Association between KRAS mutation and lung metastasis in advanced colorectal cancer

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Background: KRAS mutations have been associated with lung metastases at diagnosis of metastatic colorectal cancer (mCRC), but the impact of this mutation on subsequent development of lung metastasis is unknown. We investigated KRAS mutation as a predictor of lung metastasis development.

Methods: We retrospectively evaluated data from patients with mCRC whose tumour was tested for KRAS mutation from 2008 to 2010. The relationships of KRAS mutational status with time-to-lung metastasis (TTLM) and overall survival (OS) were analysed.

Results: Of the 494 patients identified, 202 (41%) had tumours with KRAS mutation. KRAS mutations were associated with a shorter TTLM (median 15.2 vs 22.4 months; hazard ratio = 1.40; P = 0.002) and a two-fold greater odds of developing lung metastases during the disease course in patients with liver-limited mCRC at diagnosis (72 vs 56%, P = 0.007). Overall survival did not differ by KRAS status.

Conclusions: Lung metastasis was more likely to develop during the disease course in patients whose tumour had a KRAS mutation than in those whose tumour did not have a KRAS mutation. This finding may have an impact on decision making for surgical resection of metastatic disease.

Despite advances in treatment, metastatic colorectal cancer (mCRC) remains the fourth most common cause of cancer death worldwide (Siegel et al., 2013). The lung is the most common extra-abdominal site of metastasis (Mitry et al., 2010).

Currently, the presence of KRAS mutation is the most important established predictive biomarker for resistance to anti-epidermal growth factor receptor (EGFR) antibodies cetuximab and panitumumab (Siena et al., 2009). The KRAS protein functions as an essential component of the EGFR signalling cascade. Activating mutations in the KRAS gene cause the constitutive activation of Ras GTPase, which leads to the overactivation of downstream Raf/Erk/Map kinase and other signalling pathways, resulting in cell transformation and tumorigenesis (Leevers and Marshall, 1992; Wood et al., 1992). Preclinical studies have suggested that constitutively activated mutant KRAS can promote tumour invasion and metastasis by stimulating matrix metalloproteases, cysteine proteases, serine proteases and urokinase plasminogen activator, all of which facilitate migration through the basement membrane (Jankun et al., 1991; Buo et al., 1995; Yamamoto et al., 1995). The association between KRAS mutational
status and prognosis is controversial: some studies have reported a link between KRAS mutations and poor prognosis (Lievre et al., 2006; Nash et al., 2010), whereas others have reported no association (Etienne-Grimaldi et al., 2008; Roth et al., 2010).

The pattern of CRC recurrence is partly determined by clinicopathologic features, such as primary tumour location, initial TNM stage or preoperative serum carcinoembryonic antigen level (Manfredi et al., 2006; Mitry et al., 2010; Watanabe et al., 2013). However, there are limited data evaluating whether the somatic mutation profile can have a role in the pattern of spread of metastasis in CRC patients. The few previous trials have suggested that KRAS mutations may influence and contribute to differences in the pattern of metastatic dissemination (Cejas et al., 2009; Tie et al., 2011; Kim et al., 2012). Although these studies focused on the prevalence of lung metastases, there is a clinical need for predictors of subsequent lung metastases in patients with no evidence of pulmonary involvement at the time of diagnosis of metastatic disease. We aimed to determine the potential value of KRAS mutation as a predictive factor for development of lung metastasis.

### PATIENTS AND METHODS

**Patient population.** Patients with mCRC with known KRAS status who were treated at The University of Texas MD Anderson Cancer Center from 2008 through 2010, independent of metastatic site or whether they developed metastatic disease to the lung, were selected from a prospectively maintained institutional database. A total of 494 patients were identified. The study was approved by institutional review board and ethics committee.

**Study end points.** The primary end point of this retrospective study was a comparison of the time-to-lung metastasis (TTLM). This endpoint was defined as the time from diagnosis of metastatic disease, that is, from the first metastasis in any site, to the time of the first lung metastasis, between patients whose primary tumour had no KRAS mutation (KRASwt) and patients whose tumour had a KRAS mutation (KRASmut). The secondary end point aimed to compare the pattern of lung involvement between these two groups and included the following dichotomous variables: lung as first site of metastasis, presence of lung metastasis at the end of follow-up, number of lung metastases (isolated vs multiple), lung lobes involved (1 vs >1), unilateral vs bilateral lung involvement, thoracic lymph node involvement (positive vs negative) and synchronous vs metachronous or absent lung metastasis. Synchronous metastasis was defined as metastasis diagnosed before or up to 60 days after diagnosis of primary tumour. Overall survival (OS), defined as time from the first metastasis to death from any cause, and lung metastasis-free survival (LMFS), defined as time from the first metastasis in any site to the first lung metastasis or death, also were evaluated as secondary end points. All time-to-event analyses were calculated from the time of diagnosis of metastatic disease to be consistent with time-to-event analyses commonly reported for metastatic patients. It also reflects that this cohort was collected based on their documented development of metastatic disease.

**KRAS mutation determination.** KRAS mutations (codons 12, 13, 61; Supplementary Table 1) were identified in formalin-fixed, paraffin-embedded tissue by Sanger sequencing or mass spectrometry genotyping (Sequenom, San Diego, CA, USA) in the U.S. Department of Health and Human Services Clinical Laboratory Improvement Amendments (CLIA)–compliant pathology lab as part of standard-of-care testing for the patients. Microdissection was utilised under the guidance of a clinical pathologist as required to ensure >30% tumour cellularity.

**Statistical methods and considerations.** Patient characteristics and disease factors were summarised by descriptive statistics. The categorical parameters were compared by using the two-sided Pearson χ2-test or Fisher exact test, as appropriate, and the t-test was used for continuous variables. Time-to-event variables were calculated from the time of diagnosis of first metastasis in any site according to the Kaplan–Meier method and were compared by means of the log-rank test. SPSS software (version 16.0; SPSS, Chicago, IL, USA) was used for statistical analyses. A P-value <0.05 was considered significant.

### RESULTS

**Patient characteristics.** Of the 494 patients included in this study, 203 (41%) were female. Median follow-up was 26 months. The median age was 55 years (range, 25–85 years). KRAS mutation was identified in 202 of the tumours (KRASmut, 41%), and 292 tumours were KRAS wild type (KRASwt, 59%). The groups were unbalanced according to location of primary tumour; tumours with a KRAS mutation occurred more frequently in the right side of the colon (KRASmut 43% vs KRASwt 28%) than in the left colon or rectum (57 vs 71%, P = 0.001). Other patient and disease characteristics are shown in Table 1.

**Lung and liver metastases pattern.** At the time of diagnosis of primary tumour, 60 (12%) patients had synchronous lung involvement (16% of the KRASmut patients vs 9% of KRASwt), representing a 1.9-fold greater odds of lung metastasis in the KRASmut population (P = 0.018, 95% confidence interval (CI) 1.08–3.42). The lung was the first site of metastatic disease in 18% of the KRASmut patients and 15% of the KRASwt (P = 0.287); by the end of the follow-up, lung involvement had substantially increased, with a 1.6-fold greater odds of lung metastases in the KRASmut patients (P = 0.012, 95% CI 1.09–2.43; Figure 1). The liver was less frequent as the initial metastatic organ in patients with a KRAS mutation (P = 0.004). Among the 275 patients who initially had liver as the only site of metastatic disease, KRASmut patients were two-fold more likely to develop lung metastasis (P = 0.007, 95% CI 1.2–3.5; Table 1).

Among the 315 patients with thoracic metastases, there were no differences in thoracic lymph node involvement (P = 0.18), number of lung metastases (P = 0.71), lung lobes involved (P = 0.19) or disease in bilateral lung (P = 0.27) by KRAS mutational status (Table 1). There were no statistically significant differences in lung metastasis frequency or pattern of KRAS mutations by codon (12, 13 or 61); the distribution of mutations was consistent with those reported by prior studies (Supplementary Table 1).

**Analysis of survival.** Compared with KRASwt patients, KRASmut patients had a shorter TTLM (22.4 vs 52.5 months; hazard ratio (HR) = 1.40; 95% CI 1.12–1.75; P = 0.002; Figure 2A) and LMFS (16.7 vs 29.8 months; HR = 1.27; 95% CI 1.03–1.55; P = 0.019; Supplementary Figure 1). In the cohort of patients with initially liver-limited mCRC, TTLM was also shorter in KRASmut patients (33.1 vs 15.6 months; HR = 1.82; 95% CI 1.34–2.49; P < 0.001; Figure 2B). Overall survival was not different between the groups (HR = 1.03; P = 0.6; Supplementary Figure 2).

### DISCUSSION

To the best of our knowledge, with 494 patients included, this is the largest retrospective series to analyse the role of KRAS mutational status in pattern of pulmonary metastasis in CRC patients. Lung metastasis was more common at diagnosis of mCRC in patients whose primary tumour carried a KRAS mutation and more likely
During follow-up in all patients with mCRC and during follow-up for the cohort of patients with initially liver-limited mCRC.

have shown that KRAS mutational status has a prognostic role in specific surgical populations, for example, in predicting the risk of recurrence in localised CRC (Hutchins et al, 2011) and after hepatic or lung resection (Vauthey et al, 2013; Schweiger et al, 2014). Our findings confirm the findings of Kim et al (2012) and Tie et al (2011), which identified differences in metastatic patterns according to KRAS status. Our study extends this finding to demonstrate that, in patients with liver-limited mCRC at diagnosis, lung metastasis is more likely to develop in patients with KRAS mutations. This finding provides validation of the recent report by Vauthey et al (2013) that demonstrated that rate of lung recurrence after hepatectomy is higher for tumours with a KRAS mutation, reiterating the potential clinical relevance of this mutation in selecting patients for surgical intervention.

Although our sample size is larger than those of other studies, our study does have a few limitations. First, it is a retrospective analysis of patients from a single institution and thus is subject to the bias of patterns of referral to major academic medical centres and could also explain the young median age observed, which reflects the population from the M.D. Anderson Cancer Center. However, the groups were well balanced according to age (P = 0.971–Table 1) and, therefore, it was not a confounding factor in the analysis of end points between groups (KRASWT vs KRAS MUT), with a low, if any, impact in the external validity of this study. Second, KRAS status was obtained from routinely available archival material and does not incorporate potential discordance in KRAS status between a primary tumour and its metastases. However, KRAS mutations are thought to be highly concordant during the progression of the disease (Artale et al, 2008; Ettienne-Grimaldi et al, 2008; Santini et al, 2008; Cejas et al, 2009). Third, the traditional sequencing (Sanger) method (with ~20% allele frequency as lowest threshold for detection) or a mass spectroscopy method (~15% allele frequency) for detection of KRAS mutations was used, both of which have lower sensitivity for mutation analysis than current next-generation sequencing approaches (~5% allele frequency; Jimeno et al, 2009; Plesec and Hunt, 2009). However, the relevance of the additional low-allele frequency mutations revealed by the newer methodology is not clear and awaits for further validation.

While our analysis was limited to KRAS codons 12, 13 and 61, KRAS codon 146 and NRAS codons 12, 13 and 61 may exhibit similar behaviour. Extended RAS testing including these codons has improved the predictive ability for EGFR monoclonal antibody therapy and is being incorporated into standard-of-care testing (Douillard et al, 2013; Stintzing et al, 2013). Collectively, the accumulating data suggest that the RAS mutations confer similar biology, but the association of extended RAS mutation with tropism to lungs remains to be defined. A few retrospective series to develop during the disease course, suggesting a potential metastatic tropism of CRC to the lung that may have clinical significance for treatment planning.

Despite the lack of difference in OS by KRAS mutational status in the general population of patients with mCRC, several studies
have suggested heterogeneous behaviour among the types of KRAS mutation. More recently, Modest et al (2011) showed that, compared with mCRCs that have a KRAS codon 12 mutation, those with a codon 13 mutation present as more aggressive disease, with a statistically significantly higher rate of synchronous organ metastasis. Despite this, in our cohort there was no difference in lung metastasis frequency or pattern among cases with KRAS mutation in codons 12, 13 or 61.

These results support the conclusion that KRAS status can be used to risk stratify patients for development of pulmonary metastasis. Surveillance might be more focused on lung metastasis in patients with KRASmut after curative locoregional treatment and when considering surgical options for oligometastatic disease.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and design, acquisition of data or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version to be published.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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