Targetted Therapy of Lung Adenocarcinomas with Epidermal Growth Factor Receptor (EGFR) Inhibitors

Thesis

Dr. Ferenc Pintér

Semmelweis University
Ph.D. School of Molecular Medicine

Tutor: Dr. Istvan Peták, Ph. D.

Opponenets: Dr. Sándor Kerpel-Fronius, D.Sc.
Dr. Zoltán Szöllősi, Ph.D.

Members of the final exam comittee: Dr. András Falus, D.Sc.
Dr. László Buday, D.Sc.
Dr. Balázs Döme, Ph.D.

Budapest
2008.

Introduction

Gefitinib and erlotinib are small molecule inhibitors of the tyrosine kinase domain (TKI) of the EGFR. These EGFR TKIs in non-small cell lung cancer (NSCLC) have an objective response rate of 8% to 19%, mild side effects and in some patient there was rapid and dramatic tumor shrinkage. Biomarkers and clinical characteristics with reliable predictive value remain in the focus of several investigations. Adenocarcinoma histology, nonsmoking history, Asian race and female gender were the patient characteristics that were associated with increased response to both EGFR TKIs.

Mutations in the tyrosine kinase domain of EGFR were reported in the majority of tumors with dramatic responses to gefitinib and erlotinib, and in some series presence of mutations were associated with improved survival. EGFR mutations were more common in patients with the same clinical characteristics of those associated with better treatment response. The latest advances in research of biological and clinical relevance of activating mutations have been reviewed recently. The frequencies of mutations in lung adenocarcinomas were 22-67% in Asia and 3-25% in North America, 10-24% in South Europe. The prevalence of EGFR gene mutations and copy number alterations in Eastern-Central Europe has not been published yet.

EGFR gene copy number, detected by FISH, is also associated with response to gefitinib. Gefitinib-treated patients carrying EGFR gene amplification or high polysomy (FISH+) had a statistically significant improvement in response, time to progression, and survival compared with patients with no or low genomic gain for EGFR. The efficiency of this molecular predictive marker was confirmed on a subgroup of samples of phase II study of gefitinib (S0126) and on subgroup of specimens of phase III study of erlotinib (BR.21).

Although, initial retrospective studies had suggested that the protein expression is not associated with gefitinib response, two subsequent studies reported longer survival among TKI treated patients with protein overexpression detected by immunohistochemistry (IHC). In the BR.21 study, survival among patients with protein overexpression (50-55%) was longer in the erlotinib group than in the placebo group, but there was no survival advantage among patients with EGFR IHC negative tumors.

Due to the lack of consensus on the significance of predictive diagnostic tests - in particular the mutation tests - clinical oncologists both in the US and the EU most often rely on the IHC of EGFR for patient selection. This decision is based on the assumption that detection of the molecular target protein is the most reliable way to use a molecular targeted therapeutic drug. In addition, it is also assumed that IHC positive population includes the smaller patient populations of FISH positive and tumors with activating EGFR mutations.

Aims

Following successful BR 21 phase III examination, erlotinib – as the only EGFR inhibitor – became accessible as secondary and tertiary treatment of the EGFR positive, non-small-cell lung cancer. Based on the clinical experiences, the treatment in our country was restricted to the adenocarcinomas. The exact definition of EGFR positivity, as it appears in the register too, and the most appropriate examination to test for the predictability of the efficiency of the EGFR TKI treatment are remained undetermined.

Our primary objective was to assess the precise EGFR status of the lung-adenocarcinomas and to define the relationship between one another and the efficiency of the EGFR TKI therapy by analysing retrospectively 9 samples of patients responding well to the EGFR TKI and by investigating prospectively 127 specimen of patients prior to the treatment. It was important to answer the question as to whether the overproduction of the EGFR protein necessarily goes hand in hand with the activating mutation of the EGFR or with the gene copy elevation and the TKI therapy-sensitivity.

Materials and methods

Tumor Specimens: 9 samples retrospectively after and 127 lung adenocarcinóma tissues prospectively before EGFR TKI therapy were examined. In case of prospective analysis the specimens of 90 primer tumors and 37 metastases were obtained from 21 Hungarian pathologic departments. There were 7 bronchoscopic biopsies and 2 small brain biopsies. The others were samples of surgical resections of the standard size. Each specimen was reviewed by pathologist and only those with ≥ 30% tumor component were used for DNA mutation analysis in the prospective examination. In retrospective analysis 2 specimens with <30% tumor component were reanalyzed by mutant-enriched PCR and sequencing. For treatment 250 mg gefitinib or 150 mg erlotinib was daily administered. Tumor response was evaluated in accordance with Response Evaluation Criteria in Solid Tumors (RECIST).

PCR and Sequencing: After DNA extraction from the paraffin embedded specimens and biopsy smears, exons 18, 19 and 21 of the EGFR gene were amplified using nested PCR with „touch-down" protocol. After purification and bi-directional sequencing reactions using the 2^{nd} step primers sequencing fragments were detected with ABI Prism 310 Genetic Analyzer (Applied Biosystems). High quality sequence variations were confirmed from both directions in two independent PCR reactions of the original DNA samples.

Mutant-Enriched PCR: Mutant-enriched PCR is a multi-step PCR with intermittent restriction digestion to eliminate wild-type genes selectively, thus enriching the genes with exon 19 deletion or L858R exon 21 point mutation. We used a modified protocol described by Asano et al.

Fluorescent in Situ Hybridization (FISH): Gene copy number per cell was investigated by FISH using the Vysis EGFR probe and semi-automated or manual procedure. In the former procedure the tissue was pretreated by Discovery Automatic Hybridizator (Ventana). The classification was done according to the six FISH categories defined by Cappuzzo et al and was also used in the BR.21 study. Samples with a high EGFR gene copy number (high polysomy or amplification) were considered to be FISH-positive.

Immunohistochemistry (IHC): The expression of EGFR protein was determined by IHC using Dako EGFR PharmDx kits (DakoCytomation). For evaluation the same categorization was used as in the BR.21 study: samples with more than 10 percent of tumor cells showing membranous (partial or complete) staining of any intensity were stated as positive for EGFR. For semi-quantitation we also used the scoring system defined by Capuzzo et al with the modification that we evaluated only membranous staining and determined 4 level of intensity (0, 1+, 2+ = control slide of the kit, 3+) according to the vendor (Dako). The IHC score was calculated by multiplying the staining intensity and the fraction of the positive cells (0-100%).

Statistical Methods: Relationships between EGFR statuses and clinical characteristics and between mutation type and CR/PR+SD rate were analyzed by chi-square or Fisher's exact test. Age differences of various subpopulations were compared with the use of t-test for independent samples. Correlation between IHC score and other EGFR statuses was analyzed using Mann-Whitney U test. All reported p values are two-sided.

Results

Retrospective Analysis

To evaluate which biomarker analysis is associated most with the responsiveness to EGFR TKIs we analyzed the EGFR status in tumors of 9 Hungarian lung cancer patients who had responded on erlotinib or gefitinib therapy (**Table 1**). Eight patents were treated with erlotinib and one patient was a gefitinib responder. Three patients showed complete response (CR), 5 had partial response (PR) and 1 patient had stable disease (SD).

Seven samples were formalin fixed and paraffin embedded, but only smears were available from 2 patients. After IHC the smears were scraped for mutation analysis, therefore FISH could not be performed in these cases. The mutation analysis was successful in all cases. Eight (89%) patients had mutant and only 1 had wild type tyrosine kinase domain. Six mutations were exon 19 deletions and 2 were point mutations of exon 21. In bronchoscopic biopsy specimens (two cases) the proportion of

Table 1. Characteristics of patients who responded on EGFR TKI treatment and EGFR TK mutant patients designated by prospective analysis.

*, ** Bronchoscopic biopsy specimen with **25% and ***<10% tumor cell proportion. Mutant-enriched PCR assay was applied..

Ana-lysis	Case	Histo-logy	Age	Sex	Smoking status	EGFRstatus			TKI	Respon-se
						Phenotype	FISH	IHC (score)		
Retrosp	Rtr 1	ADC	58	F	never	del747-753insS	+	+ (150)	gefitinib	**CR**
	Rtr 2	ADC	68	F	never	del746-750	NI (kenet)	- (0)	erlotinib	**CR**
	Rtr 3	adsq. c.	62	F	former	L858R	+ (ampl)	+ (160)	erlotinib	**CR**
	Rtr 4	ADC	66	F	never	del746-750	NI (kenet)	- (5)	erlotinib	PR
	Rtr 5	ADC	50	M	never	del746-751insA	+ (ampl)	+ (120)	erlotinib	PR
	Rtr 6	ADC	65	F	never	L858R	+	+ (70)	erlotinib	PR
	Rtr 7	ADC	72	F	never	del746-750	NI	- (5)	erlotinib	PR
	Rtr 8	anap. c.	54	M	former	del746-750*	+	- (0)	erlotinib	SD
	Rtr 9	anap. c.	60	F	?	wild type**	+	+ (200)	erlotinib	PR
Prospective	Pr 1	ADC	61	F	never	del747-750insP	+	+ (150)	gefitinib	**CR**
	Pr 2	ADC	45	F	never	del746-752insV	+ (ampl)	+ (240)	gefitinib	**CR**
	Pr 3	ADC	50	M	former	del747-753insS	-	+ (60)	erlotinib	**CR**
	Pr 4	ADC	55	F	never	del746-750	+	- (0)	erlotinib	**CR**
	Pr 5	ADC	59	F	never	L858R	NI	+ (120)	gefitinib	**CR**
	Pr 6	ADC	67	F	never	del746-750	-	+ (15)	erlotinib	PR
	Pr 7	ADC	53	F	never	L858R	-	ND	erlotinib	PR
	Pr 8	ADC	67	M	never	L858R	+	- (0)	erlotinib	PR
	Pr 9	ADC	64	F	never	L858R	-	- (0)	erlotinib	PR
	Pr 10	ADC	55	M	never	L858R	+ (ampl)	+ (270)	erlotinib	primer: PR, meta: SD
	Pr 11	ADC	62	M	never	L858R	+	+ (270)	erlotinib, ceased	-
	Pr 12	ADC	57	F	never	del746-750	+ (ampl)	+ (180)	-	-
	Pr 13	ADC	54	F	?	del747-751	+ (ampl)	+ (270)	-	-
	Pr 14	ADC	43	F	never	del746-750	+	- (5)	-	-
	Pr 15	ADC	56	F	smoker	del747-753insS	-	+ (180)	-	-
	Pr 16	ADC	62	F	never	L861Q	+	+ (240)	-	-
	Pr 17	ADC	81	M	?	A743T	-	ND	-	-

tumor cells was low. We could not identify mutations in these samples by the standard PCR and sequencing method. However after mutant-enriched PCR of exons 19 and 21, an exon 19 deletion was found in the sample, which contained about 25% tumor cells.

In the specimen where tumor cell content was less than 10% we were not able to identify any mutation.

Prospective Analysis

The EGFR statuses in paraffin-embedded lung adenocarcinoma samples of 127 Hungarian patients were evaluated. The results of the prospective EGFR status analysis are in **Table 2**.

EGFR Mutation Analysis: The evaluation was successful in almost all (99%) cases (126). EGFR kinase domain mutation was identified in 17 cases (13,5%). Characteristics of the mutant patients are in **Table 1**. Nine mutations were 19 exon deletions and 7 were exon 21 point mutations (6x T2573G>L858R, 1x T2582A>L861Q). Beside these mutations, that have been reported to be associated with responsiveness to EGFR TKIs, we also identified a new point mutation of exon 19 (G2227A>A743T).

There was conspicuously higher mutation rate in never smokers than in current or former smokers, (42% vs. 4%, p<0,001) and in female patients (16% vs. 10%, non signficant). The frequency of mutations were higher, but not significantly, in FISH positive cases (26% mutation rate in FISH+ vs. 10,5% in FISH-, p=0,051; 67% in amplified vs. 13,3% in non amplified).

Using the 10% as a cutoff criteria for IHC positivity the frequency of mutations was higher (16%) in IHC+ than in IHC- tumors (8%),but this difference was not significant (p=0,204). The frequency of mutations was 8,1% in the tumors with low IHC score (0-99), 16% in the middle range (100-199) and 31% in tumors with the highest IHC score (200-299) by semi-quantitative score analysis. The correlation between the IHC score and the frequency of mutations is significant (p=0,025).

Additional 36, mostly not published sequence variations was found in 28 (22%) samples. However, these sequence alterations could not be confirmed from the repeated second and third independent PCR reactions, therefore these alterations were not considered valid mutations.

FISH Analysis: EGFR gene copy number was assessed by FISH analysis in 118 patients and was successful in 97 cases (82%). It was increased in 39 samples (40%

Table 2. Results of the prospective analysis.

Parameter Y ↓	All	Mutation+		FISH+		*Amplified*		IHC+	
		% (N_{mut+}/ $N_{Y\text{-examined}}$)	*P*	% (N_{FISH+}/ $N_{Y\text{-examined}}$)	*P*	% (N_{amp+}/ $N_{Y\text{-examined}}$)	*P*	% (N_{IHC+}/ $N_{Y\text{-examined}}$)	*P*
Total	127	**13,5** (17/126)		**40** (39/97)		***6*** *(6/97)*		**59** (68/116)	
Sex:									
Female	74	**16** (12/74)	*0,286*	**42** (24/57)	*0,649*	***7*** *(4/57)*	*1,000*	**58** (39/67)	*0,916*
Male	53	**10** (5/52)		**38** (15/40)		***5*** *(2/40)*		**59** (29/49)	
Smoking status									
Current / former	52	**4** (2/51)	***<0,001***	**42** (16/38)	*0,354*	***5*** *(2/38)*	*0,366*	**64** (32/50)	*0,388*
Never	31	**42** (13/31)		**54** (13/24)		***13*** *(3/24)*		**73** (22/30)	
Female	*25*	***40*** *(10/25)*	*0,676*	***47*** *(9/19)*	*0,327*	***11*** *(2/19)*	*0,521*	***71*** *(17/24)*	*1,000*
Male	*6*	***50*** *(3/6)*		***80*** *(4/5)*		***20*** *(1/5)*		***83*** *(5/6)*	
Unknown	44	**5** (2/44)		**71** (25/35)		**3** *(1/35)*		**39** (14/36)	
Age (year) +/-	54,9	58,3/54,0	*0,086*	54,1/56.7	*0,163*	*50,3/56,0*	*0,135*	55,0/54.6	*0,795*
Mutant	17			**63** (10/16)	*0,051*	***25*** *(4/16)*	***0,007***	**73** (11/15)	*0,204*
Wild type	109			**36** (29/80)		***2,5*** *(2/80)*		**56** (56/100)	
FISH+	39	**26** (10/39)	*0,051*					**66** (25/38)	*0,377*
Amplified	*6*	***67*** *(4/6)*	***0,007***					***100*** *(6/6)*	*0,078*
FISH-	58	**10,5** (6/57)						**57** (30/53)	
IHC+	68	**16** (11/67)	*0,204*	**45** (25/55)	*0,377*	***11*** *(6/55)*	*0,078*		
IHC-	48	**8** (4/48)		**36** (13/36)		***0*** *(0/36)*			
IHC score									
0-99	74	**8,1** (6/74)		**31** (17/55)		**0** (0/55)		**35** (26/74)	
100-199	25	**16** (4/25)	***0,025***	**57** (12/21)	*0,077*	**4,8** (1/21)	***<0,001***	**100** (25/25)	*<0,001*
200-300	17	**31** (5/16)		**60** (9/15)		**33** (5/15)		**100** (17/17)	

FISH+). In 6 (6%) of them the EGFR gene was amplified. One sample showed both EGFR amplification and polysomy (≥ 4 chromosome 7 centromeres). The other 33 (34%) FISH+ samples had high polysomy without amplification. Disomy for the EGFR gene was present in 42 (43%), low trisomy in 1, high trisomy in 14 samples and low polysomy in 1 specimen. These populations were categorized as FISH- (60%).

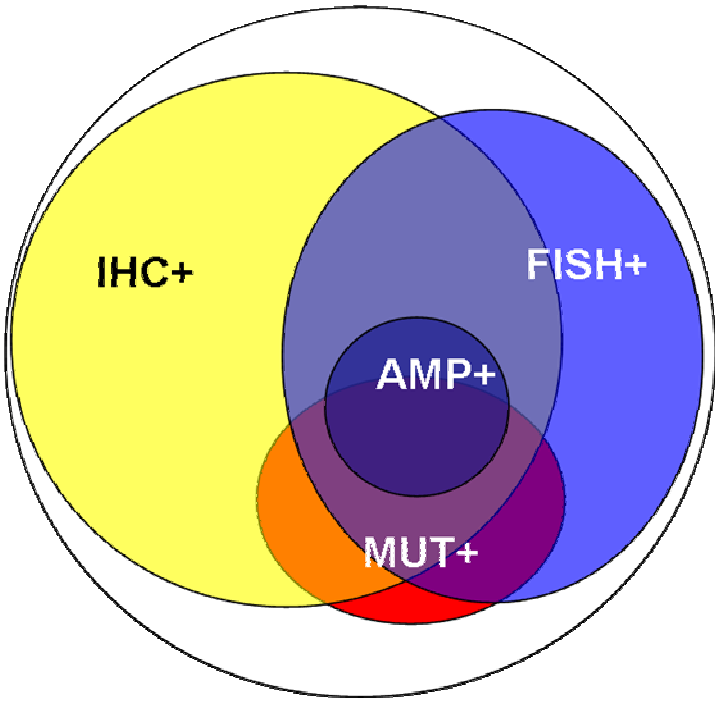
There were no significant differences in the rate of FISH positivity and gene amplification between groups with different clinical characteristics (age, sex, smoking status). A significant association was observed between gene amplification and mutation (25% EGFR gene amplification in mutant patients vs. 2,5% in wild type patients). Although the prevalences of amplification and FISH+ were higher in case of IHC positivity (11% amplif./45% FISH+) than in case of IHC negativity (0% amplif./36% FISH+), these associations did not reach significance.

IHC Analysis: EGFR protein expression was successfully evaluated in 116 patients (success rate: 99%). Overexpression was found in 68 cases (59%). There were no significant differences in the rate of IHC positivity in patients of different age, sex and smoking status. Only the semi-quantitatively estimated protein expression, using the IHC score showed significant association with the frequency of mutation. Although all gene amplification caused a very strong immunohistochemical membrane staining, statistically the protein expression was unrelated to gene copy number.

Samples analyzed by all three methods: In case of 90 patients' sample all three analyses were successfully performed, so this population can be divided into 8 subgroups based on the EGFR status. The independence of the three methods in the analysis of the EGFR status manifests again. None of the subgroups of the mutant, FISH+ and IHC+ patients fully overlapped the other, so neither method can serve as a substitution or even preselection of the other. Only 24% of the lung adenocarcinomas had a totally negative EGFR status by all three methods (**Figure 1**).

Clinical Responses of EGFR Mutant Tumors to Gefitinib or Erlotinib: Ten patients were treated with either gefitinib or erlotinib after detection of the EGFR TK domain mutation (**Table 1**). All (100%) permanently treated EGFR mutant patients responded to therapy. Five patents showed complete response (CR), 5 had partial response (PR). Patients with exon 19 deletion were more sensitive to TKI treatment than L858R

mutants. While 4 of the 5 patients with exon 19 mutations showed complete response, only one of the 5 L858R mutants showed complete response and the other 4 had partial response.



MUT	FISH	IHC	*n*	frequency
+	+	+	*7*	**7,8%**
+	+	-	*3*	**3,3%**
+	-	+	*3*	**3,3%**
+	-	-	*1*	**1,1%**
-	+	+	*18*	**20%**
-	+	-	*10*	**11,1%**
-	-	+	*26*	**28,9%**
-	-	-	*22*	**24,4%**
			90	**100%**
AMP +	**MUT +**		*4*	**4,4%**
AMP +	**MUT -**		*2*	**2,2%**

Figure 1. Subgroups of lung adenocarcinomas designated by the three EGFR status analyses and their relations to each other.

Five EGFR mutant responders were FISH positive (two had EGFR amplification) and 4 were FISH negative. In one case the FISH analysis wasn't

successful. The EGFR protein was overexpressed in six cases. In three cases, however, mutant responders were negative by IHC. One sample from a partial responder patient was not determined by IHC due to technical failure. This tumor had also a negative FISH status. Another patient who had a partial response as a result of erlotinib treatment had normal EGFR gene copy number (disomy, FISH-) and protein expression (IHC-), but showed T2573G>L858R mutation as the only EGFR abnormality.

Discussion

Prospective, randomized, placebo-controlled clinical trials are currently underway to determine the exact role of different predictive biomarkers in selection of non-small-cell lung cancer patients for EGFR TKI treatment, in particular, erlotinib therapy. It will take probably years to complete these trials. At the present, oncologists can only rely on the existing reports of clinical benefit in patient populations positive for different biomarkers related to the increased activity of EGFR, protein overexpression, increased gene copy number and presence of activating mutations.

Our results provide evidence that patient populations positive by these different EGFR diagnostics only partly overlap. Most importantly, patients which do not overexpress EGFR protein therefore presenting negative EGFR immunohistochemical reaction, can carry activating mutations and have excellent response to EGFR TKI therapy, including erlotinib.

Although no conclusion can be reached about the negative predictive value of the absence of activating mutations due to lack of clinical information of all patients, the 93% (13/14) response rate to erlotinib in the patients carrying activating mutations is remarkable higher then it was reported in the study published by Tsao et al. Tsao et al. found higher, but only 2 out of 8 (25%) response rate in EGFR (classical mutations) mutant patients, in comparison to the 9% response rate in the whole population (BR.21 study).

In the BR21 trial, only EGFR IHC positive patients gained a significant survival benefit from erlotinib treatment. Based on this study, erlotinib was registered by the

FDA for the second and third line treatment of NSCLC independently from any biomarker analysis. However, the registration by the EMEA in the EU included an important remark: “the survival benefit of erlotinib is not proven in EGFR negative patients”.

The advantages of IHC analysis are: a) the most cost efficient and easiest method. b) most *in vitro* diagnostic units can perform this test, c) this test is the most frequently positive - in our study 59% - therefore excludes the least patients form therapy. The disadvantage of IHC lays in the difficulties of standardization. In our laboratory, we use the same standardized IHC protocol used by Tsao et al., and the same evaluation criteria of 10% positivity as the threshold for positive EGFR IHC status. The sensitivity of the IHC reaction can be increased, but the altering the standardized protocol result abolishes the quantitative information of expression levels and interlaboratory comparability. These technical problems are discussed for HER2 testing. However there is no similar consensus for the guidelines of EGFR IHC and FISH testing in lung cancer.

The FISH analysis is more expensive and difficult method. In 61 samples the FISH signals were already valuable after the first FISH procedure. However, in case of 36 samples we had to repeat the reaction with different extent of digestion. In case of 21 specimens we couldn’t gain sufficient signal intensity for the adequate diagnosis even after multiple attempts with modified FISH protocol. After extensive optimalization of the protocol we reached 82% success rate, but it is still less then the 99% success of the IHC analysis. However, FISH analysis is a more reliable method than IHC since the presence of the normal signals of the normal karyotype (disomy) exclude the possibility of false negativity. The quantitative evaluation of FISH is also more objective. These technological differences may have contributed to the better predictive value of FISH in comparison to IHC in the retrospective analysis of BR21 similarly to results found previously with gefitinib.

In our study FISH was less frequently positive (40%) than IHC (59%). Since, the survival benefit was significant in the larger population of IHC positive patients of the BR21 trial, it is not warranted to exclude FISH negative but IHC positive patients from erlotinib treatment. All of the 6 patients with intrachromosomal gene amplifications were also 3+ IHC positive similarly to HER-2 in breast cancer, but 41% of the FISH

defined by polysomy were IHC negative. The FISH positive patients significantly benefited from erlotinib regardless of the IHC status in BR21. Therefore, IHC negative but FISH positive patients can not be regarded as “EGFR negative”.

The sequence analysis of archived tissue samples by automatic sequencing is considered the gold standard for mutation detection but also a very labor intensive and difficult assay. However, in our laboratory EGFR DNA sequence could be determined in 99% of surgical biopsies.

Activating mutations of the EGFR may increase the receptor activity even in the absence of protein overexpression which can lead to oncogene dependence. In order to test this hypothesis and to explore the potential clinical significance of these mutations in the absence of IHC positive protein, we obtained clinical information of 10 mutant patients who received either gefitinib or erlotinib treatment following our diagnosis. Remarkably, there was 5 CR and 5 PR in this group of patients regardless of IHC positivity. In addition, we retrospectively analyzed the samples of 9 patients reportedly having an exceptionally good response to EGFR TKI treatment. We found activating mutations in all except one of these tumors. We have no clinical data of the non-mutant patients, but these results can be evaluated in consideration of the low frequency (13,5%) of mutant tumors in our set of 127 tumor samples, and in comparison to the 13,9% response rate of the BR21 trial in case of unselecteded lung adenocarcinomas.

Most importantly 7 of the mutant patients who responded to EGFR TKI treatment were EGFR IHC negative. 5 of these samples were completely IHC negative (0%) and 2 did not meet the 10% threshold criteria. Alterations from the standard IHC protocols can increase the ratio of IHC positive samples but it is already 59%, and further increase in sensitivity would diminish the quantitative information on EGFR expression. Previous studies have reported lack of correlation between EGFR mutation and IHC positivity, and EGFR mutant tumors with absent IHC positivity in gefitinib treated NSCLC patients, but this is the first study which focuses on this phenomenon in lung adenocarcinomas treated with both erlotinib and gefitinib.

The evaluation of the clinical significance of EGFR mutations always suffers from the low number of patients which under power the statistical analysis. The BR21 trial which led to the market authorization of erlotinib included only 8 erlotinib-treated

patients with classic activating EGFR mutations. There were only 1 CR and 1 PR (25%) in those patients which far below to the 65-100% response others reported with EGFR-TKI treated EGFR mutant patients. In this study we provide clinical data of 18 (14 erlotinib treated, 4 gefitinib) EGFR mutant patients. We found 94% PR or CR rate in these patients. The reason for this difference may lay in the different laboratory practices in different laboratories.

The BR21 study also concluded that patients carrying EGFR mutations have better prognosis independently from EGFR TKI treatments. The median survival of EGFR mutant patients in the control arm was 9,1 months, the median survival in control arm of the unselected population was 4,7 months. In contrast, several others have reported the average survival of EGFR mutant patients receiving EGFR-TKI treatment over 30 months. Although, these patient populations can not be directly compared and new randomized prospective studies will be necessary to draw final conclusion. Our observation is that EGFR mutant patients in this study received the EGFR TKI treatment at a very late stage of their disease, therefore the major reduction, often complete elimination of the tumor burden most evidently prolong their survival, although further follow up (probably years) is required to determine median survival time. The first EGFR mutant patient in this study started the gefitinib treatment four years ago against multiple brain metastasizes in a moribund clinical stage, and she is still tumor free and enjoys a good quality life.

Therefore, in our view, although further studies will be required to decide which biomarker analysis is the most suitable for individualized EGFR-TKI treatment, at the present, this important clinical decision can not solely be based on a single method, immunohistochemistry, but molecular diagnostic methods, particularly DNA sequence analysis, should be part of the biomarker analysis of EGFR status of NSCLC patients. This report also includes 3 EGFR mutant former smoker patients with excellent therapeutic response. Most importantly, this study is underpowered to suggest that only EGFR mutant patients should be exclusively selected for EGFR TKI therapy, but provide strong evidence that all EGFR mutant lung adenocarcinoma patients should be treated regardless of other biomarkers or smoking status.

Publications

Publications related to thesis

1. **Pinter F**, Papay J, Almasi A, Sapi Z, Szabo E, Kanya M, Tamasi A, Jori B, Varkondi E, Moldvay J, Szondy K, Keri G, Dominici M, Conte P, Eckhardt S, Kopper L, Schwab R, Petak I. Epidermal growth factor receptor (EGFR) high gene copy number and activating mutations in lung adenocarcinomas are not consistently accompanied by positivity for EGFR protein by standard immunohistochemistry. J Mol Diagn. 2008 Mar;10(2):160-8. **IF (2007): 3,478**
2. Schwab R, **Pinter F**, Moldavy J, Papay J, Strausz J, Kopper L, Keri G, Pap A, Petak I, Oreskovich K, Mangel L. Modern treatment of lung cancer: case 1. Amplification and mutation of the epidermal growth factor receptor in metastatic lung cancer with remission from gefitinib. J Clin Oncol. 2005 Oct 20;23(30):7736-8. **IF: 11,81**
3. Varkondi E, **Pinter F**, Robert K, Schwab R, Breza N, Orfi L, Keri G, Petak I. Biochemical assay-based selectivity profiling of clinically relevant kinase inhibitors on mutant forms of EGF receptor. J Recept Signal Transduct Res. 2008;28(3):295-306. **IF (2007): 1,815**
4. Schwab R, Peták I, **Pintér F**, Szabó E, Kánya M, Tamási A, Várkondi E, Almási A, Szokolóczi O, Pápay J, Moldvay J, Kéri G, Kopper L. Epidermal growth factor receptor (EGFR): therapeutic target in the treatment of lung adenocarcinoma. Orv Hetil. 2005 Nov 13;146(46):2335-42.
5. Varkondi E, **Pinter F**, Petak I. The role of epidermal growth factor receptor family int he molecularrly targeted therapy and diagnostics of epithelial malignancies. Orvosképzés. 2006;81(3):159-167.

Other publications

1. Elek G, Gyökeres T, Schäfer E, Burai M, **Pintér F**, Pap A. Early diagnosis of pancreatobiliary duct malignancies by brush cytology and biopsy. Pathol Oncol Res. 2005;11(3):145-55. **IF: 1,162**