Review Article

Targeted therapy in non-small cell lung cancer: a focus on epidermal growth factor receptor mutations

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Author's introduction: Gerard Milano, PhD, is currently Associated Director of the Nice Cancer Center, in charge of Scientific Affairs. He also drives the Oncopharmacology unit of this institute as well as the university associated Unit EA 3638. Gérard Milano is a pharmacologist in cancer area, senior author of more than 400 peer-review international publications. His main fields of interest are: preclinical pharmacology of anti-cancer agents, clinical pharmacokinetics, pharmacogenetics, tumor genomics. Gérard Milano loves mountain biking, flie trout fishing and blues music.

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Abstract: The main molecular targeting of lung cancer [non-small cell lung cancer (NSCLC)] concerns mutations of epidermal growth factor receptor (EGFR). The awaited responsiveness of tumors carrying these mutations is high with for instance 60% to 80% with tyrosine kinase inhibitors hitting EGFR mutations. The EGFR T790M as a secondary mutation is responsible for the occurrence of a resistance phenomenon. A multitude of drugs have been produced and tested with the property of a specific binding at the EGFR T790M site. There is currently an evolution oriented to a robust genotyping methods allowing the identification of given molecular anomalies (pyrosequencing for instance) towards the consideration of a much larger set of molecular anomalies under the form of a global genotyping realized with the use of next-generation sequencing (NGS). This phase of whole genome analysis necessitates the introduction of a specialized staff for data treatment. A possible substitution plasma/tumor for the mutation analyses is perceptible in lung cancer, a preference being however given to the intratumoral direct investigation when

this is feasible. EGFR mutations as targetable anomalies are illustrative examples, that the management of NSCLC is currently drawing a significant benefit from personalized therapy.

Keywords: Targeted therapy; epidermal growth factor receptor pathway (EGFR pathway); tyrosine kinase inhibitors (TKIs); lung cancer; tumor mutations

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Lung cancer represents the main tumoral pathology with a high mortality (1). The last ten years have seen the emergence of histology (squamous cell vs. non squamous cell) as a determining factor for the management of lung cancer. But, above all, an important proportion of patients may now benefit of a molecular characterisation of their tumoral lesions which can be treated with targeted therapy (on mutations, on fusion genes). Currently, the majority of the molecular targets concerned by this therapeutic strategy are found in tumors which are of adenocarcinoma type.

Background

The main molecular targeting of lung cancer [non-small cell lung cancer (NSCLC)] concerns mutations of epidermal growth factor receptor (EGFR). The first applied tyrosine kinase inhibitors (TKIs) like erlotinib and gefitinib have a preferential activity against activating EGFR mutations of lung cancer, these agents have been the first to open the era of targeted therapy of lung cancer in the beginning of 2000 (2). Of note, the presence of these mutations is globally at relatively low frequency in NSCLC with the occurrence in 17% of Caucasian patients and 40% of Asian patients of targetable EGFR mutations and around 6% of patients with the ALK translocation. The awaited responsiveness of tumors carrying these mutations is high with for instance 60% to 80% to TKIs hitting EGFR mutations (3). After an initial and satisfactory response to EGFR TKIs, almost all patients present a phenomenon of resistance manifested by tumoral progression evident after 9 to 12 months (4). Focused genotyping analyses performed on biopsy samples of resistant patients with acquired resistance have put the light on the EGFR T790M as a secondary mutation as responsible for the occurrence of this resistance phenomenon. This secondary mutation is occurring in almost 60% of resistant tumors (4). The mechanism of action by which the resistance is playing involves a conformational modification in the ATP pocket of the EGFR itself giving to the active site more affinity towards ATP than gefitinib or erlotinib. As a primary site of acquired resistance EGFR T790M was an evident tempting target for drug developers facing an important medical need. In principle, a drug which would impact preferentially the mutant EGFR would spare adverse events carried by the presence of WT-EGFR. Not surprisingly a multitude of drugs have been produced and tested with this property of a specific binding at the EGFR T790M site. Afatinib is among these emerging drugs showing activity on this specific form of EGFR (4). More recently (5), a 3rd generation of drugs targeting specifically T790M were made available (AZD9291, CO1686…). To summarize at this stage, most EGFR mutations concern exon 19 deletions (Del 19) and L858R mutation in exon 21, they represent globally 90% of all mutations and are linked with sensitivity to EGFR TKIs. At the opposite, lung cancers exhibiting exon 20 insertions or T790 M in exon 20 are shown to be resistant to these drugs (5).

ALK targeting with crizotinib is offering 50% to 60% of objective response rate in patients whose tumor is carrying the ALK anomaly (3). A new generation of ALK TKIs are now of clinical use with ceritinib and alectinib. These drugs allow a new phase of therapeutic gain to be obtained in cases of resistance to crizotinib (6). Work is in progress in order to identify predictive factors for a resistance to crizotinib with candidates being numerous including growth factors, kinases, interacting proteins, transcription factors but no one among this large list is emerging currently with sufficient evidence (6). A second-generation of ALK inhibitors, with ceritinib as a concrete example, can overcome several crizotinib-resistant mutations and has shown efficacy both *in vitro* and *in vivo* with the use of pertinent laboratory models of acquired

Table 1 Lung cancer—druggable targets (from INCa data 2012)

Target	Function of the marker	Drug	Activity of drug
EGFR activating mutations	Molecular target	Gefitinib Erlotinib	Reversible inhibitors of EGFR
		BIBW 2992	Irreversible inhibitor of EGFR and HER2
		PF00299804/PF299	Irreversible inhibitor of EGFR and HER2
Primary resistance to EGFR targeting	Molecular target + (resistance TKI-EGFR)	BIBW 2992	Irreversible inhibitor of EGFR and HER2
		PF00299804/PF299	Irreversible inhibitor of EGFR and HER2
Mutations in exon 20 of HER2	Molecular target + (resistance TKI-EGFR)	BIBW 2992	Irreversible inhibitor of EGFR and HER2
EML4-ALK translocations	Molecular target + (resistance TKI-EGFR)	PF-02341066	Double inhibitor MET/ALK
KRAS mutations	Prediction of response + (resistance TKI-EGFR and TKI EGFR irreversibles)	AZD6244/ARRY-142886	MEK inhibitor
	Prediction of response + (resistance TKI-EGFR irreversibles)	GSK1120212	MEK inhibitor
	Prediction of response + (resistance TKI-EGFR irreversibles)	Ridaforolimus (AP 23573, Deforolimus)	mTOR inhibitor

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitors.

resistance to crizotinib. This is consistent with recent clinical data showing an evident activity of ceritinib in patients with crizotinib-resistant disease (7).

Mutation analyses

In France, the National Cancer Institute (INCa) is playing a preponderant role for putting at disposal and unifying the methods for the practice of molecular testing with clinical applications for the larger number possible of patients (see *Table 1*). The French territory is covered with regional platforms dedicated to the practice of molecular biology testing under the auspices of the INCa. Thus, the analytical need for the determination of molecular anomalies of therapeutic interest is taken into consideration and this is particularly true for lung cancer. The analysis is to be considered in its totality including not only the analytical aspect with a specific equipment but also the biological sample itself on which the analysis is applied. There is currently an evolution from the use of robust genotyping methods allowing the identification of given molecular anomalies (pyrosequencing for instance) towards the consideration of a much larger set of molecular anomalies under the form of a global genotyping realized with the use of next-generation sequencing (NGS) necessitating in the whole analysis the introduction of specialized step for data treatment. Currently the precise field of utilization of NGS between research and routine use remains to be elucidated. As said above another consideration to be paid to these molecular analyses concerns the tumoral material itself. This is particularly true in the domain of lung cancer where it is often difficult to obtain a tumoral sample in adequate conditions (access, optimal volume) when keeping also in mind the inherent problem of the intra-tumoral heterogeneity. In this context a perspective of amelioration is perceptible. This ray of hope is brought by the use of so-called "liquid biopsies" which is, practically speaking, the possibility to get tumoral DNA isolated from a blood sample. A recent work by Douillard *et al.* (8) is particularly illustrative on these aspects. The authors have compared, on the basis of almost one thousand of patients, the results of the analysis of EGFR mutations classically performed on the solid tumor in place (deletions exon 19 and point mutation L858R, as the most frequent ones) with those arising from tumoral DNA extracted from blood in parallel in the same patient. The authors reported an interesting high level of concordance higher than 90% for the cases in comparison (652 in total). These data

Table 2 Gene mutations of clinical interest in NSCLC [2015]

Gene	Exon	Method	Type of analysis	Molecular analysis
EGFR	18	Pyrosequencage	Targeted	p.E709*, p.G719*
EGFR	19	Pyrosequencage	Targeted	Del19
EGFR	20	Pyrosequencage	Targeted	p.T790*, pS.S768*
EGFR	21	Pyrosequencage	Targeted	p.L858*, p.L861*
KRAS	2	Pyrosequencage	Targeted	p.G12*, p.G13*
KRAS	3	Pyrosequencage	Targeted	p.Q61*
KRAS	4	NR		NR
BRAF	15	Pyrosequencage	Targeted	p.V600*, p.G464E, p.G466v, p.G469A
PI3KCA	9	Direct sequencage	Global	
PI3KCA	20	Direct sequencage	Global	
HER2	20	Direct sequencage	Global	
ALK		FISH		Translocation ALK
		IHC		Translocation ALK
		RT-PCR		ALK-EML4 (V1,2,3a,3b,5)

NSCLC, non-small cell lung cancer; EGFR, epidermal growth factor receptor; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry; RT-PCR, reverse transcription polymerase chain reaction.

have led the authors to conclude to a possible substitution plasma/tumor for the mutation analyses in lung cancer, a preference being however given to the intratumoral direct investigation if this one is feasible. Following the publication of these results, European health authorities have confirmed this possibility for the delivery of Iressa and Tarceva (EGFR TKIs on the market).

Conclusions

In total one can consider EGFR mutations in NSCLC as an illustrative example for targeted therapy in cancer care. In France this personalized treatment is made possible to a large number of patients thanks to the concrete and constant implication of the INCa. *Table 2* is providing a complete list of gene mutations, all validated by the INCa, of concerns for the management of NSCLC with targeted therapy.

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None.

Footnote

Conflicts of Interest: Gérard A. Milano, Honoraria (Merck Serono, Pierre Fabre Oncology, Roche), Consultancies (ONXEO, Nordic Pharma).

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