Poster discussion session
Advanced NSCLC

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**Poster discussion session: advanced NSCLC**

**EGFR mutation**

- Clinical application of liquid biopsy
  - EGFR mutation detection in plasma of lung tumor patients in the absence of contributive tissue is a relevant alternative for prescription of tyrosine kinase inhibitors in a routine clinical setting *(Denis et al)*

- EGFR-TKI resistance mechanisms
  - An integrative analysis of the putative gefitinib-resistant genes in a lung cancer cell line model system *(Han et al)*
EGFR mutation detection in plasma of lung tumor patients in the absence of contributive tissue is a relevant alternative for prescription of tyrosine kinase inhibitors in a routine clinical setting

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EGFR mutation detection in plasma of lung tumor patients in the absence of contributive tissue is a relevant alternative for prescription of tyrosine kinase inhibitors in a routine clinical setting, Denis et al

Aim

- EGFR alterations can be detected in the plasma of lung cancer patients mutated in their tumor

- The European Medicines Agency has recently allowed the use of gefitinib in patients tested positive in blood if a tumor sample is not evaluable

- But there has been no prospective report evaluating the efficacy of tyrosine kinase inhibitors (TKI) in patients only tested in circulating DNA (ctDNA) in a routine clinical setting
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**Methods**

- Extraction of circulating DNA from plasma (EDTA) with the PureLink Virus Kit on an iPrep purification instrument (Life Technologies) – 40 min
- Detection of EGFR alterations using the approved Therascreen EGFR RGQ kit (Qiagen) – 2 h
EGFR mutation detection in plasma of lung tumor patients in the absence of contributive tissue is a relevant alternative for prescription of tyrosine kinase inhibitors in a routine clinical setting, Denis et al.

RESULTS

- 3 clinical cases, insufficient tumor tissue for EGFR mutation determination
  - The 3 were EGFR mutation positive in cDNA and were treated with EGFRTKI
  - No correlation with tumor sample determination

- No p outcomes described, although there are images of radiological tests pre and during EGFRTKI treatment showing good response

- Authors’ conclusion: when tumor sample is not available for molecular testing, detection of an EGFR alteration in circulating DNA (plasma) is a relevant alternative for TKI prescription in routine clinical practice
Liquid biopsies

- Non-invasive blood tests that detect circulating CTCs and fragments of tumor DNA that are shed into the blood from the primary tumor and from metastatic sites

- Cell-free tumour DNA (cfDNA) is emerging as an effective alternative to CTCs, with the benefits of easier collection and analysis

- May capture the heterogeneity of the disease

- May allow the detection of additional mutations from emerging subclones, including those involved in the development of AR

- At some point, these tests may be used in the early diagnosis of cancer
EMA extended the drug label of gefitinib to include the detection of EGFR mutations in ctDNA obtained from plasma when tumor sample is not evaluable

- Comparison tumor/matched plasma DNA for EGFR-mut in 1060 p screened in a 1st-line gefitinib study (Douillard JTO 2014)
  - Baseline tumour samples in 1033 p (118+/859 mutation status known; 13.7%)
  - 94.3% concordance between tumor and matched plasma in 652 p with a sensitivity of 66.7% and specificity of 99.8%
  - 96.9% concordance between two plasma specimens from the same p (224 matched specimens)

- 1st-line gefitinib, ORR and PFS
  - Mutation+ tumor: 70% and 9.7 mo
  - Mutation+ tumor and plasma: 76.9% and 10.2 mo
EURTAC trial: association of *EGFR* L858R mutation in circulating free DNA with survival

- *EGFR* mutations in serum, 2\textsuperscript{nd} end-point
- cfDNA obtained in 57\% of p
- *EGFR* mutations identified in cfDNA in 78\% of p
- *EGFR* in cfDNA did not predict RR in either arm (erlotinib vs CT)

- L858R mutation in tumor tissue (HR, 1.85; *P* = .007) and in cfDNA (HR, 2.70; *P* < .001) related to shorter OS

*Karachaliou JAMA 15*
EGFR mutation detection in plasma of lung tumor patients in the absence of contributive tissue is a relevant alternative for prescription of tyrosine kinase inhibitors in a routine clinical setting, Denis et al

COMMENTS

- EGFR mutation results in plasma rapidly obtained
- Real-time analysis determination
- False negative?
- Possible role in prognosis should be explored
- Potential value in monitoring the course of the disease and for determination of resistance mechanisms (T790M)
  - The group reported two clinical cases with sequential determinations of EGFR mutation in plasma samples that correlate with clinical outcome (Marc JTO 14)
An integrative analysis of the putative gefitinib-resistant genes in a lung cancer cell line model system

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15-18 April 2015, Geneva, Switzerland
**An integrative analysis of the putative gefitinib-resistant genes in a lung cancer cell line model system, Han et al**

**METHODS/RESULTS**

- Analyses of the H1650 cell line, and the gefitinib resistant H1650GR cell line
  - *Exome-seq*: there were 8 unique missense mutation of H1650GR, including 3 genetic mutations that might influence the coding protein structure: EPHB6, LAX1 and TRPC6
  - *SNP chip*: 47 up-regulated genes in H1650GR amplification region 3q13.1-3q19 and 61 down-regulated genes in the deletion region 18q12.1-18q23

- EPHB6 gene mutation detected in H1650GR by Sanger sequencing
  - Drug resistance mechanism of EPHB6 gene mutation might lie in enhancing AKT protein phosphorylation level and inhibiting cell apoptosis
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CONCLUSIONS

• EPHB6 gene mutation might play an important role in gefitinib drug resistance and the drug resistance mechanism of EPHB6 gene mutation might lie in enhancing AKT protein phosphorylation level and inhibiting cell apoptosis

• Further mechanistic studies are warranted to explore how the genes, such as EPHB6, LAX1 and TRPC6 are involved in the gefitinib resistance to NSCLC
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COMMENTS

• AR, a major limitation of EGFRTKI activity in EGFR-mutated p

• ~30% of AR cases lack identifiable molecular alteration

• H1650 NSCLC cells display primary resistance to EGFRTKIs although they have a deletion mutation on exon 19 of the EGFR gene
  - Gefitinib-resistant H1650 cells are FGFR1-dependent for growth and proliferation (Ware Oncogenesis 13)
  - PTEN loss and activation of AKT signaling pathway contributed to erlotinib resistance in EGFR-mutant NSCLC cell line H1650 (Han, Zhongguo Fei Ai Za Zhi. 12)
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**COMMENTS**

- EPHB6 gene, background
  - Encodes a member of a family of transmembrane proteins that function as receptors for ephrin-B family proteins
  - The Eph family of receptors is the largest known subfamily of receptor tyrosine kinases; ligands are called ephrins
  - Expression of EPHB6 gene significantly higher in tumors of non-smokers vs smokers (p=0.0033) (*Szymanowska-Narloch Adv Med Sci. 2013*)
  - Mutations in EPHB6 occurring in NSCLC might lead to a pro-metastatic phenotype (*Bull PLoS One 2012*)

- EPHB6 gene may play a relevant role in EGFRTKI resistance and should be further investigated
**Poster discussion session: advanced NSCLC EGFR mutation**

- **Clinical application of liquid biopsy** *(Denis et al)*
  - In absence of suitable tumor tissue, determination of EGFR mutation in cDNA could be used to treat p
  - Non-invasive methods for monitoring outcome and resistance should be further developed

- **EGFRTKI resistance mechanisms** *(Han et al)*
  - The identification of mechanisms of resistance is relevant to the treatment strategy
  - EPHB6 gene mutation might be further investigated as a mechanism of EGFRTKI resistance in EGFR-mutated p
Thanks!!!

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