradictory results.[3–6] There is currently no meta-analysis in patients with resectable CLM that could synthesize these variable results and thus conclude whether PTL is prognostic. As such, whether PTL plays a prognostic role in CLM remains largely unknown.

In an effort to explore why outcomes differ, we compared the design of the respective studies in resectable and unresectable mCRC. The main methodologic difference was that in the former, the KRAS mutational status was unknown and therefore not accounted for in the survival analysis, with the exception of 2 retrospective studies.[7,8] In contrast, the majority of the studies in unresectable mCRC were conducted in homogenous cohorts of wild-type patients treated in the context of randomized controlled trials.[9,10]

Thus, the aim of the study was to explore the hypothesis that KRAS mutational status influences the prognostic association of PTL in resectable CLM. To answer this question, we decided to use the large international, multi-institutional database of the International Genetic Consortium for Colorectal Liver Metastasis (IGCLM), which has been specifically assembled to capture patients with genetic data such as KRAS mutations.

METHODS

Study Design

All patients who underwent curative-intent surgery for colorectal cancer liver metastases (CRLM) between January 1, 2000, and December 31, 2016, and had genetic data were retrospectively identified from the patient records of 9 tertiary academic centers from the United States (The Johns Hopkins University, Baltimore, MD; Stanford University School of Medicine, Stanford, California; Digestive Disease Institute, Cleveland Clinic, Cleveland, OH), Europe (Medical University of Vienna, Vienna, Austria; Medical University of Graz, Graz, Austria; Charite — University of Berlin, Berlin, Germany; Haukeland University Hospital, Bergen, Norway) and Japan (Yokohama City University Graduate School of Medicine, Yokohama, Japan; Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan). The institutional review boards of all participating institutions approved the study. Of note, this is a unique study cohort as patient data from 2 more institutions (Yokohama and Kumamoto) have been added to the originally constructed database of the IGCLM.

Patient Selection

The database was queried to identify the study cohort. Patients who did not have data on their KRAS mutational status were excluded. Those who had a primary cancer of the rectum or the transverse colon were excluded as well. Demographic, clinicopathologic, and genetic data from the IGCLM database were used either to describe the baseline characteristics of the cohort(s) or as co-variates for the survival analysis because of their known prognostic relevance.

Definition of Right Versus Left PTL

We excluded patients with transverse colon primary tumors (with the exception of ''hepatic flexure'' tumors, which were included in the RS group) for 2 reasons. First, it would allow for comparison with the largest study to date on PTL in resectable CLM, which employed this exclusion criterion.[8] Second, it is often impossible to determine retrospectively from the pathologic report whether the primary tumor was located before or after the point that separates the first two-thirds from the final third of the transverse colon. Importantly, location compared to that point determines the embryologic origin (midgut vs hindgut) of the tumor.

KRAS Mutation Profiling

All patients underwent sequencing of the following KRAS codons: 12, 13, and 61, with the exception of the patients from Haukelund University, who only underwent sequencing of codons 12 and 13. Patients from the early 2000s were not tested for all mutations in exons 3, and none of the patients was tested for exon 4 mutations. Standard molecular biology techniques that have been previously described were employed. Either primary or metastatic tissue was used for the measurements, as a high concordance of the KRAS mutational status between primary and corresponding metastases has been reported.[11]

Sub-Analysis

We performed a sub-analysis after excluding patients who underwent concurrent resection and intraoperative ablation for 2 reasons. First, although still under debate, many reports concur that radio-frequency ablation results in inferior long-term outcomes compared to hepatic resection in patients with CLM, and is applied only when surgical resection is not feasible.[12] Second, it would allow for comparison with the largest study to date on PTL in resectable CLM, which employed this exclusion criterion.[8]

Statistical Analysis

Demographic, clinicopathologic, and perioperative features of the study population were stratified according to the primary tumor site and KRAS mutational status. Summary statistics were presented as totals and frequencies for categorical variables or as median values with interquartile ranges (IQRs) for continuous variables. Categorical variables were assessed using the chi-square or the Fisher exact test, as appropriate. Overall survival (OS) was measured from the date of hepatic resection until the date of death or last follow-up. Survival curves were generated using the Kaplan-Meier method, and differences between curves were evaluated with the log-rank test.

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Univariable and multivariable analyses to identify predictors of survival were performed by using Cox proportional hazards regression models. Variables with $P < 0.05$ in univariable analysis were entered into multivariable analysis. Last, we performed nearest neighbor propensity score matching for potential confounders using the MatchIt package for R 3.5.1. Confounders were defined as variables unevenly distributed across LS and right-sided (RS) cancers in the wild-type population ($P < 0.1$). Logistic regression was used to estimate the distance measure and the caliper was set to 0.2. All analyses were performed using STATA version 13 (StataCorp, College Station, TX) and R 3.5.1. (https://cran.r-project.org/). A P value of <0.05 (2-tailed) defined statistical significance.

RESULTS

Patient Characteristics

Overall, 1568 patients were identified from the IGCLM database. From this cohort, patients with unknown KRAS mutational status (n = 270), rectal primaries (n = 515), or transverse primaries (n = 65) were excluded (Fig. 1). The remaining 718 patients formed the study cohort with 277 (38.6%) RS and 441 (61.4%) LS primary tumors. Detailed demographic, clinicopathologic, and genetic data were stratified by PTL and are presented in Table 1. Patients with RS tumors were more likely to be older (63.9 vs 60.7 years old, $P < 0.001$) and female (47% vs 38%, $P = 0.014$). As expected, they were also more likely to have KRAS mutated tumors (39% vs 21%, $P < 0.001$). The frequency of liver bilobar disease was lower in the RS patients (29% vs 39%, $P = 0.006$), although they were more likely to receive posthepatectomy adjuvant chemotherapy (65% vs 52%, $P = 0.003$). Lastly, patients with RS tumors were less likely to receive anti-epidermal growth factor receptor (anti-EGFR) therapies (2% vs 7%, $P = 0.001$).

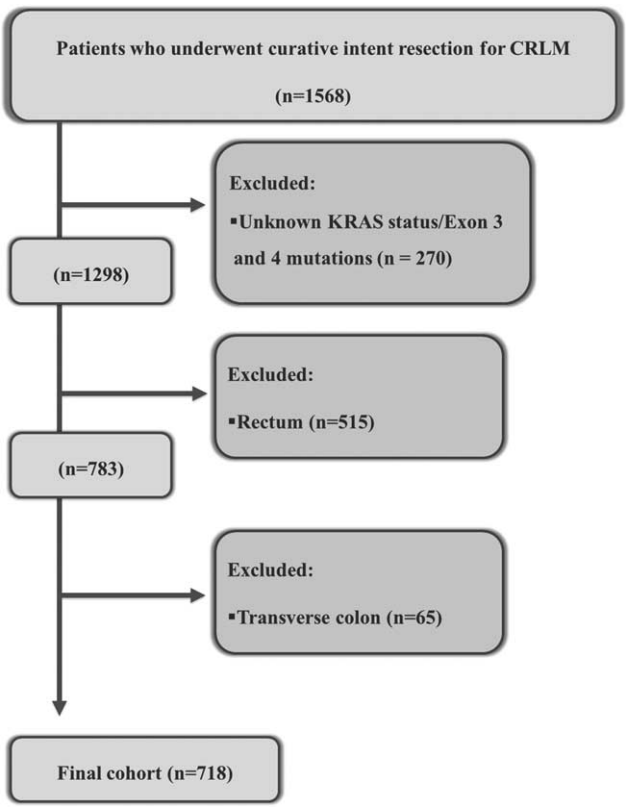

FIGURE 1. Patient selection flowchart.

Following the comparison of RS versus LS patients, we also compared demographic, clinicopathologic, and genetic data, stratified this time by both KRAS mutational status and PTL (Table 2). Among those with wild-type tumors, patients with RS tumors were more likely to be older and female. The frequency of liver bilobar disease and of the receipt of anti-EGFR therapies was also lower in those patients. In contrast, among patients with KRAS mutated tumors, age and sex were equally distributed between patients with RS versus LS tumors. The only differences were a higher rate of both nodal disease and posthepatectomy chemotherapy for those with RS tumors.

OS in the Entire Cohort

At a median follow-up of 30.4 months (IQR, 16.5–52.7 months), median survival for the entire cohort was 53.6 months and 5-year survival was 46.4%. Fig. 2A demonstrates that survival after resection of CLM was significantly different ($P = 0.035$) when stratified by PTL, with a median OS of 45.5 months for those with RS tumors versus 61.6 months for those with LS tumors. Five-year OS for RS tumors was 39.4% compared to 50.8% for LS tumors. Importantly, after controlling for known prognostic clinicopathologic and genetic factors on multivariable analysis, RS remained independently associated with a higher risk of death [hazard ratio (HR): 1.45, $P = 0.042$] (Table 3). Furthermore, KRAS mutational status, extrahepatic disease, receipt of prehepatectomy chemotherapy, concurrent intraoperative ablation, and number of metastases >3 were independently associated with a higher risk of death.

OS in the Wild-Type Patients

At a median follow-up of 30.7 months (IQR, 16.5–53.6 months), patients with wild-type tumors had a median survival of 65.9 months and a 5-year survival of 51.6%. Fig. 2B demonstrates that survival after resection of CLM was significantly different when stratified by PTL, with a median OS of 47.2 months for those with RS tumors versus 67.8 months for those with LS tumors ($P = 0.023$). Five-year OS for RS tumors was 43.7% compared to 55.5% for LS tumors. Importantly, RS remained independently associated with a higher risk of death even after controlling for other prognostic factors (HR: 1.49; 95% confidence interval: 1.09–2.04; $P = 0.01$) (Table 4). Nodal disease of the primary tumor, receipt of prehepatectomy chemotherapy, and extrahepatic disease were also independently associated with a higher risk of death.

OS in the KRAS Mutated Patients

At a median follow-up of 30.1 months (IQR, 15.8–51.5 months), median survival for patients with KRAS mutated tumors was 39.7 months and 5-year survival was 33.6%. Fig. 2C demonstrates that survival after resection of CLM was similar between patients with RS versus LS tumors, with a median OS of 39.7 months for those with RS tumors versus 40.1 months for those with LS tumors ($P = 0.383$). Five-year OS for RS tumors was 32.8% compared to 34.0% for LS tumors. Extrahepatic disease and a CEA higher than 100 ng/mL was independently associated with a higher risk of death (Table 5).

Sub-analysis of OS After Excluding Patients Who Underwent Resection and Concurrent Ablation

The findings of the main analysis were corroborated in the sub-analysis after excluding the 73 patients who underwent concurrent resection and ablation. When considering the entire cohort, RS was not associated with a higher risk of death, even in univariable analysis (HR: 1.25, $P = 0.08$). Upon stratifying patients by mutational

TABLE 1. Patient Demographic, Clinicopathologic, and Genetic Characteristics Stratified by PTL (n = 718)

Patient Characteristics		Left-sided Primary	Right-sided Primary	*P*
n		441	277	
Age at the time of surgery [median (IQR)]		60.7 (50.9, 67.1)	63.9 (55.4, 71.4)	**<0.001**
Sex (%)	Male	275 (62)	147 (53)	**0.014**
	Female	166 (38)	130 (47)	
Primary T stage (%)	T1-T2	44 (11)	31 (12)	0.564
	T3-T4	372 (89)	227 (88)	
Primary lymph node (%)	No	156 (36)	96 (35)	0.711
	Yes	274 (64)	179 (65)	
Synchronous CRLM (%)	No	216 (50)	152 (55)	0.136
	Yes	220 (50)	123 (45)	
CEA >100 ng/mL (%)	No	323 (86)	198 (89)	0.247
	Yes	53 (14)	24 (11)	
CLM number ≥3 (%)	No	280 (64)	176 (64)	0.947
	Yes	159 (36)	101 (36)	
CLM size >3 cm (%)	No	186 (51)	141 (58)	0.085
	Yes	181 (49)	103 (42)	
Bilobar liver disease (%)	No	260 (61)	189 (71)	**0.006**
	Yes	166 (39)	76 (29)	
Extrahepatic disease (%)	No	394 (90)	244 (89)	0.483
	Yes	42 (10)	31 (11)	
KRAS-mutated (%)	No	348 (79)	168 (61)	**<0.001**
	Yes	93 (21)	109 (39)	
R0 resection (%)	No	297 (81)	197 (81)	0.926
	Yes	68 (19)	46 (19)	
Bevacizumab (%)	No	229 (65)	145 (65)	0.973
	Yes	124 (35)	79 (35)	
Prehepatic resection chemotherapy (%)	No	147 (34)	100 (36)	0.489
	Yes	286 (66)	174 (64)	
Posthepatic resection chemotherapy (%)	No	192 (48)	88 (35)	**0.003**
	Yes	212 (52)	160 (65)	
Anti-EGFR (%)	Yes	31 (7)	5 (2)	**0.001**

Bold fond indicates statistical significance ($P < 0.05$).
CLM indicates colon cancer liver metastases; IQR, interquartile range; PTL, primary tumor location.

status; however, 2 distinct trends were uncovered. In patients with wild-type tumors, RS remained independently associated with a higher risk of death even after controlling for all other prognostic factors (HR: 1.45; 95% confidence interval: 1.01–2.06; $P = 0.04$). In contrast, in patients with KRAS mutated tumors, RS was not associated with a higher risk of death, even in univariable analysis (HR: 0.76, $P = 0.20$).

Sensitivity Analyses

Sensitivity analyses were performed after excluding patients with extrahepatic metastases (Supplemental Tables 1–3, http://links.lww.com/SLA/B721), those who received anti-EGFR agents (Supplemental Tables 4 and 5, http://links.lww.com/SLA/B721), those who underwent operations when modern chemotherapeutics (ie, oxaliplatin-based, irinotecan-based, anti-EGFR agents, and anti-vascular endothelial growth factor agents) were available (Supplemental Tables 6–8, http://links.lww.com/SLA/B721), or after including patients with KRAS exon 3 or 4 mutations (Supplemental Tables 9–11, http://links.lww.com/SLA/B721). Importantly, the 2 major findings of the study did not change in these additional analyses. Specifically, among patients with wild-type tumors, those with RS colon cancer had a shorter survival, whereas among those with KRAS mutated tumors, tumor laterality was not prognostic. Furthermore, a propensity score analysis to adjust for possible confounders confirmed that prognostic survival difference between right and left primary colon cancer was still detected only in patients harboring KRAS wild-type tumors (Supplemental Table 12, http://links.lww.com/SLA/B721 and Supplemental Figure 1, http://links.lww.com/SLA/B721).

DISCUSSION

In this international, multi-institutional analysis of patients with resected CLM, we found that among patients with wild-type tumors, those with RS colon cancer had a shorter survival (Fig. 2B). This result persisted in the multivariable analysis. In contrast, among those with KRAS mutated tumors, tumor laterality was not prognostic even in the univariable analysis (Fig. 2C). Importantly, these findings were corroborated in a sub-analysis after excluding patients who underwent concurrent resection and ablation. Furthermore, these findings were corroborated in a second sub-analysis which included only patients who underwent operations when all modern chemotherapeutics were available (ie, oxaliplatin-based, irinotecan-based, anti-EGFR agents, and anti-vascular endothelial growth factor agents).

Another interesting finding of the study was that prehepatectomy chemotherapy was independently associated with worse OS. This result merits further attention, particularly because publications from Memorial Sloan Kettering Cancer Center and MD Anderson have reported similar findings.[13,14] Interestingly, Andreou et al from MD Anderson reported that patients treated with FOLFOX before CRLM resection had a higher rate of KRAS mutation, thus suggesting a mechanism for the selection of more aggressive disease.[15] Nonetheless, the retrospective design of the study, the heterogeneity of the employed chemotherapy protocols, and the lack of

TABLE 2. Patient Demographic and Clinicopathologic Characteristics Stratified by the KRAS Mutational Status and PTL (n = 718)

		Wild-Type			KRAS-Mutated		
Patient Characteristics		**Left-sided Primary**	**Right-sided Primary**	***P***	**Left-sided Primary**	**Right-sided Primary**	***P***
n		348	168		93	109	
Age at the time of surgery [median (IQR)]		61.2 (51.3, 67.5)	64.2 (56.2, 71.5)	**0.001**	58.4 (50.4, 66.7)	63.0 (54.8, 70.7)	0.089
Sex (%)	Male	221 (64)	89 (53)	**0.022**	54 (58)	58 (53)	0.489
	Female	127 (36)	79 (47)		39 (42)	51 (47)	
Primary T stage (%)	T1-T2	38 (12)	17 (11)	0.814	6 (7)	14 (14)	0.156
	T3-T4	291 (88)	140 (89)		81 (93)	87 (86)	
Primary nodal disease (%)	No	114 (34)	64 (39)	0.297	42 (45)	32 (29)	**0.02**
	Yes	223 (66)	102 (61)		51 (55)	77 (71)	
Synchronous CRLM (%)	No	167 (49)	92 (55)	0.154	49 (53)	60 (55)	0.738
	Yes	176 (51)	74 (45)		44 (47)	49 (45)	
CEA >100 ng/mL (%)	No	254 (86)	115 (89)	0.313	69 (87)	83 (89)	0.698
	Yes	43 (14)	14 (11)		10 (13)	10 (11)	
CLM number ≥3 (%)	No	223 (64)	113 (67)	0.53	57 (61)	63 (58)	0.614
	Yes	123 (36)	55 (33)		36 (39)	46 (42)	
CLM size >3 cm (%)	No	146 (49)	88 (59)	0.05	40 (59)	53 (56)	0.757
	Yes	153 (51)	62 (41)		28 (41)	41 (44)	
Bilobar liver disease (%)	No	201 (60)	121 (76)	**0.001**	59 (65)	68 (65)	0.991
	Yes	134 (40)	39 (24)		32 (35)	37 (35)	
Extrahepatic disease (%)	No	311 (91)	147 (88)	0.354	83 (89)	97 (90)	0.896
	Yes	32 (9)	20 (12)		10 (11)	11 (10)	
KRAS-mutated (%)	No	348 (100)	168 (100)	NA	0 (0)	0 (0)	NA
	Yes	0 (0)	0 (0)		93 (100)	109 (100)	
R0 resection (%)	No	239 (81)	121 (81)	0.906	58 (84)	76 (81)	0.597
	Yes	57 (19)	28 (19)		11 (16)	18 (19)	
Bevacizumab (%)	No	178 (65)	83 (63)	0.613	51 (63)	62 (67)	0.541
	Yes	94 (35)	49 (37)		30 (37)	30 (33)	
Prehepatic resection chemotherapy (%)	No	112 (33)	60 (36)	0.447	35 (38)	40 (37)	0.891
	Yes	228 (67)	105 (64)		58 (62)	69 (63)	
Posthepatic resection chemotherapy (%)	No	148 (47)	54 (38)	0.074	44 (51)	34 (32)	**0.011**
	Yes	169 (53)	89 (62)		43 (49)	71 (68)	
Anti-EGFR (%)	Yes	31 (9)	4 (2)	**0.005**	0 (0)	1 (1)	0.35

Bold fond indicates statistical significance ($P < 0.05$).
CLM indicates colon cancer liver metastases; IQR, interquartile range; PTL, primary tumor location.

randomization preclude any reliable interpretation. Randomized controlled trials should further explore the optimal role and timing of chemotherapy in patients with CRLM.

The results from the previous studies regarding PTL in resectable CLM are contradicting. Some studies showed that PTL is prognostic for both OS and recurrence-free survival (RFS), others that it is prognostic for either OS or RFS, and some that it is not prognostic for either outcome. Interestingly, the common limiting factor across these contradicting studies has been the lack of KRAS data. For example, a study by the Memorial Sloan Kettering Cancer Center group found that although an LS primary was independently associated with an improved median OS, it was not associated with a difference in RFS and long-term survival.[3] The authors acknowledged the lack of data on KRAS mutational status as one of the major limitations of the study. Similarly, the

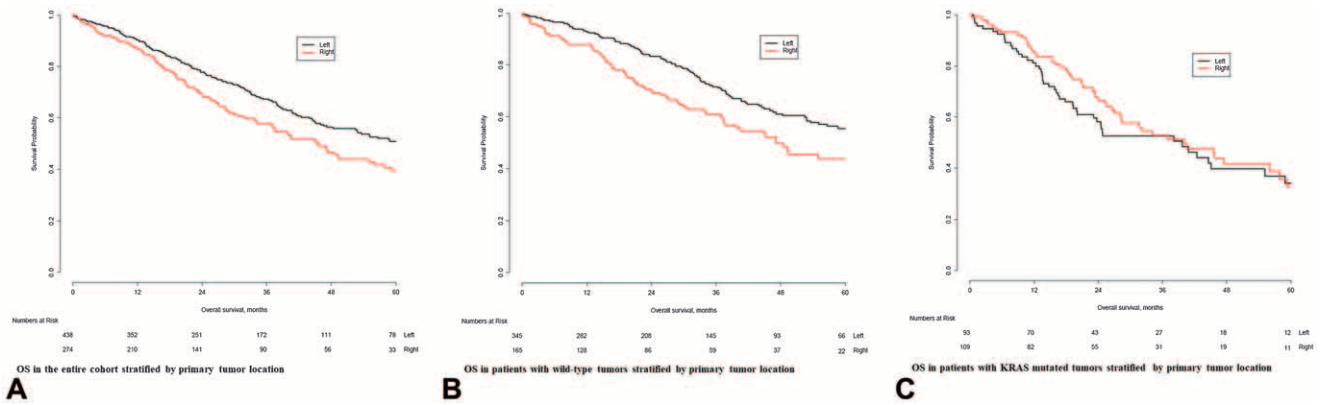

FIGURE 2. Kaplan-Meier curve of overall survival. A, In the entire cohort stratified by primary tumor location. B, In wild-type patients stratified by primary tumor location. C, In KRAS mutated patients stratified by primary tumor location.

TABLE 3. Univariate and Multivariate Survival Analysis in the Entire Cohort (n = 456)

	Univariate		Multivariate	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
KRAS mutated tumors				
Wild-type tumors	Ref.		Ref.	
Mutated	1.62 (1.28–2.06)	<0.001	1.99 (1.30–3.06)	**0.002**
Left-sided primary	Ref		Ref	
Right-sided primary	1.28 (1.02–1.61)	0.03	1.45 (1.01–2.07)	**0.042**
Interaction between laterality and KRAS mutation variable	0.61 (0.37–0.99)	0.045	0.63 (0.34–1.17)	0.141
Patient age at the time of surgery	1.002 (0.99–1.01)	0.76	—	
Female gender	1.00 (0.79–1.25)	0.98	—	
Primary tumor stage				
T3 and T4 versus T1 and T2 stage	1.31 (0.89–1.93)	0.17	—	
Nodal disease of the primary	1.37 (1.08–1.75)	0.010	1.36 (1.00–1.85)	0.05
CEA >100 ng/dL	1.50 (1.06–2.11)	0.023	1.45 (0.95–2.21)	0.083
Prehepatectomy chemotherapy	1.3 7 (1.06–1.77)	0.016	1.90 (1.34–2.70)	**<0.001**
Synchronous liver metastases	1.09 (0.86–1.36)	0.47	—	
Extrahepatic disease	2.20 (1.57–3.10)	<0.001	1.94 (1.24–3.03)	**0.004**
Liver tumor diameter >3 cm	1.11 (0.86–1.42)	0.41	—	
Liver tumor number >3	1.48 (1.18–1.86)	<0.001	1.38 (1.20–1.88)	**0.037**
Bilobar liver disease	1.20 (0.95–1.52)	0.12	—	
R1 resection	1.54 (1.14–2.08)	0.005	1.27 (0.90–1.79)	0.166
Posthepatectomy chemotherapy	0.73 (0.58–0.93)	0.009	0.84 (0.63–1.13)	0.248
Intraoperative ablation	1.47 (1.08–2.00)	0.015	1.49 (1.03–2.15)	**0.035**
Anti-EGFR	1.26 (0.72–2.20)	0.42		

Bold fond indicates statistical significance ($P < 0.05$).
CI indicates confidence interval; HR, hazard ratio.

authors of several studies on the prognostic value of PTL in CLM have acknowledged the lack of data on KRAS mutational status as one of the major limitations of their studies.[4–6,16]

Importantly, only 2 studies on PTL in resectable CLM completely accounted for RAS mutations in their analysis. The first study came from our group at Johns Hopkins Hospital, and the other study from Yamashita et al followed soon after.[7,8] Both studies concluded that patients with RS colon cancer had a shorter survival. Neither study stratified patients by their KRAS mutational status before assessing the impact of PTL on survival. Instead, they controlled for RAS mutations in the multivariable analysis, and PTL remained independently associated with survival. Although controlling for confounders using multivariable analysis is a valid statistical method, it can be misleading in the case of collinearity of two variables.[17] Collinearity occurs when 2 prognostic factors correlate not only with prognosis, but also with each other. In this case, it is likely that right sidedness and RAS mutations are collinear variables, and thus controlling for RAS mutations in the multivariable analysis may not necessarily mean that PTL is an independent prognostic factor. This is because RAS mutations are more common in RS tumors, and thus positive mutational status can be predicted from tumor laterality. In turn, the higher risk of death for patients with RS tumors may have been falsely attributed to laterality instead of the associated RAS mutations.

In contrast, we chose to account for confounders by not only using multivariable analysis, but also by stratifying patients by their KRAS mutational status before assessing the prognostic impact of PTL. This minimizes the chance of residual confounding. Among patients with wild-type tumors, we found that those with RS colon cancer had a shorter survival. In contrast, among those with KRAS mutated tumors, PTL was not prognostic even in the univariable analysis. Although no other study in resectable CLM has stratified patients by their KRAS mutational status, similar stratifications have been performed in 2 studies on unresectable mCRC. The first study utilized data from a randomized, multicenter phase II trial (AIO KRK-0104) and had similar findings to our study.[18] Specifically, LS tumors were associated with significantly longer OS and PFS as compared to RS tumors, but only in patients with KRAS codon 12/13 wild-type tumors (HR OS: 0.42; HR PFS: 0.54). In contrast, PTL was not prognostic in those with KRAS codon 12/13 mutant tumors (HR OS: 1.3; HR PFS: 1.01). The authors concluded that there existed a significant interaction between KRAS status and PTL, which in turn impacted OS and PFS.[18] In the second study, Loupakis et al explored a cohort of 546 patients with unresectable KRAS mutated mCRC, and found that PTL was not prognostic in KRAS mutants (HR = 0.99, $P = 0.964$).[19] Perhaps reflecting those studies, Stinzing et al stated in a recent review that "Currently, data on RAS-MT LCC versus RCC are limited; therefore, the prognostic and predictive value of the primary tumor site within the RAS MT population still requires evaluation."[20]

BRAF mutation is the only somatic mutation that is prognostic in CLM and not accounted for in our analysis, which was a limitation of this study.[21] However, studies in unresectable mCRC suggest that the prognostic value of PTL is independent of the BRAF mutational status.[22,23] Nevertheless, given that BRAF mutations occur mostly in KRAS wild-type tumors and on the RS, it is possible that unaccounted BRAF mutations in KRAS wild-type tumors may be responsible for the worse survival in patients with RS versus LS tumors. Future studies will need to account for other genetic mutations (eg, BRAF mutation) in KRAS wild-type patients and address whether PTL remains prognostic in wild-type patients after accounting for them. Another limitation of the current study was the lack of data on histology and specifically on mucinous histology, which is more commonly observed in RS tumors and has been previously shown to negatively impact prognosis in CLM.[24] However, evidence in the mCRC setting also suggests that the prognostic value of PTL is independent of histology.[23,25] As a retrospective analysis, the study is subject to inherent bias regarding patient selection and follow-up. For example, we cannot rule out that RS patients with "worse" cancers (poor differentiation, more nodal disease, etc) never make it to

TABLE 4. Univariate and Multivariate Survival Analysis in Wild-type Patients (n = 422)

	Univariate		Multivariate	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Primary tumor laterality				
Left-sided primary	Ref.			
Right-sided primary	1.40 (1.05–1.86)	0.023	1.49 (1.09–2.04)	**0.013**
Patient age at the time of surgery	1.00 (0.99–1.02)	0.73	—	
Female gender	0.96 (0.72–1.27)	0.77	—	
Primary tumor stage				
T3 and T4 versus T1 and T2	1.32 (0.83–2.10)	0.24	—	
Nodal disease of the primary	1.41 (1.04–1.92)	0.02	1.42 (1.02–1.97)	**0.038**
CEA >100 ng/dL	1.30 (0.85–1.99)	0.23	—	
Prehepatectomy chemotherapy	1.39 (1.01–1.92)	0.04	1.79 (1.21–2.64)	**0.003**
Synchronous liver metastases	1.04 (0.78–1.37)	0.80	—	
Extrahepatic disease	2.03 (1.32–3.10)	0.001	1.79 (1.11–2.87)	**0.016**
Liver tumor diameter >3 cm	1.30 (0.96–1.74)	0.09	—	
Liver tumor number >3	1.43 (1.08–1.89)	0.01	1.21 (0.87–1.69)	0.26
Bilobar liver disease	1.08 (0.81–1.45)	0.59	—	
R1 resection	1.47 (1.02–2.10)	0.04	1.33 (0.91–1.94)	0.14
Posthepatectomy chemotherapy	0.76 (0.57–1.00)	0.06	—	
Intraoperative ablation	1.51 (1.04–2.19)	0.03	1.43 (0.94–2.17)	0.09
Anti-EGFR	1.44 (0.80–2.59)	0.23		

Bold fond indicates statistical significance ($P < 0.05$).
CI indicates confidence interval; HR, hazard ratio.

surgery. In turn, this selection bias may to a certain extent account for the equivocal worse survival of patients with RS KRAS mutated tumors. Of note, different independent prognostic factors were observed between the KRAS-wild and KRAS-mutant sub-cohorts. This is probably a reflection of the nonlinearity in prognostic models. Specifically, mathematical realities suggest that the interactions among different prognostic factors are far from linear, and that some variables gain or lose significance due to the absence or presence of other variables — in this case in the presence or absence of KRAS mutation.[26] Last, as mentioned in the methods section, patients from the early 2000s were not tested for all mutations in exons 3, and none of the patients were tested for exon 4 mutations. As such, some of these mutations may not have been captured. Given that a recent study reported on a variable prognostic impact of those mutations, future studies should account for them.[27]

The IGCLM is by definition an international, multi-institutional database that includes patients with resectable stage IV CRC, and in particular, liver metastases (with or without concurrent extrahepatic disease). As such, it was not feasible to analyze all different stages of CRC. Because the study findings concern only resectable CRLM (with or without concurrent resectable extrahepatic disease), they should not be extrapolated to nonmetastatic resectable CRC (stage I-III) or unresectable metastatic CRC. Interestingly, although our findings are in line with those in studies on

TABLE 5. Univariate and Multivariate Survival Analysis in KRAS Mutated Patients (n = 129)

	Univariate		Multivariate	
	HR (95% CI)	***P***	**HR (95% CI)**	***P***
Primary tumor laterality				
Left-sided primary	Ref			
Right-sided primary	0.84 (0.57–1.24)	0.38	—	
Patient age at the time of surgery	1.003 (0.98–1.02)	0.88	—	
Female gender	0.99 (0.67–1.46)	0.96	—	
Primary tumor stage				
T3 and T4 versus T1 and T2	1.24 (0.62–2.47)	0.54	—	
Nodal disease of the primary	1.36 (0.90–2.05)	0.15	—	
CEA >100 ng/dL	2.95 (1.61–5.42)	<0.001	3.16 (1.26–7.96)	**0.014**
Prehepatectomy chemotherapy	1.42 (0.93–2.17)	0.10	—	
Synchronous liver metastases	1.23 (0.84–1.81)	0.29	—	
Extrahepatic metastases	2.97 (1.65–5.34)	<0.001	2.92 (1.29–6.58)	**0.01**
Liver tumor diameter >3 cm	0.82 (0.51–1.31)	0.41	—	
Liver tumor number >3	1.50 (1.02–2.21)	0.04	1.68 (0.87–3.23)	0.12
Bilobar liver disease	1.51 (1.01–2.23)	0.042	1.04 (0.54–2.01)	0.90
R1 resection	1.84 (1.06–3.19)	0.029	1.43 (0.77–2.66)	0.25
Posthepatectomy chemotherapy	0.62 (0.42–0.93)	0.021	0.99 (0.54–1.82)	0.98
Intraoperative ablation	1.40 (0.79–2.47)	0.237		

Bold fond indicates statistical significance ($P < 0.05$).
CI indicates confidence interval; HR, hazard ratio.

unresectable metastatic CRC, current literature on nonmetastatic resectable CRC is controversial. Most studies reported on a poorer survival of RS tumors, whereas others like Weiss et al and Warschkow et al found no significant difference in survival or even a better prognosis in those with RS tumors, respectively.[28,29] Future studies should include patients with non-metastatic resectable CRC (stage I-III) and metastatic resectable CRC, and assess whether our findings apply to all stages after stratifying by KRAS mutational status. As is always the case with retrospective research, these results should be validated by larger, ideally prospective, studies.

This study is important for three reasons. First, for the first time in a surgical cohort of CLM, our study shows that KRAS mutational status influences the prognostic impact of PTL, which confirms findings from prior studies in unresectable mCRC. As Boeckx et al noted, given that data on the interplay between PTL and RAS mutated disease is inconclusive, this study adds to the current literature.[30] Second, the double PTL-KRAS stratification that we introduced may explain the disparate results of the previous studies on the prognostic impact of PTL in resectable CLM, which did not account for KRAS mutational status. Thus, our method may be used to better inform prognosis compared to the simple RS versus LS stratification. Third, if our findings are confirmed by others, their clinical importance extends beyond informing prognosis, and can potentially be used to tailor surgical technique and/or surgical margin width, as our group previously reported for KRAS mutation status alone.[31,32] The combination of PTL and KRAS mutational status may allow for even more tailored multimodality treatments. Importantly, a recent study using NCDB data indirectly corroborates our findings.[33] Though this study was different in its scope from the index study, a sub-analysis (Goffredo et al, 2018, Fig. 3) shows that the difference in OS between RS and LS cancers was of much greater magnitude for wild-type versus KRAS mutated patients.

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Original Study

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Effect of Primary Tumor Location on Second- or Later-line Treatment Outcomes in Patients With *RAS* Wild-type Metastatic Colorectal Cancer and All Treatment Lines in Patients With *RAS* Mutations in Four Randomized Panitumumab Studies

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Abstract

The results from the retrospective analyses of data from 4 phase III randomized panitumumab trials showed a worse prognosis for patients with right- versus left-sided *RAS* wild-type metastatic colorectal cancer (mCRC) receiving second-line or greater therapy. Furthermore, the addition of panitumumab to standard treatment provided benefit to patients with left-sided *RAS* wild-type tumors. Further research is needed to define the optimal treatment of *RAS* mutant and right-sided *RAS* wild-type mCRC.

Background: The primary tumor location has a prognostic impact in metastatic colorectal cancer (mCRC). We report the results from retrospective analyses assessing the effect of tumor location on prognosis and efficacy of second- and later-line panitumumab treatment in patients with *RAS* wild-type (WT) mCRC and on prognosis in all lines of treatment in patients with *RAS* mutant (MT) mCRC. **Patients and Methods:** *RAS* WT data (n = 483) from 2 randomized phase III panitumumab trials (ClinicalTrials.gov identifiers, NCT00339183 and NCT00113763) were analyzed for treatment outcomes stratified by tumor location. The second analysis assessed the effect of tumor location in *RAS* MT patients (n = 1205) from 4 panitumumab studies (ClinicalTrials.gov identifiers, NCT00364013, NCT00819780, NCT00339183, and NCT00113763). Primary tumors located in the cecum to transverse colon were coded as right-sided; those located from the splenic flexure to the rectum were coded as left-sided. **Results:** Of all patients, the tumor location was ascertained for 83% to 88%; 71% to 77% of patients had left-sided tumors. *RAS* WT patients with right-sided tumors did worse for all efficacy parameters compared with those with left-sided tumors. The patients with left-sided tumors had better outcomes with panitumumab than with the comparator treatment. Because of the low patient numbers, no conclusions could be drawn for right-sided mCRC. The prognostic effect of tumor location on survival was unclear for *RAS* MT patients. **Conclusion:** These retrospective analyses have confirmed that *RAS* WT right-sided mCRC is associated with a poor prognosis, regardless of the treatment. *RAS* WT patients with left-sided tumors benefitted from the addition of panitumumab in second or later treatment lines. Further research is warranted to determine the optimum management of right-sided mCRC and *RAS* MT tumors.

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Keywords: mCRC, *RAS* mutant, *RAS* WT, Treatment lines, Tumor location

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Introduction

The idea that tumor location had a link with disease biology arose in 1990, when Bufill[1] described colorectal cancer (CRC) by the primary tumor location. Right-sided colon tumors more frequently harbor *BRAF* mutations, have a higher tumor/nodes/metastases stage at presentation, and have a worse prognosis compared with left-sided colorectal tumors.[2,3] The fact that the proximal part of the colon is derived from the embryologic midgut, and the distal part and rectum are derived from the embryologic hindgut might help explain the observed differences.

Several retrospective analyses have assessed the clinical effect of epidermal growth factor receptor (EGFR)-targeted agents in patients with metastatic CRC (mCRC) according to the primary tumor location,[4-7] most of which evaluated first-line data from cetuximab trials.[5-7] These analyses reported better results for cetuximab plus chemotherapy versus chemotherapy alone or combined with bevacizumab in patients with left-sided mCRC.[5-7] In contrast, patients with right-sided tumors generally appeared to benefit more from chemotherapy combined with bevacizumab. Few data are available on the effect of the tumor location on the efficacy of later-line treatment or in patients with *RAS* mutant (MT) mCRC. Also, no studies to date have investigated the effect of tumor location on panitumumab efficacy in these settings. The first aim of the present retrospective analyses was to investigate the possible association between primary tumor location and second- or later-line panitumumab efficacy in patients with *RAS* wild-type (WT) mCRC. The second aim was to assess the effect of tumor location in patients with *RAS* MT tumors.

Patients and Methods

Study Design and Data Sources

The first analysis was performed on the *RAS* (*KRAS* and *NRAS* exon 2, 3, and 4) WT populations from 2 randomized phase III mCRC trials. The second-line 20050181 trial (ClinicalTrials.gov identifier, NCT00339183) evaluated the effect of panitumumab plus FOLFIRI (folinic acid, 5-fluorouracil, irinotecan) compared with FOLFIRI alone.[8,9] The later-line 20020408 trial (ClinicalTrials.gov identifier, NCT00113763) evaluated panitumumab plus best supportive care (BSC) versus BSC alone for patients in whom the available treatment options had failed.[10,11] This analysis assessed the effect of tumor location on clinical outcomes in the *RAS* WT and *RAS/BRAF* WT (after exclusion of all *BRAF* V600E MT patients) populations. The second analysis studied differences in the clinical outcomes for *RAS* MT patients with left- and right-sided mCRC from the 2 cited studies and from 2 additional first-line trials: PRIME (ClinicalTrials.gov identifier, NCT00364013), a phase III trial comparing panitumumab plus FOLFOX (folinic acid, 5-fluorouracil, oxaliplatin) versus FOLFOX alone,[12] and PEAK (ClinicalTrials.gov identifier, NCT00819780), a phase II trial comparing panitumumab plus FOLFOX versus bevacizumab plus FOLFOX.[13]

Assessment of Tumor Location

Tumor location information was obtained from the free-text surgery descriptions included in the case report forms and the original pathology reports. Primary tumors located in the cecum to transverse colon were coded as right-sided. Tumors located from the splenic flexure to rectum were categorized as left-sided. The assessors of the tumor location were unaware of the *RAS* and *BRAF* mutation status, treatment allocation, and clinical outcomes.

Statistical Analysis

Because these were exploratory, retrospective analyses, no formal hypothesis testing was planned. The efficacy endpoints evaluated were the response rate (RR), duration of response (DoR), progression-free survival (PFS), and overall survival (OS). These endpoints were calculated as previously reported.[14]

Data were summarized descriptively. The treatment hazard ratio (HR) for the panitumumab arm relative to the comparator arms and the associated 95% confidence intervals were estimated from a stratified Cox proportional hazard model. Wald tests were used to generate *P* values. For the *RAS* WT analysis, the Cox model was adjusted for *BRAF* status, previous adjuvant therapy, and baseline Eastern Cooperative Oncology Group (ECOG) score (study 20050181) or for *BRAF* status and baseline ECOG (study 20020408). For the *RAS* MT analysis, the Cox model was adjusted for the stratification variables as described in the respective study protocols, including region and baseline ECOG (PRIME and study 20020408), previous adjuvant oxaliplatin therapy (PEAK), and region, baseline ECOG, and previous oxaliplatin exposure (study 20050181). No adjustments for *BRAF* status were made in this population because *RAS* and *BRAF* mutations are generally mutually exclusive. Kaplan-Meier curves were generated for all time-to-event endpoints.

Results

Patient Population

The primary tumor location could be determined unequivocally in > 80% of patients in each study (PRIME, 874 of 1049 [83%]; PEAK, 197 of 228 [86%]; 20050181, 887 of 1011 [88%]; 20020408, 290 of 349 [83%]). Approximately three quarters of the patients with the side ascertainable had left-sided mCRC (Supplemental Table 1; available in the online version). In general, the left/right distribution seen in the *RAS* WT and *RAS* MT populations was similar to that in the overall study population. However, in the *RAS* MT population of PEAK, 39% of patients had right-sided mCRC. This *RAS* MT subgroup was markedly smaller in this study because enrollment in PEAK was restricted to *KRAS* exon 2 WT patients.

In the *RAS* WT populations of studies 20050181 (n = 368) and 20020408 (n = 115), *BRAF* V600E mutations were present in 4% and 6% of patients with left-sided mCRC compared with 31% and 20% of right-sided mCRC patients. No difference was found in age between the left- and right-sided mCRC patients in either the *RAS* WT (Table 1) or *RAS* MT (Table 2) populations.

Prognostic Effect of Primary Tumor Location

RAS WT. In the 20050181 and 20020408 studies, *RAS* WT patients with left-sided tumors had better OS and PFS compared with those with right-sided tumors, irrespective of the treatment received (Table 3, Figure 1). Poor survival was observed in right-sided mCRC patients, and the HRs for OS in both studies demonstrated a worse prognosis for patients with right-sided disease (Supplemental Table 2;

Table 1 Baseline Demographics and Disease Characteristics of *RAS* Wild-type Population

Characteristic	20050181				20020408			
	Pmab Arm		Comparator Arm		Pmab Arm		Comparator Arm	
	Left	Right	Left	Right	Left	Right	Left	Right
Patients	150	31	148	39	42	16	43	14
ECOG PS								
0	78 (52.0)	11 (35.5)	77 (52.0)	19 (48.7)	23 (54.8)	4 (25.0)	12 (27.9)	3 (21.4)
1	66 (44.0)	17 (54.8)	61 (41.2)	17 (43.6)	14 (33.3)	9 (56.3)	22 (51.2)	8 (57.1)
2	6 (4.0)	3 (9.7)	10 (6.8)	3 (7.7)	5 (11.9)	3 (18.8)	9 (20.9)	2 (14.3)
3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.1)
Previous adjuvant chemotherapy								
No	115 (76.7)	21 (67.7)	124 (83.8)	32 (82.1)	NA	NA	NA	NA
Yes	31 (20.7)	9 (29.0)	24 (16.2)	6 (15.4)	NA	NA	NA	NA
Sex								
Female	48 (32.0)	15 (48.4)	46 (31.1)	19 (48.7)	18 (42.9)	7 (43.8)	17 (39.5)	4 (28.6)
Male	102 (68.0)	16 (51.6)	102 (68.9)	20 (51.3)	24 (57.1)	9 (56.3)	26 (60.5)	10 (71.4)
***BRAF* status**								
Test failure	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (6.3)	1 (2.3)	1 (7.1)
Mutant	7 (4.7)	9 (29.0)	4 (2.7)	13 (33.3)	3 (7.1)	3 (18.8)	2 (4.7)	3 (21.4)
Wild-type	143 (95.3)	22 (71.0)	144 (97.3)	26 (66.7)	39 (92.9)	12 (75.0)	40 (93.0)	10 (71.4)
Metastatic sites								
Liver + other	102 (68.0)	20 (64.5)	90 (60.8)	27 (69.2)	NA	NA	NA	NA
Liver only	29 (19.3)	3 (9.7)	36 (24.3)	5 (12.8)	NA	NA	NA	NA
Other only	19 (12.7)	8 (25.8)	22 (14.9)	7 (17.9)	NA	NA	NA	NA
Age, y								
Median	61	60	60	62	61	55	63	62
Range	28-81	38-77	33-85	42-82	29-78	31-79	32-81	37-78

Data presented as n (%).
Abbreviations: ECOG = Eastern Cooperative Oncology Group; NA = not available; Pmab = panitumumab; PS = performance status.

Table 2 Baseline Demographic Data and Disease Characteristics of *RAS* Mutant Population

	PRIME				PEAK				20050181				20020408			
	Pmab Arm		Comparator Arm		Pmab Arm		Comparator Arm		Pmab Arm		Comparator Arm		Pmab Arm		Comparator Arm	
Characteristic	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
Patients	166	64	158	70	14	11	19	10	183	76	194	65	61	16	77	21
ECOG PS																
0	89 (53.6)	39 (60.9)	87 (55.1)	36 (51.4)	8 (57.1)	6 (54.5)	9 (47.4)	7 (70.0)	85 (46.4)	43 (56.6)	97 (50.0)	31 (47.7)	31 (50.8)	7 (43.8)	32 (41.6)	5 (23.8)
1	71 (42.8)	20 (31.3)	65 (41.1)	30 (41.1)	6 (42.9)	5 (45.5)	10 (52.6)	3 (30.0)	88 (48.1)	31 (40.8)	84 (43.3)	30 (46.2)	22 (36.1)	6 (37.5)	34 (44.2)	13 (61.9)
2	6 (3.6)	5 (3.6)	6 (3.8)	6 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (5.5)	2 (2.6)	13 (6.7)	3 (4.6)	8 (13.1)	3 (18.8)	11 (14.3)	2 (9.5)
3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8)
Previous adjuvant chemotherapy																
No	139 (83.7)	19 (29.7)	132 (83.5)	69 (98.6)	12 (85.7)	10 (90.9)	15 (78.9)	9 (90.0)	142 (77.6)	56 (73.7)	158 (81.4)	54 (83.1)	NA	NA	NA	NA
Yes	27 (16.3)	45 (70.3)	26 (16.5)	1 (1.4)	2 (14.3)	1 (9.1)	4 (21.1)	1 (10.0)	37 (20.2)	19 (25.0)	35 (18.0)	11 (16.9)	NA	NA	NA	NA
Sex																
Female	59 (35.5)	19 (29.7)	68 (43.0)	28 (40.0)	8 (57.1)	4 (36.4)	8 (42.1)	3 (30.0)	83 (45.4)	32 (42.1)	73 (37.6)	28 (42.1)	28 (45.9)	6 (37.5)	27 (35.1)	21 (100.0)
Male	107 (64.5)	45 (70.3)	90 (57.0)	42 (60.0)	6 (42.9)	7 (63.6)	11 (57.9)	7 (70.0)	100 (54.6)	44 (57.9)	121 (62.4)	37 (56.9)	33 (54.1)	10 (62.5)	50 (64.9)	0 (0.0)
Metastatic sites																
Liver + other	113 (68.1)	49 (76.6)	112 (70.9)	54 (77.1)	4 (28.6)	8 (72.7)	7 (36.8)	6 (60.0)	143 (78.1)	45 (59.2)	85 (46.4)	43 (56.5)	NA	NA	NA	NA
Liver only	31 (18.7)	6 (9.4)	26 (16.5)	10 (14.3)	7 (50.0)	0 (0.0)	3 (15.8)	2 (20.0)	24 (13.1)	14 (18.4)	88 (48.1)	31 (40.8)	NA	NA	NA	NA
Other only	31 (18.7)	9 (14.1)	20 (12.7)	6 (8.6)	3 (21.4)	3 (27.3)	9 (47.4)	2 (20.0)	16 (8.7)	17 (22.4)	10 (5.5)	2 (2.6)	NA	NA	NA	NA
Age, y																
Median	62	66	63	62	59	64	63	65	60	63	64	65	60	64	62	61
Range	35-80	33-83	27-82	33-79	32-78	41-80	39-75	40-72	29-78	35-84	29-86	34-86	27-82	37-77	32-83	27-72

Data presented as n (%).
Abbreviations: ECOG = Eastern Cooperative Oncology Group; NA = not available; Pmab = panitumumab; PS = performance status.

Table 3 Overall Survival, Progression-free Survival, Response Rates, and Duration of Response in *RAS* Wild-type Population

Study	Treatment	Patients		OS, mo; Median (95% CI)		PFS, mo; Median (95% CI)		RR, %; Median (95% CI)		DoR, mo; Median (95% CI)	
		Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
20050181	Pmab + FOLFIRI	150/147[a]	31/30[a]	20.1 (16.5-21.7)	10.3 (5.2-13.7)	8.0 (7.3-9.1)	4.8 (3.5-7.4)	49.7	13.3	7.7 (6.1-9.5)	NE (9.5-NE)
	FOLFIRI	148/144[a]	39/38[a]	16.6 (14.8-21.2)	8.1 (6.3-12.1)	5.8 (5.2-7.3)	2.4 (1.8-5.7)	13.2	2.6	9.3 (5.7-12.3)	NE
	aHR[b]	—	—	0.96 (0.75-1.23)	1.14 (0.68-1.89)	0.88 (0.69-1.12)	0.75 (0.45-1.27)	6.49[c] (3.52-12.26)	5.69[c] (0.51-287.73)	—	—
	P value	—	—	.7388	.6193	.3086	.2859	—	—	—	—
20020408	Pmab + BSC	42/42[a]	16/16[a]	9.4 (7.3-11.7)	3.1 (2.0-12.0)	5.5 (2.6-5.7)	1.7 (1.0-2.8)	23.8	0	5.4 (2.8-12.0)	NA
	BSC	43/43[a]	14/14[a]	8.8 (6.4-10.4)	4.6 (0.9-6.0)	1.6 (1.2-1.8)	1.5 (0.7-1.8)	0	0	—	—
	aHR[d]	—	—	1.02 (0.64-1.63)	0.72 (0.31-1.66)	0.31 (0.19-0.50)	0.50 (0.22-1.15)	Inf[c] (3.51-Inf)	NE	—	—
	P value	—	—	.9326	.4349	<.0001[e]	.1029	—	—	—	—

Abbreviations: aHR = adjusted hazard ratio; BSC = best supportive care; DoR = duration of response; ECOG = Eastern Cooperative Oncology Group; FOLFIRI = folinic acid, 5-fluorouracil, irinotecan; Inf = infinity; NA = not available; NE = not evaluable; OS = overall survival; PFS = progression-free survival; Pmab = panitumumab; RR = response rate.

[a]Number of patients evaluable for response.

[b]Adjusted treatment HR calculated from model with factors for *BRAF* status, previous adjuvant therapy, and baseline ECOG; HR < 1 favors the Pmab arm (study 20050181).

[c]Odds ratio for treatment difference in RR presented; odds ratio > 1 favors the Pmab arm (studies 20050181 and 20020408).

[d]Adjusted treatment HR calculated from model with factors for *BRAF* status and baseline ECOG; HR < 1 favors the Pmab arm (study 20020408).

[e]Statistically significant.

available in the online version). The prognosis remained poor in the *RAS*/*BRAF* WT right-sided population compared with that for those with left-sided tumors, irrespective of the treatment (Supplemental Table 3; available in the online version).

RAS MT. In PEAK, *RAS* MT patients with left-sided tumors had markedly better OS than those with right-sided tumors; however, little to no difference was found in PRIME (Table 4). In the later-line trials (studies 20050181 and 20020408), no clear prognostic difference was evident in the *RAS* MT population. Overall, a prognostic effect of primary tumor location on the HRs for OS was not seen in the *RAS* MT population (Supplemental Table 4; available in the online version).

Predictive Effect of Primary Tumor Location in RAS *WT Patients Undergoing Second- or Later-line Treatment*

The effect of primary tumor location on the outcomes for *RAS* WT patients receiving second- or later-line treatment is shown in Table 3 and Figure 1. In study 20050181, the addition of panitumumab to FOLFIRI resulted in a numerically improved median OS (20.1 vs. 16.6 months; HR, 0.96; $P = .7388$) and PFS (8.0 vs. 5.8 months; HR, 0.88; $P = .3086$) compared with FOLFIRI alone in patients with *RAS* WT left-sided primary tumors. In right-sided mCRC patients, the HR for PFS favored panitumumab (4.8 vs. 2.4 months; HR, 0.75; $P = .2859$), but the HR for OS favored FOLFIRI (10.3 vs. 8.1 months; HR, 1.14; $P = .6193$).

In study 20020408, a significant PFS benefit (5.5 vs. 1.6 months; HR, 0.31; $P < .0001$) was seen when panitumumab was added to BSC for *RAS* WT left-sided mCRC patients. No difference was found in PFS for patients with right-sided tumors (1.7 vs. 1.5 months; HR, 0.50; $P = .1029$). The OS results in that study were difficult to interpret because most patients in the BSC arm crossed over to panitumumab at progression (44 of 57 [77%] of the BSC patients with known tumor side status crossed over to panitumumab).

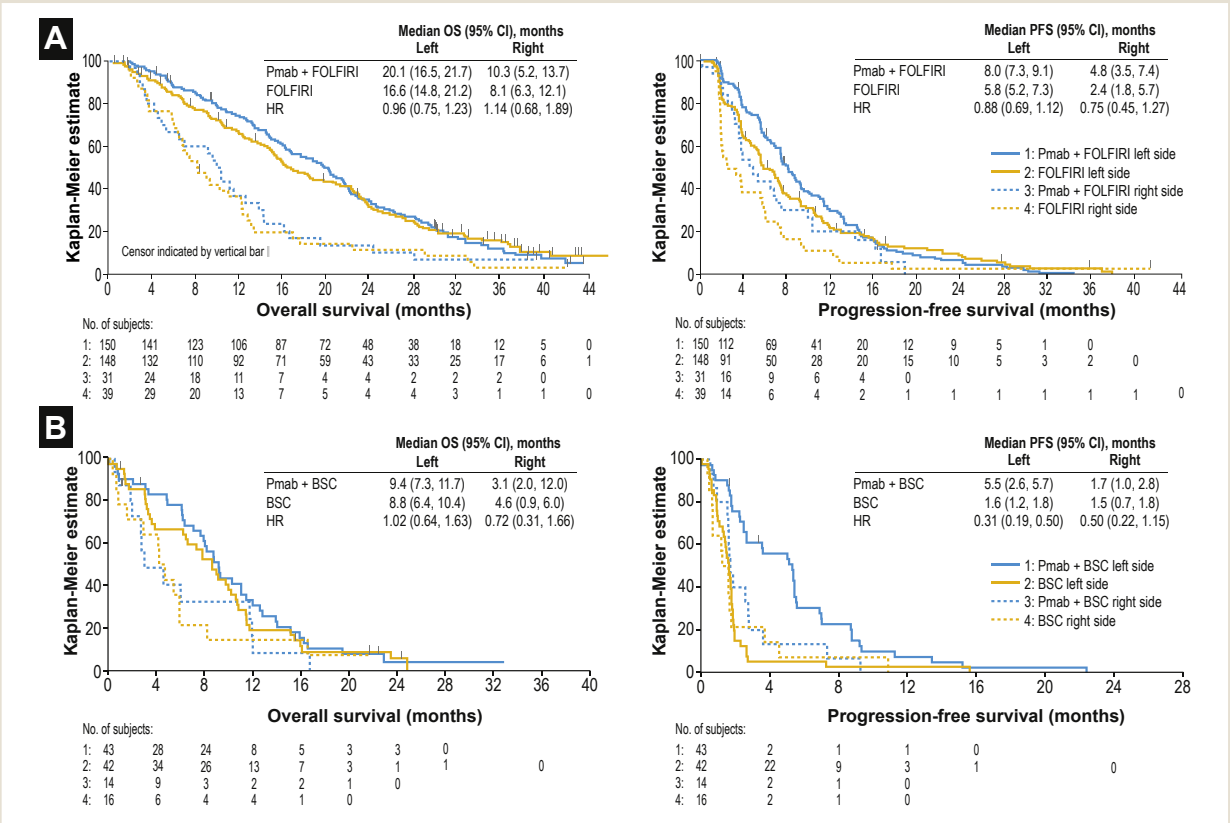
The RRs were greater for the panitumumab versus control arm in the *RAS* WT left-sided mCRC patients in the 20050181 study (50% vs. 13%) and 20020408 study (24% vs. 0%). In patients with right-sided tumors, the same effect was observed in study 20050181 (13% vs. 3%), but no responses were seen in right-sided mCRC in study 20020408, irrespective of treatment. Owing to the low number of responders with right-sided tumors (4 of 30 vs. 1 of 38 evaluable patients in the panitumumab vs. comparator arm in study 20050181 and 0 of 16 vs. 0 of 14 evaluable patients in study 20020408, respectively), no comparison could be made of the DoR stratified by treatment.

The effect of primary tumor location on the outcomes for *RAS*/*BRAF* WT patients receiving second- or later-line treatment is shown in Supplemental Table 3 (available in the online version).

PFS, OS, and RR in RAS *MT Patients*

In PRIME, patients with *RAS* MT left-sided tumors had a significantly worse median PFS in the panitumumab versus FOLFOX arm (7.5 vs. 9.4 months; HR, 1.29; $P = .0288$; Table 4), consistent with the results of the study's primary analysis. The same trend was observed for right-sided mCRC patients (7.4 vs. 8.5 months; HR, 1.37; $P = .0874$). Regarding OS, the HRs favored

Figure 1 Overall Survival (OS) and Progression-free Survival (PFS) in the *RAS* Wild-type Population From the (A) 20050181 and (B) 20020408 Studies



Abbreviations: BSC = best supportive care; CI = confidence interval; FOLFIRI = folinic acid, 5-fluorouracil, irinotecan; HR = hazard ratio; Pmab = panitumumab.

FOLFOX for both left- and right-sided *RAS* MT mCRC patients. No differences between treatments or by location group were observed with respect to RR or DoR.

In PEAK, the results were based on a very small sample size and should therefore be considered with caution. Although left-sided *RAS* MT mCRC patients had worse median PFS in the panitumumab than in the bevacizumab arm (10.2 vs. 12.0 months; HR, 1.29; $P = .4939$), the median OS was markedly longer in the panitumumab arm than in the bevacizumab arm (38.3 vs. 22.9 months; HR, 0.55; $P = .1871$). In right-sided *RAS* MT mCRC, no difference was found in PFS (7.8 vs. 8.7 months; HR, 1.20; $P = .7158$), but the median OS favored panitumumab treatment (19.8 vs. 14.1 months; HR, 0.37; $P = .0765$).

No differences in OS or PFS were observed between treatment arms for left-sided *RAS* MT mCRC patients in the 20050181 study. In patients with right-sided tumors, the panitumumab arm had better OS (14.1 vs. 10.3 months; HR, 0.57; $P = .0027$), although no difference was found in PFS (5.6 vs. 5.3 months; HR, 0.77; $P = .1500$). The median OS appeared to be better in the panitumumab arm in *RAS* MT right-sided mCRC (14.1 months) than left-sided mCRC (11.3 months).

In the 20020408 study, no difference in PFS between treatments in either *RAS* MT tumor location subgroup was observed.

Discussion

To the best of our knowledge, the present study is the first to report the effect of primary tumor location on clinical outcomes during second- or later-line panitumumab treatment. Our results also provide valuable location data for the *RAS* MT cohorts from 4 randomized panitumumab mCRC trials, which have not been explored previously.

Our analyses found prognostic effects in both patients with *RAS* WT and patients with *RAS/BRAF* WT tumors, confirming the prognostic effect of tumor location in second and later treatment lines that was previously reported for the first-line setting.[5,7,14] As was seen in the retrospective analysis of data from the first-line panitumumab studies,[15] *RAS* WT patients with right-sided primary tumors had worse prognosis than those with left-sided tumors in later lines of mCRC treatment. To the best of our knowledge, the present study is the first to demonstrate a prognostic effect beyond first-line treatment in *RAS* WT patients. The observed prognostic effect of tumor location in the second- and later-line *RAS/BRAF* WT population has confirmed that the worse prognosis of right-sided primary tumors does not only result from the presence of *BRAF* mutations, as has been reported previously.[16]

To date, most studies assessing the predictive effect of tumor location on the efficacy of anti-EGFR therapy have focused on cetuximab

Table 4 Overall Survival, Progression-Free Survival, Response Rates, and Duration of Response in *RAS* Mutant Population

Study	Treatment	Patients		OS, mo; Median (95% CI)		PFS, mo; Median (95% CI)		RR, %; Median (95% CI)		DoR, mo; Median (95% CI)	
		Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
PRIME	Pmab + FOLFOX	166/164[a]	64/60[a]	15.8 (13.5-18.4)	15.1 (11.3-19.4)	7.5 (7.1-9.0)	7.4 (6.3-9.0)	44.5	43.3	7.4 (5.7-8.9)	7.4 (5.6-9.2)
	FOLFOX	158/150[a]	70/69[a]	19.7 (16.7-22.4)	16.8 (13.2-24.0)	9.4 (7.7-10.8)	8.5 (5.7-10.4)	44.7	47.8	7.7 (5.6-9.5)	7.7 (5.5-10.9)
	aHR[b]			1.14 (0.90-1.45)	1.36 (0.94-1.98)	1.29 (1.03-1.63)	1.37 (0.96-1.96)	0.99[a] (0.62-1.59)	0.83[a] (0.39-1.77)	—	—
	P value			.2701	.1052	.0288	.0874	—	—	—	—
PEAK	Pmab + FOLFOX	14/14[a]	11/11[a]	38.3 (15.1-53.6)	19.8 (11.8-33.8)	10.2 (5.3-16.6)	7.8 (4.1-10.7)	85.7	45.5	8.5 (3.7-15.1)	5.8 (3.7-7.6)
	Bmab + FOLFOX	19/19[a]	10/10[a]	22.9 (12.6-30.0)	14.1 (3.0-19.4)	12.0 (7.7-14.9)	8.7 (1.7-11.2)	47.4	50.0	6.9 (3.7-24.2)	4.0 (3.8-12.2)
	aHR[c]			0.55 (0.23-1.34)	0.37 (0.12-1.11)	1.29 (0.62-2.70)	1.20 (0.45-3.18)	6.67[d] (0.98-73.07)	0.83[d] (0.11-6.29)	—	—
	P value			.1871	.0765	.4939	.7158	—	—	—	—
20050181	Pmab + FOLFIRI	183/181[a]	76/73[a]	11.3 (9.3-12.5)	14.1 (10.1-16.4)	5.2 (3.8-5.6)	5.6 (3.9-7.9)	14.4	19.2	6.8 (4.2-7.9)	5.6 (3.9-6.5)
	FOLFIRI	195/190[a]	65/60[a]	11.9 (10.4-13.0)	10.3 (7.9-12.5)	5.3 (3.7-5.6)	5.3 (3.4-6.6)	13.2	13.3	5.6 (3.9-8.1)	4.0 (2.7-7.4)
	aHR[e]			1.09 (0.88-1.35)	0.57 (0.40-0.83)	0.96 (0.78-1.18)	0.77 (0.54-1.10)	1.11[d] (0.59-2.09)	1.54[d] (0.55-4.59)	—	—
	P value			.4221	.0027	.6970	.1500	—	—	—	—
20020408	Pmab + BSC	61/61[a]	16/16[a]	5.2 (4.0-6.8)	4.7 (2.1-6.1)	1.7 (1.6-1.8)	1.7 (1.5-1.9)	1.6	0	3.7 (NE)	NA
	BSC	77/77[a]	21/21[a]	5.2 (4.3-7.0)	3.3 (1.3-4.4)	1.8 (1.6-1.8)	1.3 (0.7-1.9)	0	0	NA	NA
	aHR[b]			1.01 (0.70-1.44)	0.63 (0.29-1.37)	1.02 (0.72-1.46)	0.50 (0.23-1.10)	Inf[d] (0.07-Inf)	NE[d]	—	—
	P value			.9739	.2414	.9059	.0862	—	—	—	—

Abbreviations: aHR = adjusted hazard ratio; Bmab = bevacizumab; BSC = best supportive care; DoR = duration of response; ECOG = Eastern Cooperative Oncology Group; Inf = infinity; mCRC = metastatic colorectal cancer; NA = not available; NE = not evaluable; OS = overall survival; PFS = progression-free survival; Pmab = panitumumab; RR = response rate.

[a]Number of patients evaluable for response.

[b]Adjusted treatment HR calculated from model with factors for region and baseline ECOG; HR < 1 favors the Pmab arm (PRIME, 20020408).

[c]Adjusted treatment HR calculated from model with factors for previous adjuvant oxaliplatin therapy; HR < 1 favors the Pmab arm (PEAK).

[d]Odds ratio for treatment difference in RR presented; odds ratio > 1 favors the Pmab arm (PRIME, PEAK, 20050181, 20020408).

[e]Adjusted treatment HR calculated from model with factors for region, baseline ECOG, and previous oxaliplatin exposure for mCRC; HR < 1 favors the Pmab arm (20050181).

data and have yielded results similar to those from the present analyses. In the present report, we found that patients with *RAS* WT left-sided primary tumors benefitted from the addition of panitumumab to chemotherapy or BSC. In the second-line 20050181 study, despite numeric PFS and RR benefits in right-sided *RAS* WT mCRC with the addition of panitumumab, the OS HR appeared to favor FOLFIRI alone (P = NS). Patients with right-sided mCRC undergoing second-line treatment had very low RRs, especially in the FOLFIRI arm. In the 20020408 trial, the addition of panitumumab to BSC resulted in better PFS for patients with left-sided *RAS* WT mCRC, which was also reflected by an improved RR, and once again, the very poor prognosis of right-sided mCRC was confirmed.

Few data have been reported on the effect of primary tumor location in *RAS* MT mCRC. In our analyses, the prognostic effect of tumor location in patients with *RAS* mutations was not clear. Regarding the predictive effect, we found better outcomes favoring the FOLFOX arm in patients with left- and right-sided mCRC in the first-line PRIME trial. These results were not surprising, because they were in line with the study's primary analysis. In the PEAK study, the results should be considered with caution owing to the low number of patients with *RAS* MT tumors (recruitment was limited to patients with *KRAS* exon 2 WT tumors in that study). In patients with left-sided *RAS* MT primary tumors, the median OS in the panitumumab arm was > 50% longer than that seen for bevacizumab; similar results were seen for patients with right-sided primary tumors. These results were unexpected because, although *RAS* MT tumors are known to be resistant to anti-EGFR therapy, this small subgroup of patients did not appear to clearly benefit more from the addition of bevacizumab. These results are consistent with those reported from the first-line CALGB/SWOG (Cancer and Leukemia Group B/Southwestern Oncology Group) 80405 trial[7] and FIRE-3 (FOLFIRI plus cetuximab vs. FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer) trials,[17] in which OS was not significantly different statistically between cetuximab and bevacizumab. In the 20050181 study, the OS for patients with right-sided primary tumors appeared better for the panitumumab arm than for the FOLFIRI arm in *RAS* MT patients. This could be a chance finding, but an alternative hypothesis is whether first-line treatment might induce clonal selection, making some patients more sensitive to anti-EGFR treatment. Validation of these findings in other cohorts is necessary to draw definitive conclusions regarding the optimum treatment of patients with *RAS* MT tumors.

The present study was limited by its retrospective nature and the relatively small number of patients with right-sided primary tumors. Therefore, definitive conclusions could not be drawn regarding the optimum treatment of right-sided mCRC. It would also be useful to assess the effect of biomarkers other than *RAS* and *BRAF*, because these could also affect clinical outcomes. These analyses were, nonetheless, strengthened by the high tumor location and *RAS*/*BRAF* ascertainment rates. The assessors of tumor location were also kept unaware of the *RAS*/*BRAF* mutation status, treatment allocation, and clinical outcomes.

Conclusion

Panitumumab plus chemotherapy or BSC provided better clinical outcomes compared with chemotherapy or BSC alone in *RAS* WT patients with left-sided primary tumors receiving second- or later-line treatment. Because of the relatively small number of patients with right-sided tumors, it was not possible to draw definitive conclusions on the optimal treatment. In view of these and other recently reported findings, tumor location should be considered during treatment decision-making. Further research is needed regarding the optimal treatment of patients with right-sided primary tumors and those with *RAS* MT mCRC.

Clinical Practice Points

- During the past decade, several studies have investigated the clinical effect of primary tumor location in CRC, and it has been reported that patients with right-sided disease have a worse prognosis than patients with left-sided disease.
- Recently, researchers also evaluated the predictive value of tumor location in the treatment of CRC, with most of these studies focusing on data from first-line cetuximab trials.
- In addition, another study from our research group has addressed the effect of primary tumor location on panitumumab treatment in 2 first-line studies.
- We have reported tumor location data from 2 studies of panitumumab after the first treatment line; to the best of our knowledge, ours is the first study to investigate the effect of tumor location in second- and later-line panitumumab studies.
- The results of these analyses have confirmed the negative prognostic effect of right-sided disease in *RAS* WT patients undergoing second- and later-line treatment.
- In addition, we found that patients with *RAS* WT left-sided disease benefit from the addition of panitumumab to chemotherapy or BSC compared with chemotherapy or BSC alone.
- These results are in line with those recently reported from first-line cetuximab and panitumumab studies, showing that patients with left-sided disease benefit from the addition of cetuximab or panitumumab, respectively.
- Our data on right-sided and *RAS* MT disease are inconclusive and require further investigation.
- Nevertheless, it is clear that tumor location is clinically important and should be considered during treatment decision-making.

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Disclosure

R.K. is an employee of Amgen Ltd. C.R. has received research funding (institutional) from Novartis and Sanofi, has acted as a consultant for Mylan and Oncompass, and has undertaken speaking engagements for Boehringer Ingelheim, MSD, and Novartis. S.S. is a member of advisory boards for Amgen, Bayer, Celgene, Eli Lilly, Merck, Merrimack, Novartis, Roche, and Sanofi. J.T. has had

advisory roles for Amgen, Bayer, Boehringer Ingelheim, Celgene, Chugai, Lilly, MSD, Merck Serono, Novartis, Pfizer, Roche, Sanofi, Symphogen, Taiho, and Takeda. J.Y.D. has participated in steering committees on behalf of Amgen and Bayer, participated in advisory boards and symposia, acted as a consultant for Amgen, Merck Serono, Roche, Sirtex and Takeda, participated in advisory boards for Boehringer Ingelheim, and Sanofi, and received research funding from Merck Serono. T.A. has acted as a consultant for Amgen, Bristol-Myers Squibb, and Roche and has had advisory roles for Bayer, Boehringer Ingelheim, Celgene, Eli Lilly, Novartis, Roche, Sanofi Aventis, and Xbiotech. M.P. has received research funding and acted in consultancy/advisory roles for Amgen, received research funding from Roche and Sirtex, and received research funding and participated in symposia for Merck Serono and Servier. The remaining authors declare that they have no competing interests.

Supplemental Data

The supplemental data accompanying this article can be found in the online version at https://doi.org/10.1016/j.clcc.2018.03.005.

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Supplemental Table 1 General Patient Distribution According to Tumor Location and *RAS* Mutation Status in Different Studies

Variable	First Analysis (*RAS* WT), n (%)						Second Analysis (*RAS* MT), n (%)					
	20050181			20020408			PRIME			PEAK		
	Left	Right	Total	Left	Right	Total	Left	Right	Total	Left	Right	Total
Total population	676 (76.2)	211 (23.8)	887 (100)	223 (76.9)	67 (23.1)	290 (100)	652 (74.6)	222 (25.4)	874 (100)	140 (71.1)	57 (29.4)	197 (100)
RAS WT	298 (81.0)	70 (19.0)	368 (100)	85 (73.9)	30 (26.1)	115 (100)	328 (78.8)	88 (21.2)	416 (100)	107 (74.8)	36 (25.2)	143 (100)
RAS MT	378 (72.8)	141 (27.2)	519 (100)	138 (78.9)	37 (21.1)	175 (100)	324 (70.7)	134 (29.3)	458 (100)	33 (61.1)	21 (38.9)	54 (100)

Abbreviations: MT = mutant; WT = wild-type.

Supplemental Table 2 Overall Survival and Associated Adjusted Hazard Ratios for Patients With Right- Versus Left-sided Tumors (*RAS* Wild-type Population)

Variable	20050181	20020408
Panitumumab arm	Panitumumab + FOLFIRI	Panitumumab + BSC
Median OS (95% CI), mo		
Right-sided	10.3 (5.2-13.7)	3.1 (2.0-12.0)
Left-sided	20.1 (16.5-21.7)	9.4 (7.3-11.7)
aHR[a] (95% CI)	2.01 (1.29-3.13)	1.89 (0.95-3.76)
Comparator arm	FOLFIRI	BSC
Median OS (95% CI), mo		
Right-sided	8.1 (6.3-12.1)	4.6 (0.9-6.0)
Left-sided	16.6 (14.8-21.2)	8.8 (6.4-10.4)
aHR[b] (95% CI)	1.51 (0.96-2.37)	2.41 (1.21-4.81)

Abbreviations: BSC = best supportive care; CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; aHR = adjusted hazard ratio; OS = overall survival.
[a]Adjusted treatment HR calculated from a model with factors for *BRAF* status, previous adjuvant therapy, and baseline ECOG (20050181); OS HR > 1 indicates worse prognosis for right-sided tumors.
[b]Adjusted treatment HR calculated from a model with factors for *BRAF* status and baseline ECOG (20020408); OS HR > 1 indicates worse prognosis for right-sided tumors.

Supplemental Table 3 Overall Survival and Progression-free Survival in the *RAS* Wild-type/*BRAF* Wild-type Population

Study	Treatment	Patients, n		OS, mo; Median (95% CI)		PFS, mo; Median (95% CI)	
		Left	Right	Left	Right	Left	Right
20050181	Pmab + FOLFIRI	143	22	19.7 (16.2-21.5)	11.9 (6.4-16.0)	8.0 (7.3-9.1)	6.7 (3.7-10.3)
	FOLFIRI	144	26	17.9 (14.9-23.4)	10.9 (6.7-13.0)	5.8 (5.2-7.3)	3.7 (2.0-5.9)
	aHR[a]	—	—	0.95 (0.70-1.29)	0.84 (0.43-1.62)	0.82 (0.63-1.06)	0.61 (0.31-1.19)
	P value	—	—	.7421	.5937	.1272	.1481
20020408	Pmab + BSC	39	12	9.4 (8.1-12.3)	6.1 (2.0-12.2)	5.5 (2.8-5.7)	1.7 (1.0-3.7)
	BSC	40	10	8.8 (6.4-10.8)	5.2 (0.7-6.0)	1.6 (1.3-1.8)	1.6 (0.5-1.8)
	aHR[b]	—	—	0.87 (0.54-1.40)	0.66 (0.25-1.77)	0.29 (0.18-0.48)	0.54 (0.21-1.39)
	P value	—	—	.5579	.4097	<.0001	.1980

Abbreviations: BSC = best supportive care; ECOG = Eastern Cooperative Oncology Group; aHR = adjusted hazard ratio; mCRC = metastatic colorectal cancer; OS = overall survival; PFS = progression-free survival; Pmab = panitumumab.
[a]Adjusted treatment HR calculated from a model with factors for region, baseline ECOG, and previous oxaliplatin exposure for mCRC (20050181).
[b]Adjusted treatment HR calculated from a model with factors for region and baseline ECOG (20020408).

Supplemental Table 4 Overall Survival and Associated Hazard Ratios for Patients With Right- Versus Left-sided Tumors (*RAS* Mutant Population)

Variable	PRIME	PEAK	20050181	20020408
Panitumumab arm	Panitumumab + FOLFOX	Panitumumab + FOLFOX	Panitumumab + FOLFIRI	Panitumumab + BSC
Median OS, mo				
Right sided	15.1 (11.3-19.4)	38.3 (15.1-53.6)	14.1 (10.1-16.4)	4.7 (2.1-6.1)
Left sided	15.8 (13.5-18.4)	19.8 (11.8-33.8)	11.3 (9.3-12.5)	5.2 (4.0-6.8)
aHR[a,b,c]	1.17 (0.85-1.61)	2.24 (0.87-5.78)	0.84 (0.63-1.11)	1.26 (0.67-2.36)
Comparator arm	FOLFOX	Bevacizumab + FOLFOX	FOLFIRI	BSC
Median OS, mo				
Right sided	16.8 (13.2-24.0)	14.1 (3.0-19.4)	10.3 (7.9-12.5)	3.3 (1.3-4.4)
Left sided	19.7 (16.7-22.4)	22.9 (12.6-30.0)	11.9 (10.4-13.0)	5.2 (4.3-7.0)
aHR[a,b,c]	1.09 (0.81-1.48)	2.8 (1.05-7.43)	1.46 (1.09-1.96)	1.60 (0.95-2.68)

Data in parentheses are 95% confidence interval.
Abbreviations: BSC = best supportive care; ECOG = Eastern Cooperative Oncology Group; aHR = adjusted hazard ratio; mCRC = metastatic colorectal cancer; OS = overall survival.
[a]Adjusted treatment HR calculated from a model with factors for region and baseline ECOG (PRIME, 20020408); OS HR > 1 indicates worse prognosis for right-sided tumors.
[b]Adjusted treatment HR calculated from a model with factors for previous adjuvant oxaliplatin therapy (PEAK); OS HR > 1 indicates worse prognosis for right-sided tumors.
[c]Adjusted treatment HR calculated from a model with factors for region, baseline ECOG, and previous oxaliplatin exposure for mCRC (20050181); OS HR > 1 indicates worse prognosis for right-sided tumors.

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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Left-sided primary tumors are associated with favorable prognosis in patients with *KRAS* codon 12/13 wild-type metastatic colorectal cancer treated with cetuximab plus chemotherapy: an analysis of the AIO KRK-0104 trial

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Abstract
Purpose AIO KRK-0104 investigated first-line therapy of metastatic colorectal cancer (mCRC) with cetuximab, capecitabine and irinotecan versus cetuximab, capecitabine and oxaliplatin. This analysis investigated the impact of primary tumor location on outcome of patients.
Patients and methods Left-sided primary tumors were defined as tumors from rectum to left flexure, while tumors in the remaining colon were regarded right sided. Overall survival (OS), progression-free survival (PFS) and response rate were correlated with primary tumor location. A Cox regression model was used to evaluate interaction between primary tumor location and *KRAS* mutation.
Results Of 146 patients of the AIO KRK-0104 trial, 100 patients presented left-sided (of those 68 *KRAS* codon 12/13 wild-type) and 46 patients right-sided primary tumors (of those 27 *KRAS* codon 12/13 wild-type). Left-sided tumors were associated with significantly longer OS ($p = 0.016$, HR = 0.63) and PFS ($p = 0.02$, HR = 0.67) as compared to right-sided tumors. These effects were present in the *KRAS* codon 12/13 wild-type population (HR OS: 0.42; HR PFS: 0.54), while no impact of primary tumor location was evident in patients with *KRAS* codon 12/13 mutant tumors (HR OS: 1.3; HR PFS: 1.01). A significant interaction of *KRAS* status and primary tumor location concerning OS and PFS was observed.
Conclusion Our findings suggest that primary tumor location and *KRAS* codon 12/13 mutational status interact on the outcome of patients with mCRC receiving cetuximab-based first-line therapy. Left-sided primary tumor location might be a predictor of cetuximab efficacy.

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Keywords Colorectal cancer · Primary tumor location · CAPIRI plus cetuximab · CAPOX plus cetuximab · *KRAS* mutation status

Introduction

The idea of personalized medicine was introduced to the treatment of metastatic colorectal cancer (mCRC) when *KRAS* codon 12/13 mutations were identified as negative predictors of anti-EGFR-antibody (EGFR-mAB) treatment. Consequently, only patients with *KRAS* codon 12/13 wild-type tumors were subjected to cetuximab or panitumumab treatment (Douillard et al. 2013; Huang et al. 2012; Modest et al. 2012; Douillard et al. 2010; Bokemeyer et al. 2011; Amado et al. 2008). This *KRAS* codon 12/13 wild-type population already excluded about 40 % of all patients and was associated with improved response rates (objective response rates, ORRs), progression-free survival (PFS) and overall survival (OS) in patients receiving EGFR-mABs. However, ORR in clinical trials investigating EGFR-based first-line regimens was usually <60 %, indicating that *KRAS* codon 12/13 wild-type alone was not a sufficient condition to predict response (Douillard et al. 2013; Modest et al. 2012; Van Cutsem et al. 2011; De Roock et al. 2010; Stintzing et al. 2009). The identification of additional negative predictors such as *KRAS* exon 3/4 and *NRAS* exon 2–4 mutations created a new target population for EGFR-mABs: patients with RAS wild-type tumors. This population comprises about 50 % of all patients with mCRC with a benefit in median OS following EGFR-targeted first-line therapy of 5–7 months (Douillard et al. 2013; Stintzing et al. 2009).

Taking into account that even RAS wild-type tumors potentially do not define the perfect marker for response to EGFR-mABs, additional biomarkers are needed. This question was recently addressed by retrospective evaluations of patients receiving cetuximab treatment in further treatment lines. The efficacy of cetuximab was determined to be modulated by the location of the primary tumor (Missiaglia et al. 2013; Brule et al. 2013). Due to this initial evidence, the question was raised whether the location of the primary tumor in colorectal cancer can serve as a prognostic marker and potentially as a predictive marker for treatment with EGFR-mABs. To our knowledge, the effect of primary tumor location on outcome has not been shown in a mCRC study population receiving first-line treatment with cetuximab.

The AIO KRK-0104 trial randomized patients to CAPIRI plus cetuximab or CAPOX plus cetuximab. With reference to this design, we hypothesized that primary tumor location of the left colon might have a favorable prognostic effect in patients with *KRAS* wild-type tumors, but not in patients with *KRAS* mutant tumors.

Methods

Study design

Data for this analysis were obtained from the AIO KRK-0104 trial. This study was a randomized, multicenter phase II trial to investigate the efficacy of cetuximab plus CAPIRI versus cetuximab plus CAPOX as first-line chemotherapy in patients with mCRC and recruited patients from 2004 to 2006. The primary analysis and the molecular subgroups analysis have been published elsewhere (Modest et al. 2012; Moosmann et al. 2011). Primary endpoint of the AIO KRK-0104 study was ORR. This investigation refers to the population of 146 patients with central assessment of *KRAS/BRAF* mutations as published before (Modest et al. 2012).

Definition of right-sided versus left-sided tumors

The primary tumor location was defined in the study reports and was extracted from the central database. Tumors located in rectum, sigma, descending colon and the left flexure were defined as left-sided tumors. All tumors from cecum to the distal part of the transverse colon were categorized as right-sided tumors.

Treatment schedule

In both arms, cetuximab was given at an initial dose of 400 mg/m^2 as a 120-min infusion, followed by weekly infusions of 250 mg/m^2 over 60 min. Patients in arm A received chemotherapy with CAPIRI (i.e., oral capecitabine 800 mg/m^2 twice daily on days 1 through 14, followed by a 1-week rest period plus irinotecan 200 mg/m^2 as a 30-min intravenous infusion on day 1). In patients older than 65 years, doses were further reduced by 20 %. Patients in arm B received chemotherapy with CAPOX (i.e., capecitabine 1,000 mg/m^2 twice daily on days 1 through 14, followed by a 1-week rest period plus oxaliplatin 130 mg/m^2 as a 120-min intravenous infusion on day 1). Treatment cycles were repeated every 3 weeks until disease progression or unacceptable toxicity (Moosmann et al. 2011).

Patients

The patient population of the AIO KRK-0104 trial was described in recent reports (Modest et al. 2012; Moosmann et al. 2011). Patients with *BRAF* mutant tumors were analyzed as *KRAS* wild-type tumors. One patient presenting a tumor with *BRAF* and *KRAS* mutation was regarded as *KRAS* mutant in this analysis. In two patients, two primary tumors were located in the left-sided colon (sigma and rectum; descendent colon and sigma); these cases were

analyzed as left-sided colorectal cancer. In another patient, one primary tumor was located in the right part of the colon (cecum), while another primary tumor was observed at the left side (rectum); this case was classified as right-sided colorectal cancer.

Endpoints

The present investigation was performed as an exploratory analysis using response rates (ORR = complete and partial remission), PFS and OS as parameters for outcome in patients with tumors of right-sided and left-sided origin. Tumor assessment was performed every two cycles (6 weeks). A final update on overall survival was conducted in 2011, and the statistical analysis plan was published in detail (Modest et al. 2012; Moosmann et al. 2011).

Statistical analysis

In this retrospective, exploratory investigation, OS and PFS were stratified by primary tumor location and were estimated using the Kaplan–Meier method. Possible differences were evaluated by log-rank test and Cox regression analysis. A Cox regression model was used to evaluate interaction between primary tumor location and *KRAS* mutation as explanatory variables. χ^2 tests compared response rates. A p value <0.05 was regarded significant. For interaction test, a p value <0.10 was regarded significant. SPSS PASW 21.0 (SPSS Inc., Chicago, Illinois) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA) were used for statistical analysis.

KRAS mutation detection

KRAS/BRAF testing was performed in a German reference laboratory for *KRAS* analysis (University of Munich, Department of Pathology) as described before (Modest et al. 2012; Moosmann et al. 2011).

Results

Study population and tumor characteristics

In all 146 patients of the pathological analysis-set, the primary tumor location was assessable. Out of the full population, 100 patients presented with left-sided tumors, whereas 46 patients presented primary right-sided tumors. In detail, tumors were located in rectum ($n = 49$), sigma ($n = 40$), descending colon ($n = 7$), left flexure ($n = 2$), transverse colon ($n = 11$), ascending colon ($n = 18$), cecum ($n = 16$) and double primary location ($n = 3$). Out of the 100 patients presenting left-sided colorectal tumors, 68 tumors presented *KRAS* codon 12/13 wild-type tumors, and 32 tumors had *KRAS* codon 12/13 mutations. Out of 46 tumors of right-sided origin, 27 tumors were diagnosed with *KRAS* codon 12/13 wild-type status, while 19 patients presented a *KRAS* codon 12/13 mutant tumor. Distribution of patients with left-sided versus right-sided tumors to the treatment arms of the AIO KRK-0104 trial (CAPIRI plus cetuximab/CAPOX plus cetuximab) was comparable (52/48 % vs. 50/50 %; Table 1).

Baseline patient characteristics

Baseline patient characteristics are shown in Table 1. No major imbalances associated with primary tumor location in the left or right part of the colon were present in our cohort. However, a trend toward more female patients was observed in the group of right-sided tumors when compared to the group of patients with left-sided primary tumors (39 % vs. 23 %, $p = 0.05$; Table 1).

Effect of primary tumor location on overall survival (OS)

The whole study population reached a median OS of 21.1 months. Survival times by exact tumor locations are shown in Fig. 1a. If analyzed as right vs. left colon, median OS of patients with right-sided tumor was 14.8 months, while median OS in patients with left-sided tumor was 26.3 months ($p = 0.016$, HR = 0.63), (Fig. 1b). In patients with *KRAS* codon 12/13 wild-type tumors, median OS was 13.0 months in patients with right-sided versus 29.0 months in patients with left-sided mCRC ($p < 0.001$, HR: 0.42). The effect of primary tumor location on OS in patients with *BRAF* V600E mutant tumors seemed consistent with the observation in the *KRAS* codon 12/13 wild-type cohort. In patients with *KRAS* codon 12/13 mutant tumors, no significant difference was present when OS of patients with right-sided and left-sided mCRC was compared (Figs. 1c–d and 3a).

Effect of primary tumor location on progression-free survival (PFS)

Median PFS in all patients was 7.0 months. PFS by exact tumor location is shown in Fig. 2a. In patients with right-sided tumors, median PFS was 5.2 months, and in patients with left-sided-tumors, it was 7.8 months ($p = 0.02$, HR = 0.67). If *KRAS* status was taken into account, in accordance to OS, a significant difference in PFS associated with primary tumor location was only evident in patients with *KRAS* codon 12/13 wild-type tumors (4.6 vs. 8.4 months, $p = 0.007$, HR = 0.54), but not in patients presenting a mutation in these loci. In patients with *BRAF* mutant mCRC, the effect of primary tumor location seemed

Table 1 Baseline characteristics of patients and tumors

	Patients with left-sided mCRC		Patients with right-sided mCRC		*p* value
	n	%	*n*	%	
Patients	100	68	46	32	
Age					
Median	63		61		0.50
Range	32–77		47–74		
Sex					
Female	23	23	18	39	0.05
Male	77	77	28	61	
Performance status (Karnofsky)					
100 + 90	73	73	32	70	0.55
80 + 70	25	25	14	30	
Not reported	2	2	0	0	
Prior therapy					
Chemotherapy	21	21	5	11	0.17
Radiotherapy	12	12	1	2	0.06
Disease sites					
Liver	84	84	42	91	0.31
Lung	32	32	19	41	0.35
Lymph node	30	30	20	43	0.13
Peritoneum	12	12	3	7	0.39
Treatment arm					
CAPIRI plus cetuximab	52	52	23	50	0.86
CAPOX plus cetuximab	48	48	23	50	
Tumor mutation status					
KRAS codon 12/13 wild-type	68	68	27	59	0.35
KRAS codon 12/13 mutant	32	32	19	41	
BRAF V600E mutant	6	6	11	24	0.004

p values: Chi-square test/Fisher's exact test except for age: Mann–Whitney *U* test

again consistent with the effects observed in patients with *KRAS* codon 12/13 wild-type tumors (Figs. 2b–d and 3b).

Interaction of *KRAS* codon 12/13 mutation and primary tumor location

Cox regression models with hazard ratios for primary tumor location, *KRAS* mutation and interaction respectively as explanatory variables were analyzed. For OS, the hazard ratio of primary tumor location was 0.160 in favor of left-sided ($p = 0.002$, 95 % CI 0.050–0.511), 0.640 in favor of wild-type ($p = 0.159$, 95 % CI 0.344–1.191) for *KRAS* mutation, and 0.372 ($p = 0.013$, 95 % CI 0.171–0.810) for interaction between primary tumor location and *KRAS* mutation. For PFS, the hazard ratio of primary tumor location was 0.252 in favor of left-sided tumors ($p = 0.013$, 95 % CI 0.085–0.745), 0.780 in favor of wild-type ($p = 0.411$, 95 % CI 0.432–1.140) for *KRAS* mutation, and 0.487 ($p = 0.056$, 95 % CI 0.233–1.017) for interaction between primary tumor location and *KRAS* mutation.

Influence of *BRAF* V600E mutations in this study

In our cohort, BRAF mutation was more frequent in right-sided compared with left-sided primary tumors (24 vs. 6 %). After removing *BRAF* V600E mutant tumors from the *KRAS* codon 12/13 wild-type cohort, median PFS of patients with *KRAS/BRAF* wild-type tumors ($n = 79$) was 5.9 months in patients with right-sided versus 8.2 months with left-sided tumors ($p = 0.47$, HR = 0.81). Median OS was 16.2 months in patients with right-sided compared with 27.3 months in patients with left-sided tumors ($p = 0.11$, HR = 0.60; Fig. 3a, b).

Effect of primary tumor location on response rate

Response rates were analyzed based on non-missing data and did not show significant differences. ORR was 58 % in left-sided and 53 % in right-sided tumors ($p = 0.70$) in the whole study population. In *KRAS* codon 12/13 wild-type tumors, right-sided tumors were associated with a lower ORR when compared to left-sided tumors (43 % vs. 64 %), and this difference did not reach significance ($p = 0.12$). When comparing response rates in patients with *KRAS* codon 12/13 mutant mCRC, ORR was 65 % in right-sided and 45 % in left-sided tumors ($p = 0.23$; Table 2).

Discussion

Personalized treatment of mCRC patients is entering daily routine in clinical practice. The more tumor sub-classifications based on molecular markers are defined, the higher the chance is to identify positive and negative predictors and consequently to specify different strategies of therapy. Clinical data have proven that mutant RAS genes are negative predictive biomarkers and that patients with a *KRAS/NRAS* mutation do not benefit from an EGFR-mAB-based therapy (De Roock et al. 2010; Peeters et al. 2013a, b; Andre et al. 2013). Therefore, mutations of *KRAS* and *NRAS* represent an established negative predictor of EGFR-mAB efficacy. The role of *BRAF* in first-line treatment of mCRC is described as a negative prognostic marker, but not as a predictive marker in terms of EGFR-mAB therapy (Douillard et al. 2013). Recently, it has been suggested that, in addition to RAS mutations, the primary tumor location might play a crucial role for efficacy of EGFR-mABs (Douillard et al. 2013; Missiaglia et al. 2013).

For this reason, we hypothesized that in the AIO KRK-0104 trial primary tumor location in the left colon might

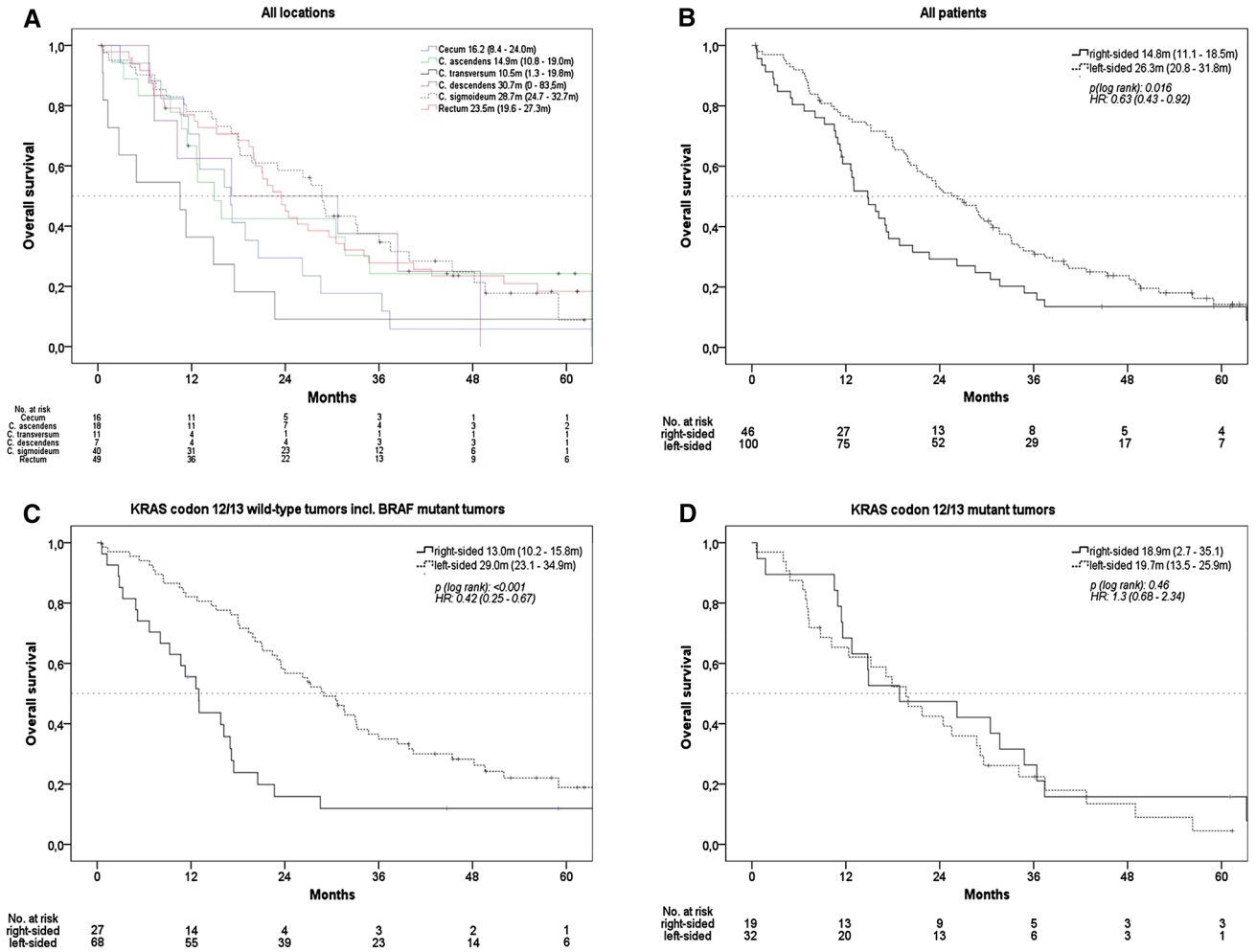

Fig. 1 Overall survival (OS), **a** according to exact primary tumor location, patients with double primary tumors and with primary tumor of left flexure were excluded due to sample size, **b** according to right-sided versus left-sided mCRC, **c** patients with KRAS codon 12/13 wild-type tumors, **d** patients with KRAS codon 12/13 mutant tumors

have a favorable prognostic effect in patients with *KRAS* wild-type tumors, but not in patients with *KRAS* mutant tumors when receiving cetuximab-based first-line therapy. In fact, OS and PFS differed significantly when comparing left- to right-sided tumors. This effect was driven by patients with *KRAS* codon 12/13 wild-type tumors and seemed also present in those patients that presented with *KRAS* codon 12/13 wild-type but *BRAF* V600E mutant tumors. By contrast, in patients with *KRAS* codon 12/13 mutant tumors, the primary tumor location was not associated with significant differences in terms of OS or PFS. This interaction of *KRAS* mutation and primary tumor location was found to be significant for both OS and PFS. No significant impact of primary tumor location on response rates was observed in this study. Taking the difference in patients with *KRAS* wild-type tumors into account (64 % vs. 43 % in patients with left-sided vs right-sided primary tumor), this could be interpreted as a consequence of missing sample size for this endpoint.

Our results are supported by a recent analysis of the NCIC CTG CO.17 trial (that investigated cetuximab plus best supportive care (BSC) versus BSC alone), which reported less striking cetuximab-induced effects in patients with *KRAS* codon 12/13 wild-type, right-sided tumors as compared with patients bearing a left-sided tumor (Brule et al. 2013). A similar observation was reported by Missiaglia and colleagues who observed a longer PFS in refractory patients that received cetuximab treatment if the primary tumor was left-sided as compared to right-sided tumors (Missiaglia et al. 2013).

As described above, we grouped patients in left-sided versus right-sided tumors, which included tumors from cecum to the distal part of the transverse colon. This distinction corresponds to the midgut versus hindgut definition

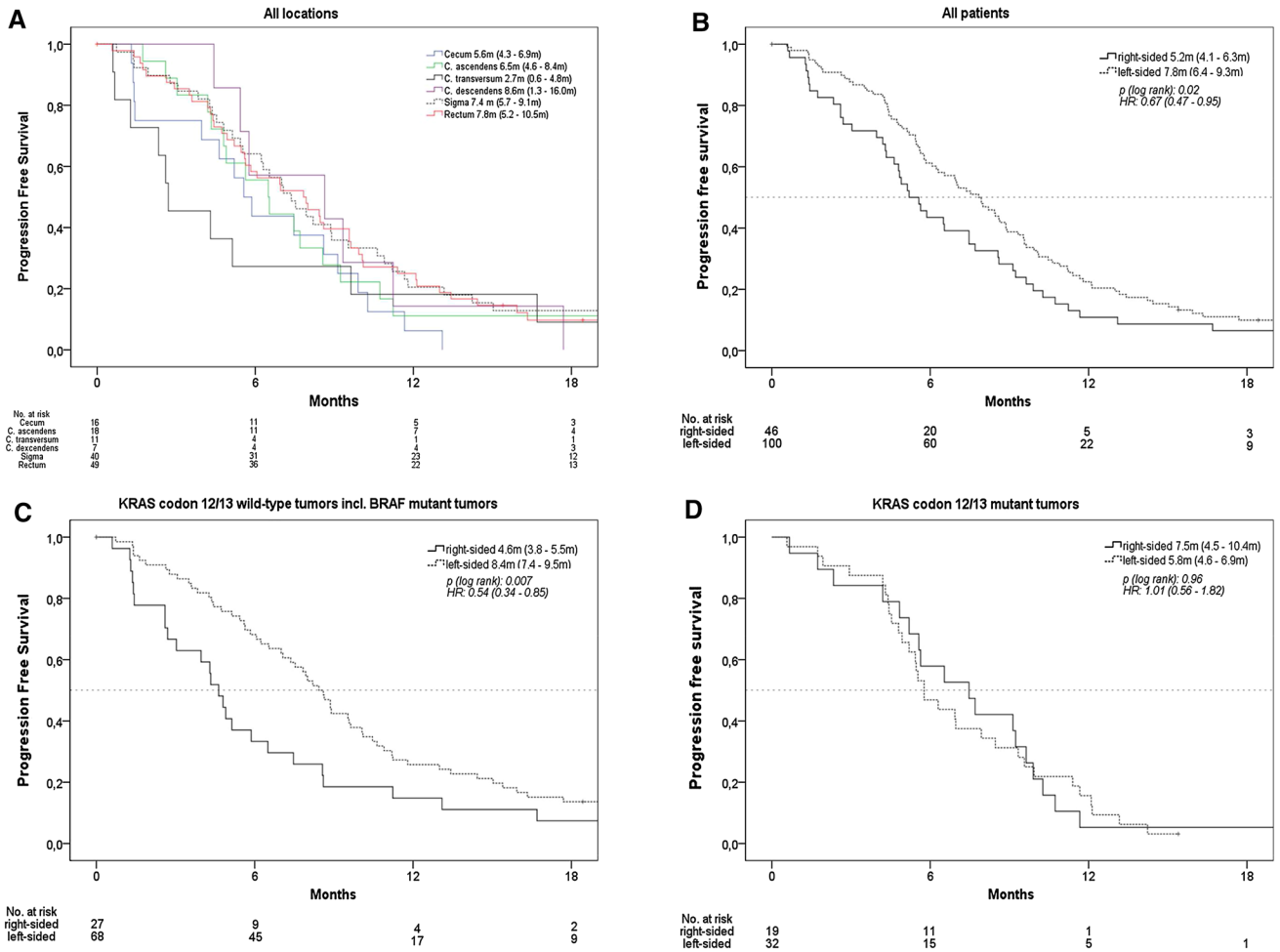

Fig. 2 Progression-free survival (PFS), **a** according to exact primary tumor location patients with double primary tumors and with primary tumor of left flexure were excluded due to sample size, **b** according to right-sided versus left-sided mCRC, **c** patients with KRAS codon 12/13 wild-type tumors, **d** patients with KRAS codon 12/13 mutant tumors

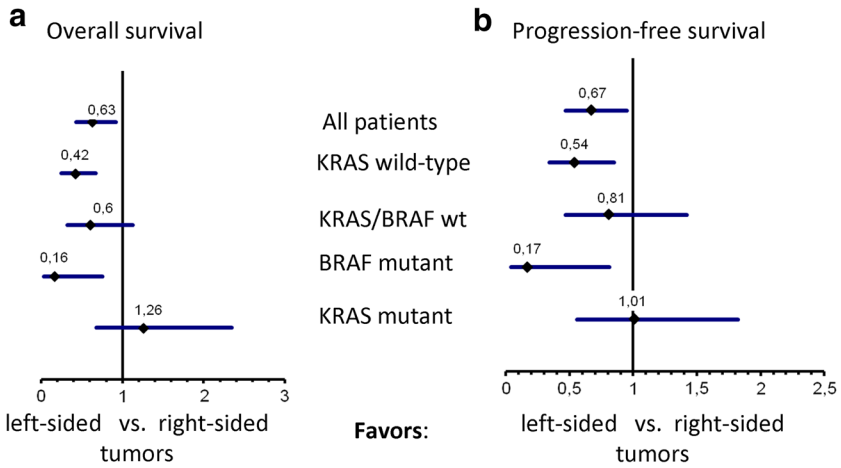

Fig. 3 Hazard ratios of molecular subgroups, error bars indicating the 95 % confidence interval. **a** Overall survival, **b** progression-free survival

and is modified counting the total colon transversum as right-sided colon. Nevertheless, the strict separation of different tumor locations is questioned by the "continuum hypothesis," which postulates that molecular features of the tumor gradually change along bowel subsides, rather than change abruptly at splenic flexure (Yamauchi et al. 2012a,

Table 2 Response to treatment

Parameter	L-mCRC	R-mCRC	L-mCRC KRAS wt	R-mCRC KRAS wt	L-mCRC KRAS mut	R-mCRC KRAS mut
No. of patients	100	46	68	27	32	19
ORR evaluable (no. of pts)	85	38	56	21	29	17
n.a. (no. of pts)	15	8	12	6	3	2
ORR % (95 % CI)	58 (47–68)	53 (37–68)	64 (51–77)	43 (24–65)	45 (27–65)	65 (40–86)
p value	0.70		0.12		0.23	

R-mCRC patients with right-sided mCRC, *L-mCRC* patients with left-sided mCRC, *wt* wild-type, *mut* mutant; *ORR* (CR + PR) overall response rate, *CI* confidence interval, *n.a* not assessable due to any reason. *p* value: Fisher's exact test. ORR calculation based on non-missing data (patients evaluable for response)

b). As shown in Figs. 1a and 2a, a trend toward specific OS and PFS could possibly be derived from the exact primary tumor location of the colon, but possibly not according to the physiological course of the colon. Clearly, our data concerning this issue are limited by sample size.

As samples from the AIO KRK-0104 trial were only tested for *KRAS* exon 2 codon 12/13 mutations, but not for *KRAS* exon 3, 4 or *NRAS* mutations, our data might contain a bias of approximately 10 % hidden mutations that we cannot identify due to lacking tumor material. In our cohort, the impact of left- versus right-sided tumors was specifically strong in *BRAF* mutant tumors. This finding might be explainable by sample size and MSI/MSS status that is unknown for the tumors of AIO KRK-0104 trial cohort. It might have been suspected that the whole side effect might be influenced by *BRAF*/MSI/MSS status, since *BRAF* mutations are known to be more frequent in right-sided colorectal cancer (Pai et al. 2012; Popovici et al. 2013). However, even after excluding *BRAF* mutant tumors from the *KRAS* codon 12/13 wild-type cohort, the strong prognostic effect of left-sided primary tumors seemed still present (hazard ratio for OS: 0.60). It might be concluded that in mCRC *BRAF* mutation interacts in the left- versus right-sided tumor story, but is not the only reason for the observed differences.

Our data are limited by several aspects. As discussed above, we distinguished between *KRAS* mutant and non-mutant only and did not take other RAS mutations into account. Furthermore, this study only consists of a rather small population that might lead to biases, especially in the *KRAS* mutant cohort. Furthermore, treatment differences between oxaliplatin- and irinotecan-treated patients could not be excluded.

In conclusion, our data correspond favorably with other publications investigating EGFR-mAB use and primary tumor location in mCRC. The interaction of primary tumor location and *KRAS* mutations suggests that primary tumor location might be an additional biomarker for EGFR-mABs. Corresponding pathological findings to explain this phenomenon are still lacking and could be more complex than RAS mutations (Missiaglia et al. 2013; Maus et al. 2013). Data from randomized phase III trials such as CRYSTAL, PRIME and FIRE 3 are necessary to draw definite conclusions concerning the restriction of EGFR-mAbs to patients with RAS wild-type left-sided mCRC.

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Conflict of interest D.P.M.: research grant, travel support and honoraria for lectures: Merck Serono, Roche, Amgen. Advisory boards: Amgen; V.H.: research grant, travel support, advisory boards and honoraria for lectures: Merck Serono, Roche, Amgen; S.S: research grant, travel support and honoraria for lectures: Merck Serono, Roche, Amgen. Advisory boards: Merck Serono, BMS; CG: travel support: Roche; M.M.: travel support: SIRTeX; P.A.: travel support: Pharma Mar; M.H.: travel support, honoraria for lectures: Celgene, Roche; T.D.: travel support and honoraria for lectures: Roche, Amgen and All other authors have declared no conflict of interest.

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The Oncologist®

Brief Communications

Another Chapter of the *Right Versus Left Story*: Is Primary Tumor Location a Prognostic Feature in *RAS* Mutant Metastatic Colorectal Cancer?

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Disclosures of potential conflicts of interest may be found at the end of this article.

Abstract

Background. The prognostic value of primary tumor location (PTL) in patients with metastatic colorectal cancer (mCRC) was reported by recent analyses in *RAS* wild-type patients. Here, we investigated the prognostic value of PTL in *RAS* mutated mCRC patients.

Materials and Methods. PTL was defined as left or right if distal or proximal to the splenic flexure. Primary endpoint was overall survival (OS) according to PTL. Subgroup analyses were conducted according to time to metastases and *RAS* mutation subtypes.

Results. Five hundred sixty-four patients were included. Left- and right-sided cases were 65% and 35%, respectively. No difference in OS was detected according to PTL (hazard ratio [HR] = 0.99, p = .964). No difference in OS was observed in right versus left when looking at synchronous (HR 0.92, p = .557) or metachronous (HR 1.07, p = .807) patients.

Conclusion. No OS difference was detected in *RAS* mutated mCRC. Molecular and clinical features able to improve prognosis and treatment strategies in this setting are needed. ***The Oncologist*** 2019;24:e77–e79

Introduction

In the last years, many data emerged leading to the identification of right- and left-sided colorectal cancer (CRC) as two distinct clinical, pathological, and molecular diseases [1].

Many post hoc analyses and recent metanalyses [2–5] deriving from randomized trials of molecularly selected patients showed a negative prognostic role and a negative predictive value to anti-epidermal growth factor receptor's response for right cancer (RC).

According to the most recent guidelines, primary tumor location (PTL) is a fundamental feature in the definition of patients' prognosis and therapeutic approaches. However, no specific data are available regarding the population of *RAS* mutated patients, as highlighted by a recent editorial by Ciombor et al. [6]. A major putative confounder in prognostic studies on PTL for metastatic CRC (mCRC) could theoretically be the later diagnosis occurring in RC, and a possible different impact on prognosis for different *RAS* mutation has been reported. On these bases we analyzed the prognostic impact of PTL in a population of *RAS* mutated mCRC patients, but also considering the time to metastases development and according to the specific *RAS* mutation subtype.

Materials and Methods

Clinical and molecular data of mCRC patients referred to Medical Oncology 1, Veneto Institute of Oncology from January 1, 2010, to December 31, 2016, were collected. Patients were evaluable for the present study if a *RAS* mutation (*KRAS* and *NRAS* exons 2, 3, and 4) was detected either on primary tumor and/or metastasis.

PTL was defined as "right" or "left" if located proximally or distally to the splenic flexure. Time to metastasis was defined as "synchronous" if metastases appeared

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Table 1. Baseline patient characteristics

Characteristic	Total = 564, *n* (%)	Left-side = 365, *n* (%)	Right-side = 199, *n* (%)	*p* value
Sex				
Female	123 (39)	143 (39)	80 (40)	.858
Male	341 (61)	222 (61)	119 (60)	
Age, years				
Median (range)	63 (22–90)	63 (22–85)	63 (24–90)	—
Age, years (70 years cutoff)				
>70	178 (32)	109 (30)	69 (35)	.258
≤70	386 (68)	256 (70)	130 (65)	
Baseline ECOG PS				
≤1	452 (93)	286 (93)	166 (93)	1.000
≥2	33 (7)	21 (7)	12 (7)	
NA	79	58	21	
Primary tumor resected				
Yes	462 (82)	296 (81)	166 (84)	.495
No	102 (18)	69 (19)	33 (16)	
Presentation of mets				
Synchronous	393 (70)	243 (67)	150 (75)	.028[a]
Metachronous	171 (30)	122 (33)	49 (25)	
Sites of mets at diagnosis				
Liver	405 (72)	254 (70)	151 (77)	.017[a]
Lung	144 (25)	106 (29)	38 (19)	
Distant nodes	100 (18)	55 (15)	45 (23)	
Peritoneum	94 (17)	52 (14)	42 (21)	
Other	44 (8)	27 (7)	17 (9)	
Metastatic sites, *n*				
1	383 (68)	258 (71)	125 (63)	.073
≥2	181 (32)	107 (29)	74 (37)	
KRAS mutation				
Cod 12	340 (60)[b]	216 (59)	124 (62)	.937
Cod 13	86 (15)	57 (16)	29 (15)	
Other codons	56 (10)	35 (10)	21 (11)	
Double mutation	3 (1)	2 (1)	1 (1)	
Data not available	44 (8)	27 (7)	17 (8)	
No	35 (6)	28 (7)	7 (3)	
NRAS mutation				
Yes	36 (6)[b]	28 (8)	8 (4)	.238
No	528 (94)	337 (92)	191 (96)	

[a]Statistically significant.
[b]One patient had KRAS codon 12 mutation plus NRAS codon 61 mutation.
Abbreviations: —, no data; ECOG PS, Eastern Cooperative Oncology Group Performance Score; NA, not applicable.

within 6 months from primary tumor diagnosis or as "metachronous" if metastases appeared after 6 months.

Median overall survival (OS) and 95% confidence interval (CI) were calculated using Kaplan-Meier method. Cox proportional hazards regression analyses were used to estimate the association between PTL and survival according to time to metastases. Categorical clinical features were compared by means of chi-square test.

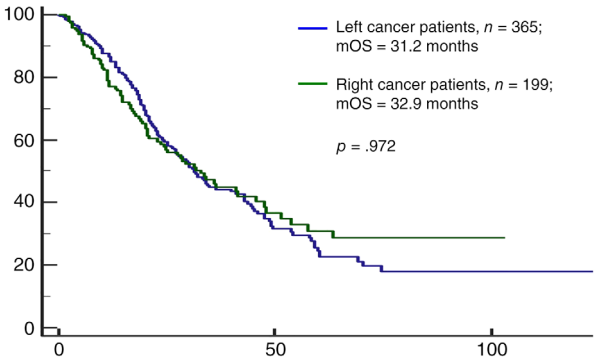

Figure 1. Median overall survival results according to primary tumor location.
Abbreviation: mOS, median overall survival.

Results

Clinical and molecular data were available from 1,319 patients referred to our institution in the prespecified time frame. The study population included 564 patients. Specific features were observed in LC (left cancer) compared with RC patients, as shown in Table 1.

Univariate analysis showed no difference in median OS (mOS) according to PTL (mOS was 31.2 months for RC vs .32.9 months for RC, hazard ratio [HR] = 1.00, 95% CI 0.77–1.29, p = .972; Fig. 1). In synchronous patients, mOS in LC and RC was 26.2 and 29.6 months, respectively (HR 0.92, p = .557). Among metachronous patients, mOS in LC and RC was 45.3 and 47.5 (HR 1.07, p = .807). No differences were observed when looking at specific *RAS* mutations subtypes (Table 2).

Discussion

For the first time, our analysis evaluated the prognostic value of PTL in patients with *RAS* mutated mCRC. This study showed that in *RAS* mutant patients, tumor location does not affect prognosis. The same results were observed when looking at synchronous and metachronous mCRC patients separately and looking at *RAS* subtypes.

Interestingly, Taieb et al. evaluated the prognostic role of PTL in stage III CRC patients, and no differences were identified in the subgroup of *RAS* mutant patients in terms of OS, but also in *RAS* and *BRAF* wild-type patients [7].

Our findings underline the need of accurate and wide large study populations and subgroup analyses in the evaluation of potential prognostic clinical and molecular features in order to avoid misleading conclusions. Due to the exploratory nature of our work, the present results might be further validated in modern clinical trials of mCRC patients receiving first-line chemotherapy with known RAS status and PTL.

From a practical point of view, PTL has been proposed as a stratification factor for future clinical trials and as a driver of therapeutic choices in mCRC; however, in the *RAS*

Table 2. Univariate analyses according to primary tumor side looking at time to metastases and specific RAS mutation

Time to metastases	Primary tumor side	*n* (%)	Median OS, months	Overall survival		
				HR	95% CI	*p* value
Overall	Left	365 (65)	31.2	1	—	—
	Right	199 (35)	32.9	1.00	0.77–1.29	.972
Synchronous	Left	243 (62)	26.2	1	—	—
	Right	150 (38)	29.6	0.92	0.68–1.23	.557
Metachronous	Left	122 (71)	45.3	1	—	—
	Right	49 (29)	47.5	1.07	0.63–1.82	.807
KRAS mut						
Cod 12	Left	216 (64)	31.9	1	—	—
	Right	124 (36)	31.6	1.07	0.77–1.48	.700
Cod 13	Left	57 (66)	22.7	1	—	—
	Right	29 (34)	45.5	0.68	0.32–1.43	.313
Other codons	Left	35 (63)	23.7	1	—	—
	Right	21 (37)	NR	0.76	0.32–1.80	.528
NRAS mut						
All codons	Left	28 (78)	45.3	1	—	—
	Right	8 (22)	47.9	0.84	0.27–2.65	.769

Abbreviations: —, no data; CI, confidence interval; HR, hazard ratio; NR, not reached; OS, overall survival.

mutant population, features other than PTL should be identified and considered in the future. Moreover, our results suggest the need for a constant and careful integration of new clinical prognostic markers with known molecular determinants and vice versa.

Conclusion

Extensive and modern clinical and molecular characterization is key to understanding both *RAS* wild-type and mutant CRC biology and clinical behavior and to identifying new prognostic determinants and targeted treatment strategies. In particular, we can indirectly hypothesize that still-unrevealed molecular drivers other than *RAS* and *BRAF* mutations might be responsible for the prognostic impact of PTL in *RAS* wild-type patients.

Acknowledgments

This study was approved by the Ethics Committee of Istituto Oncologico Veneto and was conducted according to ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. This study was funded by Regione Veneto – RP-2014-00000395.

Disclosures

The authors indicated no financial relationships.

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JAMA Oncology | Brief Report

Association of Prognostic Value of Primary Tumor Location in Stage III Colon Cancer With *RAS* and *BRAF* Mutational Status

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IMPORTANCE We know of no data on the prognostic value of primary tumor location (PTL) according to *BRAF, RAS*, and microsatellite instability (MSI) status in patients who have undergone resection for colon cancer (CC) and have been treated with current standard adjuvant chemotherapy.

OBJECTIVE To determine the prognostic and predictive value of PTL according to *BRAF*, *RAS*, and MSI status in patients with stage III CC receiving adjuvant treatment with FOLFOX (folinic acid [leucovorin calcium], fluorouracil, and oxaliplatin) with or without cetuximab.

DESIGN, SETTING, AND PARTICIPANTS This post hoc analysis included patients with available tumor blocks of resected stage III colon adenocarcinoma who participated in the Pan-European Trials in Alimentary Tract Cancer (PETACC)-8 phase 3 randomized trial. Among the 2559 patients who underwent randomization, 1900 were screened by next-generation sequencing, which showed that 1869 had full information concerning PTL. We categorized primary tumor site as located proximal (right) or distal (left) to the splenic flexure.

MAIN OUTCOMES AND MEASURES The associations between PTL (right- vs left-sided) and disease-free survival (DFS), survival after relapse (SAR), and overall survival (OS) were assessed by Cox models and adjusted for clinical and pathological features, treatment, and MSI, *BRAF*, and *RAS* status.

RESULTS Among the 1869 patients (1056 [57%] male; mean [SD] age, 59.4 [9.5] years) with full molecular data analyzed, 755 (40%) had a right-sided tumor, 164 (10%) had MSI, 942 (50%) had *RAS* mutations, and 212 (11%) had *BRAF* mutations. Right-sided tumor location was not prognostic for DFS in the whole population but was associated with a shorter SAR (hazard ratio [HR], 1.54; 95% CI, 1.23-1.93; $P = .001$) and OS (HR, 1.25; 95% CI, 1.02-1.54; $P = .03$). When looking at DFS in the different molecular subgroups, we found similar results for microsatellite-stable tumors and tumors with MSI; a better DFS in right-sided vs left-sided tumors in patients with *RAS* mutations (HR, 0.80; 95% CI, 0.64-1.00; $P = .046$); and a worse DFS in right-sided vs left-sided tumors in patients with *RAS* and *BRAF* double wild type (HR, 1.39; 95% CI, 1.01-1.92; $P = .04$). These results were found independently of the treatment received, and no beneficial effect of cetuximab on DFS or OS was observed in left-sided tumors.

CONCLUSIONS AND RELEVANCE Although right-sided tumor location is associated with poor survival in patients with metastatic CC as previously reported, the association with disease recurrence appears to vary for patients with stage III CC and *RAS* or *BRAF* mutations vs those with double wild type.

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Supplemental content

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Group Information: The PETACC-8 Investigators are listed at the end of the article.

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Biological and molecular factors such as microsatellite instability (MSI) and *KRAS* and *BRAF* mutational status have recently been proposed as prognostic factors in nonmetastatic colorectal cancers and may play a role as stratification factors in future adjuvant trials.[1]

While the prognostic value of primary tumor location (PTL) for overall survival (OS) in metastatic colorectal cancer (mCRC) seems clear and consistent in reports in recent decades, the prognostic impact of PTL for stage III nonmetastatic colorectal cancer remains unclear. Moreover, as many recent publications on PTL deal with patients with mCRC treated with anti-epidermal growth factor receptors, very few data sets report results in patients with *RAS* mutations. Finally, greater effectiveness of anti-epidermal growth factor receptors in left-sided tumors has also been suggested in patients with mCRC.[2]

We therefore examined the relationship between PTL and disease-free survival (DFS), OS, and survival after recurrence (SAR) in patients with stage III colon cancer (CC) who received adjuvant FOLFOX (folinic acid [leucovorin calcium], fluorouracil, and oxaliplatin) alone or combined with cetuximab. These data were then assessed according to MSI, *RAS*, and *BRAF* status and treatment received.

Key Points

Question Is primary tumor location prognostic or predictive, according to *BRAF*, *RAS*, and microsatellite instability status, in patients with stage III colon cancer receiving adjuvant FOLFOX (folinic acid [leucovorin calcium], fluorouracil, and oxaliplatin) with or without cetuximab?

Findings In this study of 1869 patients with tumor blocks of resected stage III colon cancer, for those patients with *RAS* mutant or *BRAF* mutant genotype, disease-free survival was better with right- vs left-sided tumors; for patients who had *RAS* and *BRAF* double wild type, disease-free survival was worse in those with right-sided tumors. No predictive effect of sidedness for cetuximab efficacy was found.

Meaning The association between sidedness and disease recurrence varied between patients with *RAS* or *BRAF* wild type and those with a mutation; no beneficial effect of cetuximab on disease-free survival and overall survival in patients with left-sided tumors was seen in the adjuvant setting.

Methods

The Pan-European Trials in Alimentary Tract Cancer (PETACC-8) study was done in accordance with the Declaration of Helsinki (amended 2000) and the International Conference on Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH) Note for Guidance on Good Clinical Practice and approved by the appropriate ethics committees. All patients analyzed gave their informed consent for translational research projects in addition to the informed consent given for the therapeutic trial. Patients with histologically proven stage III resected colon adenocarcinoma for the previously reported PETACC-8 trial were randomly assigned to receive 6 months of FOLFOX or FOLFOX plus cetuximab.[3] We categorized PTL as on the right or the left of the splenic flexure.

Tumor samples were prospectively banked. Methods for MSI, *KRAS, NRAS*, and *BRAF* assessments were previously described.[4,5]

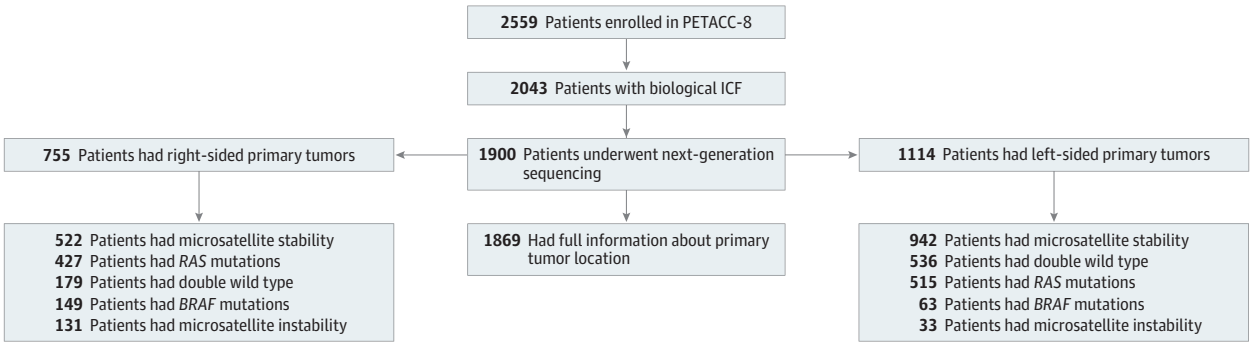
The DFS, OS, and SAR curves were estimated with the Kaplan-Meier method. Differences between groups of patients were analyzed using log-rank tests and Cox models with the SAS statistical software package version 9.4 (SAS Institute). A *P* value (1-sided) of less than .05 was considered significant.

Results

Study Population

Of 2559 patients who underwent randomization, 1900 were screened by next-generation sequencing, which showed that 1869 patients had full information concerning PTL (**Figure 1**). Of those 1869 patients, 755 (40%) had right-sided tumors, 164 (10%) had MSI, 942 (50%) had *RAS* mutations, and 212 (11%)

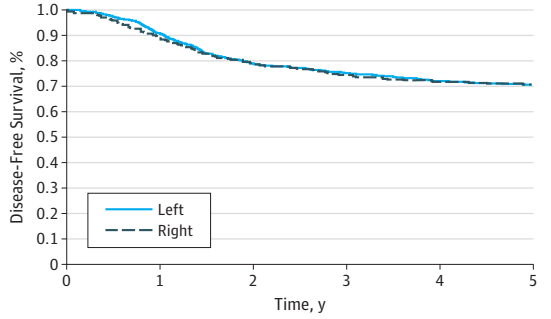
Figure 1. Flow of Patients in PETACC-8 Trial Molecular Study



Of 2559 patients who underwent randomization, 1900 were screened by next-generation sequencing, which showed that 1869 had full information concerning PTL. Of those 1869 patients, 755 (40%) had right-sided tumors, 164 (10%) had microsatellite instability, 942 (50%) had *RAS* mutations, and 212 (11%) had *BRAF* mutations.

Figure 2. Disease-Free Survival, Overall Survival, and Survival After Recurrence in the Whole Population According to Primary Tumor Location

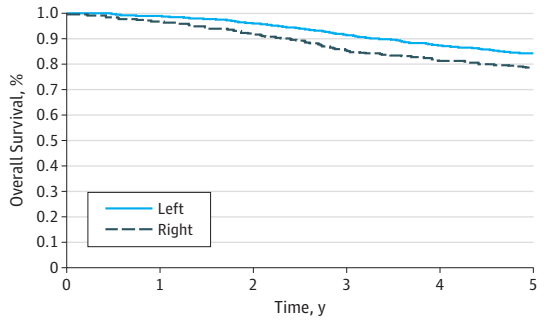
A Disease-free survival



No. at risk						
Left	1114	1000	862	798	699	528
Right	755	666	582	534	470	350

	Left localization (n = 1114)	Right localization (n = 755)
Events, No.	334	224
3-y Disease-free survival, % (95% CI)	75.5 (72.5-77.6)	74.3 (71.0-77.3)
HR (95% CI); *P* value, log-rank	1.00 (0.85-1.19); *P* = .98	
Adjusted HR (95% CI): *P* value, Wald	0.91 (0.75-1.11); *P* = .33	

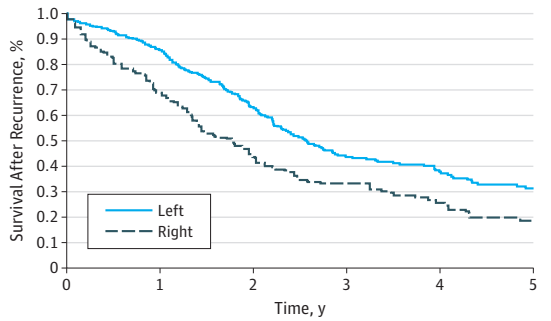
B Overall survival



No. at risk						
Left	1114	1091	1047	983	836	821
Right	755	723	883	813	535	393

	Left localization (n = 1114)	Right localization (n = 755)
Events, No.	203	165
5-y OS, % (95% CI)	84.2 (81.8-86.3)	78.6 (75.4-81.5)
HR (95% CI); *P* value, log-rank	1.25 (1.02-1.54); *P* = .03	
Adjusted HR (95% CI): *P* value, Wald	1.22 (0.96-1.55); *P* = .11	

C Survival after recurrence



No. at risk						
Left	298	235	158	90	64	37
Right	187	124	72	47	27	14

	Left localization (n = 1114)	Right localization (n = 187)
Events, No.	175	136
5-y OS, % (95% CI)	31.1 (24.9-37.6)	18.6 (12.3-25.8)
HR (95% CI); *P* value, Log-rank	1.54 (1.23-1.93); *P* = .001	
Adjusted HR (95% CI): *P* value, Wald	1.78 (1.13-1.92); *P* = .005	

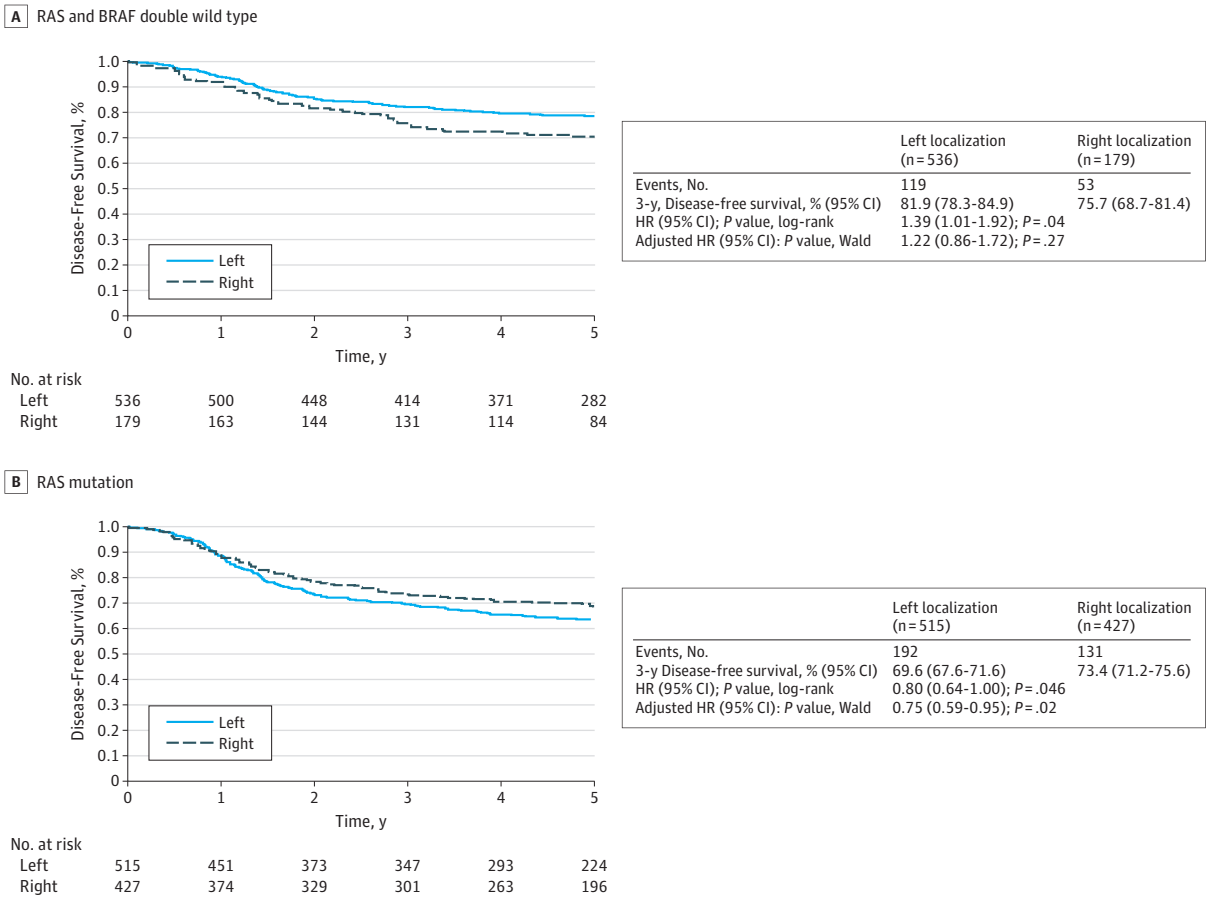
A, No difference was noted in disease-free survival when comparing right-sided with left-sided stage III colon cancer in the whole study population. B, Overall survival was significantly better for left-sided tumors. C, Survival after recurrence was significantly better for left-sided tumors. HR indicates hazard ratio.

had *BRAF* mutations. Demographic and clinical characteristics of the patients in the molecular study (n = 1869) were not statistically different from those excluded from the molecular study (n = 690) (eTable 1 in the Supplement). All demographic and molecular characteristics according to sidedness are summarized in eTable 2 and eTable 3 in the Supplement.

Outcome in the Whole Population

No difference was noted in DFS when comparing right-sided with left-sided stage III CC in the whole study population (**Figure 2**A). However, SAR (hazard ratio [HR], 1.54; 95% CI, 1.23-1.93; *P* = .001) and OS (HR, 1.25; 95% CI, 1.02-1.54; *P* = .03) were significantly better for left-sided tumors, and 5-year SAR and

Figure 3. Disease-Free Survival in Patients With *RAS* and *BRAF* Double Wild Type and *RAS* Mutations According to the Primary Tumor Location



	Left localization (n = 536)	Right localization (n = 179)
Events, No.	119	53
3-y, Disease-free survival, % (95% CI)	81.9 (78.3-84.9)	75.7 (68.7-81.4)
HR (95% CI); *P* value, log-rank	1.39 (1.01-1.92); *P* = .04	
Adjusted HR (95% CI): *P* value, Wald	1.22 (0.86-1.72); *P* = .27	

	Left localization (n = 515)	Right localization (n = 427)
Events, No.	192	131
3-y Disease-free survival, % (95% CI)	69.6 (67.6-71.6)	73.4 (71.2-75.6)
HR (95% CI); *P* value, log-rank	0.80 (0.64-1.00); *P* = .046	
Adjusted HR (95% CI): *P* value, Wald	0.75 (0.59-0.95); *P* = .02	

A, In patients with *RAS* and *BRAF* double wild type, those with right-sided primary tumors had a shorter disease-free survival compared with those with left-sided tumors. B, For *RAS*-mutated tumors, patients with right-sided primary tumors had a longer disease-free survival compared with those with left-sided tumors.

OS rates were 31.1% vs 18.5% and 84.2% vs 78.6% for left-sided vs right-sided tumors, respectively (Figure 2B and C).

Multivariable Analysis

In multivariable analysis, the following were associated with shorter DFS: histopathology grades 3 and 4; TNM categories pT3, pT4, and pN2; *RAS* mutations; Eastern Cooperative Oncology Group Performance Status (ECOG PS) 1 and 2; and bowel obstruction and/or perforation. Overall survival was also worse in patients with histopathology grades 3 and 4; TNM categories pT3, pT4, and pN2; *RAS* mutations; ECOG PS 1 and 2; and bowel obstruction and/or perforation. Moreover, patients with MSI tumors had better OS. Only right-sided, grade 3 or 4, pN2, and *BRAF*-mutated tumors were associated with shorter SAR (eTable 4 in the Supplement).

Outcome in Different Molecular Subgroups

All molecular subgroups' outcomes are summarized in eTable 5 in the Supplement.

No difference in DFS was reported for right-sided vs left-sided tumors in microsatellite-stable or MSI tumors (eFigure 1 in the Supplement).

In patients with double wild-type genome, those with right-sided primary tumors had a shorter DFS compared with those with left-sided tumors (HR, 1.39; 95% CI, 1.01-1.92; *P* = .04), with 3-year DFS rates of 75.7% and 81.9%, respectively (**Figure 3**A). However, these results were not significant (HR, 1.22; 95% CI, 0.86-1.72; *P* = .26) when adjusted for histopathological grade, pT, pN, ECOG PS, and bowel obstruction and/or perforation.

For *RAS*-mutated tumors, patients with right-sided primary tumors had a longer DFS compared with those with left-sided tumors (HR, 0.80; 95% CI, 0.64-1.00; *P* = .046), with 3-year DFS of 73.4% and 69.6%, respectively (Figure 3B). These results were still significant (HR, 0.75; 95% CI, 0.59-0.95; *P* = .02) when adjusted for histopathological grade, pT, pN, ECOG PS, and bowel obstruction and/or perforation in a multivariable model. The same trends were observed when looking at *BRAF*-mutated tumors (eTable 5 in the Supplement).

Treatment Outcomes in Right-Sided vs Left-Sided CC

When separately analyzing patients treated with FOLFOX alone or FOLFOX plus cetuximab, the association of PTL with DFS,

OS, and SAR was comparable to that described in the general population (eFigure 2 in the Supplement).

In patients with double wild type, there was no statistical difference in DFS when comparing FOLFOX plus cetuximab vs FOLFOX alone in both right-sided tumors (HR, 0.89; 95% CI, 0.78-1.26; P = .94) and left-sided tumors (HR, 0.84; 95% CI, 0.59-1.21; P = .34) (eFigure 3 in the Supplement).

Discussion

These data describe the prognostic value of PTL in stage III CC treated with FOLFOX with full *RAS*, *BRAF*, and MSI assessment. We confirmed historical data that right-sided tumors were older and more likely to be poorly differentiated, exhibit vascular invasion or lymphatic infiltration, and have MSI and *BRAF* mutation.[6,7] Similarly, at disease relapse, right-sided tumors had a worse prognosis. This result is similar in all molecular subgroups presently characterized and is in accordance with the currently published results of PTL prognostic value in the metastatic setting.[2]

However, when looking at disease recurrence, patients with *RAS*-mutated and/or *BRAF*-mutated right-sided tumors had a better DFS than left-sided tumors. Right-sided tumors remained of poor prognosis regarding DFS only in patients with double wild type.

Some recent studies demonstrated that PTL may be considered as a predictive factor in *RAS* wild-type mCRC treated with chemotherapy and cetuximab.[2,8-11] In the present work, no benefit of adding cetuximab to FOLFOX was observed in our population of patients with stage III left-sided tumors; nor was any detrimental effect of adding cetuximab observed in right-sided tumors.

The major strengths of our study are that patients are coming from a randomized, prospective, registration-designed phase 3 trial; that all patients were treated with the current standard FOLFOX chemotherapy regimen (with or without cetuximab) in this setting; and that a full *RAS* and *BRAF* mutational profile using next-generation sequencing for all included patients (not limited to *KRAS* and *BRAFV600E*) was determined together with the MSI assessment.

Limitations

Our work also has limitations. The post hoc design of the present analysis and the limited number of patients with *BRAF* mutations and MSI tumors led to small subgroups for some of the analyses performed.

Conclusions

Although PTL does not seem to be associated with DFS in the whole study population, opposite sidedness prognostic values are observed for *RAS* and *BRAF* wild-type and mutant tumors. A larger analysis that uses all currently available adjuvant trials with complete molecular analysis and takes into account other prognostic molecular data from the consensus molecular subtypes[12] is needed to confirm these first results and to better determine the molecular differences explaining them.

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Original Research

Impact of primary tumour location and RAS/BRAF mutational status in metastatic colorectal cancer treated with first-line regimens containing oxaliplatin and bevacizumab: Prognostic factors from the AIO KRK0207 first-line and maintenance therapy trial

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Abstract ***Background:*** The major prognostic relevance of primary tumour location (LPT) in advanced colorectal cancer was shown in large retrospective studies, but quantitative estimates are highly heterogeneous, and there is still limited information about its impact within the framework of biomarker-guided treatment strategies. Therefore, we analysed LPT in relation

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location;
RAS mutation;
BRAF mutation;
Bevacizumab;
Overall survival;
First-line therapy

to other clinical and molecular parameters, based on mature survival data from the recent randomised AIO KRK0207 trial.

Methods: Patients uniformly received first-line induction treatment with a combination of bevacizumab, oxaliplatin and fluoropyrimidine. LPT was retrospectively determined using surgical reports, pathology reports and endoscopy reports. The prognostic analyses were performed using Kaplan–Meier estimations and log-rank tests, while hazard ratios (HRs) and multivariable results were derived from Cox models.

Results: Among 754 patients with unequivocal information on LPT, patients with left-sided tumours showed a median overall survival of 24.8 months compared with the right-sided cohort with 18.4 months (HR: 1.54, 95% confidence interval: 1.30–1.81, $P < 0.0001$). In a multivariable model, LPT proved to be the strongest prognosticator (HR 1.60), with performance status, number of metastatic sites, baseline carcinoembryonic antigen (CEA) and platelets independently retaining prognostic significance. In the subgroup of patients with known RAS/BRAF status (n = 567, 75%), a BRAF mutation showed the greatest unfavourable impact (HR 3.16). Although BRAF is strongly correlated to LPT, the latter remained a significant prognosticator in the BRAF wild-type subgroup. In contrast, no major impact of LPT was seen on tumours carrying RAS mutations.

Conclusions: Within the framework of a uniform treatment strategy according to the current standards, LPT proved to have an important, although not solely dominating, relevance for survival prognosis. Its impact seems to be low in tumours with a RAS mutation.

Registration: ClinicalTrials.gov NCT00973609.

1. Introduction

Colorectal cancer (CRC) ranks among the most frequent types of malignant neoplasms in both sexes and accounts for a high proportion of cancer mortality worldwide [1]. Therefore, numerous study cohorts and retrospective series have been analysed to predict the survival probability based on the characteristics of the patient and his/her cancer. In the prebiomarker era, this resulted in the development of prognostic scores for stage IV patients, such as those of Köhne [2] or GERCOR [3], established in the first decade of the 21st century. However, owing to the improvement of the diagnostic and therapeutic armamentarium in the recent decades, the overall survival (OS) times after the detection of distant metastases showed a distinct increase, from a median of about 1 year in the 5-fluorouracil (5-FU) era to about 30 months nowadays [4].

As a consequence, the relative prognostic impact of various patient and tumour characteristics may also have changed over time. The era of exploding development of knowledge and procedures on the molecular and cellular level resulted in a waterfall of biomarker information of potential prognostic and predictive relevance. Nevertheless, up to now, few molecular biomarkers could be shown to have major prognostic impact [5]. On the other hand, surprisingly, the importance of a relatively 'trivial', easy-to-collect dichotomous information, readily available in all patients after initial staging, had been missed in the established prognostic profiling systems mentioned previously. Only since the beginning of this decade, the location of the primary tumour (LPT) on the right or left side of the colorectal anatomy was recognised as of utmost importance.

A recently published large meta-analysis on tumour sidedness and prognosis, based predominantly on retrospective series [6], showed a statistically convincing result on the bottom line, but an extreme level of effect heterogeneity between the individual studies. This remained largely unexplained by the authors despite subgrouping and meta-regression procedures, focussing on race, stage, pretreatment, study design and sample size or decade of diagnosis. Further meta-analyses have focussed on RAS wild-type patients [7,8] and on the efficacy of adding bevacizumab to first-line chemotherapy in relation to tumour sidedness [5]. Data on patients treated with first-line oxaliplatin plus fluoropyrimidine (FP), with RAS and BRAF status available but including also patients carrying the respective mutations, are rare. Although sidedness data are available from the NO16966 trial, one has to keep in mind that this trial was recruiting more than a decade ago when RAS mutational analysis was not yet available. Therefore, further prognostic profile analyses of data from large controlled trials including well-characterised patients treated according to the current standards are warranted.

As previously published [9], the main objective of AIO KRK0207 was the randomised comparison of three different maintenance strategies in patients having achieved at least a stabilisation of metastatic disease after induction chemotherapy: either no continuation of

therapy or bevacizumab alone or any FP plus bevacizumab. With respect to OS, the results were comparable in the three arms. However, all patients were enrolled and adequately documented after the initial diagnosis of dissemination and uniformly received 24 weeks of a combination therapy including FP, oxaliplatin and bevacizumab. Thus, our data allowed for the prognostic analysis of OS in a representative population of patients with metastatic CRC without any biomarker-based selection, receiving a three-drug combination corresponding to the present guidelines, including a vascular endothelial growth factor (VEGF)–targeted component. Because this is a major difference to the majority of recently published analyses of randomised trials on the impact of LPT, focussing on the RAS wild-type subpopulation only [8,10,11], detailed prognostic factor analyses of our study population should give rise to further important insights.

2. Material and methods

2.1. Study design and participants

AIO KRK0207 was an open-label, randomised multicentred phase 3 trial. Study design, eligibility criteria, ethical approvals, informed consent, treatment regimens and additional information on diagnostic, therapeutic and follow-up procedures as well as the primary outcome of the trial, comparing the different maintenance strategies, were reported elsewhere [9].

Eligible patients were registered for the trial before starting an induction regimen consisting of any FP (infusional or capecitabine), oxaliplatin and bevacizumab, eventually followed by randomised maintenance and reinduction strategies of differing intensity. OS data were updated by December 2016, extending the data lock of the initial publication (December 2014). Except for LPT, all data were retrieved from the main trial database of KRK0207 [9].

2.2. Categorisation of the primary tumour localisation/ biomarker data

LPT was retrospectively determined using reports on surgery, pathology and endoscopy. Right-sided primary tumours were defined as located in the caecum, ascending colon and transverse colon up to the splenic flexure. Left colon was defined as splenic flexure, descending and sigmoid colon and rectum, in accordance with recent publications [10,11]. Patients with synchronous tumours located in the left and right colon or patients in whom primary tumour location could not be determined were scored as undetermined or missing. Although not required by the initial protocol, tumour samples were retrieved for central examination of the RAS and BRAF status (Institute of Pathology, Ruhr-University Bochum, Germany), whenever possible. Formalin-fixed paraffin-embedded tissue was microdissected and cut. DNA was extracted using the DNA Purification Kit (Promega; Madison). Mutational analysis was performed stepwise using the pyrosequencing technique (Q24, Qiagen, Hilden). In the first step, the mutational status of the codon 12 and 13 of the KRAS gene was determined. In the second step, the mutational status of codons 59, 61, 117 and 146 and the mutation hotspots of the NRAS gene in exons 2–4 were analysed (p.G12, p.G13, p.A59, p.Q61, p.K117 and p.A146).

2.3. Statistical aspects

All analyses, including the resulting *P*-values, are of exploratory nature and hypothesis generating. Therefore, adjustments for multiple testing were neither preplanned nor performed. Nevertheless, within the scope of this analysis, the term 'significant' is used in case of $P < 0.05$.

OS was calculated from the study recruitment (or date of randomisation in the analyses of the maintenance subpopulation) to death or censored at the time point of the last valid observation without the respective event. Survival curves and medians were estimated according to Kaplan–Meier. Univariable analyses were performed using the log-rank test. The Cox proportional hazard model [12] was applied for multivariable analysis, based on the patients with a complete set of covariates, with *P*-values derived from Wald tests and initially including all covariates with a prospectively defined univariable P level < 0.1. Subsequently, the model was reduced by stepwise exclusion of the covariate with the highest *P*-value, until only factors with $P \leq 0.1$ remained. All hazard ratios (HRs) and confidence intervals (CIs) reported are derived from Cox models. All presented *P*-values are two sided.

3. Results

3.1. Correlation of location of the primary tumour with other baseline characteristics

In 754 of 825 enrolled patients, LPT could be unequivocally allocated to the left (n = 525, 70%) or right side (n = 229, 30%). The distribution of major baseline characteristics of potential prognostic relevance in the subgroups according to anatomic side is displayed in Table 1. LPT was more often on the right in women. An impaired performance status and synchronous distant metastases, usually determining a higher risk, showed a discernable, albeit only weak to moderate, association with LPT on the right. On the other hand, high carcinoembryonic antigen (CEA) values were more often found in patients with tumours on the left. The most distinct correlation was detected in the molecular level,

Table 1
Correlation of location of the primary tumour with other baseline characteristics.

Characteristic	Left side (n = 525)	Right side (n = 229)	Side undefined, unknown (n = 71)	Total (n/%)	p[a]
Age (years)					
Mean ± SD	63.4 ± 10.1	64.2 ± 10.5	64 ± 9.9	63.7 ± 10.2	
Median	65	66	67	65	
Range	32–82	23–83	43–81	23–83	
≤70 years	364 (69%)	147 (64%)	45 (63%)	556 (67%)	
>70 years	161 (31%)	82 (36%)	26 (37%)	269 (33%)	0.18
Sex					
Male	353 (67%)	129 (56%)	47 (66%)	529 (64%)	
Female	172 (33%)	100 (44%)	24 (34%)	296 (36%)	0.0050
Performance status (n = 796)					
ECOG 0	301 (59%)	108 (49%)	39 (57%)	448 (56%)	
ECOG 1–2	207 (41%)	111 (51%)	30 (43%)	348 (44%)	0.015
Time of metastasis					
Synchronous	418 (80%)	199 (87%)	59 (83%)	676 (82%)	
Metachronous	107 (20%)	30 (13%)	12 (17%)	149 (18%)	0.018
Adjuvant chemotherapy					
No	448 (85%)	213 (93%)	65 (92%)	726 (88%)	
Yes	77 (15%)	16 (7%)	6 (8%)	99 (12%)	0.0036
No. of metastatic sites (n = 819)					
1	223 (43%)	107 (47%)	27 (39%)	357 (44%)	
>1	300 (57%)	120 (53%)	42 (61%)	462 (56%)	0.26
CEA at baseline (n = 737)					
≤20 ng/ml	156 (33%)	85 (41%)	16 (28%)	257 (35%)	
>20 ng/ml	315 (67%)	123 (59%)	42 (72%)	480 (65%)	0.056
Platelets at baseline (n = 815)					
≤ULN	376 (72%)	159 (71%)	46 (65%)	581 (71%)	
>ULN	143 (28%)	66 (29%)	25 (35%)	234 (29%)	0.66
RAS/BRAF status (n = 567)					
Wild type	175 (46%)	34 (22%)	12 (39%)	221 (39%)	
RAS mutation	191 (51%)	85 (54%)	17 (55%)	293 (52%)	0.51
BRAF mutation	12 (3%)	39 (25%)	2 (6%)	53 (9%)	<0.0001
RAS or BRAF mutation	203 (54%)	124 (78%)	19 (61%)	346 (61%)	<0.0001

ECOG, Eastern Cooperative Oncology Group; ULN, upper limit of the site-specific normal range; CEA, carcinoembryonic antigen.
[a] Fisher's exact test for association with the left or right side; patients with undefined/unknown side were excluded.

with BRAF mutations being a rare finding in left-sided tumours. In contrast, RAS mutations of any subtype were equally common in both cohorts.

3.2. Univariable prognostic analyses on OS

With a maximum follow-up duration of more than 80 months and the death event recorded in 702/825 patients (85%), the mature data on OS from recruitment, in the total evaluable study population, showed a median of 21.5 months. As to be expected, the 472 patients qualifying for randomisation lived distinctly longer with a median of 26.8 months compared with a median of 13.2 months in the remaining 353 patients not randomised to maintenance strategy, for various reasons.

Among 754 patients with unequivocal information on LPT, patients with left-sided tumours showed a median OS of 24.8 months compared with the right-sided cohort with 18.4 months. The corresponding univariable HR was 1.54 (95% CI: 1.30–1.81, $P < 0.0001$; Fig. 1A). This and other variables with a statistically significant prognostic impact are shown in Table 2. Neither age cohorts nor previous adjuvant chemotherapy were of prognostic relevance. In the subcohort of 576 patients (70%) with information on the RAS/BRAF(V600E) mutational status available from centralised reference pathology, both mutation of RAS (any location) and, outstandingly, of BRAF proved to be associated with poor prognosis (Table 2; Fig. 1B).

3.3. Multivariable prognostic analyses on OS

In the Cox model including all univariably significant parameters (excluding molecular markers because of the relatively high proportion of missing information), female sex and synchronous metastasis did not exhibit independent prognostic relevance (Table 2). However, the other five parameters, that is, performance status, number of metastatic sites, LPT, CEA and platelets at baseline, retained their impact on mortality risk. Location of the primary tumour seems to be the factor of utmost importance; however, the resulting impact sizes, that is, HRs, all fell within a rather narrow range from 1.32 to 1.60.

This result of rather comparable, independent importance suggested to allocate risk points, one to each

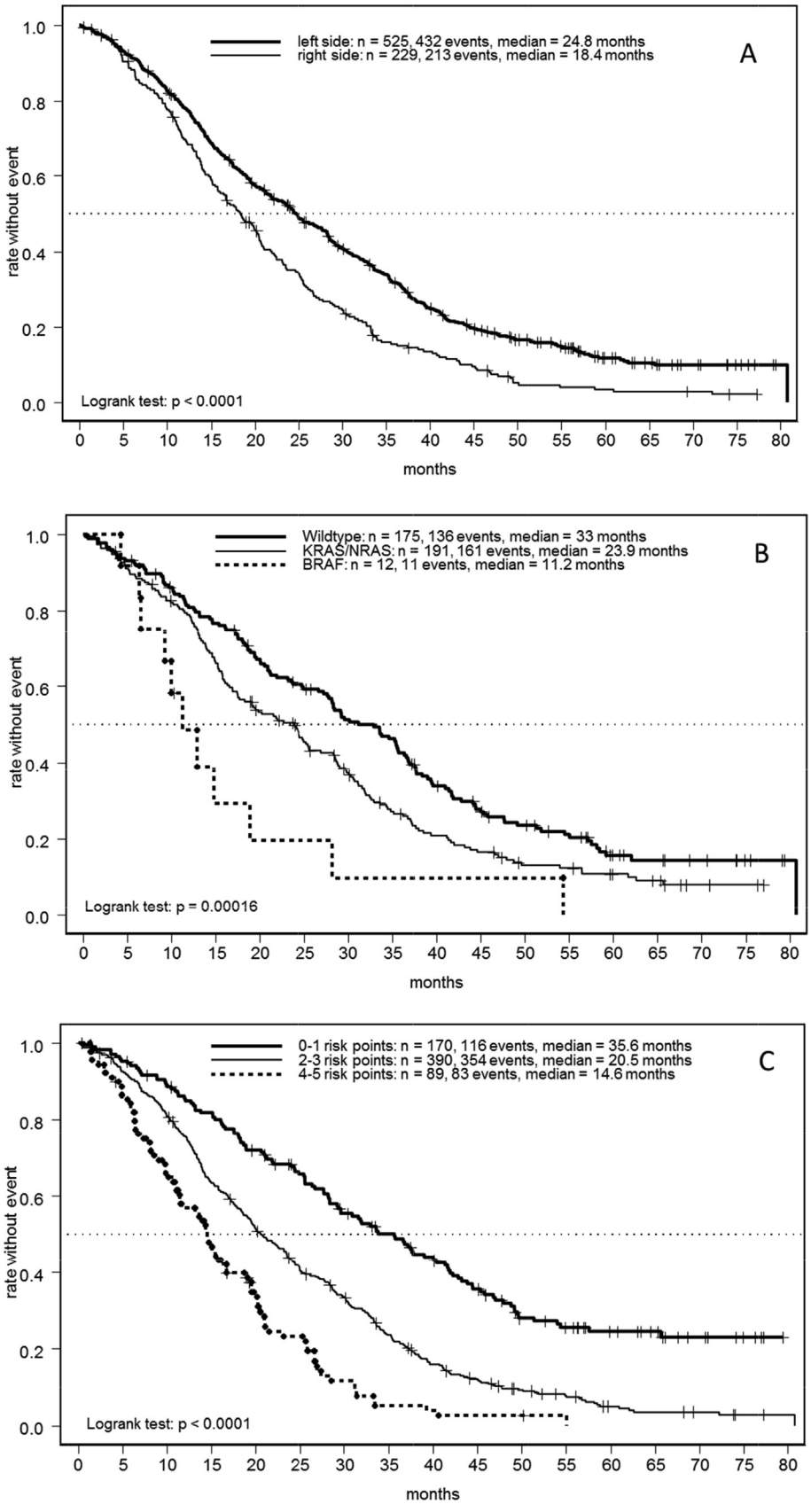

Fig. 1. Kaplan–Meier estimation of overall survival (A) by location of the primary tumour (left versus right), (B) by any RAS mutation, BRAF (V 600E) mutation and wild-type tumours and (C) by prognostic score group.

Table 2
Analysis of prognostic factors for overall survival from recruitment for first-line therapy.

Prognostic factor (first group mentioned is reference for HR)	Univariable[a] median; months (95% CI), *P*	Multivariable, full model (n = 649); HR (95% CI), *P*	Multivariable, reduced model (n = 649); HR (95% CI), *P*
Sex: male versus female	23.2 (20.7–25.1) versus 19.5 (17.4–23.2), $P = 0.030$	1.02 (0.86–1.22), $P = 0.81$	–
ECOG performance: 0–1 versus 2-4	25.6 (24.2–28.4) versus 17.0 (15.1–19.8), $P < 0.0001$	1.48 (1.25–1.76), $P < 0.0001$	1.49 (1.25–1.77) $P < 0.0001$
Time of metastasis: synchronous versus metachronous	20.5 (18.8–22.4) versus 28.5 (24.4–33.6), $P = 0.0015$	0.89 (0.71–1.12), $P = 0.33$	–
Number of metastatic sites: 1 versus > 1	24.6 (21.3–28.2) versus 19.8 (18.4–22.6), $P = 0.00014$	1.42 (1.19–1.69), $P < 0.0001$	1.42 (1.19–1.69), $P < 0.0001$
CEA at baseline: ≤ 20 ng/mL versus > 20 ng/mL	28.2 (23.9–32.8) versus 19.5 (17.2–21.0), $P < 0.0001$	1.53 (1.27–1.84), $P < 0.0001$	1.55 (1.29–1.86), $P < 0.0001$
Platelets at baseline: ≤ ULN versus > ULN	23.9 (21.8–26.4) versus 18.1 (15.7–20.0), $P < 0.0001$	1.29 (1.06–1.57), $P = 0.012$	1.32 (1.10–1.60), $P = 0.0036$
Primary tumour location: left versus right	24.8 (21.9–28.2) versus 18.4 (15.8–20.5), $P < 0.0001$	1.58 (1.32–1.90), $P < 0.0001$	1.60 (1.33 – 1.92), $P < 0.0001$
Mutational status: all wild type versus RAS versus BRAF	33.0 (27.9–36.6) versus 23.9 (17.7–28.4) versus 11.2 (9.3- undefined), $P < 0.0001$	–	–

ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; CI, confidence interval; ULN, upper limit of the site-specific normal range; –, not included in model.
[a] Only variables with $P < 0.1$ were presented (see text). The number of patients varies between 825 and 576 (molecular markers) due to missing values in some parameters.

of the factors, adding up to a scale ranging from zero to five. Fig. 1C shows the clear-cut OS distinction between patients with 0–1, 2–3 or 4–5 risk points ($P < 0.0001$).

The negative impact on survival of any RAS or BRAF mutation in the overall population was highly significant (HR = 1.49, 95% CI: 1.24–1.80, $P < 0.0001$). If patients with a BRAF mutation were excluded from the analysis, any RAS mutation showed a lesser impact, albeit still significant (HR = 1.35, 95% CI: 1.11–1.64; $P = 0.0022$). This was similarly detected in the subgroup of left-sided tumours (Fig. 2A), with an HR of 1.42 for all wild-type versus any RAS mutation (95% CI: 1.13–1.79; $P = 0.0026$). Remarkably, this trend could not be shown, or is numerically even reversed, in right-sided tumours (Fig. 2B; HR = 0.89, 95% CI: 0.58–1.35; $P = 0.57$). The other way round, patients with an all–wild-type tumour exhibited a strong prognostic impact of LPT (HR = 1.83, 95% CI: 1.23–2.72), while in tumours with a RAS mutation, no impact of LPT could be confirmed (HR = 1.09, 95% CI: 0.83–1.43).

In a Cox model of 465 patients with all the respective covariates available, including the five independent parameters described in Table 2, the additionally incorporated term of any RAS/BRAF mutation showed an HR of 1.32 (95% CI: 1.06–1.63), with all of the other five covariates remaining significant (Supplementary Table S1, available at the *European Journal of Cancer* [*EJC*] online). However, if RAS and BRAF mutations were addressed as separate factors, the latter had the by far strongest unfavourable impact (HR = 3.16, 95% CI: 2.17–4.60; $P < 0.0001$), while the former did not retain statistical significance (HR = 1.22, 95% CI: 0.98–1.52; $P = 0.080$).

As tumour sidedness and BRAF mutational status are strongly correlated (Table 1), we multivariably addressed the relevance of the anatomical site in patients with BRAF wild-type tumours only: right-sided location remained an indicator of poor prognosis even in this subpopulation (HR = 1.40, 95% CI: 1.09–1.79; $P = 0.0084$), with all the other four factors identified above similarly retaining their significance (Supplementary Table S2, available at the *EJC* online).

4. Discussion

There is a growing consensus that primary tumour location is an important factor for the general prognosis of a patient and is also contributing to the guidance of targeted therapy. However, LPT only presents a surrogate marker for complex, and still heterogeneous, molecular patterns associated predominantly with the respective location, with details not yet fully understood [13,14]. The rate of 30% of right-sided tumours detected in our study corresponds well to the findings in other large studies focussing on patients selected for VEGF- or epidermal growth factor receptor (EGFR)–targeted combinations [5,7]. In an exclusively RAS wild-type population, the proportion seems to be somewhat lower, that is, about 24% [8].

The prognostic impact of LPT seems to be rather independent of, or not fully captured by, other conventional or molecular variables. Although shown qualitatively in most series, its quantitative effect size is subject to a huge amount of heterogeneity, as has become evident in recent reviews [15] and meta-analyses [6,7]. Beyond the biomarker-driven selection of data

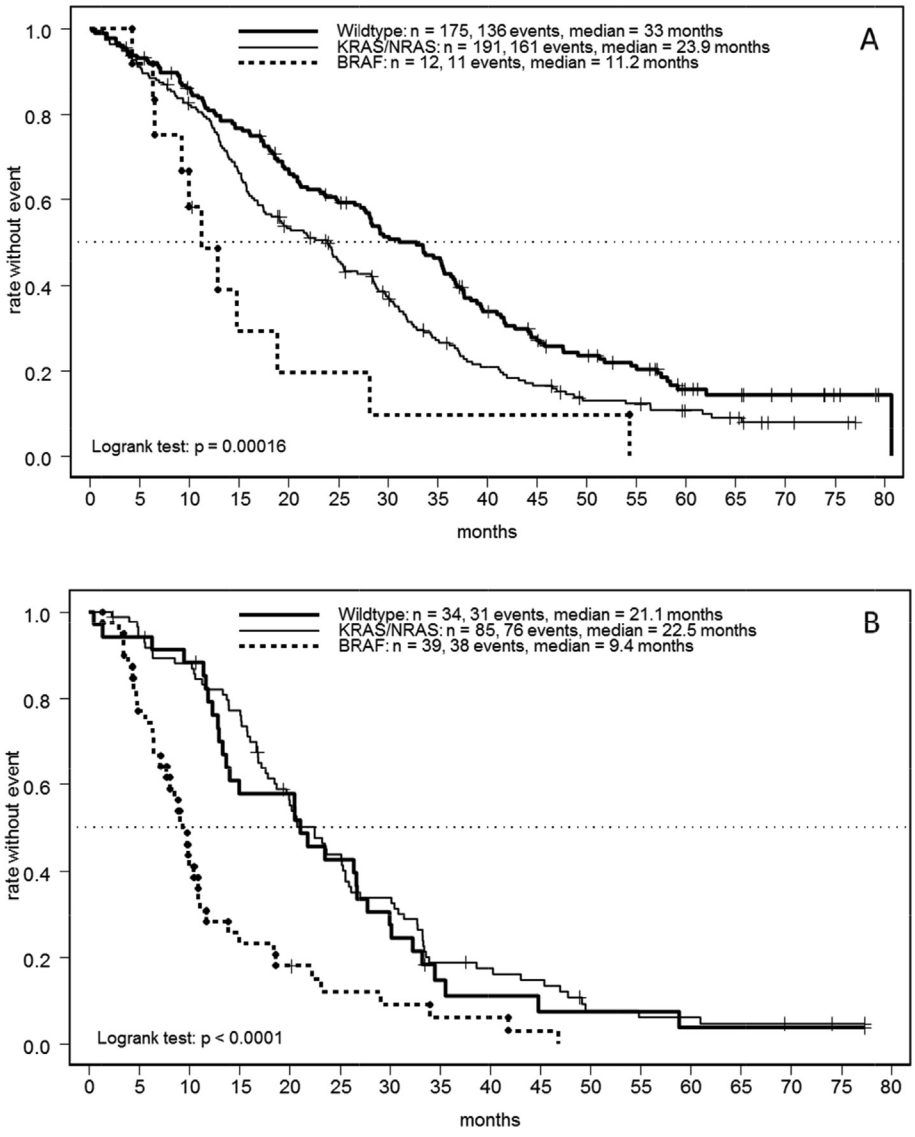

Fig. 2. Kaplan–Meier estimation of overall survival by any RAS mutation, BRAF (V 600E) mutation, (A) in the subgroup of left-sided tumours and (B) in the subgroup of right-sided tumours.

sources, this heterogeneity may also be due to the therapeutic approach in the respective studies, that is, with/without targeted agents or whether addressing the EGFR or VEGF target [7,16].

The largely unrestricted inclusion of a total of 66 studies in the large meta-analysis by Petrelli *et al.* [6] resulted in a rather small increase of mortality risk for right-sided tumours of 22%, but this estimate is hampered by an unexplained heterogeneity of $I^2 = 97\%$. The recent meta-analysis by Holch *et al.* [7], exclusively focussing on prospective first-line studies in metastatic disease, showed a much larger prognostic difference. Although the disposition of trials is somewhat disproportionally inclined to a RAS wild-type population, the finding of an HR = 1.54 (fixed-effect model), with acceptable heterogeneity ($I^2 = 16\%$), corresponds well to the findings of our analysis (1.54 univariably and 1.60 multivariably).

In studies investigating VEGF-targeted first-line treatment, the univariable impact sizes of a right location were HR = 1.41, 1.82 and 2.27 in the studies NO16966, AVF2107g and PROVETTA, respectively [5], mirroring the findings of our analysis. NO169966 had an oxaliplatin backbone, similar to this study. The similar prognostic information (HR 1.41 and 1.54, respectively) could be suggestive of a differential effect of different chemotherapy backbones. As all of our analysed patients received bevacizumab, we cannot contribute to the still somewhat contradictory evidence on a possible interaction between VEGF antibody treatment and LPT [13,16].

Furthermore, our trial is the first one to provide data on the prognostic impact of RAS/BRAF mutational status in this unselected cohort of patients. The data suggest that the strong prognostic effect of LPT is mainly located in the RAS wild-type population.

Although most tumours with BRAF mutations are located proximally, an additional unfavourable impact of LPT was retained in this biomarker subgroup.

Several prognostic scores for metastatic CRC have been developed during the last two decades, markedly differing in the individual parameters incorporated [2,3,17]. While the most established one, the Köhne Score, was based on data from the 5-FU era, it proved to be also valid in populations treated with irinotecan or oxaliplatin [18,19]. Nevertheless, even before taking LPT into account, its relevance in the era of targeted biotherapies was strongly questioned [20,21], notably due to a lack of discrimination between the low and intermediate risk group. The numerical combination of 'risk points' derived from the five independent (conventional) prognostic factors in our multivariable model yielded a distinct separation into three groups. However, this approach requires prospective confirmation in a validation sample.

A major limitation of this analysis is its retrospective nature. Some variables, notably LPT and molecular markers, were not available for all patients, due to the retroactive data collection, leading to decreased event numbers in multivariable models. As in most studies on tumour sidedness, the demarcation line between left and right may be suboptimally defined and differing from the underlying embryology.

In conclusion, our study enlarged the body of evidence on tumour sidedness in patients with metastatic CRC, who were prospectively selected, observed and uniformly treated with a triple combination, specifically including an oxaliplatin-based chemotherapy with bevacizumab. It confirmed the important, although not solely dominating, relevance of LPT for the survival prognosis, but at the same time suggests interactions with biomarkers, such as the RAS mutational status. A plethora of recent [5,14,22,23] and ongoing activities will further elucidate the molecular variability of advanced CRC originating from the different anatomic sites.

Conflict of interest statement

S.H.-B. is an advisory board member of Amgen, Chugai, BMS, Lilly and Merck. S.N.-D. received honoraria from Baxalta, BMS and Ipsen and is an advisory board member of BMS. A.H. received honoraria from Roche. U.G. received honoraria from Roche, Sanofi, Amgen, Merck and Servier. A.R.-S. received honoraria from Amgen, Roche, Pfizer, Sanofi-Aventis and Merck and is an advisory board member of Amgen, Roche, Pfizer, Sanofi-Aventis and Merck. B.K. received honoraria from BMS and Roche and travel costs from Janssen. S.-E.A.-B. received honoraria and research grants Roche . A.T. received honoraria from Amgen, Roche, Merck Serono and Sanofi-Aventis. D.A. received honoraria from Bayer, Biocompatibles, Lilly, Merck, MSD, Roche, Sanofi, Servier and Sirtex and is an advisory board member of Bayer, Lilly, Merck, Roche, Sanofi, Servier, Sirtex and Terumo. The other authors declare that they have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejca.2018.06.015.

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