MOLECULAR PREDICTIVE MARKERS OF LUNG CARCINOMA: KFSH&RC EXPERIENCE

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Lung Cancer

Leading cause of cancer mortality

- 1.4 million death/year worldwide (WHO, 2007)
- 160,000 death/year in USA (25% of all cancer death in USA)
- 5-year survival of lung cancer 6-15%
DISTRIBUTION OF 20 MOST COMMON MALIGNANCIES
1975 - 2011 (TOTAL CASES = 72,557)

- Breast
- Leukemia
- NHL
- Thyroid
- Colon, Rectum
- Brain, CNS
- Oral Cavity
- Hodgkin’s Lymphoma
- Lung, Bronchus
- Liver
- Nasopharynx
- Bladder
- Soft Tissue
- Esophagus
- Stomach
- Bone
- Kidney, Urinary
- Other Skin Cancer
- Ovary
- Cervix Uteri

KFSH&RC Tumor Registry, 2011
<table>
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<th>SITE</th>
<th>AGE GROUP</th>
<th>No</th>
<th>%</th>
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<td>15 - 39</td>
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<td>15 - 39</td>
<td>166</td>
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<td>40 - 60</td>
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<tr>
<td>NON-HODGKIN’S LYMPHOMA</td>
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<td>BRAIN, CNS</td>
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<td>3.5%</td>
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</tr>
<tr>
<td>LUNG, BRONCHUS</td>
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<td>BONE</td>
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<td>SKIN MELANOMA</td>
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<tr>
<td>PROSTATE (% to MALES)</td>
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<td>29.3%</td>
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### DISTRIBUTION OF 20 MOST COMMON MALIGNANCIES
#### 2011 ANALYTIC CASES (TOTAL CASES = 2,292)

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<td>Liver</td>
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<tr>
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<tr>
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<td>Kidney, Urinary</td>
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<tr>
<td>Lung, Bronchus</td>
<td>46</td>
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<td>Bladder</td>
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<td>Hodgkin's Lymphoma</td>
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<tr>
<td>Bone</td>
<td>29</td>
<td>3.0%</td>
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<tr>
<td>Soft Tissue</td>
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<tr>
<td>Other Skin CA</td>
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<table>
<thead>
<tr>
<th>Female</th>
<th>Count</th>
<th>Percentage</th>
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<tbody>
<tr>
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<tr>
<td>Cervix Uteri</td>
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<tr>
<td>Liver</td>
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<td>2.3%</td>
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<tr>
<td>Soft Tissue</td>
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<td>2.3%</td>
</tr>
<tr>
<td>Kidney, Urinary</td>
<td>30</td>
<td>2.3%</td>
</tr>
<tr>
<td>Hodgkin's Lymphoma</td>
<td>30</td>
<td>2.3%</td>
</tr>
<tr>
<td>Bone</td>
<td>26</td>
<td>2.0%</td>
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<tr>
<td>Stomach</td>
<td>23</td>
<td>1.7%</td>
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<tr>
<td>Lung, Bronchus</td>
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<td>1.7%</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>16</td>
<td>1.2%</td>
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<td>1.2%</td>
</tr>
<tr>
<td>Eye</td>
<td>16</td>
<td>1.2%</td>
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</table>
WHO Classification of Lung Cancer

1967
Written by pathologists
for pathologists

1981

1999

2004 Genetic and clinical information introduced (not current anymore)

2015 5th Edition
Lung Carcinoma

- Small cell carcinoma
- Non-small cell lung carcinoma (NSCLC)
  - Adenocarcinoma (including bronchioalveolar carcinoma BAC)
  - Adenosquamous carcinoma
  - Squamous cell carcinoma
  - Large cell carcinoma
  - Large cell neuroendocrine carcinoma
WHO Classification of Lung Cancer

Classification is based on resected specimen. On small biopsy, the differentiation of various subtypes of NSCLC is not reliable in many cases.

NSCLC - NOS

WHO Classification of Lung Cancer, 2004
Revised classification that emphasizes:

- Integrated multidisciplinary approach for classification is needed
- Classification in small biopsies and cytology specimen (was not addressed in 2004 WHO Classification)
- Tissue management by pathologists
Major Changes of Proposed Classification

- Stop usage of “bronchioalveolar carcinoma”
- Addition of minimally invasive carcinoma
- Classification of invasive carcinoma according to predominant subtype

Journal of Thoracic Oncology, Vol. 6, Number 2, February 2011
IASLC/ATS/ERS Classification of Lung Adenocarcinoma in Resection Specimens

- Preinvasive lesions
  - Atypical adenomatous hyperplasia
  - Adenocarcinoma in situ (≤ 3 cm formerly BAC)

- Minimally invasive adenocarcinoma (≤ 3 cm lepidic predominant tumor with ≤ 5 mm invasion)

- Invasive adenocarcinoma

*Journal of Thoracic Oncology, Vol. 6, Number 2, February 2011*
Lepidic predominant pattern

Acinar adenocarcinoma

Papillary adenocarcinoma

Solid adenocarcinoma

Micropapillary adenocarcinoma

*Journal of Thoracic Oncology, Vol. 6, Number 2, February 2011*
Differentiate primary pulmonary adenocarcinoma from metastatic carcinoma

Differentiate adenocarcinoma from squamous cell carcinoma

Distinguish adenocarcinoma from mesothelioma

Determine the neuroendocrine status of the tumor

NCCN Guidelines Version 2.20, 2013
<table>
<thead>
<tr>
<th>Resection Diagnosis by WHO Criteria (No. Cases)</th>
<th>Biopsy IHC Number Positive/Total Stained (%)</th>
<th>Biopsy Diagnosis After IHC (No. Cases)</th>
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<tr>
<td>AC (20)</td>
<td>19/19 (100)*</td>
<td>AC (16/20)</td>
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<td>SCt (15)</td>
<td>9/15 (60)</td>
<td>NSCLC, NOS (4/20)</td>
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<td>LCC (4)</td>
<td>3/4 (75)</td>
<td>SCC (14/15)</td>
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<td>NSCLC, NOS (1/15)</td>
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<td></td>
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<td>AC (2/4)</td>
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<tr>
<td></td>
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<td>NSCLC, NOS (2/4)</td>
</tr>
</tbody>
</table>

*No tumor was present on the immunostained slide in 1 case.

AC indicates adenocarcinoma; IHC, immunohistochemistry; LCC, large cell carcinoma; NSCLC, Non-small cell lung carcinoma; NOS, not otherwise specified; SCC, squamous cell carcinoma; WHO, World Health Organization.
Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: The NCI’s Lung Cancer Mutations Consortium (LCMC)

- **KRAS** 107 (25%)
- **EGFR** 98 (23%)
- **ALK** rearrangements 14 (6%)
- **BRAF** 12 (3%)
- **PIK3CA** 11 (3%)
- **MET** amplifications 4 (2%)
- **HER2** 3, (1%)
- **MEK1** 2(0.4%)
- **NRAS** 1 (0.2%)
- **AKT1** 0(0%)

In 60% tumor driver mutation detected

*J Clin Oncol 29: 2011 (suppl; abstr CRA7506)*
Lung Adenocarcinoma
Activating Oncogenes

- Deletion and point Mutations
  - KRAS (30%)
  - EGFR (15%)

- Gene Amplification
  - EGFR (6-9%)

- Chromosomal rearrangement
  - EML4-ALK (5%)
  - ROS1 (2%)

EGFR, EML 4-ALK and KRAS are mutually exclusive
Molecular Testing Guideline for EGFR and ALK Tyrosine Kinase Inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology.

*Archives of Pathology & Laboratory Medicine*
*June 2013, Vol. 137, No. 6, pp. 828-860*
Testing for EGFR mutations and ALK gene rearrangements is recommended in the NCCN NSCLC guidelines for adenocarcinoma patients.

NCCN Guidelines Version 2.20, 2013
Distinction is critical between:

- Adenocarcinoma
- Pure squamous cell carcinoma
- Pure small cell carcinoma
- Pure neuroendocrine carcinoma

For EGFR and Alk testing
Lung carcinoma with mixed histology (adenosquamous, adeno/small cell) can have EGFR mutation or Alk rearrangement. Testing is required if possibility of adenocarcinoma component cannot be excluded.
It is important to retain sufficient tissue for molecular testing after establishing diagnosis of adenocarcinoma.
Molecular testing results should be available within 2 weeks of receiving samples to molecular labs.
EGFR mutations are seen more common (50%) in:

- Women
- Never smoker
- Asian

Selection of patients for EGFR mutation testing is dependent on subtype of lung cancer not on clinical information.
Common Mutations Identified in *EGFR* Gene

*EGFR* transcript

- Exons 1–16
- Exon 17
- Exons 18–24
- Exons 25–28

*EGFR* TK domain (exons 18-21)

- Exon 18
- Exon 19
- Exon 20
- Exon 21

Mutations:
- L858R
- L861
- G719
- D770_N771 insNPG
- T790M

Reference:
EGFR TK Mutations

**Common**

- Exon 19 in-frame deletion
- Exon 21 L858R mutation (Lysine to Arginine)

Both mutations result in activation of TK domain and associated with sensitivity to TKI.
EGFR Mutations

- Exon 18 Gly719 (sensitive)
- Exon 19 deletion (sensitive)
- Exon 20 insertion (resistance)
- Exon 20 Thr790Met (acquired resistance)
- Exon 21 Leu858Arg (sensitive)
Frequency of EGFR Mutations in Lung Adenocarcinoma

- 32% in East Asia
- 7-15% in Caucasians
- 2% in African America
- About 30% in Saudi population (unpublished data)

EGFR Mutation Testing in Saudi Arabian Lung Adenocarcinoma

Dr. Fouad Al Dayel,* Dr. Hamad Husaini,** Dr. Asma Tulbah,* Dr. Shamayel Mohammed,* Dr. Prashant Bavi, ***Dr. Halah Abalkhail*. *Pathology and Laboratory Medicine, **Oncology Center, ***Research Center
King Faisal Specialist Hospital and Research Centre

Introduction

Lung cancer is the fifth leading cause of cancer in males in Saudi Arabia. As per current World Health Organization (WHO), lung carcinoma is subdivided into small cell and non-small cell carcinoma (NSCLC). The latter compromise 70-80% of lung carcinoma and consists of heterogeneous groups that is further divided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. Due to poor prognosis of lung cancer, there is an increasing need to find molecular biomarkers which can be used for diagnosis, risk stratification, early detection, treatment selection, prognosis and monitoring for recurrence. Increasing interest in adenocarcinoma of lung has been raised lately for various reasons. One reason is the increasing incidence of adenocarcinoma, which is now the most predominant histologic subtype. Other reason is the possible use of targeted therapy in cases showing EGFR mutations or ALK rearrangements. Adenocarcinoma comprise approximately 70% of primary lung cancer in Saudi population.

Objectives

The aim of this study is to review the incidence of EGFR mutation in lung adenocarcinoma in Saudi patients.

Materials & Methods

Clinical data and genomic DNA was available from a cohort of 37 primary lung adenocarcinoma diagnosed at King Faisal Specialist Hospital and Research Centre (KFSH&RC) clinics. Ethical approval for the study was granted by the Research Advisory Council (RAC) at KFSH&RC.

The diagnosis was established histologically and confirmed by Immunohistochemistry. DNA from paraffin embedded tissue was manually extracted and was paired with histology-guided tissue macro-dissection to target tumor cells. The mutation status of EGFR exons 18-21 was evaluated using Polymerase chain reaction (PCR). Amplified products were purified and sequenced on an Applied Biosoftware Genetic Analyzer 3730xl. Analysis and mutation nomenclature was based on GeneBank NM_005226.

Results

EGFR mutation was detected in 10 cases (27%). Of the 10 cases, 80% of mutations (deletions) were located in exon 19 and 10% in exons 20 and 21 respectively. All mutations detected conferred increased sensitivity to tyrosine kinase inhibitors (TKI).

Pathology Images

Figure 1 A) Representative H&E of Adenocarcinoma of lung B) IHC stains for TTF-1 that shows nuclear staining in primary lung adenocarcinomas

Mutation Analysis

Figure 2. Mutation analysis of EGFR. A representative chromatogram from normal control (A) and case submitted for mutation analysis (B) exhibiting L858R in exon 21.

Conclusion

The incidence of EGFR mutation in lung adenocarcinoma in our patients (27%) is slightly higher than western population (15-23%). To our knowledge, this is the first molecular analysis of EGFR gene mutational analysis in lung adenocarcinoma in Saudi Arabia.
Resistance to EGFR-TKIs

- **Primary resistance**
  - KRAS mutations and Alk gene rearrangement
  - EGFR mutations not sensitive to EGFR TKIs (rare, ~2%) – ex 20 insertion
  - BRAF mutations (rare, ~3%)

- **Acquired resistance**
  - Second EGFR mutation: T790M (50% of cases)
  - MET amplification (some)
  - Pi3k mutations
  - Transformation to small cell lung ca
Tissue Sampling Methods in NSCLC

- Three main methods of obtaining tumour samples:
  - Excised during surgery
  - Bronchoscopic biopsy (for central lesions)
  - Guided needle biopsy (for peripheral lesions)

- Preservation of DNA is essential (e.g. formalin-fixed, paraffin-embedded tumour sample)

- Preferably use primary tumour tissue:
  - when this is not available, may consider metastatic tissue, pleural effusion or blood
Testing for Mutation

1. Tumor Sample Collection
2. Sectioning (at least 50% tumors)
3. DNA Extraction
4. Amplification
5. Sequencing
EGFR Testing Method

- Direct (Sanger) sequencing
- Pyrosequencing
- High resolution melting analysis
- Polymerase chain reaction (PCR), allele specific hybridization
- Real time PCR
- Whole exome sequencing
- Whole genome sequencing
Limitations of Mutation Detection by Direct Sequencing

- Sequencing will not detect proportions of tumor cells below the sensitivity level (25%).
- Microdissection routinely used to increase tumor content (eliminate non-neoplastic areas).
- Blocks or unstained sections for DNA extraction should be from the most cellular areas with >50% tumor cells.
- Select sections without excessive inflammatory response.
Adequacy of EGFR Testing

- Adequacy is determined by malignant cells content and DNA quality and not sample type
- Specimen should be fixed in 10% NBF for 6-48 hours
- Cell blocks are preferred over smears for cytology samples
ALK-rearranged Adenocarcinoma

- 2-7% of adenocarcinomas
- Younger patients
- Never smoking
- Higher stage
- Solid tumor growth, frequent signal cells with abundant intracellular mucin

Similar to EGFR mutation positive patient except they are younger and male
The diagram illustrates the genomic region of 2p23.2, focusing on the 300 kb to 442 kb area. It shows the normal state and the altered state due to an inversion of approximately 12.5 Mb. The ALK and EML4 genes are involved in this process, leading to the formation of an EML4-ALK fusion. The fusion occurs at the 5' and 3' ends of the genes, as indicated by the arrows and the color-coding of the gene segments.
SIGNAL CLASSIFICATION

Patterns observed in native ALK
- Red and green separated by <2 signal diameters
  - Classified as negative
- Red and green separated by ≥2 signal diameters
  - Classified as positive

Patterns observed in split 3'-5' ALK
- Red and green separated by ≥2 signal diameters
  - Classified as positive
- Red and green separated by <2 signal diameters
  - Classified as negative

SPECIMEN CLASSIFICATION

Nonrearranged tumors:
- Rearrangement-positive cell rate <15% of cells

Rearranged tumors:
- Rearrangement-positive cell rate ≥15% of cells
IHC

Negative 0/1+
- Reported as ALK negative

Equivocal 2+
- CISH/FISH
- Reported as ALK positive

Positive 3+
- Reported as ALK positive
NSCLC → ALK testing → IHC

IHC:
- Negative
- Positive (1+, 2+, 3+)
  → FISH
   - Negative
   - Positive → ALK rearrangement

No ALK rearrangement
Frequency of ALK Gene Rearrangement in Saudi Lung Cancer

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Introduction
Lung carcinoma is the fifth common cancer affecting Saudi men. Recently, translocation of the anaplastic lymphoma kinase (ALK) gene is found to play a predictive role in adenocarcinoma tumor genesis. The ALK gene codes for trans membrane glycoprotein with tyrosine kinase activity. In frame rearrangement with the known fusion partners places the ALK kinase domain under the control of different gene promoter, which results in chimeric protein with constitutive tyrosine kinase activity. ALK gene rearrangement can identify patients with adenocarcinoma who are sensitive to ALK inhibitors. However, no data are available on the prevalence of ALK rearrangements changes in Middle Eastern population. Therefore, we carried out this study to evaluate the prevalence of ALK rearrangements in lung adenocarcinoma of Saudi patients.

Materials & Methods
ALK gene rearrangements were studied using fluorescence in situ hybridization (FISH) on 97 adenocarcinoma samples utilizing tissue microarray format. ALK gene translocations identified by BAC clone RP11-328L16 were studied by the break point probe from Vysis (Abbott Molecular, II, USA) to detect chromosome 2p23 rearrangements.

Results
Ninety seven (97) lung adenocarcinoma cases were evaluated. There were 3 cases exhibited ALK gene rearrangement (3%). All of these 3 cases was moderately differentiated adenocarcinoma. None of our cases showed signet cells or abundant intracellular mucin.

Pathology Images

Figure 1 A) Representative H&E of Adenocarcinoma of lung B) IHC stains for TTF-1 that shows nuclear staining in primary lung adenocarcinomas

Figure 2. In situ hybridization method for the detection of ALK translocation in a ethically unique cohort of Saudi lung cancer patients. The findings of this study show that incidence of ALK adenocarcinoma is similar to the published western data and these patients can benefit from targeted therapy like Crizotinib a dual ALK and MET inhibitor that has shown promising results in clinical trials.

Conclusion
This is the first study that reveals frequency of ALK translocation in a ethically unique cohort of Saudi lung cancer patients. The findings of this study show that incidence of ALK adenocarcinoma is similar to the published western data and these patients can benefit from targeted therapy like Crizotinib a dual ALK and MET inhibitor that has shown promising results in clinical trials.
Cancer is a disease of genome. Today we have the technology to understand the alterations of these genes using exome sequencing, transcriptome sequencing and whole genome sequencing.