Predictive and prognostic markers in

Lung adenocarcinoma -

The EGFR and KRAS mutation status and correlations with the prevalence of bone metastases

PhD. Thesis

Nóra Bittner MD.

Supervisor: Lajos Géczi MD. PhD.

University of Pécs, Medical School

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National Institute of Oncology

Chemotherapy for Outpatients Department

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Nora Bittner MD.

University of Pécs, Medical School

Clinical Medical Science, Doctoral School

Leader: Sámuel Komoly MD. PhD. Dsc.

Surgery and its border fields program

Program leader: Péter Örs Horvath MD. PhD. DSc.

National Institute of Oncology

Supervisor: Lajos Géczi MD. PhD.

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1, Introduction

Lung cancer is the leading cause of cancer related mortality all over the world and a number of developments have indicated future clinical benefit recently. The development of molecular pathology methods has become increasingly important in the prediction of chemotherapy sensitivity and mutation analysis to identify driver mutations as important targets of new therapeutic agents.

The most significant changes in the treatment of NSCLC revealed in new pathologic classification and in the introduction of molecularly targeted therapies, which include monoclonal antibodies and small molecule tyrosine kinase (TK) inhibitors.

In the advanced disease the hope of cure is less than 3 % but improvements in survival have been clearly achieved. Some years ago the median lung cancer survival rate was 10–12 months, now in case of available specific molecular targets a significant increase in median survival rates to 24–36 months has been achieved. These agents give an opportunity to provide a new standard of care. Therefore testing EGFR mutations and ALK rearrangements in patients with advanced lung adenocarcinoma should be incorporated into routine clinical practice.

This review focuses on the rationale for targeted agents and new treatment possibilities in case of advanced lung adenocarcinoma.

Correlations between bone metastases and lung adenocarcinoma mutation status:

Bone is the most frequent type of distant metastases in case of Non Small Cell Lung Cancer (NSCLC). During the disease this is developing 30-40%. The most important features are: pain in bones, Sceletal Releated Events (SRE), compression of neuralroots, and hypercalcemia. Because of the short survival (6 months) the treatment possibilities were not in the aim of scope. After the changes of treatment guidelines – first the platinum based chemotherapy, later the step of EGFR TK inhibitors therapy – the Overall Survival (OS) became more longer. There are different imaging methods to identify bone lesions : X-Ray, bone scintigraphy, CT,

MRI, PET/CT and of course biopsy from detected bone. The relevant clinical studies concluded that: bone metastases and (SRE) are frequently observed in men, heavy smokers, with non adenocarcinoma histology, and without treatment of EGFR TK inhibitors. In the absence of EGFR mutations and treatment without of EGFR TK inhibitors the prevalence of SRE is significant higher (P=0,02) therefore it is a pretictive factor. To compare the treatment effectivity within lung adenocarcinoma's patients they used in one arm cytotoxic chemotherapy and in other arm EGFR TK inhibitors specially for those patient who are EGFR mutation positive. The investigators detected a relatively new phenomen and correlations in the pathophysiology of bone involvement within EGFR mutation positive patients. In those patients who were treated with cytotoxic chemotherapy because of advanced lung cancer the ratio of SRE was much higher than in those who were treated by EGFR TK inhibitors. The theory behind this phenomen is that: lost of bone density is related with cytotoxic chemotherapy. Another new observation in clinical studies, within the EGFR mutation positive patients is that the incidence of osteoblastic bone lesion is much more higher than the osteolytic bone metastases. The osteblastic type of bone metastases were observed rarely in NSCLC before this study but nowdays the prevalence of osteoblastic metastases is more frequent mostly in lung adenocarcinoma.

Correlations between the EGFR TK inhibitors and bone remodelling

a: Direct inhibiton of tumor growth – especially for EGFR mutant carcinomas inhibits proliferation and induce apoptosis.

b: Regulation of release of pro-osteoclastogenic factors by mesenchymal sterm cells.

c: Gefitinib inhibits ability of human bone marrow stromal cells to induce osteoclast differentiation. Induction of osteoblast differentiation from mesenchymal sterm cells , EGFR signalling prevents differentiation of mesenchymas sterm cells into osteoblast, therefore EGFR TKI treatment might favour osteoblast formation.

d: Secretion reduction of pro-angiogenic factors by both tumor and mesenchymal sterm cells: EGFR blockade effects on secretion of angiogenic growth factors in carcinoma cells.

The presence of osteoblastic metastases or the evolution to osteoblastosis from previous osteolytic metastases should always be noted since it might represent and important predictive factor of response to EGFR TKI treatment.

In this thesis we analysed retrospectively 224 patient data who suffered from lung adenocarcinoma from 01/Jan/2008 to 31/Dec/2010. We have collected the most important clinical data and investigated the correlations between the mutation status (EGFR, KRAS) of bone metastases and survival rate. Based on these results we have concluded that EGFR and KRAS mutation status are both predictive factors for the treatment efficacy and are prognostic factors for the disease progression, but these are not predictors of the presence of bone metastases. The presence of bone metastates is an independent prognostic marker wich is correlates with the poor performace and worse Quality of Life (QL).

It is known that, the carcinogenesis of non-smokers is different from the heavy smokers and this fact helps us to choose the best treatment for the patients. Lung cancer is a complex heterogenous tumor type. The special molecular pathology signs on one hand are predictive markers for the treatment efficacy and in other hand prognostic markers for the disease progression.

In this thesis I try to find some correlations between the mutation status (EGFR, KRAS) of lung adenocarcinoma and the appearance of bone metastases.

The Scientific background of our Hypothesis:

1, At the time of the diagnosis more than 60% of lung cancer is advanced disease (Stage: III/B-IV). The most frequent metastases are controlateral lung, liver and bone metastases. The ratio of these metastases in between 30-60%.

2, The clinical experience shows that after the appearance of bone metastases the tumor progression becomes rapidly faster, the survival time decreases faster in lung

adenicarcinoma, compared with the hormonal sensitive breast and prostate cancers. The similar treatment possibilities for bone metastases shows higher effectivity and gives longer survival.

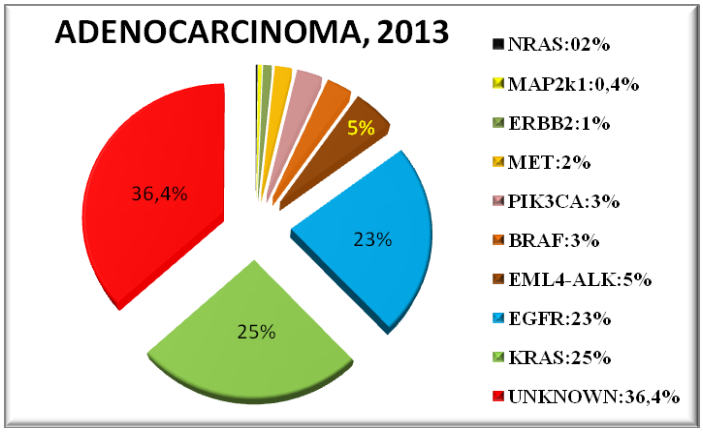
3, The presence of driver mutations in the lung cancer disease progression is detected. After the choice of EGFR TKI based on EGFR mutation status, there is much longer survival time for the patients suffering from advanced lung adenocarcinoma. The EGFR mutation status is a predictiv marker for the efficacy of theratment, and is a prognostic marker also for the disease progression.

2. AIMS OF THESIS

I, In this 3 years retrospectíve study we have analysed the frequency of the EGFR, KRAS mutation status related to smoking.

II, We have investigated the correlations betweeen the EGFR, KRAS mutation status and the appearance of bone metastases. We have wondered whether this new correlation have a predictive or prognostic value.

III, What about the patients Quality of Life and survival time after the cytotoxic chemotherapy as well as after the EGFR TKI therapy.



1.Fig. Mutations ratio at lung adenocarcinoma

Non Small Cell Lung Cancer (NSCLC) is classifiled by histology types. In the lung adenocarcinoma so called 'driver mutations' are verified wich meansthat in case of the presence of these mutations the tumor progression becomes faster. These mutations are mutually excluding each other, except of the *PIK3CA* mutation.

3. TREATMENT POSSIBILITIES IN LUNG ADENOCARCINOMA

3.1. Cytotoxic chemoterapy:

According to the NCCN and ESMO guidelines the first line therapy for the advanced Stage III/B- IV disease is the platinum based (cis/carbo) chemotherapy combined with other second or third generation cytotoxic drugs (paclitaxel, docetaxel, gemcitabin, vinorelbin, pemetrexed). When the therapy result is Stable Disease SD) we can continue the treatment as a maintenance therapy giving the patients longer survival time.

3.2. Targeted therapy:

The so called "driver mutations" indicate a special signal sign in the nucleus, causing the faster proliferation and survival of tumor cells.

The goal of combined targeted therapy is to prevent or deley the treatment resistency. These results are coming with the additive and synergistic effects influencing the angiogenesis, apoptosis, tumorgenesis and also the tumor growth.

3.2.1. Different signal trasduction pathways in NSCL: The EGFR signal trasduction

EGFR mutations are more common in NSCLC tumors in women, Asians and never smokers. Approximately 10-15 % of all NSCLCs Caucasians and 20-30 % of all NSCLCs East-Asian harbour EGFR mutations with the prevalence increasing to 50 % or more in never smokers with NSCLC. The more frequent mutations are at the gen 18-21- exon. The three types of mutations are the exon 19 deletion, exon 20 insertion and exon 18-21-es mutation. The most common mutations are exon 19 and exon 21- mutations. The metaanalysis showed a therapeutic response over 70% in cases of EGFR mutation of adenocarcinoma (exon 19, 21 mutation). EGFR mutation is therefore a significant predictive factor in cases where EGFR TK

inhibitors are used. There is a key role of KRAS mutations in the EGFR signal. KRAS mutation is detected in 25-35% within the TKI treatment resistant patients. KRAS mutation is more frequent in smokers. This mutation is found 17%- in Afro-American patients and 26% -in Caucasians patients. Although the KRAS mutation is a bad prognostic factor, but not independent predictor of treatment result of EGFR TKI. Up to now the EGFR mutation status is the only one significant predictor of EGFR TKI treatment.

EGFR mutation and the Drug resistance

Approximately 30 % of patients still do not experience disease responses despite harboring EGFR mutant disease, and less than 5 % experience a complete respons. Most driver mutations are present in resistant tumors. Furthermore, EGFR mutations, ALK gene rearrangements, and KRAS mutations rarely coexist in treatment-naive NSCLC tumors. Acquired resistance to EGFR TKIs in the metastatic setting is inevitable. The average PFS is 10–16 months. The drug resistance remains a major clinical problem in the daily practice.
The mechanisms of primary and secondary resistance to EGFR TKIs should be separated.

4. PATIENTS AND METHODS

Between 01.Jan.2008-31.Dec.2010. we have collected and retrospectively analyzed 224 lung adenocarcinomas patient's data. The ratio of patients was the following : 113 patients were from the Medical University of Pécs, Pulmonology Department, and 111 patients were from the National Institute of Oncology. In this study for the homogenous patient population we have collected only the advanced Stage IIIB/IV patients data who suffered from lung adenocarcinoma. The closing date of survival time was: 31.Dec.2013. We have used the Kaplan Meier method. The statistical analysis was done by SPSS method (version.20.0).

The list of collected clinical parameters:

- Age, Gender

- Smoking habit

- Time of diagnosis

- Type of Biopsy

- EGFR status

- KRAS status

- Stage (IIIB/IV)

- Metastases

- Treatment of Bone Metastases

- Treatment possibilities (1st line, 2nd line, 3rd line)

- Concomittant disease

- Survival

RESULTS OF THE ANALYSED DATA:

4.1. Demographic data

The ratio of 224 patients was: 108 (48%) women and 116 (52%) men.

The overall age: 60, 54 year (39-85)

These data are confirmed that the previous dominance of men suffering from lung cancer have decreased and the prevalence of women is increasing rapidly, especially in younger ages. The cause of this change might be the spread of smoking habits with “light” cigarettes.

4.2. Biopsy

At that time of diagnosis in case of 83 patients (37%) citology was done, and in case of another 141 patients (62%) histological biopsy was done. For the best results of molecular pathology diagnosis histological biopsy is the recommended option.

4.3. Smoking habits

From the all 224 patients 156 (70%) patients were smokers. Most of men were heavy smokers: from 116 men 87 (75%) were smokers, and the rest 29 men (25%) were never smokers. From the analysed 108 women 69 (64%) were smokers and 39 (36%) never smoked. These data are supporting the strong wellknown correlation between the smoking habit and the incidence of lung cancer. We all know that smoking is increasing not only the risk of COPD but is the first risk factor in cases of all types of lung cancer. From the results of molecular pathology research it is clear that smoking is playing a very important role in developing KRAS mutations at early stage of carcinogenesis with absolutely different signal pathway than in non-smokers.

4.4. Ratio of Mutation status

In Hungary very strict finance rules are prepared by National Health Insurance (OEP). According these rules during this period we can use the EGFR TKI treatment possibilities only for those patients who are representing KRAS wild type (without any mutation). This is the real reason why during this period the majority of molecular pathology methods identified KRAS mutation status. We also have to mention that during this period the EGFR TKI treatment was used only in 2^{nd} and or 3^{rd} line, after the chemotherapy. The examination of EGFR 19 and 21 mutations were done in 117 patients (52%) out of 224 patients. In case of 56 patients (25%) mutations were detected and in case of 61 patients (27%) wilde type (Wt) was detected. In Hungary we can use EGFR TKI inhibitor, specially erlotinib in the case of 2^{nd} line treatment of EGFR Wt. Dominantly KRAS examination was done in case of 187 patients (84%) out of 224 patients. KRAS mutation was detected in case of 104 patients (55%) , they were not eligible for the EGFR –TKI treatment and only 83 patients (44 %)- were eligible for the treatment with EGFR TKI (Wt).

4.5. Concomittant Diseases

Treatment possibilities are not only due to the Stage and the hystologial types but the performace status and other concomitants diseases. In this study we have collected clinical data of the most important and most frequent chronic diseases like: Ischemic Heart Disease, Diabetes mellitus, Hypertension and COPD. Out of 224 patients only in case of 154 patients

(69%) were other diseases documented, in case of the remaining 70 patients (31%) there were no information any concomitted disease. Hypertension was the most frequent concomitant disease (in case of 54 patients both women and men). The second most frequent disease was COPD it was documented in case of 50 patients. The third one was Ischemic Heart Disease and was documented in case of 34 patients (15%). Diabetes mellitus was found in case of 16 patients.

4.6. Treatment possibilities

4.6.1. First line therapies

During this period the everyday practice was the use of cytotoxic drugs for Stage IIIB/IV patients as following: bevacizumab+paclitaxel (42), gemcitabin+cisplatin (68), gemcitabin+carboplatin (26), paclitaxel+carboplatin (21), pemetrexed+cisplatin (31), docetaxel+ cisplatin (23), gemcitabin (13). The first line therapy was used for all 224 patients, in case of 116 men and 108 women. From this result we have concluded that, all 224 patients were in good general condition, ECOG: 0-2.

4.6.2. Second line therapies

The second line therapy was used for 85 patients (38%), in case of 36 men and 49 women. It is very important message that for the second line therapy less than 40% of all patients were eligible. Behind this reason there are different things: disease progression, death, decrease of performance status (ECOG ≥2), and sometimes the patients decisions. As I mentioned before during this period we could use EGFR TKI erlotinib in second line, and only for those patients who were KRAS wilde type for 38 patients (17%). The second more frequent therapy was pemetrexed. Docetaxel monoterapy was used in case of 10 patients and paclitaxel+ carboplatin treatment was used in case of 3 patients.

4.6.3. Third line therapies

The patient number is low, as only 18% of patients were eligible. The reasons are similar like incase of second line was: disease progression, death, decrease of performance status (ECOG ≥2), and sometimes the patients decisions. From the treated 41 patients more were women (23), and less 18 were men. In this group 19 patients were treated with erlotinib,

and 14 patients with pemetrexed and for more than 8 patients docetaxel was the therapeutic choice.

4.7. Distant Metastases

Lung cancer symptoms are very poor this is the one of reasons why more than 60% of lung cancer have distant metastases at the time of diagnosis. From the analysed total 224 patients in case of 174 patients (78%) distant metastases were verified. Most of patients, 72 (42%)had at least one bone lesion detected, in case of 42 patients (24%) brain metastases and in case of 28 patients (16%) liver metastases were found. Both brain and bone metastates were verified in case of 32 patients. When the brain metastases appears we can use complex oncotherapy but the death is coming so fast. The progression time of bone metastases is much more longer but the appearance of strong pain and SRE decrease the Quality of Life.

4.7.1. Ratio of Bone metastases within EGFR mutant patients

Bone lesions are detected by:

Bone Scintigraphy : 75% Computer Tomograph (CT) : 23% X-Ray: 2%,

We could realised in case of 174 patients distant metastases out of 224 patients. Bone metastases are the most frequent 42% followe by brain metastases as: 24%. Syncron bone and brain metastases were verified as 18-%. Liver metastases were less only 16% were detected. Quality of life decreased rapidly with the prevalence of bone metastases because of strong bone pain as well as with life-threatening SRE. There are complex oncotherapy possibilities what we can use step by step. Giving Bisphosphonat to the patient to turn back the bone remodelling is the most frequent therapeutic process, this was used at 36%. Radiotherapy is very important because not only stabilises the bone structure but decrease the bone pain. This method was used at 20%. Best Supportiv Care (BSC) therapy was done for 32% of the patients. Considering the EGFR mutation status is a wellknown prognostic and predictive factor in case of of lung adenocarcinoma as well we have tried try to find some correlations between the prevalence of bone metastases and EGFR mutations status. From the 72 patients with verified bone metastases we could detect EGFR mutation in case of 38

patients, and in other 34 patients EGFR mutation was not detected. Suprisingly the ratio was similar within those patients who have not suffered from bone metastases. In this group, from 152 patients the ratio was: 78 patients were EGFR mutant, and 74 patients were wilde type.

> **I. Statement**: *We can conclud that there is no significant correlation between appearance of bone metastases and EGFR mutation status. (p=0,59).*

4.7.2. Bone and Brain Metastases appearance within KRAS mutant patients

Carcinogenesis of lung cancer has a long pathway. During this process lots of genetics and epigenetics abnormalities are accumulated in the normal lung tissue, which leads to the malignant disease. KRAS mutations is one of the most important and very early mutation wich correlates with smoking habits. The other very important signal transduction pathway is the activation of EGFR mutations. Those patients who are heavy smokers the KRAS mutation status is very high and they will not respond for EGFR TK inhibitors therapy. This analysis is based on these correlations. In this study, from 72 patients who were suffering from bone metastases in case of 44 patients KRAS mutation (62%) was detected and in case of 28 patients (38%) there were no mutations (Wt). In case of those 85 patients (56%) who has no verified bone metastases there were no KRAS mutations detected. In this study we have found syncron bone and brain metastases in case of 32 patients. Duplex metastases were documented at 60% in case of 19 patients and they were verified as KRAS mutant patients, opposite the 40% who were KRAS Wilde type. The prevalence of duplex metastases decreased rapidly the Quality of life and Survival time also.

> **II. Statement:** *The results of this analysis showed that there is a higher ratio, (60%) of bone metastases within patients where KRAS mutation was detected. These patients are mostly heavy smokers, and they are not eligible for EGFR TK inhibitors therapy according to Hungarian Health Insurance rules.*

In other hand, ratio of bone metastases was lower within non-smokers (40%) and mostly (56%) for those patients who not representing KRAS mutation, so they were KRAS wilde type. According to the Hungarian Health Insurance Rules these patients are eligible for the treatment with EGFR TK inhibitors. In the scientific literature a complex therapeutical effects is mentioned. In this case the targeted agents, like EGFR TK inhibitors are influencing the bone remodelling system, changing the osteolytic metastases into osteoblastic metastases, and there is a pain killer effects as well.

4.8. Survival data

The survival results of the three years analysis which was done from 01.Jan.2008 to 31.Dec. 2010 are the following. Closing time of survival data collection was at 31.12.2013. Survival times were calculated using Kaplan-Meier method. We have used SPSS statistic modell (Versio 20.0). At that time from the total of 224 patients only 42 patients (18%) were alive most of the patients - 182 patient (82%) has died. The gender ratio of between 42 survivor was: 24 women and 18 man.

Out of 182 patients who had died, 98 patients (54%) were man and 84 patients (46%) were women.

The therapeutic decision making KRAS mutation status was the following: From those patient who had died, 60 % detected KRAS mutation, so they were not treated by EGFR TK inhibitors. The rest of patients who had died 40% where KRAS wilde type. From the 42 survivors 31 patients were KRAS mutant and 71 (58%) were wilde type. In this study according to the Hungarian health Insurance Policy we could use erlotinib only for 2^{nd} and 3^{rd} lines, for KRAS Wt patients. The smokers were not eligible for this EGFR TK inhibitor therapy because of higher presence of KRAS mutation. Cytotoxic chemotherapy was given to all these patients as a 1^{st} line therapy, so there is some overlap in these two types of therapy. Chemotherapy was give for 167 patients because of KRAS mutant status they were not eligible for EGFR TKI therapy. In conclusion we have realised that the EGFR TKI treatment

possibilities gives longer life for the selected pupulations. This is clear that these good results are coming from the very strict eligible criteria (For 2nd and 3rd line, for ECOG 0-2 patients).

III. Statement: *Those patients lived longer who were never smokers, who were KRAS wild type and who were treated with EGFR TK inhibitors. Good performance status (ECOG 0-2) which was a selection criteria by Hungarian Health Insurance has a great influence this results.*

5. RESULTS

In this study we have retrospectively analysed clinical data of 224 patients. From this group 57 patients received EGFR TK inhibitor treatment as 2nd and 3rd lines therapies. The treatment choice was based on the erlotinib label and the Hungarian Health Insurance rules.

I. These data validated the fact that ratio of EGFR mutation is decreasing within heavy smokers and the ratio of KRAS mutant patients is higher because of smokig. In our data more than 70% of patients were smokers.

II. The presence of bone metastases at that time of diagnosis suggest a faster disease progression. In this case to start the targeted EGFT TK inhibitor treatment and the complex oncotherapy is mostly recommended. In this retrospective analysis we use common diagnostic process to recognise bone metastases. (X-Ray, bone sctintigraphy, CT, MRI).

III. We have investigated the survival times of patients treated with cytotoxic chemotherapy during bone metastases. These data are confirming the hypothesis that treatment results with EGFR TK inhibitors for selected patients (EGFR mutant or KRAS wilde type) gives survival benefit in that case of developed bone metastases. There are different reasons: On one hand, according to the Hungarian Health Insurance Rules EGFR TK Inhibitors treatment is allowed only for good performace patients (ECOG: 0-2) therefore the survival chance is longer. The results of this study has confirmed that. On the other hand this good results are coming from not only as result of signal transduction blockade pathways in

nucleus, but also from the new recognition that EGFR TK inhibitors are able to change osteolytic metastases into osteblatis metastases. They are decreasing the risk of SRE and increasing the Quality of Life. As a result of all this inhibition of tumor progression has became more effective. Our data verify that the bone metastases is not independent prediktiv marker's of EGFR TKI treatment, but very strong prognostic factor of disease progression.

In our retospectiv study we were not able to differentiate the osteolytic or osteoblastic metastases because there were no clinical data on it. From this study we are not able to confirm the osteobastic change during EGFR TK inhibitor treatment. There are more EGFR TK inhibitors available from the time of this analysis (afatinib, erlotinib, gefitinib). Nowaday we can use them in 1st line setting. There are more treatment choices for the metastatic bone lesions. First recommedntation is bisphosphonat treatment (parenteral or per os). The penetration of hydroxy-apatit into the bone mineral compound makes the bone stronger. New treatment option is the RANK ligand decoy - denosumab (Xgeva). Denosumab inhibits the contact of osteoclast with the bone surface.Thinking about these results lead to some open questions.

6. CONCLUSIONS

1, Suggested to compare the ratio of SRE without EGFR TK inhibitors and ratio of SRE during cytotoxic chemotherapy as based on scientific literature this is considered as a predictive factor.

2, Further investigations are recommended to compare the bone metastases and the pain killers need during EGFR TKI therapy.

3, Smoking is not only the most important risk factor of lung cancer but an independent risk factor of SRE also. The osteoblastic metastases are more frequent in EGFR mutation positive lung adenocarcinoma, therefore it is suggested to be investigated within the EGFR mutant

patients what is the result of denosumab + EGFR TKIs combination and how does it correlate with SRE and survival times.

4, The result of this study has showed that the mutation status of EGFR and KRAS is influencing the treatment possibilities but is not prediktiv for the appearance of bone metastases and an independent prognostic factors of disease progression. Based on the scientific literature, sometimes the mutation status has changed in the lung adenocarcinoma (trend from osteolytic change to osteoblastic metastases) and it gives longer survival chance. In this retrospective study we were not able to investigate it and additional study is recommended.

There was a big improvement in the treatment possibilities for lung cancer because more and more information is detected from the tumor biology background. More and more “drivers” mutations are verified, and the biggest problem is the early detection of mutation status and the ability to find the eligible patients especially in case of rare 1-7% mutations.

In the future more and more clinical research is need to help the patients to find the best solution and therefore to have longer survival.

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Soli Deo Glória!

8 . RELEVANT ARTICLES GROUNDING THE THESIS

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