PhD thesis

Clinical relevance of KRAS mutational status and tumor location in bone-metastatic lung adenocarcinoma

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Budapest

2022

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List of Abbreviations

ALK: anaplastic lymphoma kinase

BRAF: v-raf murine sarcoma viral oncogene homolog B

CI: confidence interval

CT: computed tomography

CTx, chemotherapy

DNA: deoxyribonucleic acid

ECOG: Eastern Cooperative Oncology Group

EGFR: epidermal growth factor receptor

ERK1/2: extracellular signal-regulated kinase 1/2

GTP: guanosine triphosphate

GTPase: small guanine triphosphatase

HR: hazard ratio

KEAP1: Kelch-like ECH-associated protein 1

KRAS: Kirsten rat sarcoma viral oncogene homolog

LADC: lung adenocarcinoma

MEK: mitogen-activated protein kinase

MET: mesenchymal–epithelial transition

MRI: magnetic resonance imaging

MUT: mutant

NCCN: National Comprehensive Cancer Network

NFE2L2: nuclear factor erythroid 2-related factor 2

NSCLC: non-small cell lung cancer

ORR: objective therapeutic response rate

OS: overall survival

PCR: polymerase chain reaction

PD-1: programmed cell death protein 1

PET: positron emission tomography

PFS: progression-free survival

RFS: recurrence-free survival

RTx: radiation therapy

SCC: squamous-cell carcinoma

SCLC: small cell lung cancer

SD: Standard Deviation

SEER: Surveillance Epidemiology and End Results

STK11: serine/threonine kinase 11

TKI: tyrosine kinase inhibitor

TP53: tumor antigen 53

VEGF: vascular endothelial growth factor

WT: wild-type

1. Introduction

1.1. Lung cancer and KRAS mutations

Lung cancer, the leading cause of cancer-related deaths, is the most frequently diagnosed cancer worldwide in both sexes (accounting for approximately 11.6% of the total cases) [1]. The most common histologic subtype of lung cancer is lung adenocarcinoma (LADC), which comprises 40-50% of all lung cancer cases [2, 3].

With the development of molecular pathology and precision medicine, significant progress has been made in the treatment and prognosis of advanced non-small cell lung cancer (NSCLC) over the past two decades [4]. In LADC, the most common gain-of-function alteration is the Kirsten rat sarcoma viral oncogenic homolog (KRAS) mutation, which accounts for about 25-30% of LADCs in Western countries and approximately 10-15% of Asian LADCs [5-8]. The clinicopathological significance of different KRAS mutations is currently intensively studied, as both their prognostic and predictive role is controversial [4].

The KRAS protein, encoded by the KRAS protooncogene, is a small guanine triphosphatase (GTPase) that serves as a binary linker in the signal transduction of most receptor tyrosine kinases, including epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor (MET), or anaplastic lymphoma kinase (ALK), and thus plays a crucial role in tumor progression [4, 9]. Oncogenic mutations in the KRAS gene occur primarily at codon 12 of exon 2, less frequently at codon 13 (3–5%), and very rarely at codon 61 in exon 3 (less than 1%) [4]. These changes are missense mutations that result in impaired ability of KRAS to hydrolyze GTP, leading to constitutive activation of effector pathways and thus malignant transformation and progression [10].

Due to the frequency of LADC, several preclinical and clinical studies have been conducted to explore effective therapeutic options for KRAS mutation. Nevertheless, to date, no effective RAS inhibitors are used in routine clinical practice for LADC except for the KRAS G12C mutation [11].

1.2. Clinicopathological importance of KRAS mutations in LADC

Specific demographic and clinicopathological characteristics of LADC patients correlate with the presence and incidence of KRAS mutations. Recent international surveys suggest that KRAS mutations are most common in Caucasian or African-American patients,

while their frequency is much lower in Asian patients [4, 6, 12]. Additionally, a comprehensive analysis of resected NSCLC tumors found that KRAS mutations are significantly more common in women and younger patients (although only the latter remained statistically significant in multivariate analyzes [p = 0.044]) [13, 14].

Interestingly, smoking also leaves a molecular fingerprint on KRAS status: transitions (G12D) are more common among never smokers, while transversions (G12C and G12V) are more frequently detected among former or current smokers [15, 16]. In addition, smokers generally develop more genetically complex KRAS mutant tumors, characterized by a higher mutational burden and a higher frequency of cellular tumor antigen 53 (TP53) and serine/threonine kinase 11 (STK11) mutations [5, 15, 17].

Recently, several publications investigated the specific histological features of KRAS mutant lung cancer. Although initial studies [18, 19] have reported that KRAS mutations may be present not only in LADC but also in squamous cell carcinoma (SCC), subsequent analyses using the latest state-of-the-art differential diagnostic criteria suggest that KRAS mutations does not occur in pure pulmonary SCCs but only in the LADC component of mixed adenosquamous carcinomas [20]. Another critical issue is the clinical relevance of specific KRAS mutations and their co-occurrence with other mutations. It is well known that different KRAS mutation subtypes have been associated with different biological behaviors [21, 22]. For example, tumors with KRAS G12C mutations show increased extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation compared to KRAS G12D tumors [6, 23]. A recent study on a KRAS-mutant mouse model showed that the mitogen-activated protein kinase (MEK) inhibitor selumetinib is more effective in KRAS G12C than in KRAS G12D tumors [23]. Accordingly, different KRAS mutations can cause differentiated induction of signaling cascades, thus leading to specific drug susceptibility profiles [24]. Regarding co-occurring mutations/conversions, double mutants (KRAS and EGFR/ALK/v-raf murine sarcoma viral oncogene homolog B1 (BRAF)) are rare in LADC, and KRAS mutations are typically present as a single mutation [25-27]. However, KRAS mutations often co-occur with tumor suppressor genes (e.g., TP53, STK11, and Kelch-like ECH-associated protein 1 (KEAP1)/ nuclear factor erythroid 2-related factor 2 (NFE2L2), and there is increasing evidence that these co-occurring mutations are associated with unique tumor characteristics and biological behaviors [28]. In summary, different amino acid substitutions and contiguous mutations

in the KRAS oncogene highlight the need for genotype-specific analysis to identify clinically relevant subgroups of patients [6].

1.3. Prognostic role of KRAS mutation in NSCLC

The prognostic significance of the different KRAS mutations remains controversial in NSCLC. Previous publications from the late 1980s found that KRAS mutation is a negative prognostic factor in NSCLC [29, 30]. However, the majority of these studies were characterized by processing very heterogeneous patient material, both in terms of tumor stage and methodology [31-35]. The most relevant early publications examined prognosis in surgically resected LADC cases, while stage IIIB-IV NSCLC patients were included in later studies [30-32, 35-37]. Mascaux et al. performed a meta-analysis of 53 studies and found that the KRAS mutation was associated with a significantly worse prognosis than the wild type. (Hazard ratio [HR]: 1.40; $p = 0.01$; HR: 1.5 in LADC; $p = 0.02$). This is strongest evidence of KRAS being a negative prognostic factor in NSCLC [38]. In contrast, in another study, Villaruz et al. analyzed the data from 998 LADC patients (from which 318 patients had a KRAS-mutant tumor), and they found that the presence of KRAS mutation was not an independent prognostic factor [39]. Similar results were yielded by a meta-analysis summarizing the results of four separate clinical trials. After analyzing the data of 1500 NSCLC patients (300 with KRAS-mutant tumor), the authors concluded that KRAS status does not influence the survival outcomes in surgically treated patients receiving adjuvant chemotherapy [14]. Another study examining the role of the KRAS mutation in circulating tumor DNA showed worse progression-free survival (PFS) and overall survival (OS) for KRAS mutant genotypes [40].

A meta-analysis of 41 clinical trials with 6939 patients also assessed the KRAS mutation in NSCLC as an adverse prognostic factor (HR: 1.39; 95% CI: 1.24–1.55). Among other things, the authors compared the outcome in each ethnic group. They found that HR was significantly higher in Asian patients, suggesting that KRAS mutations had a worse prognosis in Asian patients compared to non-Asians [41]. This was recently supported by another meta-analysis involving more than 9000 patients [42].

Several studies have concluded that due to the heterogeneity of KRAS mutations, specific mutation subtypes might have different effects on survival and therapeutic response. In a

mutation subtype-specific survey of 505 patients diagnosed with stage III-IV LADC and treated with chemotherapy (CTx), there were no significant differences in either PFS or OS. However, when analyzed separately, patients with G12V mutant tumors showed a better therapeutic response and a moderately longer PFS compared to those with other codon 12 KRAS mutations (G12X) [36]. In addition, two retrospective studies have found that the OS is shorter in the case of the G12C mutation [43]. In their work, Garassino et al. revealed further important information about the role of KRAS mutational subtypes. The susceptibility of tumor cells carrying different KRAS subtype mutations to CTx was studied *in vitro*. Their results showed that G12D transformation led to paclitaxel resistance and increased sensitivity to sorafenib. In contrast, G12C reduced the effect of cisplatin while it sensitized cells to paclitaxel and pemetrexed. In addition, G12V mutations also increased the sensitivity of cells to cisplatin [24]. A partial result of a study by Villaruz et al. showed that G12C moderately increased OS compared to other subtypes [39]. These results discussed above are shown in Table 1.

Table 1. Prognostic relevance of KRAS status in NSCLC

Studies	Results (KRAS as a prognostic factor)	Patients	Treatment	Study format
Slebos et al., 1990 [30]	Negative prognostic factor RFS p=0.038 Deaths due to cancer p<0.001	Stage I-IIIA ADC n = 69	Surgery	single center, case series
Mascaux et al., 2005 [38]	Negative prognostic factor OS (HR 1.5 for ADC)	NSCLC n = 5216	various	meta-analysis (53 studies)
Ihle et al., 2012 [37]	Not significant G12V + G12C (p=0.046) are negative prognostic factors	Stage IV ADC n = 215	CTx + EGFR TKI	data from the phase II study, BATTLE trial
Shepherd et al., 2013 [14]	Not significant HR 1.04 G12x HR 1.01 G13x	Stage I-III ADC n = 1543	Surgery / adjuvant CTx	meta-analysis (4 studies)
Guan et al., 2013 [31]	Negative prognostic factor for OS but not for PFS OS (HR 2.69; p<0.001), PFS (p=0.27)	NSCLC n = 273	Surgery (n=112) / CTx (121) / CTx-RTx (12) / EGFR TKI (75)	single center, retrospective, case matching
Villaruz et al., 2013 [39]	Not significant OS (p=0.612) PFS (p=0.89).	Stage I-III ADC n = 988	various	single center, retrospective
Meng et al., 2013 [41]	Negative prognostic factor HR: 1.45 (95% CI: 1.29–1.62) Especially for early stage and Asian ethnicity	NSCLC n=6939	various	meta-analysis (41 studies)
Cserepes et al., 2014 [36]	Not significant Only for G12V (p=0.016)	Stage IIIB-IV ADC n = 505	CTx	single center, retrospective
Izar et al., 2014 [32]	Negative prognostic factor OS (p=0.0001) and DFS (p<0.0001)	Stage I ADC n = 312	Surgery	single center, retrospective
Ohtaki et al., 2014 [35]	Negative prognostic factor 2-year survival (18% KRAS vs 81% EGFR vs 47% WT)	Stage I-IV ADC n = 58	Surgery	single center, case series
Renaud et al., 2016 [21]	Not significant Only in G12V (OS: 26 vs 60 months; PFS: 15 vs 24 months)	Stage I-IIIA NSCLC n=841	Surgery / adjuvant CTx	single center, retrospective
Fan at al., 2017 [40]	Negative prognostic factor PFS (HR=1.83, 95% CI 1.40-2.40, p<0.0001) and OS (HR=2.07, 95% CI 1.54-2.78, p<0.00001)	NSCLC n=2293	EGFR TKI	meta-analysis (13 studies) circulating tumor DNA

1.4. Predictive value of KRAS mutations

In the treatment of NSCLC, platinum-based CTx remains the most commonly used systemic therapy. Most studies do not consider the KRAS mutation to be a predictive factor for CTx. The role of KRAS has been studied in patients with advanced-stage, metastatic NSCLC receiving definite CTx [44] and in patients receiving adjuvant CTx and radiotherapy (RTx) after surgery [45]. Of particular note is the Phase III TRIBUTE study, in which erlotinib or placebo was added to first-line carboplatin/paclitaxel [46]. However, in none of these studies did the KRAS mutation prove to predict therapeutic response, PFS, or OS. In contrast, the results of the JBR10 trial, which examined the effect of postoperative vinorelbine or cisplatin in resected stage IB or II NSCLC, were recently reported. Although the benefit of CTx was seen only in KRAS wild-type (WT) patients, the difference was not significant ($p = 0.29$) [47]. The phase III IFCT-0002 study comparing neoadjuvant carboplatin/paclitaxel and cisplatin/gemcitabine combinations should be also mentioned. KRAS mutant tumors have been shown to respond less well to CTx in the univariate analysis. However, this was not subsequently confirmed by multivariate analysis [48]. A further retrospective study also found the KRAS mutation to predict poorer outcomes in patients with advanced lung cancer receiving cytotoxic CTx [49]. An exciting result of this study was that it was shown that the co-mutation of TP53 and KRAS further worsened the outcome [14]. It is noteworthy that several studies have shown a potential adverse effect of codon 13 mutation [14, 24], suggesting that the KRAS mutation may be a negative predictor of CTx, but presumably not for all KRAS mutations subtypes.

The predictive value of KRAS mutation is also unclear in case of targeted therapies. Most studies have been performed with EGFR tyrosine kinase inhibitors (TKIs). Most published research includes a meta-analysis summarizing 22 publications, showing that the KRAS mutation is a significant negative predictive factor [46, 50-52]. Accordingly, patients with KRAS mutant tumors treated with the EGFR TKI had a worse objective therapeutic response rate (ORR), PFS, and OS than patients with KRAS WT tumors. [46, 51, 52]. However, despite the convincing results, contradictions persist, and not all studies reach a similar conclusion [53, 54]. One possible explanation for this might be that the response to EGFR TKIs may be influenced by the presence or absence of KRAS

mutations and the type of KRAS codons and amino acid substitutions. [4, 55]. This is supported by a recently published study on the treatment of EGFR-TKI in advanced lung cancer, where worse therapeutic efficacy was reported for G12C and G12V subtypes (vs. G12D and G12S mutational subtypes) [56]. All in all, patients with KRAS-mutant tumors generally show worse survival outcomes with EGFR-TKI therapy than those with KRAS WT tumors.

Although it is well known that the RAS signaling pathway affects the expression of the vascular endothelial growth factor (VEGF) molecules [57], very few studies have analyzed the effects of different KRAS mutations on the efficacy of angiogenesis-inhibiting therapies. Two groups published their findings that G12V, G12A [58], and G12D [59] mutations were associated with worse survival in colorectal cancer patients treated with bevacizumab. With regards to NSCLC, a phase II study examined the efficacy of bevacizumab in combination with neoadjuvant docetaxel and cisplatin, and found that none of the ten patients carrying KRAS mutation showed a pathological response to these therapeutic agents, whereas significant tumor regression was observed in 35% of KRAS WT patients [60]. We recently published a retrospective study in advanced NSCLC, which revealed that KRAS mutations (especially the G12D mutation) are associated with worse PFS and OS in patients treated with platinum-based CTx plus bevacizumab. In a multivariate analysis, the G12D KRAS mutation was an independent adverse prognostic factor [61].

Regarding the novel immune checkpoint inhibitor therapies emerging data show that the expression of programmed cell death protein 1 (PD-1) associates with KRAS status ($p=0.006$) [62]. Accordingly, it has recently been suggested that determining KRAS mutational status could also predict the efficacy of immunotherapy. This is supported by the fact that a clear survival advantage has been achieved with immune-checkpoint inhibitors in KRAS mutant patients [63]. In another study, Gettinger et al. observed good response and more prolonged survival with nivolumab monotherapy, but EGFR or KRAS driver mutations did not show this effect [64]. Further clinical trials are needed to resolve this issue.

The results analyzing the predictive value of the KRAS mutation are summarized in Table 2.

Table 2. Predictive role of KRAS mutations in NSCLC

Study	Pts tested for KRAS	KRAS status		Treatment	Endpoint	KRAS status	
		KRAS mutant	KRAS WT			KRAS mutant	KRAS WT
Rodenhius et al., 1997 [44]	62 (stage III-IV)	16	46	carboplatin + ifosfamide + etoposide	ORR %	19	26
					PFS*	4	5
					OS*	8	9
Schiller et al., 2001 [45]	184 (stage II-IIIA)	44	140	cisplatin + etoposide	OS	24.7	42
Eberhard et al., 2005 [46]	133 (advanced stage)	25	108	carboplatin + paclitaxel + erlotinib	ORR%	23	26
					PFS	6	5.4
					OS	13.5	11.3
Khambata-Ford et al., 2010 [65]	202 (stage IIIB, IV)	35	167	taxane + carboplatin + cetuximab	ORR%	30.80	32.90
					PFS	5.60	5.10
					OS	16.8	9.7
Ludovini et al., 2011 [66]	166 (stage III, IV)	11	151	EGFR TKI	ORR%	0	35.7
					PFS	2.7	5.6
					OS	19.3	28.6
Fiala et al., 2013 [67]	448 (stage IIIB, IV)	69 (G12C: 38)	379	EGFR TKI	PFS (weeks)	4.3 (G12C) vs 9.0 (non-G12C)	
					OS (weeks)	12.1 (G12C) vs 9.3 (non-G12C)	
Zer et al., 2016 [56]	785 (stage IIIB-IV)	155	630	EGFR TKI (pooled analysis)	OS	4.5	6
Hames et al., 2016 [49]	150 (stage IV)	80	70	standard CTx	PFS	4.7	5.7
					OS	8.8	13.5
Dong et al., 2017 [63]	34 (not specified)	8	26	pembrolizumab	ORR%	25	6.6
	20 (not specified)	5	15	pembrolizumab or nivolumab	PFS	14.7	3.5
Gettinger et al. 2018 [64]	129 (advanced stage)	8	13	nivolumab	5-year survival	18%	25%
Ghimessy et al. 2019 [61]	247 (IIIB-IV)	95	152	standard CTx + bevacizumab	PFS	7.03	8.63
					OS	14.23	21.57

1.5. Clinical importance and management of bone metastases in LADC

Notably, the majority of LADC patients already have metastatic disease at diagnosis, with different distant organ metastases. Skeletal metastases are the most frequently diagnosed extrathoracic metastases in LADC since about 25-40% of all advanced-stage patients present with bone metastases [68-70].

The development of bone metastases is a significant oncological problem, as their appearance dramatically influences not only the treatment algorithm but also the patient's quality of life. Furthermore, pain from bone metastases, bone remodeling, spinal cord compression, and pathological fractures (and consequent loss of function) also significantly shorten a patient's OS (which in most cases is less than a year after bone metastasis diagnosis) [68-70]. Choosing the right treatment strategy to increase the OS and reduce the loss of function can be essential for bone metastases. In terms of treatment guidelines, today, the most commonly used therapeutic agents are various bisphosphonates, which are indicated regardless of the mutational status of the tumor [71]. These synthetic pyrophosphate analogs reduce the rate of bone resorption and stimulate bone formation by inhibiting osteoclast function and reducing osteoblast proliferation [71]. However, a study on NSCLC cell lines has shown that bisphosphonate therapy (BTx) has significant antitumor activity when used alone by inhibiting proliferation, inducing apoptosis, and regulating the immune microenvironment [72]. Another form of treatment for pain-inducing bone metastases is radiotherapy (RTx), which is initially primarily symptomatic but can later be used in fractionated doses [73]. During spinal cord compression (if a spinal surgical solution is not feasible), RTx may also be justified [73].

2. Objectives

In a previous study, our research team demonstrated that KRAS mutation is associated with significantly shorter survival (compared to KRAS WT) in LADC patients with bone metastases [74]. However, the therapeutic relevance of KRAS status is currently unknown in this patient population. Furthermore, we demonstrated in preclinical NSCLC models that KRAS WT LADC cell lines are more sensitive to zoledronic acid-induced prenylation inhibition and consequent inhibition of proliferation both *in vitro* and *in vivo*. At the same time, those carrying KRAS mutations are resistant to this inhibitory effect [75]. Therefore, we aimed to investigate the significance of KRAS mutational status according to BTx and RTx in LADC patients diagnosed with bone metastases.

Furthermore, although the presence of distant organ metastases is a significant factor for an unfavorable prognosis in LADC patients, metastatic patterns and their influence on survival have not been extensively analyzed with regards to the localization of the primary tumor (i.e., bronchoscopic, side-specific and region-specific). Our group previously found that bone metastases were more frequent in patients with central tumors, whereas lung metastases in those with peripheral LADCs [69]. Additionally, central LADCs were also associated with early metastatic spread [69]. However, to date, the bone metastasis pattern is still largely unexplored in patients with bone-only metastases. Therefore, our cross-sectional study aimed to examine the impact of primary tumor location on bone metastasis site, type of affected bones and survival in a large comprehensive cohort of advanced-stage LADC patients diagnosed with skeletal metastases. This information may help to guide early surveillance for bone metastasis detection or interventions in high-risk groups to improve the patients' survival and quality of life.

3. Results

3.1. The effects of bisphosphonate and radiation therapy in bone metastatic lung adenocarcinoma

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 3. 93 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). 83 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 mutational status (Table 3 and Figure 1C, respectively). In contrast, patients receiving BTx were significantly more likely to have ECOG 0 and RTx (Table 3).

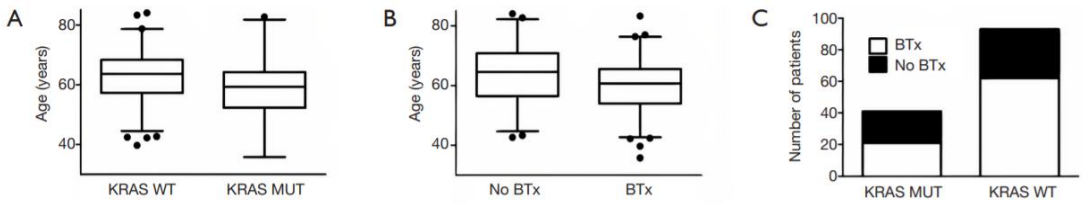

Figure 1. Patient characteristics according to KRAS mutational status and therapeutic modalities.

(***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 and the administration of BTx.

Table 3. Patient characteristics grouped by KRAS mutation and bisphosphonate treatment

	All patients	KRAS status		p	Bisphopshonate therapy		p
		wild-type	mutant		yes	no	
	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							
Female	49 (36.5%)	31 (33.3%)	18 (43.9%)	0.25	31 (37.3%)	18 (35.3%)	0.86
Male	85 (63.5%)	62 (66.7%)	23 (56.1%)		52 (62.7%)	33 (64.7%)	
ECOG							
0	84 (62.7%)	57 (61.3%)	27 (65.8%)	0.7	64 (77.1%)	20 (39.2%)	**<0.0001**
1	50 (37.3%)	36 (38.7%)	14 (34.2%)		19 (22.9%)	31 (60.7%)	
Radiotherapy							
yes	53 (39.5%)	34 (36.5%)	19 (46.3%)	0.34	40 (48.2%)	13 (25.5%)	**0.01**
no	81 (60.5%)	59 (63.5%)	22 (53.7%)		43 (51.8%)	38 (74.5%)	

KRAS mutation associates with inferior overall survival

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 months vs. 5.1 months, respectively; Figure 2A, Table 4). Kaplan-Meyer curves demonstrated longer median OS in patients who received BTx (10.1 months vs. 4.3 months in BTx-naive; Figure 2B). Notably, patients receiving second-generation BTx exhibited significantly superior OS compared to those receiving first-generation BTx (median OSs were 13.2 months vs. 7.1 months, respectively; p=0.041; Figure 3). In regards with RTx, the median OS was higher among the patients receiving RTx compared to RTx-naive patients (11 months 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 and 2C), 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 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.

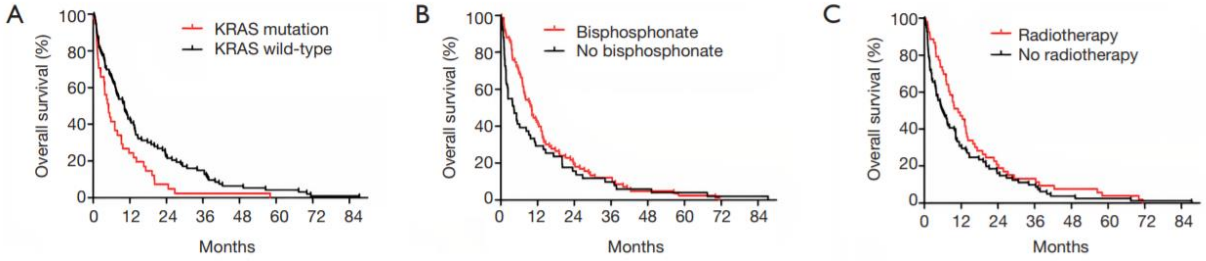

Figure 2. Kaplan-Meier estimates for OS in bone metastatic LADC patients according to KRAS mutational status and therapeutic modalities including BTx and RTx.

(***A***) LADC patients with tumors harboring KRAS mutations had significantly shorter median OS than those with KRAS WT tumors (median OSs were 5.1 months 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 months 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).

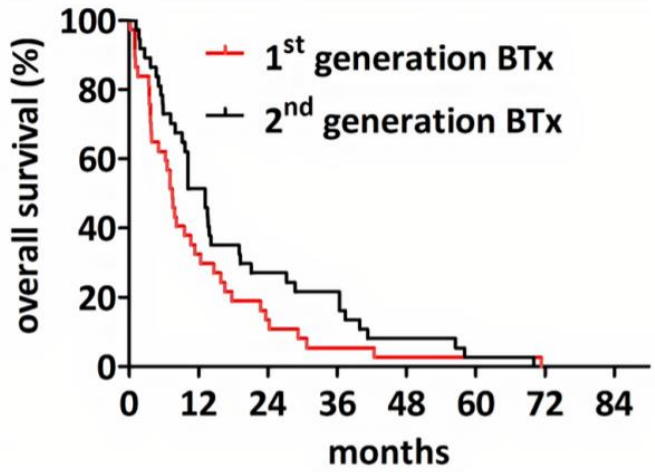

Figure 3. Kaplan-Meier estimates for OS in bone metastatic LADC patients according to different generations of BTx.

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.

Table 4. Prognostic impact of KRAS mutation, radiotherapy and bisphosphonate treatment

		OS months	**Univariable analysis**			**Multivariable analysis**		
			HR	95% CI	p*	HR	95% CI	p
KRAS status	wt mutant	10.2 5.1	1 0.535	0.349-0.820	**0.008***	1 0.564	0.382-0.833	**0.004**
Radiation therapy	yes no	11.0 5.9	1 0.763	0.541-1.076	**0.021***	1 0.737	0.505-1.078	0.115
Bisphosphonate therapy	yes no	10.1 4.3	1 0.781	0.541-1.127	**0.007***	1 0.953	0.647-1.404	0.810

***** Gehan-Breslow-Wilcoxon test

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 months vs. 5.8 months, respectively; p = 0.023; Figure 4A). 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 months vs. 7 months, respectively; p = 0.0168, Figure 4B).

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 months vs. 5.2 months, respectively; p=0.032, Gehan-Breslow-Wilcoxon test); Figure 4A). As for patients with KRAS mutant tumors, the difference in median overall survival between patients with or without BTx did not reach statistical significance (median OSs were 5.8 months vs. 3.1 months, respectively; p=0.35; Figure 4A).

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 months vs. 7.4 months; p=0.032; Figure

4B). 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 4B).

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 4C).

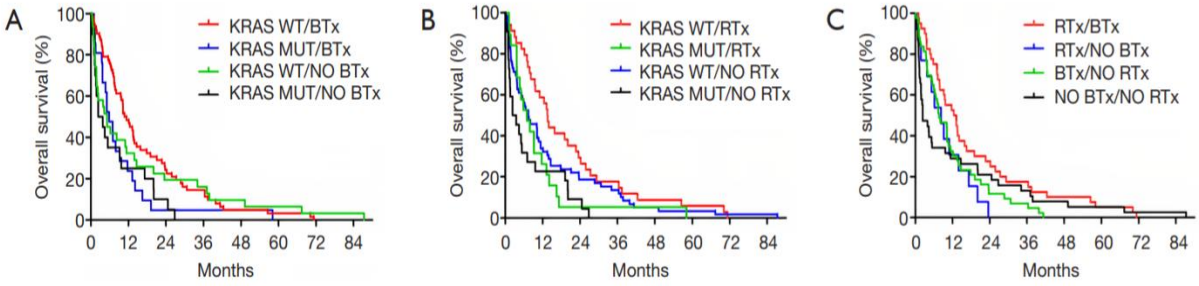

Figure 4. Kaplan-Meier estimates for OS in bone metastatic LADC patients according to KRAS mutational status and specific therapeutic approaches.

(***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 months 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 months 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 months 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 months 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 months 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 months 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).

3.2. Bone-specific metastasis pattern

Patient characteristics and metastatic sites

In total, 209 LADC patients with synchronous isolated bone metastases were enrolled in this study whose clinicopathological characteristics are summarized in Tables 5 and 6. The median age was 62 years (range 34–84). All patients had Caucasian backgrounds and 113 of them were male (54%) (Table 5). Peripheral tumors occurred more frequently than centrally-located tumors (59% vs. 41%). Right-sided LADCs were found in 57% (vs. left-sided, 43%) and upper region tumor location in 70% (vs. lower region 30%) of the patients. The general clinicopathological characteristics did not differ significantly with regards to the localization of the primary tumor. As for the localization of metastases (Table 6), the most frequent metastatic sites were the spine (n=103), the ribs (n=60), the pelvis (n=36), and the femur (n=22), followed by humeral (n=17), skull (n=13), sternal (n=10), and clavicular or scapular (n=10) metastases. We identified 163 patients with single-bone metastatic disease and 46 with metastases affecting multiple bones at the time of diagnosis. With regards to specific bisphosphonate agents 67, 29 and 57 patients received clodronate, pamidronate and zoledronic acid, respectively (of note, no data was available on the exact type of administered bisphosphonate agent in case of 15 patients). Palliative external beam RTx was applied in case of 66 patients. Regarding major comorbidities 53 individuals had COPD, whereas hypertension was detected in 117 patients.

Table 5. Patient characteristics and tumor localization in LADC patients with consecutive bone metastases

All patients		Localization of the primary tumor											
		Central	Peripheral	N/A	P value[a]	Left-sided	Right-sided	N/A	P value[a]	Upper or middle lobe[c]	Lower lobe	N/A	P value[a]
Age (years)													
<65	125	55	68	2	0.196[b]	52	69	4	0.986[b]	85	30	10	0.200[b]
≥65	84	30	54	0		36	48	0		53	28	3	
Gender													
Male	113	44	68	1	0.572[b]	51	60	2	0.342[b]	80	27	6	0.142[b]
Female	96	41	54	1		37	57	2		58	31	7	
Smoking history													
Never smoker	23	8	15	0	0.332[b]	10	11	2	0.652[b]	14	6	3	0.852[b]
Ex-smoker	50	25	25	0		19	31	0		37	12	1	
Current smoker	66	26	40	0		30	36	0		45	18	3	
N/A	70	26	42	2		29	39	2		42	22	6	

[a]p values refer to differences between patient characteristics and tumor localization; [b]χ^2 test; [c]in the right lung: upper and middle lobes; in the left lung: upper lobe and ligula;

Table 6. General clinical characteristics of different metastatic sites in bone-metastatic LADC patients

All patients	Bone metastasis site							
	Clavicle or scapula	Sternum	Skull	Humerus	Femur	Pelvis	Rib	Spine
Total[a]	10	10	13	17	22	36	60	103
Age (years)								
<65	6 (60.0%)	7 (70.0%)	5 (38.5%)	13 (76.5%)	16 (72.7%)	21 (58.3%)	36 (60.0%)	58 (56.3%)
≥65	4 (40.0%)	3 (30.0%)	8 (61.5%)	4 (23.5%)	6 (27.3%)	15 (41.7%)	24 (40.0%)	45 (43.7%)
Gender								
Male	9 (90.0%)	6 (60.0%)	4 (30.8%)	12 (70.6%)	10 (45.5%)	17 (47.2%)	39 (65.0%)	52 (50.5%)
Female	1 (10.0%)	4 (40.0%)	9 (69.2%)	5 (29.4%)	12 (54.5%)	19 (52.8%)	21 (35.0%)	51 (49.5%)
Smoking history								
Never smoker	2 (20.0%)	2 (20.0%)	3 (23.1%)	1 (5.9%)	3 (13.6%)	4 (11.1%)	5 (8.3%)	15 (14.6%)
Ex-smoker	1 (10.0%)	3 (30.0%)	3 (23.1%)	3 (17.6%)	4 (18.2%)	11 (30.6%)	17 (28.3%)	21 (20.4%)
Current smoker	3 (30.0%)	0 (0.0%)	2 (15.4%)	7 (41.2%)	7 (31.8%)	10 (27.8%)	20 (33.3%)	30 (29.1%)
N/A	4 (40.0%)	5 (50.0%)	5 (38.5%)	6 (35.3%)	8 (36.4%)	11 (30.6%)	18 (30.0%)	37 (35.9%)

[a]The total number of included patients is 209, but given that a single patient does not necessarily have metastasis in exactly one bone, the overall number of metastases might be higher.

Primary tumor location is associated with bone-specific metastatic site

Investigating the impact of the primary tumors' localization on the metastatic site, we found that femoral (OR 3.486, 95%CI 1.09-14.71, p=0.022) and rib (OR 2.338, 95%CI 1.16-4.86, p=0.012) metastases were more frequently associated with peripheral tumors (Figure 5A), whereas centrally located LADCs were associated with humeral metastases (OR 0.262, 95%CI 0.06-0.83, p=0.018; Figure 5A). Importantly, we also found that left-sided tumors give rise to skull metastases more often than right-sided primary tumors (OR 4.836, 95%CI 1.19-28.19, p=0.018; Figure 5B). These results remained significant at a 0.05 significance level with the use of Bonferroni correction. Of note, there was no significant association between the primary LADC region (i.e., lower vs. upper region tumors) and the bone-specific metastatic site (Figure 5C). With regards to the type of affected bones, metastases in flat bones were more commonly found in patients with peripheral tumors (vs. central LADCs), yet these results were not statistically significant (p=0.202; Figure 6A). Likewise, as shown in Figure 6B and 6C, the side- and region-specific localization of the primary tumor did not influence the type of bone metastases either. The localization of the primary tumors did not have an impact on the number of metastatic bones (i.e., single- vs. multiple-bone metastatic spread) at diagnosis (data not shown).

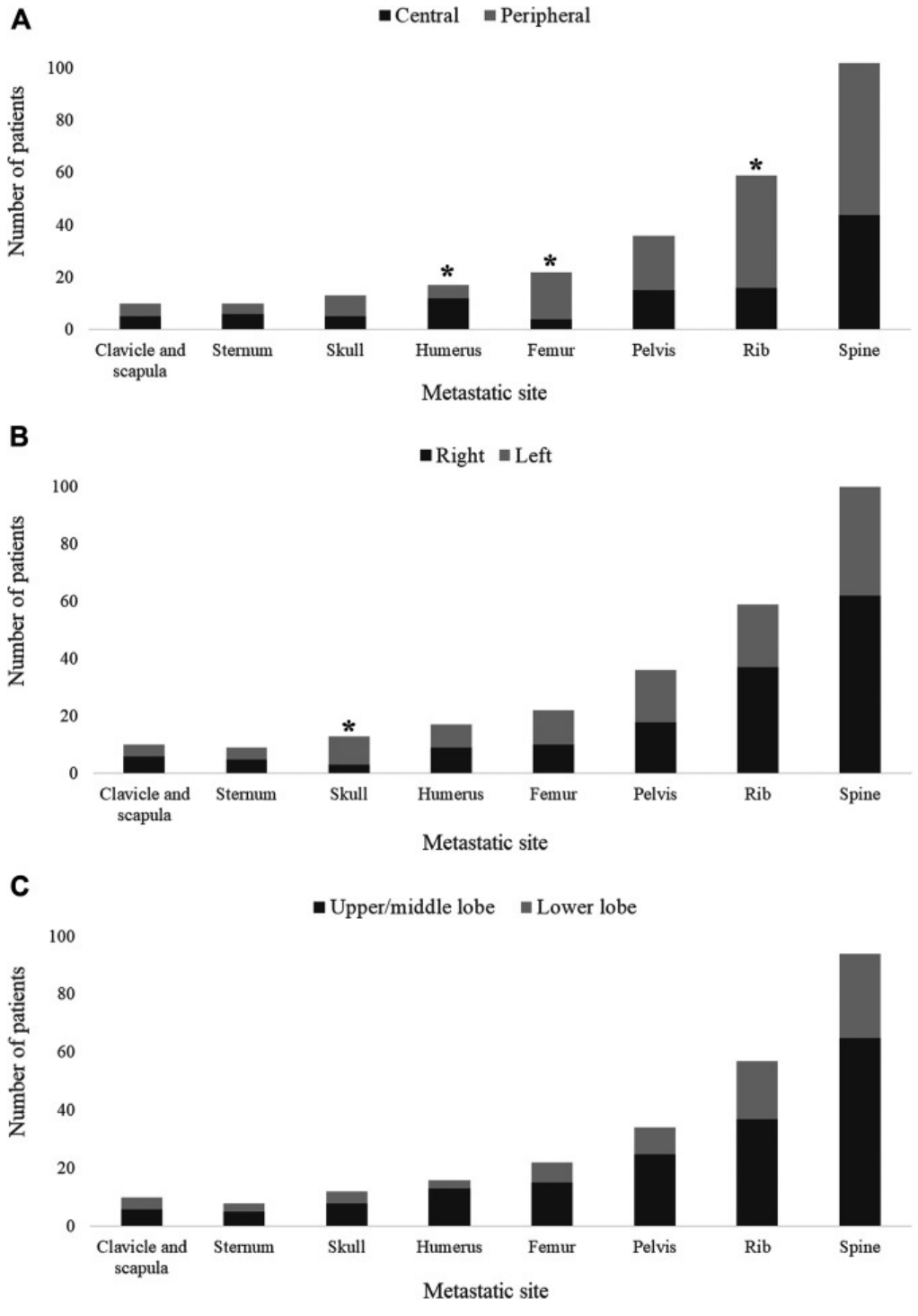

Figure 5. Primary tumor location and metastatic site in bone-metastatic LADC patients.

(***A***) Peripherally located primary tumors are associated with femoral (OR 3.486, 95%CI 1.09-14.71, p=0.022) and rib (OR 2.338, 95%CI 1.16-4.86, p=0.012) metastases, whereas central LADCs give rise to humeral metastases (OR 0.262, 95%CI 0.06-0.83, p=0.018). (***B***) Left-sided tumors are more frequently associated with skull metastases compared to right-sided primary LADCs (OR 4.836, 95%CI 1.19-28.19, p=0.018). (***C***) No significant differences were found in metastasis pattern with regards to upper/middle lobe vs. lower lobe classification.

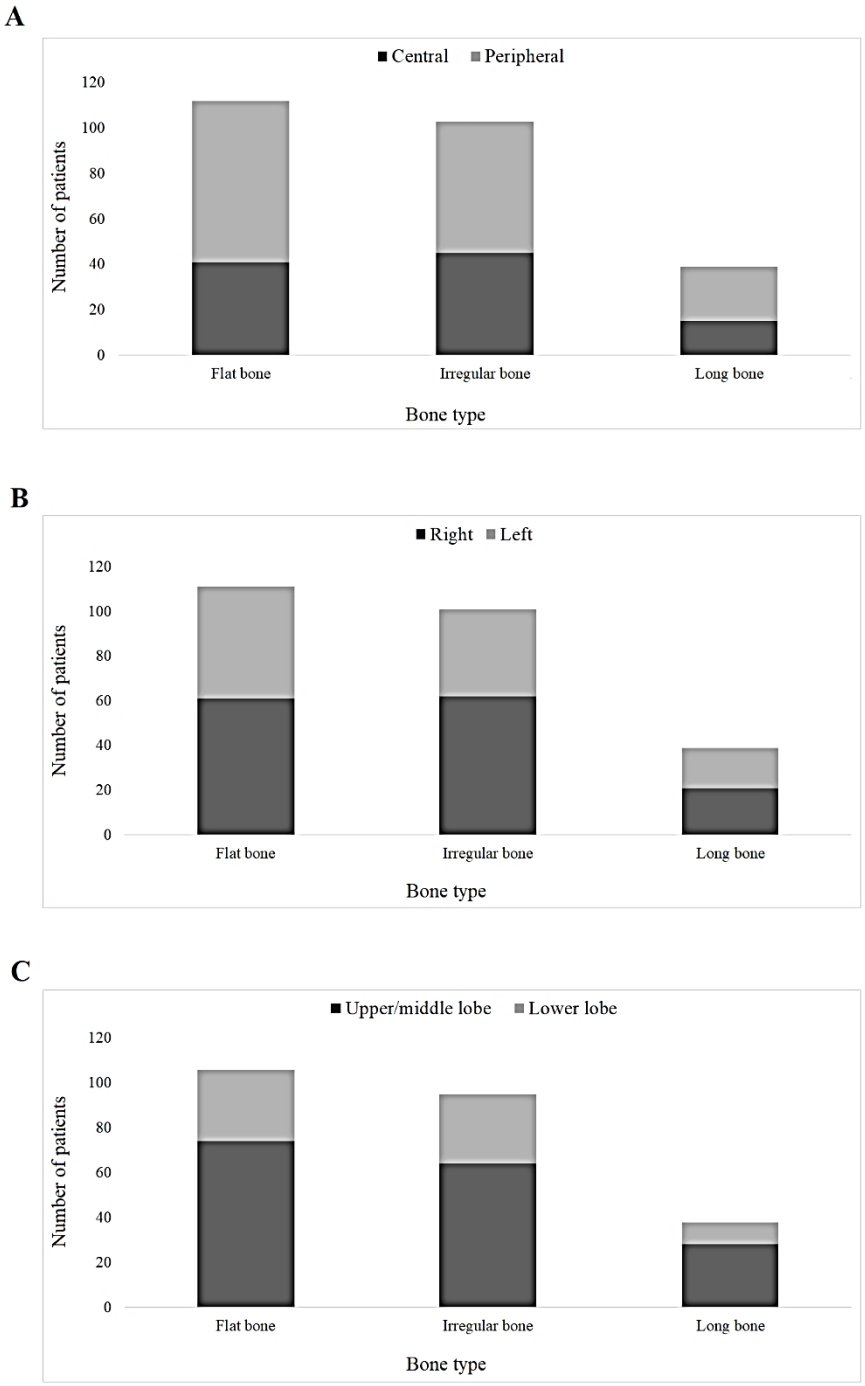

Figure 6. Primary tumor localization and type of affected bone.

(A) The percentage of LADC patients with metastases in flat bones (vs. other bone type) was non-significantly higher in case of peripheral tumors (vs. central LADCs; p = 0.202). **(B, C)** The side- and region-specific localization of the primary tumor did not influence the type of bone metastases.

Prognostic parameters and clinical outcome

The median follow-up time for the total cohort of 209 bone-metastatic LADC patients was 33.7 weeks (of note, survival data was not available in case of 10 patients). Patients with centrally located primary LADCs had worse survival outcomes compared to those with peripheral tumors (median OS, 25.1 vs. 36.2 weeks, HR 1.359, 95%CI 1.020-1.810, p=0.035, Figure 7A). No significant differences in OS have been observed for patients with right vs. left (p=0.941; Figure 7B) or upper vs. lower region (p=0.238; Figure 7C) located primary tumors. Next, we compared the number of metastatic sites with survival outcomes and found that the number of affected bones did not influence the median OS (p=0.436; data not shown). When comparing the survival outcomes of LADC patients with solitary bone metastases, we found that the site of bone metastases did not influence survival significantly (p=0.307; Figure 8A). Importantly, however, patients with femoral metastases tend to have better survival outcomes than those with other bone metastases (p=0.064; Figure 9). Although the median OS was visibly longer in patients with bone metastases affecting the long bones (vs. flat bones vs. irregular bones), this tendency does not appear to be statistically significant either (p=0.269; Figure 8B). With regards to specific therapeutic approaches, as expected, BTx-naive patients had significantly worse median OS than those receiving BTx (median OS, 12.0 vs. 40.2 weeks, HR 2.101, 95%CI 1.462-3.020, p<0.001, Figure 10A). Similarly, CTx also conferred a significant benefit for OS when compared to CTx-naive patients (median OS, 50.2 vs. 17.4 weeks, HR 0.545, 95%CI 0.410-0.726, p<0.001, Figure 10B). In order to assess if the prognostic value of tumor location (i.e., central vs. peripheral) was independent of other prognostic factors, we performed a multivariate Cox regression analysis (Table 7). Importantly, we found that the peripheral location of primary LADCs was still significantly associated with a benefit in OS (HR 0.589, p=0.001, Table 7). Besides, as expected, Cox regression analysis revealed that the specific therapeutic approaches (BTx and CTx) also influence the survival outcomes independently (p<0.001).

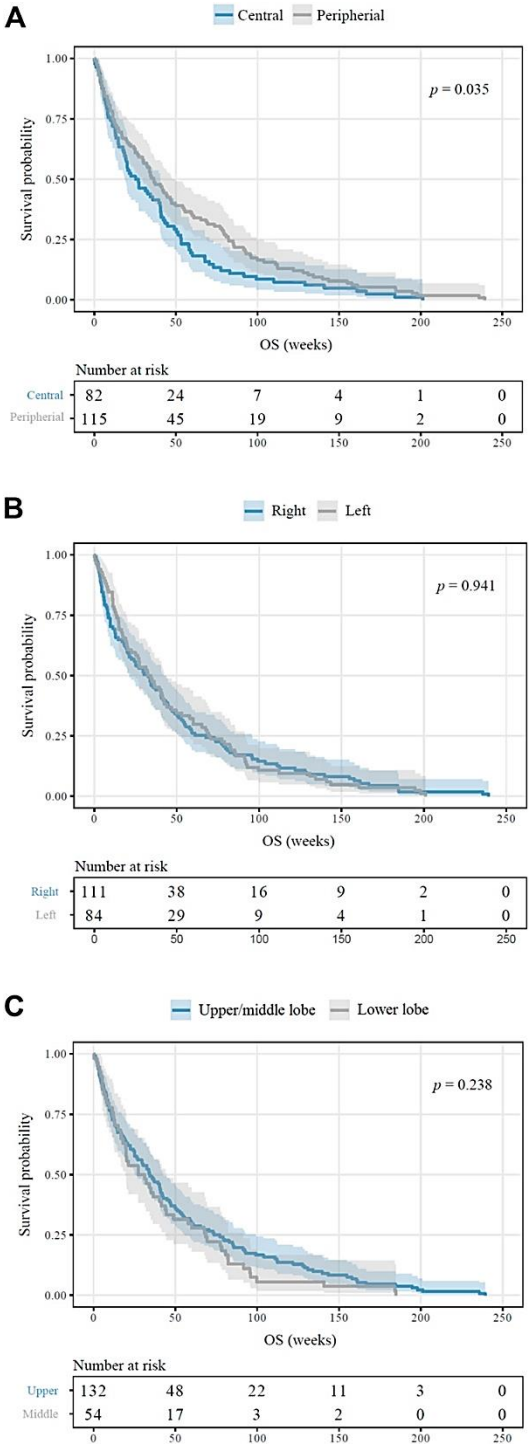

Figure 7. Survival outcomes of bone-metastatic LADC patients according to the localization of the primary tumor.

(***A***) Patients with centrally located primary LADCs exhibited significantly inferior OS compared to those with peripheral tumors (median OSs were 25.1 vs. 36.2 weeks,

respectively; HR 1.359, p=0.035). (***B***) Side-specific tumor localization did not have any impact on OS (p=0.941). (***C***) No significant differences in OS have been observed for upper/middle vs. lower lobe (p=0.238).

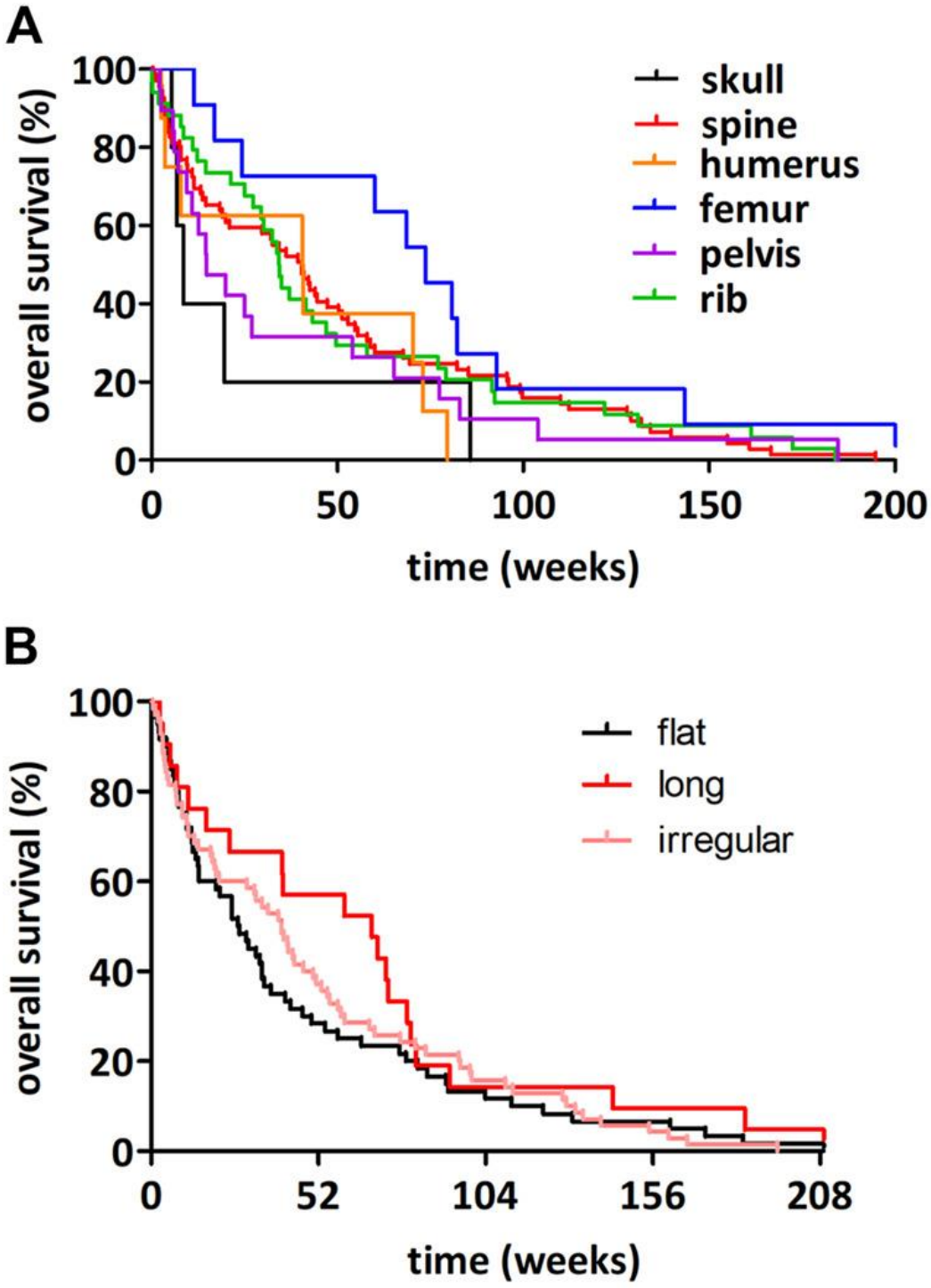

Figure 8. Kaplan-Meier plots for OS in LADC patients with solitary bone metastases according to affected bones.

(***A***) The site of bone metastases did not influence the OS significantly (p=0.307). (***B***) LADC patients with bone metastases in long bones have non-significantly longer median OS (vs. irregular bone vs. flat bone metastatic patients; median OSs were 68.5 vs. 40.4 vs. 27 weeks, respectively; p=0.269).

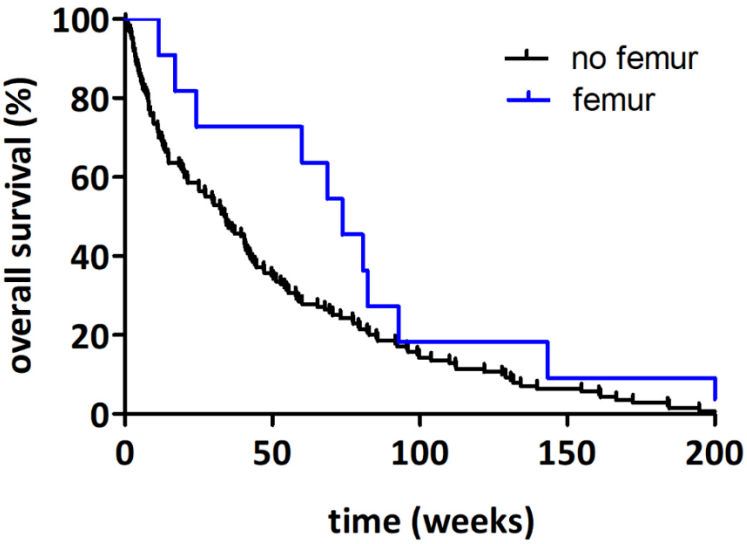

Figure 9. Survival outcomes of LADC patients with femoral metastases.

Patients with femoral metastases exhibited non-significantly superior OS compared to those with other bone metastases (median OSs were 73.7 vs. 33.7 weeks, respectively; p=0.064).

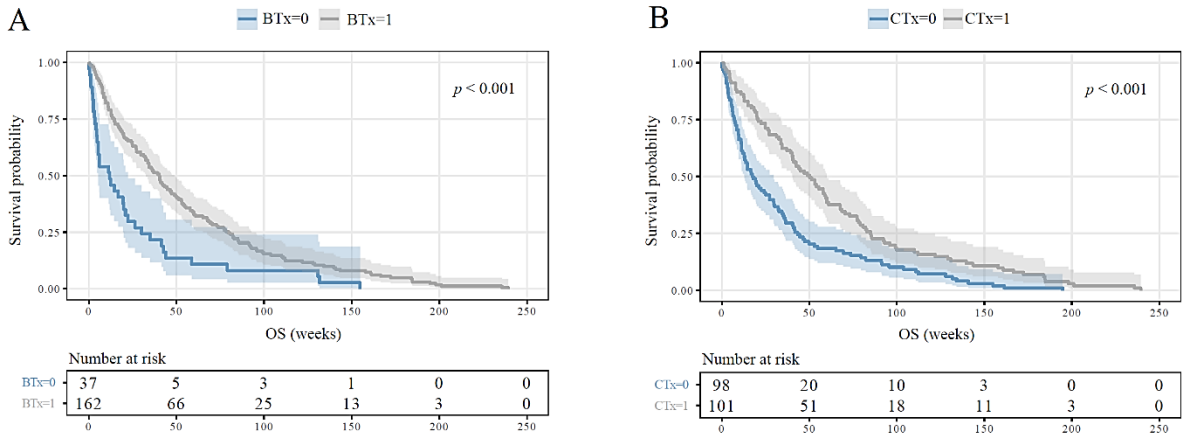

Figure 10. Kaplan-Meier estimates for OS according to specific therapeutic approaches.

(*A*) BTx-naive patients had decreased median OS (vs. BTx-treated individuals; median OSs were 12.0 vs. 40.2 weeks, respectively; HR 2.101, p<0.001). (***B***) Patients treated with CTx had significantly longer OS than those not receiving CTx (median OSs were 50.2 vs. 17.4 weeks, respectively; HR 0.545, p<0.001).

Table 7. **Multivariate Cox Regression model for clinicopathological variables influencing the OS.**

Clinicopathological variable	OS
Localization (central vs. peripheral)	
HR	0.589
95% CI	(0.438-0.794)
p	0.001
BTx (no vs. yes)	
HR	0.425
95% CI	(0.292-0.619)
p	<0.001
CTx (no vs. yes)	
HR	0.515
95% CI	(0.383-0.692)
p	<0.001

4. Discussion

The prognosis of LADC patients with bone metastases remains poor despite new treatment strategies (median survival is less than one year from diagnosis of metastases) [68, 76, 77]. Their main features include pain and decreased motility (due to pathological fractures, spinal cord compression and other skeletal-related events), and neurological and gastrointestinal symptoms (due to metabolic syndromes and hypercalcemia) [70, 78]. Although these syndromes significantly affect the patients' quality of life [70, 78], few data on appropriate treatment algorithms for oncogenic driver mutations are available. In LADC, the most common gain-of-function alteration described in Western countries are the KRAS mutations [7, 8]. However, the clinical and therapeutic relevance of KRAS mutation in patients with LADC diagnosed with bone metastases is mainly unknown. Therefore, the first part of the dissertation aimed to investigate the effects of KRAS mutation on survival in bone metastatic LADC patients according to BTx and RTx.

In our study, we retrospectively analyzed the data of 134 Caucasian patients from Hungary with isolated bone metastases at diagnosis. The incidence of KRAS mutations was 30.6%, which is in line with the results of studies conducted in similar patient populations [74, 79, 80] and is consistent with the overall incidence of KRAS mutations [5]. The association between KRAS mutation status and the appearance of bone metastases remains an open question. Zhao et al. [81] found that the incidence of bone metastases is significantly higher in KRAS mutant tumors than in patients with KRAS WT tumors. However, others failed to confirm this association [82, 83]. One possible explanation for these conflicting results might be that besides the pure absence or presence of the mutation, the appearance of bone metastases is also influenced by the KRAS mutational subtype. Indeed, Kuijpers et al. found that the frequency of bone metastases is higher only in case of KRAS G12A mutation, while the other subtypes do not significantly affect the incidence [84]. Examining the effects of KRAS mutation status on survival, we found that the median OS was significantly higher in patients with KRAS WT tumors than in those with KRAS-mutant LADCs. This finding is in line with the results of our previous studies [74]. However, it should be emphasized that the prognostic relevance and clinical significance of KRAS oncogenic mutations remain controversial and that ethnicity, tumor stage, and the therapeutic algorithms used can all influence the results [4, 36, 85]. Nevertheless, the present study demonstrated that KRAS mutation is

an independent negative prognosticator in bone metastatic LADC by using a relatively homogeneous group of patients. It is important to note that the prognostic significance of KRAS mutation was not affected by different therapeutic approaches, and KRAS WT tumors were associated with better survival outcomes regardless of therapeutic modality (i.e., BTx or RTx). As the present project is the first to investigate the prognostic relevance of KRAS mutational status in bone metastatic LADC regarding BTx and RTx further studies are warranted to confirm our findings. Next, we examined the impact of KRAS mutation status on BTx and RTx efficacy (in terms of OS). Bisphosphonates are synthetic analogues of pyrophosphates that regulate bone metabolism, inhibit osteoclast-mediated bone resorption, reduce osteoblast proliferation, and stimulate bone formation and differentiation [70, 86]. In addition, *in vitro* and *in vivo* studies have shown that BTx has direct antitumor effects in many tumor types (including breast, pancreatic, and prostate tumors and NSCLC) by inhibiting proliferation, inducing apoptosis, and regulating the immune microenvironment [72, 87-90]. Meanwhile, RTx is primarily used to relieve pain, prevent pathological fractures and spinal cord compression, and improve the patients' quality of life [91, 92]. Overall, both therapeutic modalities play a significant role in the treatment of bone metastatic LADC patients [92, 93]. In our study, BTx- and RTx-treated patients with KRAS WT tumors had significantly higher OS than those who did not receive either therapy. In contrast, in the presence of a KRAS mutation, neither BTx nor RTx significantly improved the survival. In preclinical proliferation assays, our collaborators demonstrated that KRAS WT NSCLC cell lines were more sensitive to zoledronic acid-induced prenylation inhibition, whereas those carrying the KRAS mutation were found to be resistant [75]. These findings suggest that the antiproliferative effect of bisphosphonates is limited to KRAS WT cells [75]. To our knowledge, our study was the first to examine the efficacy of BTx and RTx in clinical settings according to KRAS mutation status. Based on our results, it can be concluded that KRAS mutation status might be a valuable predictive marker in bone metastatic LADC concerning BTx and RTx. Examining the effect of BTx and RTx on survival regardless of KRAS mutation status, we found that both therapies increase the patients' survival. It should be emphasized that the best survival rates were observed in patients who received both BTx and RTx. This is in line with the results of previous preclinical studies [94-96]. The additive and superadditive effects of systemic BTx and RTx were first detailed by Hoskin

[86] and Steel [97]. However, the exact pathomechanism of these effects is currently largely unknown [98]. One possible explanation of this superadditive effect might be that both BTx and RTx exert their inhibitory effects mainly on osteoclast activity [82]. Furthermore, research on osteosarcoma cell lines has shown that the direct cytotoxic effects of BTx and RTx also accumulate when co-administered [98].

In the second part of our works, in order to investigate the bone-specific metastasis pattern, we included 209 LADC patients with concomitant skeletal metastases. The most frequent metastatic sites in our cohort were the vertebrae, followed by the ribs and the pelvis. These results are in line with the findings of Tsuya et al. who also found that the most commonly affected bones are the spine and ribs [99]. Vertebral metastases are of great clinical importance since they might contribute to pathological fractures and spinal cord compression [100]. The pathomechanism of vertebral metastases is the subject of intensive research. Importantly, by enhancing lytic bone destruction through the activation of osteoclasts, the RANK and RANKL relationship play a central role in this pathomechanism [101, 102]. The anastomoses between the lung, rib, and vertebral veins and the minimal distance between them might play a key role in the high incidence of vertebral and rib metastases in patients with lung cancer [68, 103]. LADCs in peripheral tumors can quickly spread to the ribs through hematogenous dissemination and direct invasion. The metastatic pattern according to the localization of the primary tumors has been studied in several tumor types including NSCLC [69, 104, 105], small cell lung cancer (SCLC) [106], colorectal tumor [107-109], and pancreatic cancer [110]. Accordingly, primary tumors in the body or tail of the pancreas have a less aggressive phenotype than those in the head of the pancreas [110]. Regarding colon and rectal cancer, right-sided tumors are associated with a lower rate of distant metastases [111, 112]. In lung cancer, an earlier study by our group found that peripheral LADCs are more likely to cause lung metastases, whereas centrally located tumors are associated with bone metastases [69]. In SCLC, however, we found no significant association between the bronchoscopic localization of the primary tumor and metastasis pattern [106]. In the current study, we found that femoral and rib metastases were significantly more common in patients with peripheral tumors, while centrally located LADCs were associated with humeral metastases. We also found that left-sided tumors give rise to skull metastases

substantially more frequently than right-sided tumors. However, it should be noted that the incidence of both skull and humeral metastases was relatively low in our cohort, therefore, further studies are warranted to validate our results. The region-specific localization of the tumor (i.e., upper and middle lobe tumors vs. lower lobe tumors) did not influenced the metastasis pattern. Because treatment options for patients with bone metastases may vary depending on the type of affected bones, we also examined the effect of primary tumor location on the type-specific metastatic pattern [113]. In our cohort, flat bone metastasis occurred more frequently in patients with peripheral LADC than in those with central tumors. Although, this association was not statistically significant, the observed trend might still be of clinical importance since radiofrequency ablation therapies for bone metastases in flat bones are difficult to perform [113]. Several studies report differences in tumor mortality according to side-specific localization of the primary. Right-sided localization is an independent negative prognosticator in patients with metastatic colon cancer, but the localization of the primary tumor did not affect the survival outcomes in stage II. and III. patients [114, 115]. In breast cancer, tumors located in the upper-outer quadrant of the breast have improved prognosis than those in other localizations [116]. Based on our results, the median OS of patients with centrally located primary LADCs is significantly shorter compared to patients with peripheral tumors. Importantly, we also found that the endoscopic localization of the primary tumor influenced the long-term survival outcomes independently of other clinicopathological variables. This finding is consistent with previously published data suggesting that the central location of the primary tumor is associated with significantly poorer survival outcomes in both early and advanced stage lung cancer [69, 117, 118]. However, the current study is the first to examine the effect of primary tumor location on survival in patients with isolated bone metastases. Our data suggest no differences in survival between left- vs. right-sided LADCs or upper vs. lower region tumors. Therefore, these tumors may not represent different entities and should be treated by using the same oncological principles. These results are supported by an extensive Surveillance Epidemiology and End Results (SEER) analysis, where the prognosis between right- and left-sided NSCLCs was similar in stage I – IIIA, regardless of whether the patients underwent surgery or not [119]. Furthermore, Puri et al. [120] and Whitson et al. [121] also concluded that side- and region-specific localization of the primary tumors does not

affect the survival outcomes in early-stage patients either. Regarding the prognostic relevance of bone metastasis localization and type of affected bone, we found that patients with metastases in long bones (especially in femur) exhibited longer median OS than those with cubic or flat bone metastases. Meanwhile, metastases in the flat bones (i.e., skull) were associated with a significant deterioration in survival. Although, these results were not statistically significant, a clear tendency can still be observed. A possible explanation of the poor survival in patients with cranial metastases might be that these metastases invade the adjacent anatomical structures more easily due to their localization. Accordingly, cranial bone metastases can penetrate into the dura and intradural space, resulting in an increased intracranial pressure, meningeal irritation and focal neurological symptoms that may ultimately lead to impaired survival [122]. In addition, as mentioned earlier, RTx is more difficult to achieve in flat bone metastases, while femoral metastases are relatively easy to target by radiofrequency ablation [113].

Our studies had certain limitations given by their 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. Additionally, the specific KRAS mutation subtype was also not systematically determined. Lastly, the methodology used to dichotomize the primary tumors into central and peripheral lesions based on bronchoscopic visibility might also cause bias in some results. To date, however, there are no standard definitions for centrally vs. peripherally located lung tumors. Altogether, the results presented in this dissertation have to be interpreted with caution and some of them needs to be confirmed in independent cohorts, ideally in a prospective setting.

5. Conclusions

The present dissertation describes two projects. In the first part, we examined the prognostic relevance of KRAS mutation in a large and homogenous cohort of Caucasian LADC patients diagnosed with bone metastases. We also assessed how RTx and BTx affect the OS according to KRAS mutational status. In the second part, we evaluated the impact of primary tumor localization on bone metastasis pattern and survival outcomes. Based on our findings, we conclude that KRAS mutation is an independent negative prognosticator in bone metastatic LADC. In addition, we found that the use of RTx and BTx significantly increased the OS. Of note, however, the effects of the aforementioned therapeutic modalities were considerably higher in patients with KRAS WT tumors than in those with KRAS-mutant LADCs. Co-administration of BTx and RTx conferred a significant benefit for OS regardless of KRAS mutational status. In the second part, we revealed that peripheral tumors are significantly more likely to give rise to femoral and rib metastasis. In terms of survival, centrally-located tumors were associated with poorer survival than peripheral tumors. Our results may contribute to developing new therapeutic algorithms for early diagnosis, thus improving long-term survival.

6. Summary

In Western countries, KRAS mutation is the most common gain-of-function alteration in LADC. Although the skeletal system represents one of the most frequent metastatic sites in advanced-stage LADC, the bone-specific metastasis pattern remains controversial in these patients, and there are no effective treatment guidelines based on KRAS mutational status.

First, we aimed to investigate the effects of KRAS mutation on BTx and RTx efficacy in bone-only metastatic LADC patients. In total, 134 LADC patients with known KRAS status and simultaneous bone metastases were included in our study. The therapeutic efficacy of BTx and RTx was examined according to OS. Of the total cohort, 93 patients were identified as KRAS WT and 41 as KRAS mutant patients. Importantly, the presence of KRAS mutation was associated with a significantly impaired median OS. Regardless of KRAS mutational status, both BTx and RTx conferred a significant benefit for OS. However, when patients with KRAS mutant and KRAS WT tumors were analyzed separately, the beneficial effects of both BTx and RTx on OS remained statistically significant only in case of KRAS WT patients.

Next, we assessed the impact of primary tumor location on bone-specific metastasis pattern and survival in 209 Caucasian LADC patients with bone metastases. In addition to the region- and site-specific localization, primary tumors were also classified according to their bronchoscopic visibility. We found that the most common sites of bone metastasis were the spine (n=103) and ribs (n=60), followed by the pelvis (n=36) and femur (n=22). Importantly, femoral bone metastases and rib metastases were significantly more commonly associated with peripheral tumors, whereas centrally located LADCs were associated with humeral metastases. In addition, we also found that left-sided tumors metastasized to the skull significantly more often than right-sided tumors. The localization of the primary tumor did not affect the type of involved bones. In a multivariate analysis adjusted for clinical parameters, central localization of the primary tumor proved to be an independent negative prognosticator for OS.

To sum up, KRAS mutation is an independent negative prognostic factor in bone metastatic LADC. Both BTx and RTx increase the median OS, with a significant benefit

for patients with KRAS WT tumors. Our study provides insights into unique bone-specific metastasis pattern of LADC patients concerning the localization of the primary tumor. Overall, the KRAS mutational status should be considered in the therapeutic decision-making of LADC patients with bone metastases. In addition, a better understanding of bone-specific metastasis pattern may facilitate early diagnosis, thus contributing to the development of new treatment strategies.

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8. List of publications that served as a basis for the current thesis.

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

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. Transl Lung Cancer Res. 2021 Feb;10(2):675-684. doi: 10.21037/tlcr-20-754. PMID: 33718013; PMCID: PMC7947398. **IF: 6.498**

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*shared first authorship

Therapeutic possibilities in KRAS-mutant lung adenocarcinoma.

Radeczky P*, Ghimessy Á*, Berta J, László V, Hegedűs B, Rényi-Vámos F, Fillinger J, Megyesfalvi Z[#], Döme B[#]. Magy Onkol. 2020 Sep 23;64(3):231-244. Epub 2020 Aug 6.PMID: 33196710 Free article. Hungarian. **IF: 0**

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A KRAS-mutációs státusz prediktív szerepe biszfoszfonáttal kezelt, csontáttétet képző tüdő-adenokarcinómában [Predictive relevance of KRAS mutational status in bone metastatic lung adenocarcinoma treated with bisphosphonate therapy].

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9. Other publications.

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Fazekas L, Ghimessy Á, Gieszer B, **Radeczky P**, Mészáros L, Török K, Bogyó L, Hartyánszky I, Pólos M, Daróczi L, Agócs L, Kocsis Á, Bartók T, Dancs T, Tóth KK, Schönauer N, Madurka I, Elek J, Döme B, Rényi-Vámos F, Lang G, Farkas A.Transplant Proc. 2019 May;51(4):1263-1267. doi: 10.1016/j.transproceed.2019.04.007.PMID: 31101211

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Early Postoperative Problems After Lung Transplantation: First-Year Experiences

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10. Acknowledgements

At the beginning of my professional career, I was offered an opportunity to start my training in thoracic surgery and gain insights into translational thoracic oncology research. I am grateful to a number of people who have helped me in the past years:

I will be always grateful to Ferenc Rényi-Vámos for offering me several opportunities to improve my surgical skills in thoracic cancer surgery, lung transplantation and everyday clinical practice.

I would like to thank Balázs Hegedűs, Balázs Döme, and Zsolt Megyesfalvi for helping and guiding me in the field of clinical and translational research. This dissertation would not have been possible without them.

I owe special thanks to all of my colleagues at the Department of Thoracic Surgery of the National Institute of Oncology, Budapest. It is a privilege for me to work together with such a talented and creative Team. I would like to express might sincere gratitude to László Agócs and Ákos Kocsis who were my mentors in surgical training and helped me to become a better surgeon. I would also like to thank all my co-authors and collaborators from different thoracic oncology centers across Hungary, Austria and Germany.

And finally, I am grateful to my family and friends. They have always supported me in every decision I made. Therefore, I would like to say special thanks to:

my wife, Maria Steszli for her understanding and the encouragement she gave;

my children Emma, Márk, Jakab, and Adel for their tolerance;

my parents, Rozina Radeczky and Géza Radeczky for supporting and motivating me, and for all the opportunities they gave me in life;

my brother Kristof Radeczky and my sister Anna Radeczky, who helped me in every moment of my life.

Last but not least, I would like to thank all the people who have helped me to get to this point in life.