Management of limited tissue in lung biopsy

Dr. S V Kane
Head, dept of Pathology
Tata Memorial Center
Mumbai, India
drsvkane@gmail.com

Current scenario in lung cancer

- Incidence of lung cancer increasing in developing countries [6.9% vs 15% in US]
- Histologic sub-classification is expanding
- Era of personalized therapy
- Molecular markers are emerging
- New Drug targets are developing
- Thoracic oncology field expanding

Role of pathologist is more challenging

Role of Pathologist in the management

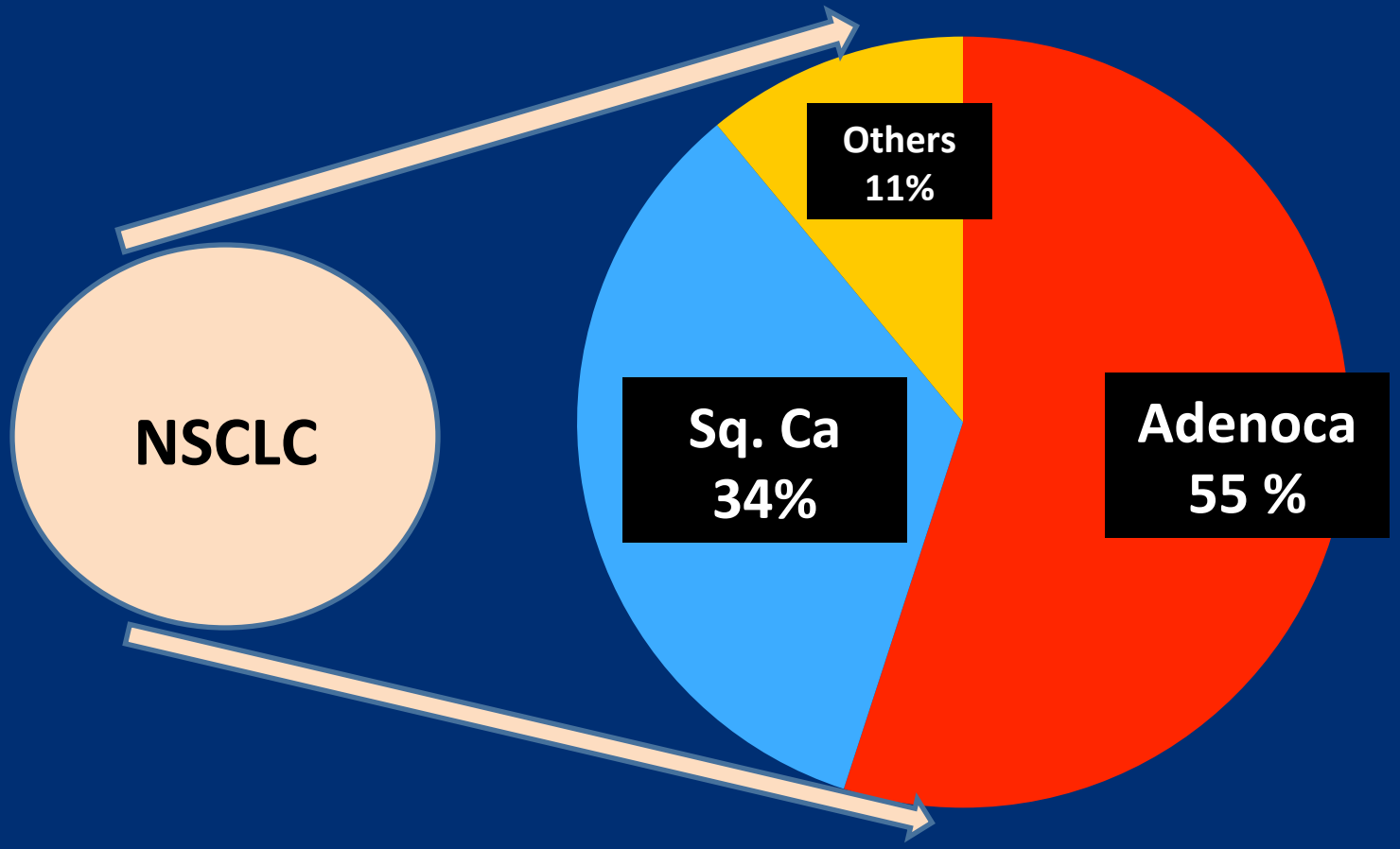
- **Diagnosis** : a] Confirmation of malignancy
  b] Typing of malignancy
  c] Prim Vs metastatic ca
- **Prognosis** : Tumour biology : SmCC VS NSCLC
  Grade of malignancy in NSCLC
  Stage of tumour

- **Prediction of therapeutic response** : expression of biomarkers & targeted therapy eg erlotinib, crisotinib ,

Traditional Classification of Lung Carcinoma

- Small cell carcinoma [SCLC] 15-20 %
- Non -small cell carcinoma [NSCLC] 80-85%
  - Squamous Carcinoma
  - Adenoca
  - Adeno sq. ca
  - Poorly diff. carcinoma
  - Large cell N E carcinoma

Common types Of NSCLC



Good reproducibility in typing & subtyping adenoca on histology

	Squamous Cell Ca	Adenocarcinoma
Age & Sex	More common in elderly Males	More common in younger Females
Frequency	30-40% of all lung cancers	40-50% of all lung cancers
Location	Central	Peripheral
Habit	Smokers	Smokers & Non-smokers
Presentation	Pancoast type/ **central - early**	Large peripheral mass- **late**
Visualization on Xray	Not easy, central cavitation	Easy
Metastasis	**Uncommon**	**Common , at presentation**
Metastasis sites	LN, Adrenal glands	**Brain, bone , pleura**
Pleural effusion	uncommon	**Common**
Types	Keratinising, non keratinising, basaloid	Varied subtypes
IHC	CK5/6, P63, P40 + VE	CK7, Napsin, TTF1 +ve

Invasive adenocarcinoma : New ISLC classification

Patterns

- **Lepidic** [replaced B-A ca]
- **Acinar-tubular**
- **Papillary-** micropapillary
- **Solid predominant**
- **Mucinous ca** -With foetal pattern
  -With colloid pattern
  -With enteric pattern
  -With signet ring features
  -With clear cell features

AIS
MIC

Lepidic Growth pattern

- **Maintains alveolar architecture**
- **No destruction or effacement**
- **No central or broad scar**
- **Often has thickened alveolar septa**
- **Single layer of Cuboidal epithelium**
- **Little to no stratification or tufting**
- **No papillary structures within**

Pathology Recommendations
AIS & MI ADCA

- Small (3 cm) solitary adenocarcinomas with pure lepidic growth termed **adenocarcinoma in situ.**
- Small (3 cm) solitary adenocarcinomas with predominant lepidic growth and foci of invasion measuring 0.5 cm termed **Minimally invasive adenocarcinoma.**
- Invasion > 0.5cm termed invasive adenocarcinoma.

Architectural Grading

By dominant pattern

- **Grade 1: Lepidic**
- **Grade 2: Acinar and Papillary**
- **Grade 3: Solid and Micropapillary, mucinous**

- **Prognostic relevance** **worst**

Squamous cell carcinoma

- Types : Keratinizing SCC , Non-keratinizing SCC
- Basaloid type SCC
- Diagnostic Implications :
- keratinising type is easy : primary or metastatic
- Non-keratinizing SCC- IHC needed to distinguish from poorly diff adenoca , solid type
- Prognostic implications : worst in basaloid type than keratinising , may need IHC

NSCLC…… Not a diagnosis

- **Means -- Poorly differentiated CA**
- **Use of ancillary techniques : IHC needed for DX**

- **Favour adenoca-------** CK7, TTF1, Napsin = +
- **Favour squamous cell ca----** CK 5/6, p63, p40 = +
- **Favour adenosquamous ca** ------- BOTH +
- **Favour NE Carcinoma** ---- 2 NE markers + [synapto, chromo, CD 56, NFP]
- NSCLC- NOS: rarely used term, all markers are negative

High grade NE CA

- SCLC & LCNEC have dismal prognosis
- Both present at high stage
- Morphology is different
- High mitosis: > 10/70
- Immunoprofile is similar [except ckit]
- At least one NE marker positive,
- high MIB1 50-80%, 40-70&
- Genomic alterations different
- LCNEC does not respond to CT like SCLC

New 2015 WHO classification of lung cancer recommendations

- ✓ **U**se of **immunohistochemistry** for typing
- ✓ a new emphasis on **genetic studies**, in particular integration of molecular testing
- ✓ a new classification for **small biopsies and cytology**

Scope of Diagnostic investigations

- Primary central Lung tumor
  - ✓ Sputum cytology
  - ✓ BAL / Bronchial brush cytology
  - ✓ Bronchoscopic biopsy
  - ✓ EBUS FNAC

- Primary peripheral lung tumour
  - ✓ CT guided Core Biopsy
  - ✓ Per cut CT Guided FNAC
  - ✓ Pleural fluid CYTOLOGY

Current trend in diagnostic investigations in lung cancer

Initial investigation in general hospital

- FNAC or bronchial cytology
- Diagnosis of cancer
- Surgery for operable cancer

- Trend is changing in cancer institutes

- *Biopsy is first line investigation*
- Parallel to breast cancer
- Cytology is less frequently used in advanced cancer

Biopsy ↑ Cytology ↓

Diagnostic challenges for Pathologists

- Bronchoscopic or CT guided Biopsy- **small tissue**
- Histopathologic classification is wide and **complex**
- High grade tumour shows necrosis / **desmoplasia**
- IHC is necessary for accurate diagnosis
- Tissue may look adequate yet can be non- representative under microscope

- Histologic typing has clinical & therapeutic implications
- Targeted therapy based on genetic analysis
- Biopsy Tissue needs to be used for multiple purposes

Tissue is crucial and that is the main issue

Recommendations for optimal tissue utilization : key issue

1. Maximum no. of tissue cores by interventional radiologist [6]
2. Avoid putting all cores in one block, make at least two blocks
3. Trained technical staff deal with the precious tissues
4. All blocks should be subjected to routine histology

5. Use the p block with **lesser amount of** tissue for routine diagnostics (HE and IHC) and save the **larger amount of tissue** for Molecular tests

Utilization of tissue

Block 1

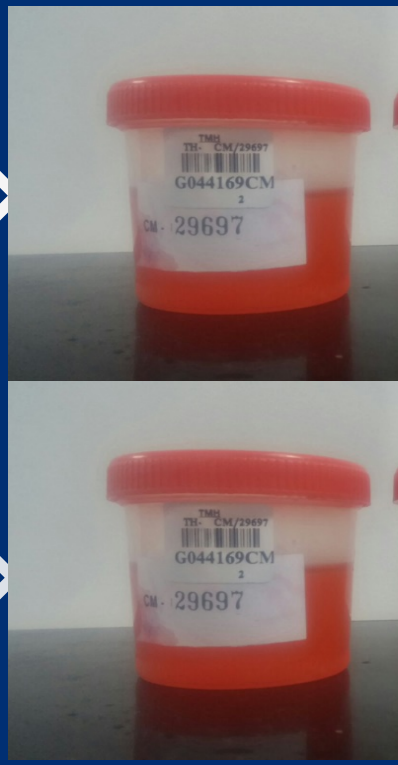
(Block with lesser tissue : 1/2 cores)

- Routine histopathology
- Immunohistochemistry

Block 2

(Block with more tissue 2/3 cores)

- Molecular pathology [EGFR / KRAS / BRAF / HER2neu / emlk 4-alk



Don’t club all the tissue cores together

Recommendations for optimal tissue utilization : key issue

6. On-site adequacy testing :core biopsy

7. Optimize IHC panel, use lung specific markers, consider step-wise IHC approach: TTF1, Napsin , CK7, P40, P63

8 . Preparation of cell block- provide additional material for diagnosis, IHC and molecular testing.

1. Multi-disciplinary : Team work imp

Diagnosis on core biopsy

- Adenocarcinoma showing acinar pattern
- Desmoplastic stroma or necrosis is seen
- IHC - TTF1 is diffusely positive,
- Ca is of pulmonary origin
- Tissue adequate for molecular testing

How much tissue necessary for molecular tests ?

- Generally advisable to have > Than 400 cells per section.
- 4 good FNA passes
- 4-5 transbronchial biopsies
- 2-3 CT-guided core needle biopsies
- Don’t exhaust tissue by doing excessive stains or levels.

Biomarkers of Current Interest

- EGFR : RT-PCR
- KRAS : RT-PCR
- EML4-ALK : FISH
- ROS-1 : FISH
- Others
  - Her2
  - BRAF
  - Met

Indications for EGFR Mutation Test

1. Adeno Ca, Favor Adeno Ca: any type

2. NSCLC Possibly Adenosquamous Ca

3. NSCLC Nos (poorly differentiated Ca, after IHC)
   Frank SCC are excluded

Incidence of EGFR higher in Asians than caucasians

EGFR Mutation Analysis

- **Activating mutations**
- Exon 21 L858R Leucine to Arginine } 85-90%
- Exon 19 microdeletions
- Several others
- Mutations which confer resistance to TKI therapy
- – Mostly exon 20 [T 790M]
- Methods include direct sequencing of exons
- 18-21, and directed amplification of commonmutations
-
- TARGETTED therapy given as first line therapy in patients with stage IV disease [inoperable] or in c/o recurrence.

Indications for ALK gene rearrangement

- Are there clinical characteristics we can use to screen?
- Young, female, Asian, non-smoker?
- – No.
- Are there histologic characteristics we can use to screen?
- – No, but close– Signet ring tumors and viral-like inclusions in EML4-AlK, mucinous tumors in KRAS.

Histopathologic criteria :

- with adenocarcinoma , Adenosquamous Ca or NSCLC N O S
- Negative EGFR mutation

EGFR/ALK testing in India – Practical Challenges

- **Adequacy of tissue** sample for testing all the biomarkers
- FNAC is often the first specimen available in lung cancer
- FNAC – not useful for biomarker testing unless it is converted to cell blocks
- Challenges in tissue sampling and tissue handling

- **Turn around time** for Test results – 10 days in metro cities and 15-20 days in TWO tier cities

Biomarker /target	Therapeutic Implications
• EGFR Mutation +	• Response to TKI
• K RAS Mutation +	• Lack of Response to TKI
• EML_4 Alk Gene rearrangement	• Response to ALK inhibitor [CRISO]
• Ros 1 expression	• Response to ALK inhibitor
• Her 2 neu Mutation	• Response to Herseptin
• BRAF Mutation	• Response Vemurafenib [Anti BRAF]
• PD- L 1, expression	• Response to Pembrolizumab
• T 790 M Mutation in EGFR	• Resistance to TKI
• MET inhibition	• Resistance to TKI

Repeat biopsy recommended when

- Dx of NSCLC made but no further typing could be done
- Bx is inconclusive but clinically strong suspicion of cancer
- Bx sufficient for dx but not for molecular testing
- Samples include prim tum bx, SC node bx, recurrent tum bx or pleural fluid cytology

BIOPSY IS THE GOLD STANDARD