Original Article

The effects of bisphosphonate and radiation therapy in bone-metastatic lung adenocarcinoma: the impact of KRAS mutation

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Background: KRAS mutation is the most common genetic alteration in lung adenocarcinoma (LADC) in Western countries and is associated with worse outcome in bone-metastatic cases. Yet, to date, no effective treatment guidelines were developed for these patients. Accordingly, our aim was to investigate the impact of KRAS mutation on bisphosphonate (BTx) and radiation therapy (RTx) in bone-metastatic LADC patients.

Methods: Clinicopathological variables of 134 consecutive LADC patients with bone metastases at diagnosis and known KRAS status were retrospectively analyzed. The effects of BTx, RTx and KRAS mutation on overall survival (OS) were investigated.

Results: Of the total cohort, 93 patients were identified as KRAS wild-type (WT) (69.4%) and 41 (30.6%) as KRAS mutant patients. The presence of KRAS mutation was associated with significantly reduced median OS (5.1 *vs.* 10.2 months in KRAS WT patients; P=0.008). Irrespective of KRAS mutational status both BTx (P=0.007) and RTx (P=0.021) conferred a significant benefit for OS. Notably, however, when analyzing the patients with KRAS-mutant and KRAS WT tumors separately, the benefit from BTx and RTx on OS remained statistically significant only in KRAS WT patients (P=0.032 and P=0.031, respectively).

Conclusions: KRAS mutation is a strong negative prognostic factor in bone-metastatic LADC patients. Both BTx and RTx can increase the OS with a pronounced benefit for patients with KRAS WT tumors. Altogether, KRAS mutational status should be considered during therapeutic decision making in bone-metastatic LADC patients.

Keywords: Bone metastases; lung adenocarcinoma (LADC); KRAS mutation; bisphosphonate therapy; radiation therapy

Submitted Jun 17, 2020. Accepted for publication Nov 23, 2020.
doi: 10.21037/tlcr-20-754
View this article at: http://dx.doi.org/10.21037/tlcr-20-754

Introduction

Lung cancer remains the most frequently diagnosed cancer worldwide (11.6% of the total cases) and the leading cause of cancer-related deaths (18.4% of the total cancer deaths) (1). Histologically, the most common type of lung cancer is adenocarcinoma [lung adenocarcinoma (LADC)], comprising around up to 40% to 50% of all lung cancer cases (2,3). Unfortunately, the majority of patients already have advanced-stage disease at diagnosis with different types of distant organ metastases (4).

Bone is a common site of metastatic cancer spread in LADC since about 25–40% of all advanced-stage LADC patients develop skeletal metastases during the course of their disease (5-7). These metastases are associated with short overall survival (OS) (usually less than 1 year from diagnosis), loss of functional independence and reduction in quality of life (5,7). Accordingly, bone metastases are often complicated by skeletal-related events (SREs), including pathological fractures, spinal cord compression, and hypercalcemia (5). It is not surprising therefore that there is an urgent necessity for the development of improved therapeutic strategies in order to mitigate the deaths and disabilities caused by bone metastases. To date, bisphosphonates are one of the most commonly used therapeutic agents to prevent and reduce the incidence and delay the onset of SREs in LADC patients regardless of mutational status (7). Bisphosphonates are specific inhibitors of the osteoclast activity thus leading to bone resorption inhibition (8). Moreover, they also decrease osteoblast proliferation and stimulate bone-forming and differentiation (8). Furthermore, based on the results of preclinical studies on non-small cell lung cancer (NSCLC) cell lines, bisphosphonate therapy (BTx) seems to have direct antitumor effects as well by inhibiting proliferation, inducing apoptosis, and modulating the immune microenvironment (9). Besides BTx, radiation therapy (RTx) is also frequently used in bone metastatic LADC for stabilization of impending pathologic fractures and treatment or prevention of spinal cord compression, pathologic fractures and bone pain (10).

In the era of precision medicine, oncogenic driver mutations have a major impact on the therapeutic strategies in LADC (11). Importantly, the most common gain-of-function alterations in LADC are the carcinogenic Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations, accounting for approximately 25% to 30% of all LADCs in Western countries and about 10–15% in Asian patients (12,13). Although the role of KRAS mutations in LADC is intensely investigated, its prognostic and predictive power in these patients remains controversial (11). Initially, KRAS mutations have been defined as a negative prognostic factor with unfavorable survival rates and disease-free survival compared to KRAS wild-type (WT) tumors (14-16). These results were further supported by the results of two meta-analyses also concluding that KRAS mutation is a negative prognosticator in LADC (17,18). Meanwhile, a more recent comprehensive study including more than 1,500 NSCLC patients from four trials of adjuvant chemotherapy (CTx) concluded that KRAS mutation has no clear prognostic or predictive relevance (19). Altogether, its general prognostic role is rather controversial, and emerging evidence suggests that the metastatic site-specific variations in LADC might as well influence the prognostic importance and potential clinical relevance of KRAS mutation (20). Accordingly, our group previously found that the presence of KRAS mutations in bone metastatic LADC patients might indeed be associated with significantly worse outcomes, but limited data is available regarding the association between KRAS mutational status and the impact of therapeutic approaches in these patients (20). Therefore, in order to better understand the influence of KRAS mutational status on therapeutic approaches, our aim was to investigate the role of BTx and RTx in KRAS WT *vs.* KRAS mutant LADC patients diagnosed with bone metastasis.

We present the following article in accordance with the REMARK reporting checklist (available at http://dx.doi.org/10.21037/tlcr-20-754).

Methods

Ethics statement

Our research was conducted in accordance with the guidelines of the Helsinki Declaration (as revised in 2013) of the World Medical Association and with the approval of the national level ethics committee (Hungarian Scientific and Research Ethics Committee of the Medical Research Council, 52614-4/2013/EKU), which waived the need for individual informed consent for this retrospective study.

Study population

Consecutive patients who were diagnosed with histologically confirmed LADC and simultaneous bone metastasis at the National Institute of Oncology, Budapest, Hungary and

National Korányi Institute of Pulmonology, Budapest, Hungary between January 1998 and November 2013 were included in this study. Of note, none of the included patients presented any other distant organ metastases at the time of diagnosis. Tumor tissue samples for routine histopathologic examination and molecular pathology testing were obtained via endobronchial biopsy or CT guided lung biopsy. The bone metastases were identified by CT scan, PET-CT or MRI of the skeleton. The demographic and clinicopathological characteristics of the patients including gender, age, CTx, BTx and RTx, KRAS mutational status and OS were retrospectively collected. OS was estimated from the time of diagnosis of bone metastasis, until death, or last available follow-up, performed in July 2013. TNM stage according to the Union for International Cancer Control (7th edition) was also recorded at the time of diagnosis (21).

KRAS mutation analysis

For the current study, all mutational analyses were performed at the 2nd Department of Pathology of the Semmelweis University or at the National Institute of Oncology, as previously described (22). In brief, tumor-rich area on H&E staining was specifically determined by pathologists prior to macrodissection from the formalin fixed paraffin-embedded (FFPE) tissue samples to include predominantly tumor cells without significant necrosis or inflammation. Based on the validated instructions of the manufacturer, DNA was extracted using the MasterPure™ DNA Purification Kit (Epicentre Biotechnologies, WI). KRAS mutational status was screened by a microfluid-based restriction fragment detection system characterized by 5% mutant tumor cell content sensitivity (22,23).

Treatment

According to the National Comprehensive Cancer Network (NCCN) clinical practice guidelines (24), only Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0 or 1 LADC patients were included, since higher PS contraindicates the use of cytotoxic CTx and RTx. Drug administration was performed in accordance with contemporary NCCN guidelines and the Hungarian health care financial regulations. Regarding CTx, patients were treated with platinum-based combination CTx either with paclitaxel and carboplatin (PC), gemcitabine and cisplatin (GC), or etoposide and cisplatin (EC). RTx mainly included palliative external beam RTx. BTx included either first-generation bisphosphonate clodronate, or second-generation bisphosphonate pamidronate or zoledronic acid administered intravenously in 4-week cycles.

Statistical analysis

Patients were grouped according to their KRAS mutational status (WT KRAS and KRAS mutant) and treatment (CTx, RTx or BTx). The correlation of clinicopathological parameters with KRAS status and therapeutic modalities was analyzed by Chi square test. OS was demonstrated by Kaplan-Meier curves and for univariate analysis both Mantel-Cox and Gehan-Breslow-Wilcoxon tests were used. Multivariate analysis was performed using a Cox regression model. Metric data is shown as median or mean and corresponding range or as median and corresponding 95% CI in case of OS. Differences between groups were considered to be statistically significant at a P value of <0.05. Statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) and with the PASW Statistics 24.0 package (Predictive Analytics Software, SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics and KRAS mutational status

A total of 134 patients diagnosed with LADC and simultaneous bone metastasis were included in this study as shown in *Table 1*. Ninety-three patients of the full cohort were identified as KRAS WT (69.4%) and 41 (30.6%) as KRAS mutant patients. The mean age of patients with KRAS mutation was found to be significantly lower than those with WT KRAS (58.9 *vs.* 62.9, respectively; P=0.029; *Figure 1A*). Eighty-three patients (62%) received BTx and the mean age was significantly lower among patients with BTx than among those without BTx (mean age 60.3±9.2 *vs.* 64.0±10.3, respectively; P=0.03; *Figure 1B*). With regards to specific bisphosphonate agents 37, 9 and 28 patients received clodronate, pamidronate and zoledronic acid, respectively. Of note, no data was available on the exact type of administered bisphosphonate agent in 9 cases. Our cohort consisted of 85 male and 49 female patients and no significant association was observed between gender and mutational status or therapeutic modality. KRAS mutation showed no association with ECOG score. The administration of RTx or BTx was also not significantly associated with KRAS

Table 1 Patient characteristics grouped by KRAS mutation and bisphosphonate treatment

Characteristics	All patients	KRAS status			Bisphopshonate therapy		
		Wild-type	Mutant	P	Yes	No	P
Total	134 (100%)	93 (69.4%)	41 (30.6%)		83 (61.9%)	51 (38.1%)	
Age (mean ± SD)	61.7±9.8	62.9±9.4	58.9±10.2	0.029	60.3±9.2	64.0±10.3	0.03
Gender				0.25			0.86
Female	49 (36.5%)	31 (33.3%)	18 (43.9%)		31 (37.3%)	18 (35.3%)	
Male	85 (63.5%)	62 (66.7%)	23 (56.1%)		52 (62.7%)	33 (64.7%)	
ECOG				0.7			<0.0001
0	84 (62.7%)	57 (61.3%)	27 (65.8%)		64 (77.1%)	20 (39.2%)	
1	50 (37.3%)	36 (38.7%)	14 (34.2%)		19 (22.9%)	31 (60.7%)	
Radiotherapy				0.34			0.01
Yes	53 (39.5%)	34 (36.5%)	19 (46.3%)		40 (48.2%)	13 (25.5%)	
No	81 (60.5%)	59 (63.5%)	22 (53.7%)		43 (51.8%)	38 (74.5%)	

KRAS, Kirsten rat sarcoma viral oncogene homolog; ECOG, Eastern Cooperative Oncology Group; SD, standard deviation.

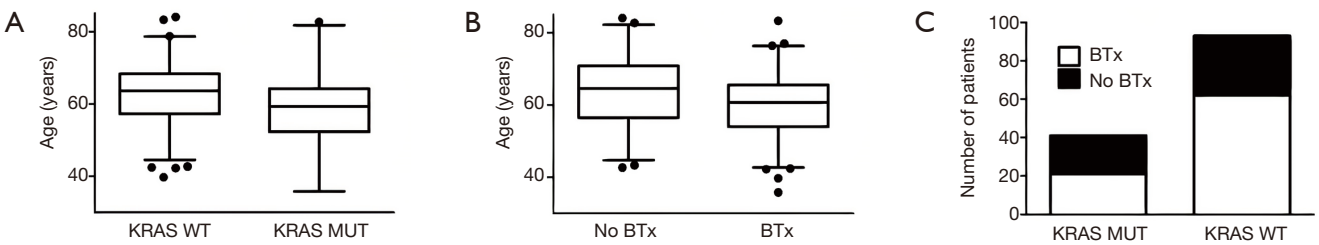

Figure 1 Patient characteristics according to KRAS mutational status and therapeutic modalities. (A) The mean age of patients with KRAS mutation was significantly lower than those with WT KRAS (58.9 *vs.* 62.9, respectively; P=0.029). (B) Patients treated with BTx had a significantly lower mean age (*vs.* patients who did not receive BTx, mean age 60.3±9.2 *vs.* 64.0±10.3, respectively; P=0.03). (C) No significant association was observed between KRAS mutational status and the administration of BTx. BTx, bisphosphonate therapy; WT, wild-type.

mutational status (*Table 1* and *Figure 1C*, respectively). In contrast, patients receiving BTx were significantly more likely to have ECOG 0 and RTx (*Table 1*).

KRAS mutation associates with inferior OS

The median OS for the entire cohort was 7.8 months. Patients with KRAS WT tumors had a significantly longer median OS compared to those with KRAS mutation (10.2 *vs.* 5.1 months, respectively; *Figure 2A*, *Table 2*). With regards to combination CTx, no significant differences in OS have been observed in patients treated with PC *vs.* GC or EC (P=0.297, Figure S1). In contrast, Kaplan-Meyer curves demonstrated longer median OS in patients who received BTx (10.1 *vs.* 4.3 months in BTx-naive patients; *Figure 2B*). Notably, patients receiving second-generation BTx exhibited significantly superior OS compared to those receiving first-generation BTx (median OSs were 13.2 *vs.* 7.1 months, respectively; P=0.041; Figure S2). In regards with RTx, the median OS was higher among the patients receiving RTx compared to RTx-naive patients (11 *vs.* 5.9 months, *Figure 2C*). Importantly, the difference in survival between the groups dichotomized by therapeutic modalities disappears for the late events (*Figure 2B*,*C*), accordingly only the Gehan-Breslow-Wilcoxon tests indicate significant differences. In contrast, KRAS mutational status curves remain separated for the entire survival range and thus KRAS status has a highly significant impact on survival both by

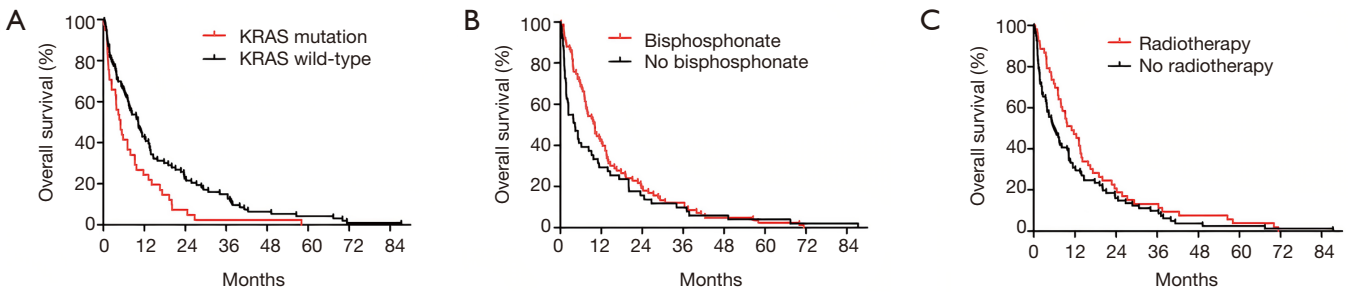

Figure 2 Kaplan-Meier estimates for OS in bone metastatic LADC patients according to KRAS mutational status and therapeutic modalities including BTx and RTx. (A) LADC patients with tumors harboring KRAS mutations had significantly shorter median OS than those with KRAS WT tumors (median OSs were 5.1 *vs.* 10.2 months, respectively; P=0.008). (B) Patients receiving BTx had significantly increased median OS (*vs.* BTx-naive patients; median OS were 10.1 *vs.* 4.3 months, respectively, P=0.007). (C) Similarly, median OS was also significantly increased in LADC patients receiving RTx compared to those who did not receive RTx (median OSs were 11 *vs.* 5.9 months, respectively P=0.021). BTx, bisphosphonate therapy; LADC, lung adenocarcinoma; OS, overall survival; RTx, radiation therapy; WT, wild-type.

Table 2 Prognostic impact of KRAS mutation, radiotherapy and bisphosphonate treatment

Variable	OS (months)	Univariable analysis			Multivariable analysis		
		HR	95% CI	P*	HR	95% CI	P
KRAS status			0.349–0.820	0.008*		0.382–0.833	0.004
Wt	10.2	1			1		
Mutant	5.1	0.535			0.564		
Radiation therapy			0.541–1.076	0.021*		0.505–1.078	0.115
Yes	11.0	1			1		
No	5.9	0.763			0.737		
Bisphosphonate therapy			0.541–1.127	0.007*		0.647–1.404	0.810
Yes	10.1	1			1		
No	4.3	0.781			0.953		

*, Gehan-Breslow-Wilcoxon test. KRAS, Kirsten rat sarcoma viral oncogene homolog; OS, overall survival; wt, wild-type; HR, hazard ratio; CI, confidence interval.

Mantel-Cox and Gehan-Breslow-Wilcoxon tests. Following univariate analysis of the impact of KRAS mutation, RTx and BTx we performed a multivariate analysis using these three factors. The presence of KRAS mutation remained a significant predictor of shorter OS.

KRAS mutation confers inferior outcome in BTx or RTx subgroups

Next, we investigated whether KRAS mutation remains a significant prognosticator in the subgroups of patients receiving BTx or RTx. We found that the OS was significantly higher in the KRAS WT BTx group (*vs.* the KRAS mutant BTx group; the median OSs were 11 *vs.* 5.8 months, respectively; P=0.023; *Figure 3A*). Similarly, KRAS mutation was a strong prognostic factor in the cohort of patients who received RTx (median OS KRAS WT *vs.* KRAS mutant were 13.5 *vs.* 7 months, respectively; P=0.0168, *Figure 3B*).

Importantly, we also found that in the KRAS WT subgroup patients with BTx had significantly increased OS compared to patients without BTx (median OSs were 11 *vs.* 5.2 months, respectively; P=0.032, Gehan-Breslow-Wilcoxon test; *Figure 3A*). As for patients with KRAS mutant tumors, the difference in median OS between patients with or without BTx did not reach statistical

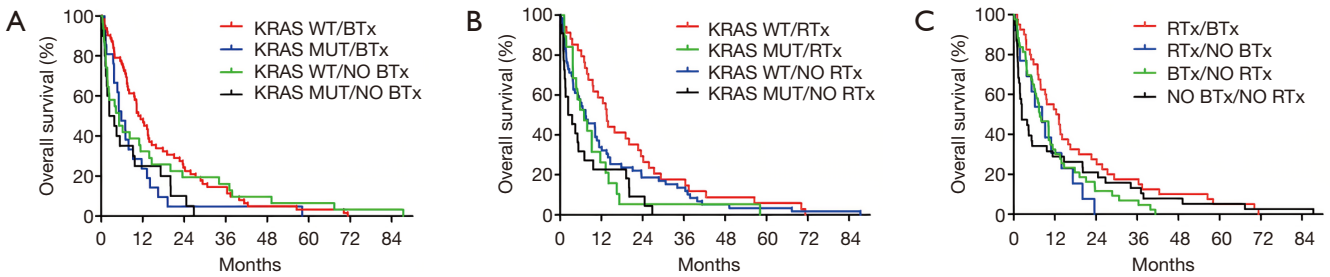

Figure 3 Kaplan-Meier estimates for OS in bone metastatic LADC patients according to KRAS mutational status and specific therapeutic approaches. (A) LADC patients with KRAS WT tumors receiving BTx had significantly increased median OS (*vs.* those with KRAS mutant tumors treated with BTx, median OSs were 11 *vs.* 5.8 months, respectively; P=0.023). With regards to KRAS mutational status, in KRAS WT LADC patients the median OS was significantly increased in patients receiving BTx compared to BTx-naive patients (median OSs were 11 *vs.* 5.2 months, respectively; P=0.032). In contrast, no significant differences in OS have been observed in KRAS-mutant LADC patients with or without BTx (median OSs were 5.8 *vs.* 3.1 months, respectively; P=0.35). (B) RTx-treated patients with KRAS WT tumors exhibited significantly superior OS compared to those with KRAS-mutant tumors (median OSs were 13.5 *vs.* 7 months, respectively; P=0.016). According to KRAS mutational status, in patients with KRAS WT tumors, RTx conferred a significant benefit for OS when compared to patients not receiving RTx (median OS; 13.6 *vs.* 7.4 months; P=0.031). The median OS did not differ significantly in KRAS-mutant LADC patients treated with or without RTx (median OSs were 7 *vs.* 3 months; P=0.12). (C) LADC patients receiving both RTx and BTx had significantly improved OS compared to those who received only RTx or BTx or none of the aforementioned modalities (P=0.031). BTx, bisphosphonate therapy; LADC, lung adenocarcinoma; OS, overall survival; RTx, radiation therapy; WT, wild-type.

significance (median OSs were 5.8 *vs.* 3.1 months, respectively; P=0.35; *Figure 3A*).

Next, we evaluated the effects of RTx in the KRAS mutational status subgroups. In the KRAS WT subgroup, RTx conferred a significant benefit for OS when compared to patients not receiving RTx (median OS; 13.6 *vs.* 7.4 months; P=0.032; *Figure 3B*). As for the patients with KRAS mutation, the median OS difference was not statistically significant (7 months for RTx and 3 months for patients without RTx; P=0.12; *Figure 3B*).

Interaction of radiation therapy and bisphosphonate treatment

Finally, when evaluating the interaction between BTx and RTx irrespective of KRAS mutational status, we found that patients who received both BTx and RTx had a significantly longer OS compared to those who received only BTx or RTx or none of the aforementioned modalities (P=0.031; *Figure 3C*).

Discussion

Despite major improvements and various new treatment modalities for advanced-stage lung cancer, patients with bone metastasis still have a rather poor prognosis (5,25). The median survival is usually less than 1 year from diagnosis of bone metastasis (5,26). In addition, skeletal metastases are often complicated by metabolic disorders such as hypercalcemia, pathologic fractures, and spinal cord compression (7,27). These skeletal related events (SREs) can substantially reduce the quality of life and increase the economic burden (27).

KRAS is the most frequently mutated oncogene in LADC in Western countries, yet limited data is available regarding the clinical relevance of KRAS mutation in LADC patients diagnosed with bone metastases. Therefore, in this cross-sectional retrospective study of bone metastatic LADC patients we evaluated the effects of KRAS mutational status on OS according to BTx and RTx.

In the current cohort of 134 Caucasian patients, we detected 30.6% KRAS mutation frequency which is similar to previous findings of Lohinai *et al.*, Confavreux *et al.*, and Bittner *et al.* (20,28,29). Of note, the KRAS mutation rate in the presented cohort is also similar to the general incidence of KRAS mutation in Caucasian LADC patients (12). Whether the incidence of KRAS mutation is associated with skeletal metastases remains an open question. While Zhao *et al.* found that patients with KRAS-mutated tumors had a higher incidence of bone metastasis than those with the WT gene, others found no significant association between KRAS mutational status and the appearance of skeletal

metastases (30-32). Notably, however, the molecular driver subtypes might also play a key role in the appearance of bone metastases, since Kuijpers *et al.* found that only patients with KRAS G12A mutation had a higher incidence of bone metastases (33). With regards to the effects of KRAS mutational status on survival, we found that patients with KRAS WT tumors had significantly improved median OS than those with KRAS mutant tumors. This observation is in line with data previously reported by us in another large LADC study (20). Nevertheless, it is also important to mention that to date, the prognostic relevance and thus the clinical utility of KRAS oncogenic mutations in LADC in general is still controversial, partly due to vast heterogeneity of the studies in terms of ethnicity, tumor stage, and treatment modality (11,22,34). In our study, however, a relatively homogeneous patient cohort was used, providing evidence that KRAS mutation is indeed a negative prognosticator in advanced-stage LADC patients with bone metastases. In support of this, multivariate analysis also confirmed the role of KRAS mutation as an independent negative prognostic factor in these patients. Importantly, the prognostic relevance of KRAS mutational status was not influenced by the therapeutic approaches, since WT KRAS status was associated with improved OS both in BTx- and RTx-treated patients. To the best of our knowledge, ours is the first detailed evaluation of the prognostic relevance of KRAS mutational status in bone metastatic LADC patients with regards to specific therapeutic approaches including BTx and RTx.

Next, we investigated if KRAS mutational status had an impact on response to BTx and RTx. Bisphosphonates are synthetic analogues of pyrophosphate, a natural regulator of bone metabolism, which inhibits osteoclast-mediated bone resorption, decrease osteoblast proliferation and stimulate bone-forming and differentiation (7,35). In addition, BTx also have direct antitumor effects by inhibiting proliferation, inducing apoptosis, and modulating the immune microenvironment in breast cancer, pancreatic cancer, prostate cancer, and NSCLC under both *in vitro* and *in vivo* conditions (9,36-39). Meanwhile, RTx mainly consists of external beam radiation and is performed primarily to relieve pain, prevent pathologic fractures and spinal cord compression, and consequently to maintain the patient's quality of life (40,41). Altogether, both BTx and RTx plays a crucial role in the management of bone metastases in LADC (41,42). Notably, in our cohort, KRAS WT LADC patients treated with BTx and RTx indeed had significantly improved OS than BTx- and RTx-naive patients, respectively. In contrast, however, neither BTx, nor RTx conferred a significant benefit for OS in patients with KRAS mutant tumors. As reported by a preclinical study, a possible explanation of this observation might be that BTx including zoledronic acid is unable to inhibit the prenylation of mutant KRAS unlike in the case of WT KRAS (43). Therefore, KRAS WT tumor cells are more likely to be inhibited by BTx, leading to reduced proliferation capacity (43). To our knowledge, this is the first study indicating distinct BTx and RTx efficacy with regards to KRAS mutational status in clinical setting. Therefore, our finding supports the proposal that KRAS mutational status should be taken into account when considering BTx and RTx in bone metastatic LADC patients.

Finally, we investigated the distinct effects of BTx and RTx on survival irrespective of KRAS mutational status and found that both therapeutic modalities improve the median OS. In addition, we also found that patients who received both BTx and RTx had longer OS compared to those who received only BTx or RTx or none of the aforementioned modalities. This finding is in line with the results of preclinical studies on multiple myeloma, breast-, prostate- and small cell lung cancer (44-46). The distinct mechanisms for the interaction of systemic BTx with RTx were originally described by Hoskin and Steel (35,47). Accordingly, a possible explanation which lies behind the additive and superadditive effect of these two treatment modalities might be that both BTx and RTx have mostly effect upon osteoclast activity (35). Consequently, through their common action on osteoclasts, a positive interaction in the affected area might be suspected (35). Moreover, based on cell line data, BTx such as zoledronic acid and RTx might also cause DNA damage and intensify cytotoxic activity when given together (48). Yet, to date, the exact mechanisms behind the additive and superadditive effect of BTx and RTx are still rather unknown (48).

The present study had certain limitations given by its retrospective nature. First, no information was available on the exact dose and cycles of the administered BTx and RTx. Due to the relatively long time period, diagnostic methods and treatment guidelines may have changed over the years which might also influence the prognosis. Another limitation was the lack of detailed clinicopathological data regarding disease history, other co-morbidities, and tumor characteristics. Of note, data on detailed smoking history, which may be associated with substitution-specific KRAS mutational status, was also not fully available in our cohort. In addition, as significantly more patients with

ECOG PS score 0 received BTx (*vs.* ECOG 1 patients), the results of the univariate analysis with regards to the efficacy of BTx might be biased. Importantly, however, ECOG 0 and 1 patients typically have similar survival outcomes (both subgroups being labeled as having "good" PS for clinical research purposes) (49). Molecular methods were focusing on the presence or absence of KRAS mutations and the KRAS WT cohort was not analyzed for additional oncogenic driver mutations. However, all EGFR mutant cases were excluded from the study. Additionally, the final number of included patients was relatively small due to our strict inclusion criteria. Nevertheless, this approach enabled us to analyze a homogenous cohort of at-diagnosis bone metastatic lung cancer patients with the same ethnicity, histology and disease stage. Finally, data was not available on specific KRAS mutational subtypes for each case preventing us from a subtype-specific analysis. Thus, altogether, to address the above limitations, our findings require independent confirmation in cohorts with larger available datasets on clinicopathological data.

Conclusions

In summary, our results indicate that KRAS mutation is a negative prognosticator in at-diagnosis bone metastatic LADC regardless of BTx and RTx. Furthermore, this is the first study that comprehensively evaluates the effects of BTx and RTx with respect to KRAS mutational status. Accordingly, both BTx and RTx can increase the OS with a pronounced benefit for patients with KRAS WT tumors. Of note, our study also suggests that the concomitant use of BTx and RTx might increase the OS irrespective of KRAS mutational status compared to those receiving only BTx, RTx, or none of the aforementioned therapeutic modalities. Altogether, our findings might not only help to improve the efficacy of BTx and RTx in bone metastatic LADC patients by improving patient selection but might as well contribute to the development of new therapeutic approaches with regards to KRAS mutational status.

Acknowledgments

The authors thank the patients and the clinical teams involved.
Funding: BD, JM and JT acknowledge funding from the Hungarian National Research, Development and Innovation Office (NAP2-2017-1.2.1- NKP-0002 and K129065 to BD and JM, KNN121510 and KH130356 to BD and K116151, NVKP16-1-2016-0004 and NVKP-16-1-2016-0020 to JT). BD and VL were also supported by the Austrian Science Fund (FWF I3522, FWF I3977 and I4677). VL is a recipient of Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences and the UNKP-19-4 New National Excellence Program of the Ministry for Innovation and Technology. ZM was supported by the UNKP-20-3 New National Excellence Program of the Ministry for Innovation and Technology.

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at http://dx.doi.org/10.21037/tlcr-20-754

Peer Review File: Available at http://dx.doi.org/10.21037/tlcr-20-754

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tlcr-20-754). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Our research was conducted in accordance with the guidelines of the Helsinki Declaration (as revised in 2013) of the World Medical Association and with the approval of the national level ethics committee (Hungarian Scientific and Research Ethics Committee of the Medical Research Council, 52614-4/2013/EKU), which waived the need for individual informed consent for this retrospective study.

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Cite this article as: Radeczky P, Megyesfalvi Z, Laszlo V, Fillinger J, Moldvay J, Raso E, Schlegl E, Barbai T, Timar J, Renyi-Vamos F, Dome B, Hegedus B. The effects of bisphosphonate and radiation therapy in bone-metastatic lung adenocarcinoma: the impact of KRAS mutation. Transl Lung Cancer Res 2021;10(2):675-684. doi: 10.21037/tlcr-20-754

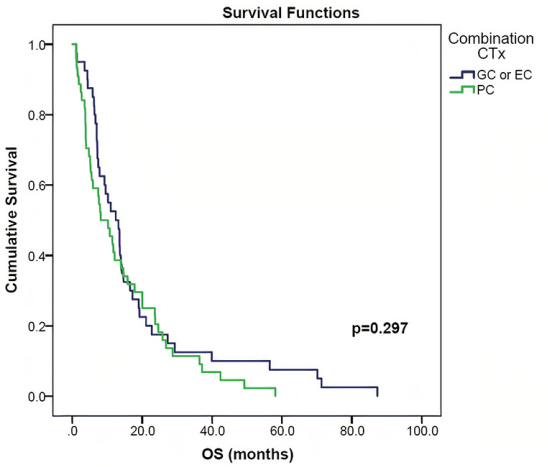

Figure S1 Kaplan-Meier plots for OS in patients with bone metastatic LADC according to combination CTx. The OS did not differ significantly between patients treated with PC *vs.* GC or EC (median OSs were 8.1 *vs.* 12.4 months, respectively; P=0.297, log-rank test). BTx, bisphosphonate therapy; LADC, lung adenocarcinoma; OS, overall survival; PC, paclitaxel and carboplatin; GC, gemcitabine and cisplatin; EC, etoposide and cisplatin.

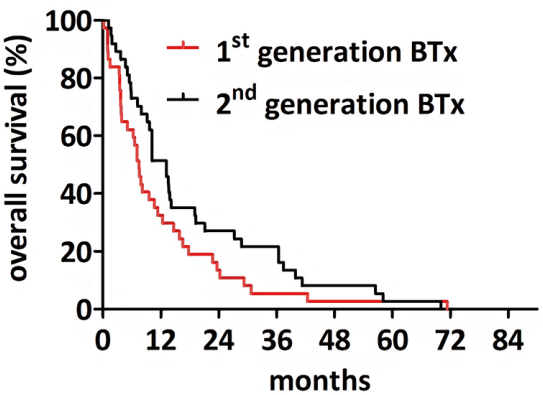

Figure S2 Kaplan-Meier estimates for OS in bone metastatic LADC patients according to different generations of BTx. LADC patients receiving second-generation BTx had significantly increased median OS (*vs.* those treated with first-generation BTx, median OSs were 13.2 *vs.* 7.1 months, respectively; P=0.041, Gehan-Breslow-Wilcoxon test). BTx, bisphosphonate therapy; LADC, lung adenocarcinoma; OS, overall survival.

http://dx.doi.org/10.21037/tlcr-20-754