“In recent years, our concepts of lung cancer have undergone a revolution”
The Molecular Biomarker Revolution in mNSCLC

Dr. Diana N. Ionescu, MD
Consultant Pathologist and AP Lead, BC Cancer
Clinical Professor of Pathology, UBC
Vancouver, Canada

Dr. Doru Paul, MD, PhD
Medical Oncologist
Associate Professor of Clinical Medicine
Weill Cornell Medical College
New York City, NY, USA
OVERVIEW

• Introduction

• Standard of Care Biomarkers

• New Biomarkers

• Emerging Biomarkers
LEARNING OBJECTIVES

Upon completion of this session, participants should be able to:

1. Describe the standard of care and emerging biomarkers in mNSCLC
2. Utilize NGS to identify the standard of care and emerging biomarkers for precision therapies in patients with mNSCLC
3. Employ strategies for optimizing workflows and turnaround times for NGS
4. Discuss the science behind resistance mechanisms in patients with mNSCLC
5. Recognize the use of emerging I-O-targeted agents combination therapies and potential new biomarkers in patients with mNSCLC
6. Develop ways to improve communication among pathologists, oncologists, and other members of the multidisciplinary lung cancer care team
WHY IS BIOMARKER TESTING ESSENTIAL?

- It is the first, essential, step in the mNSCLC diagnosis, risk stratification, treatment, and ongoing monitoring of patient response.

- Pathologists must have a thorough understanding of testing considerations for current and emerging biomarkers, such as what type of sample will yield the most actionable information, which testing methodology is the most appropriate, and which biomarkers to assay, in each patient.
STANDARD OF CARE
BIOMARKERS IN mNSCLC
~ 35-50% of Patients With Advanced Nonsquamous NSCLC Have a Targetable Driver Mutation

- **EGFR Sensitizing** 17%
- **ALK** 7%
- **MET** 3%
- > 1 Mutation 3%
- **HER2** 2%
- **ROS1** 2%
- **BRAF** 2%
- **RET** 2%
- **NTRK** < 1%
- **PIK3CA** 1%
- **MEK1** < 1%
- **NRG1** < 1%
- **Unknown Oncogenic Driver Detected** 31%
# NCCN Guidelines Version 3.2022
## Non-Small Cell Lung Cancer

**Testing Results**

- **EGFR exon 19 deletion or L858R mutation positive**
- **EGFR S768I, L858Q, and/or G719X mutation positive**
- **EGFR exon 20 insertion mutation positive**
- **KRAS G12C mutation positive**
- **ALK rearrangement positive**
- **ROS1 rearrangement positive**
- **BRAF V600E mutation positive**
- **NTRK1/2/3 gene fusion positive**
- **METex14 skipping mutation positive**
- **RET rearrangement positive**
- **PD-L1 ≥50% and negative for actionable molecular biomarkers above**
- **PD-L1 ≥1%–49% and negative for actionable molecular biomarkers above**
- **PD-L1 <1% and negative for actionable molecular biomarkers above**
Case Presentation
Clinical History

• 65 years old woman, non smoker

• Presented to the emergency room with abdominal pain and was diagnosed with acute diverticulitis by CT scan

• The incidental finding on CT scan was a lung mass
• CT chest revealed a 5.0 x 3.6 x 3.6 cm spiculated mass in the LUL, pleural and diaphragmatic nodularity, small pleural effusion, and confluent adenopathy extending into the left hilum and mediastinum.

• PET scan: LUL mass SUVmax 27.3 and a 2cm sclerotic focus in the 3rd sacral segment, suspicious for bone metastasis (confirmed by bone scan).
• Bronchoscopy: Left Upper Lobe bronchial washings, brushings, and biopsy, all positive for adenocarcinoma, TTF1 positive, consistent with lung primary
Biomarkers were performed on the transbrochial biopsy.

- IHC: ALK and ROS1 negative
- IHC: PD-L1 (22C3 PharmaDx): Tumor Proportion Score (TPS): 1-49%
- NGS gene panel (DNA Hybrid Capture, BC Cancer Oncopanel) was performed
REASON FOR REFERRAL: Oncopanel Based Testing, Non-Small Cell Lung Cancer

RESULT: EGFR (exon 20) variant detected

INTERPRETATION:

**Variants of functional significance were detected in EGFR (exon 20) and TP53 (2 variants), as described below. Though no additional variants of known functional significance were detected in any of the other genes/regions examined (including MET), one of uncertain significance was noted in MUTYH.**

---Variants of Clinical/Functional Significance---

**EGFR NM_005228.3:c.2303_2311dupGGTGGACA, p.(Ser768_Aspl770dup)**

This exon 20 variant results in the in-frame duplication of three amino acid residues and has been previously reported in lung cancer patients (Arcila (2013) PMID:23371856; Fang (2019) PMID:31208370). Exon 20 insertions are the third most common class of EGFR mutations (Arcila (2013) PMID:23371856) and have been associated with reduced sensitivity to anti-EGFR TKI therapies (Yasuda (2013) PMID:24353160). Preliminary data suggest tumors carrying EGFR exon 20 insertion mutations may respond to treatment with the pan-HER TKI potasnitib (reviewed in PMID:29162564; reviewed in Vyse (2019) PMID:30854234).

**TP53 NM_000546.5:c.637C>T p.(Arg213Ter) (VAF: 25.9%)**

This variant terminates the reading frame resulting in an unstable and/or truncated protein. Somatic loss of TP53 function is a common feature in the majority of human cancers (Olivier (2010) PMID:20182602).

**TP53 NM_000546.5:c.470T>G p.(Val157Gly) (VAF: 28.1%)**

This variant has been reported to disrupt normal TP53 function (per IARC TP53 database). Somatic loss of TP53 function is a common feature in the majority of human cancers (Olivier (2010) PMID:20182602).

---Variants of Uncertain Significance---

**MUTYH NM_001128425.1:c.67G>A p.(Gly23Arg) (VAF: 49.2%)**
EGFR: EXON 20 INSERTIONS

EGFR ins20 mutations represent approximately 10% of all oncogenic EGFR mutations. Represent the third most common class of mutations behind canonical EGFR mutations del19 and L858R. Are a heterogenous class of mutations.

EGFR Tyrosine Kinase Domain Mutations

Adapted from Journal of Thoracic Oncology 2010 51706-1713DOI: (10.1097/JTO.0b013e3181f1c8de)
There are two main ways to identify EGFR mutations

**PCR**
- PCR (single-gene testing) is typically performed sequentially in order to identify only the most common genetic alterations.
- Limited in their ability to detect molecularly heterogeneous mutations, including EGFR ex20ins mutations.
- Requires a relatively large tumor tissue sample.

**NGS**
- Simultaneously test thousands of genes without prior sequence knowledge.
- Valuable tool to identify molecularly heterogeneous sequence alterations.
- Uses only a single, smaller tumor tissue sample.

PCR identifies only half of EGFR exon 20 insertion variants identified by NGS.

*EGFR* sequencing technology used to identify *EGFR* ex20ins mutations between 2011 and 2019 in the US revealed that PCR testing rates decreased from **85.7%** to **11.3%**, and NGS testing rates increased from **0%** to **62.3%**.

Unmet need in EGFR Exon 20 insertions compared with common EGFR mutations (cEGFR)

5-year survival for ex20ins is 8% vs 19% for cEGFR

Compared with cEGFR, ex20ins were associated with:

- **75%** increased risk of death (primary endpoint)
- **93%** increased risk of progression or death (secondary endpoint)
- **60%** increased risk of shorter time to next therapy (secondary endpoint)

**Real-world Overall Survival (rwOS)**

- Adj HR, adjusted hazard ratio; cEGFR, common EGFR mutations; EGFR, epidermal growth factor receptor; ex20ins, exon 20 insertion mutation.
The patient’s exon 20ins mutation is NOT one of the EGFR sensitizing mutations and therefore the response to the 1st-3rd generation of TKI would be unsure.

Currently, there is no first-line targeted therapy approved for exon20ins mutations in NSCLC.
The patient was treated with 6 cycles of Carboplatin/Pemetrexed and CT chest showed a reduction in her disease (5.1 x 3.4 cm to 3.4 x 4.1 cm) after 2 cycles.

The patient progressed during maintenance with Pemetrexed and was switched to Monocertinib based on her Exon20ins mutation (Ser768_Asp770dup).

Good response for 17 months then showed disease progression in the lung and a new liver metastasis.

- Also had SBRT during her 17 months on Monocertinib for a single right frontal metastasis.

She was switched to Amivantamab and after 6 months there was progression in the lung, with stable liver metastasis.

- Received palliative radiation to the chest (30 Gy) and sacrum (8 Gy).
EGFR EXON20ins PATIENT

AT PRESENTATION

PARTIAL RESPONSE TO CHEMOTHERAPY FOLLOWED BY MONOCERTINIB AND AMIVANTAMAB

PROGRESSION AFTER 23 MONTHS OF TARGETED THERAPY FOR EXON20ins

November 2019, 50.2 X 35.7 MM  
September, 2021, 27.1 X 30.4MM  
June 2022, 53.9 X 49.5 MM
Mobocertinib (TAK-788) in EGFR Exon 20 NSCLC

TAK-788 in Exon 20 Insertion EGFRm+ NSCLC (n=28)

**Confirmed ORR, 43% (n=28)**

**ORR 43%**

**Median progression-free survival, mo**

<table>
<thead>
<tr>
<th>[95% CI]</th>
<th>7.3</th>
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<td>[4.4–NR]</td>
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</table>

FDA Breakthrough Designation

Trial on hold by FDA awaiting futility test

Amivantamab: EGFR-MET Bispecific Antibody

- Fully human EGFR-MET bispecific antibody with immune cell-directing activity\(^1\)\(^-\)\(^2\)
- Targets activating and resistance EGFR mutations and MET mutations and amplifications\(^3\)\(^-\)\(^4\)
- Demonstrated monotherapy activity in patients with diverse EGFRm disease including EGFR Exon19del, L858R, T790M, C797S, Exon20ins, and MET amplification\(^3\)\(^-\)\(^4\)

These are major resistance mechanisms to EGFR-TKI, and represent important biomarkers at progression

WHAT WE LEARNED…

• Exon20ins is the third most common group of EGFR mutations, and is heterogeneous.

• There are several methods to test for EGFR mutations: hot spot testing, single gene testing, or testing as part of a gene panel— it is important to know their advantages and disadvantages.

• Turnaround times are highly dependent on technology.

• Pathologists play an active role in coordinating tissue acquisition, processing, selecting the best test, and communicating findings to the multidisciplinary cancer care team.
NEW BIOMARKERS IN mNSCLC
Second Case Presentation
Clinical History and Radiological Findings

• 75 years old Caucasian woman, non-smoker, referred by family doctor to pulmonologist with a persistent cough for several months that had been progressively worse over time, with copious foamy, whitish secretions suggestive of bronchorrhea

• CT scan revealed: LLL predominant pneumonia with minimal involvement of the lingula and RLL

• Follow up CT scan was performed 6 months later and showed a worsening of previous findings in the LLL, lingual and RML, but no lymphadenopathy or new findings

• A diagnostic biopsy was performed
CT SCANS - PRIOR TO SURGERY

DECEMBER 2020

MAY 2021
Case Presentation
Pathology: Morphology and Immunohistochemistry (IHC)

- Left lower lobe biopsy and brochoalveolar lavage (BAL): Adenocarcinoma
- TTF1 positive consistent with lung primary
- Sufficient but scant for further testing
- Molecular testing performed on cell block from BAL
Biomarker testing performed on cell block from BAL

IHC: ALK and ROS1 negative

IHC: PD-L1 (22C3 PharmaDx): Inadequate (less than 100 tumor cells)

NGS gene panel (Illumina Focus Panel) was performed
  • Includes DNA-based testing for fusions
REASON FOR REFERRAL: Focus panel based mutation screen, Lung Cancer

RESULT: Tier I variant identified

INTERPRETATION:

--- TIER I: VARIANTS OF STRONG CLINICAL SIGNIFICANCE ---
GENE FUSION DETECTED: CCDC6-RET (Exons 1:12)
A RET rearrangement involving CCDC6 was identified and results in constitutive kinase activity. RET rearrangements are identified in 1-2% of patients with NSCLC. RET rearrangements in NSCLC are associated with response to Alectinib and Selpercatinib (NCCN Guidelines - NSCLC 2021, Ribeiro (2020) PMID: 31698333; Drlon (2020) PMID: 32846060).

--- TIER II: VARIANTS OF POTENTIAL CLINICAL SIGNIFICANCE ---
None identified in the genes covered by this panel

--- TIER III/IV: VARIANTS OF UNCERTAIN FUNCTION/(LIKELY) BENIGN VARIANTS ---
None identified in the genes covered by this panel

--- COPY NUMBER VARIANTS (FOR INVESTIGATIONAL USE ONLY) ---
None identified in the genes covered by this panel
SECOND PATIENT

• RET fusion was identified by NGS
• Fusion partner: CCDC6
• Reported as Tier 1 variant: STRONG CLINICAL SIGNIFICANCE
# METHODS FOR IDENTIFYING FUSIONS

**NTRK, RET, NRG1, ROS1, ALK**

<table>
<thead>
<tr>
<th>Method</th>
<th>Substrate</th>
<th>Identifies</th>
<th>$$/TAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>Protein</td>
<td>OVEREXPRESSION OF TARGET PROTEIN</td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td>DNA</td>
<td>DNA REARRANGEMENT OF A SINGLE LOCUS</td>
<td>$$</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>RNA</td>
<td>EXPRESSION OF A SINGLE REARRANGED LOCUS</td>
<td>++</td>
</tr>
<tr>
<td>Multiplex panels</td>
<td>RNA</td>
<td>EXPRESSION OF MULTIPLE ONCOGENIC GENE FUSIONS</td>
<td>++</td>
</tr>
<tr>
<td>-AMP fusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-NanoString</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-Genome Sequence</td>
<td>DNA</td>
<td>EVERYTHING</td>
<td>++++</td>
</tr>
</tbody>
</table>

**SCREENING FOR SOME FUSIONS LIKE ROS1 AND NTRK**

**COULD BE METHOD OF CHOICE FOR CANCERS WITH LOW INCIDENCE**
# Approaches of Fusions Detection by NGS

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Amplicon-based</th>
<th>Anchored Multiplex PCR</th>
<th>DNA Hybrid Capture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example</td>
<td>Thermo Scientific Oncomine Illumina Focus Panel</td>
<td>Archer Fusionplex</td>
<td>Foundation CDx MSK-IMPACT BCCA-OncoPanel</td>
</tr>
<tr>
<td></td>
<td>Illumina Ampliseq</td>
<td>Qiagen Qiaseq</td>
<td></td>
</tr>
<tr>
<td>Type of Nucleic Acid Used</td>
<td>ARN or DNA</td>
<td>Only RNA</td>
<td>Most often DNA only</td>
</tr>
<tr>
<td>Quality and Quantity of</td>
<td>Low quantity/quality</td>
<td>Moderate quantity/quality</td>
<td>High quantity/quality</td>
</tr>
<tr>
<td>Nucleic Acid Used</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusion detection</td>
<td>Will detect only known fusion partners</td>
<td>Can detect some novel fusion partners</td>
<td>Can detect novel fusion partners</td>
</tr>
<tr>
<td>Tissue Samples</td>
<td>Works very well on small or compromised samples</td>
<td>Samples are frequently insufficient</td>
<td>Can be expensive and complicated</td>
</tr>
</tbody>
</table>
The patient has undergone surgery for palliative benefit due to increasing bronchorrea.
Case Presentation
Pathology: “PALLIATIVE SURGERY”

- LEFT LOWER LOBE LOBECTOMY AND LEFT UPPER LOBE WEDGE RESECTION
  - ADENOCARCINOMA, MULTIPLE FOCI (Papillary 50%, Acinar 35%, Lepidic 10%, Micropapillary 5%)
  - Largest focus 11.5cm
  - Bronchial margin positive, vascular margin negative
  - No pleural invasion
  - No lymphovascular invasion
  - Spread through air spaces (STAS) present
  - Six negative lymph nodes (stations 12L, 11L and 9L)
  - pT4N0
Case Presentation
Pathology: “PALLIATIVE SURGERY”:
ADENOCARCINOMA, MULTIPLE FOCI
(Papillary 50%, Acinar 35%, Lepidic 10%, Micropapillary 5%)

SPREAD THROUGH AIR SPACES (STAS)
THYROID TRANSCRIPTION FACTOR (TTF1)

NAPSIN A
CT scan on abdomen, pelvis, head and bone scan revealed no distant disease

PET scan for staging was performed and was negative for finding outside the lungs. The tumor had an SUV of 1.8

The patient was referred to Medical Oncology.
Somatic RET fusions and mutations associated with oncogenesis

**RET fusion partners**
- CCDC6**
- NCOA4**
- TRIM33
- CUX1
- KIAA1217
- FRMD4A
- KIAA1468
- PRKAR1A
- FKBP15
- NCOA1
- GOLGA5
- TRIM24
- TRIM27
- KTN1
- RFG9
- ERC1
- HOOK3
- PCM1
- AKAP13
- SPECC1L
- TBLXR1
- FGFR1OP
- EML4
- EPHA5
- SQSTM1
- PARD3
- PICALM
- AFAP1L2
- PPFBP2
- ACBD5
- MYH13

** RET mutations**
- G10 S/C/R
- G633C
- C620F/R/S
- E768D
- Y806C
- G633C
- C630R/Y
- L790F
- A883F
- C609F/G/R/S/Y
- D631Y
- Y791F
- S891A
- C611F/G/S/Y/W
- C634F*/G/R/S/W/Y
- V804M*/
- M918T**
- C618F/R/S
- K666E
- V804L

**Most common fusions or mutations**
- Meningioma (5.6%)
- PTC (10-20%)
- NSCLC (1% to 2%)
- MTC (~60-90%)
- Melanoma (0.7%)
- and basal cell carcinoma (12.5%)
- Esophageal adenocarcinoma (1.4%)
- Breast carcinoma (0.2%)
- Gastric adenocarcinoma (0.7%)
- Ovarian epithelial carcinoma (1.9%)
- Ureter urothelial carcinoma (16.7%)
- Colorectal adenocarcinoma (0.7%)

**Germline RET mutations associated with oncogenesis**
- C515
- C609
- C611
- C618
- C620
- C630
- E632
- C634* (MEN 2A)
- V648
- K666
- E768
- L790
- Y791
- V804
- R833
- M848
- A883
- S891
- S904
- M918* (MEN 2B)

OUR PATIENT:
CCD6C:RET (Exons 1:12)
Efficacy of Selpercatinib in RET Fusion–Positive Non–Small-Cell Lung Cancer

Alexander Drilon, M.D., Geoffrey R. Oxnard, M.D., Daniel S.W. Tan, M.B., B.S., Ph.D., Herbert H.F. Loong, M.B., B.S., Melissa Johnson, M.D., Justin Gainor, M.D., Caroline E. McCaugh, M.D., Ph.D., Oliver Gautschi, M.D., Benjamin Besse, M.D., Ph.D., Byoung C. Cho, M.D., Ph.D., Nir Peled, M.D., Ph.D., Jared Weiss, M.D., Yu-Jung Kim, M.D., Ph.D., Yuichiro Ohe, M.D., Ph.D., Makoto Nishio, M.D., Keunchil Park, M.D., Ph.D., Jyoti Patel, M.D., Takashi Suto, M.D., Tomohiro Sakamoto, M.D., Ezra Rosen, M.D., Ph.D., Manisha H. Shah, M.D., Fabrice Barlesi, M.D., Ph.D., Philippe A. Gassier, M.D., Lyudmila Bazhanova, M.D., Filip De Braud, M.D., Elena Garralda, M.D., Vamsidhar Vamsi, M.D., Miyako Satouchi, M.D., Ph.D., Kadoaki Ohashi, M.D., Ph.D., Nathan A. Pennell, M.D., Ph.D., Karen L. Reckamp, M.D., Grace K. Dy, M.D., Jürgen Wolf, M.D., Benjamin Solomon, M.B., B.S., Ph.D., Gerald Falchook, M.D., Kevin Ebata, Ph.D., Michele Nguyen, B.S., Binoj Nair, Ph.D., Edward Y. Zhu, Ph.D., Luxi Yang, M.P.H., Xin Huang, Ph.D., Elizabeth Olek, M.D., S. Michael Rothenberg, M.D., Ph.D., Koichi Goto, M.D., Ph.D., and Vivek Subbiah, M.D.

Efficacy of Selpercatinib in RET Fusion–Positive NSCLC

PHASE 1–2 TRIAL

144 Patients with RET fusion–positive non–small-cell lung cancer (N=105)

Objective response (complete or partial response)

64% (67 patients)

95% CI, 54 to 73

Safety

Twelve of 531 patients in overall cohort (2%) discontinued because of drug-related adverse events.

The median duration of response was 17.5 mo.

N Engl J Med
Volume 383(9):813-824
August 27, 2020
Selpercatinib in *RET*-altered Cancers: LIBRETTO-001 Updated Efficacy Analysis

LIBRETTO-431 is a global, open-label, randomized, phase III trial evaluating selpercatinib vs chemotherapy (cisplatin/carboplatin and pemetrexed) ± pembrolizumab in treatment naive patients with advanced *RET*+ non-squamous NSCLC\(^2\)

<table>
<thead>
<tr>
<th>Anti-tumor activity in treatment-naive <em>RET</em> fusion-positive NSCLC (IRC), N=39(^1)</th>
<th>Anti-tumor activity in <em>RET</em> fusion-positive NSCLC Pretreated with Platinum-based Chemotherapy (IRC), N=116(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR: 85% (95% CI: 70-94)</td>
<td>ORR: 64% (95% CI: 54-73)</td>
</tr>
<tr>
<td>Naïve: ORR 85%</td>
<td>Pretreated: ORR 64%</td>
</tr>
</tbody>
</table>

Pralsetinib (BLU-667) in Advanced RET Fusion+ NSCLC: ARROW

**BLU-67 Demonstrates Substantial Antitumor Activity in RET Fusion+ Advanced NSCLC**

<table>
<thead>
<tr>
<th>Best Response</th>
<th>All (N=45)</th>
<th>Prior Platinum (N=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRR (95% CI)</td>
<td>58% (43–72)</td>
<td>60% (42–75)</td>
</tr>
<tr>
<td>CR</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PR</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>SD</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>PD</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>DCR (95% CI)</td>
<td>66% (66–99)</td>
<td>100% (90–100)</td>
</tr>
</tbody>
</table>

*All responses are confirmed on two consecutive assessments as per RECIST 1.1.

- Most responses occur at the first scan (week 8).
- 82% of responding patients remain on treatment as of the data cut-off.
- Median duration of response not yet reached.
- Patients have been on treatment up to 24 months (including dose-escalation and regardless of starting dose).

Gainor et al. ASCO 2019. Abstract 9008
EMERGING BIOMARKERS IN mNSCLC
~ 35-50% of Patients With Advanced Nonsquamous NSCLC Have a Targetable Driver Mutation

KRAS G12C IS APPROXIMATELY 40% OF KRAS MUTATIONS IN NSCLC (mainly smokers)
Association Between KRAS/STK11/KEAP1 Mutations and Outcomes in POSEIDON: Durvalumab ± Tremelimumab + Chemotherapy in mNSCLC

Solange Peters,1 Byoung Chul Cho,2 Alexander Luft,3 Jorge Alatorre-Alexander,4 Sarayut Lucien Geater,5 Sang-We Kim,6 Grygorii Ursol,7 Maen Hussein,8 Farah Louise Lim,9 Cheng-Ta Yang,10 Luiz Henrique Araujo,11 Haruhiro Saito,12 Niels Reinmuth,13 Ross Stewart,14 Zhongwu Lai,15 Ruth Doake,14 Lee Krug,16 Edward B. Garon,17 Tony Mok,18 Melissa L. Johnson19

1Centre Hospitalier Universitaire Vaudois, Lausanne University, Lausanne, Switzerland; 2Yonsei Cancer Center, Seoul, Korea; 3Leningrad Regional Clinical Hospital, St Petersburg, Russia; 4Health Pharma Professional Research, Mexico City, Mexico; 5Prince of Songkla University, Songkla, Thailand; 6Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; 7Acinus, Kropyvnytskyi, Ukraine; 8Florida Cancer Specialists – Sarah Cannon Research Institute, Leesburg, FL, USA; 9Queen Mary University of London, London, United Kingdom; 10Chang Gung Memorial Hospital, Taoyuan City, Taiwan; 11Instituto Nacional de Cancer-INCA, Rio de Janeiro, Brazil; 12Kanagawa Cancer Center, Yokohama, Japan; 13Asklepios Lung Clinic, Munich-Gauting, Germany; 14AstraZeneca, Cambridge, UK; 15AstraZeneca, Waltham, MA, USA; 16AstraZeneca, Gaithersburg, MD, USA; 17David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; 18Chinese University of Hong Kong, Hong Kong, China; 19Sarah Cannon Research Institute, Tennessee Oncology, PLLC, Nashville, TN, USA

Presented by Dr. Solange Peters, IASLC 2022 World Conference on Lung Cancer
KRAS MUTATIONS IN mNSCLC

• Are heterogenous

• Are typically considered mutually exclusive with other known activating driver mutations in NSCLC (e.g., EGFR, ALK, ROS1)

• There are specific co-mutations or alterations present exclusively in KRASG12C mutated tumors:
  • TP53 tumor suppressor gene (39%-42%)
  • serine/threonine kinase 11 (STK11; 20%-29%)
  • kelch-like ECH-associated protein 1 (KEAP1; 13%-27%)
  • ataxia-telangiectasia mutated gene (ATM; 13%)
  • hepatocyte growth factor (MET) receptor (15.4%)
  • Erb-B2 receptor tyrosine kinase 2 (ERBB2; 13.8%)

Some of these co-mutations may hold prognostic and therapeutic significance in KRASmutant NSCLC.
BAD COMPANY?
STK11, KEAP1 AND KRAS

CLINICAL IMPLICATIONS. STK11 and KEAP 1

STK11 mutations
- STK11 encodes liver kinase B1 (LKB1), a serine/threonine kinase.
- LKB1 is a tumor suppressor, due in part to its role as a master regulator of cell metabolism.
- Germline STK11 mutations are associated with Peutz-Jeghers syndrome.
- Somatic alterations in STK11 occur in approximately 3% of all human cancers but are particularly common in lung adenocarcinoma.
  - STK11 is the third most commonly mutated gene in lung adenocarcinoma, after TP53 and KRAS.
- STK11 and KEAP1 are commonly co-mutated with KRAS; these co-mutations may define unique subsets of KRAS-mutant non-small cell lung cancer (NSCLC), with differences in prognosis and response to therapy.
  - STK11 and KEAP1 mutations may rewire the metabolism of KRAS-mutant NSCLC, including increasing dependence on glutamine.
- Inhibition of glutamine metabolism is among therapeutic strategies being actively investigated for the treatment of STK11- and/or KEAP1-mutant cancers.
POSEIDON Study Design

Stage IV NSCLC
N=1013 (randomised)
• EGFR/ALKwt
• ECOG PS 0 or 1
• Treatment-naïve for metastatic disease
• Tumour biopsy* and baseline plasma sample (for ctDNA)

Stratification factors
• PD-L1 expression (TC ≥50% vs <50%)
• Histology (NSQ vs SQ)
• Disease stage (IVA vs IVB)

Primary endpoints
D+CT vs CT:
• PFS‡
• OS

Key secondary endpoints
T+D+CT vs CT:
• PFS‡
• OS

D+CT† q3w 4 cycles
T (week 16 only) + D q4w until PD
D q4w until PD

Platinum-based CT† q3w up to 6 cycles

• Durvalumab 1500mg ± limited-course tremelimumab 75mg + CT q3w for 4 cycles
  – One additional dose of tremelimumab post-CT (week 16; 5th dose)

• Followed by durvalumab q4w maintenance until PD, and optional pemetrexed q4w§

*Newly acquired or archival (<3 months); †CT options: gemcitabine + carboplatin/cisplatin (SQ), pemetrexed + carboplatin/cisplatin (NSQ) or nab-paclitaxel + carboplatin (either histology); ‡By BICR (RECIST v1.1); §Patients with NSQ histology who initially received pemetrexed only (if eligible); pemetrexed q3w also permitted in the CT arm

BICR, blinded independent central review; D, durvalumab; ECOG, Eastern Cooperative Oncology Group; NSQ, non-squamous; PD, progressive disease; PS, performance status; q3w, every 3 weeks; q4w, every 4 weeks; SQ, squamous; T, tremelimumab; TC, tumour cell
# OS by KRAS Mutation Status

**OS benefit observed for T+D+CT vs CT in KRASm with HR 0.56 and estimated 51.7% alive at 2 yrs vs 25.6%**

<table>
<thead>
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<th>KRASm</th>
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<th>KRASwt</th>
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<tr>
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<td>T+D+CT</td>
<td>D+CT</td>
<td>CT</td>
<td>T+D+CT</td>
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<tr>
<td>Events, n/N</td>
<td>38/60</td>
<td>51/69</td>
<td>43/53</td>
<td>104/148</td>
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<tr>
<td>mOS, mo (95% CI)</td>
<td>25.7 (9.9–36.5)</td>
<td>12.6 (7.5–16.9)</td>
<td>10.4 (7.5–13.6)</td>
<td>17.1 (13.4–20.1)</td>
</tr>
<tr>
<td>HR* (95% CI)</td>
<td>0.56 (0.36–0.88)</td>
<td>0.80 (0.53–1.21)</td>
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<td>0.80 (0.62–1.04)</td>
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</tbody>
</table>

*HR <1 favours D (± T) + CT versus CT (unstratified analysis)*

Assessed among mutation-evaluable patients with NSQ tumour histology; DCO, 12 Mar 2021
OS by **STK11** Mutation Status

**OS benefit observed for T+D+CT vs CT in STK11m with HR 0.56 and estimated 32.3% alive at 2 yrs vs 4.5%**

<table>
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<tr>
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<th>T+D+CT</th>
<th>D+CT</th>
<th>CT</th>
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<td>Events, n/N</td>
<td>24/31</td>
<td>29/34</td>
<td>21/22</td>
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<tr>
<td>mOS, mo (95% CI)</td>
<td>15.0 (8.2–23.8)</td>
<td>6.9 (3.6–12.9)</td>
<td>10.7 (6.0–14.9)</td>
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<tr>
<td>HR* (95% CI)</td>
<td>0.56 (0.30–1.03)</td>
<td>1.03 (0.59–1.84)</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>T+D+CT</th>
<th>D+CT</th>
<th>CT</th>
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</thead>
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<tr>
<td>Events, n/N</td>
<td>118/177</td>
<td>123/169</td>
<td>141/179</td>
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<tr>
<td>mOS, mo (95% CI)</td>
<td>17.2 (14.9–22.1)</td>
<td>17.1 (13.3–22.3)</td>
<td>13.4 (11.5–17.5)</td>
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<tr>
<td>HR* (95% CI)</td>
<td>0.73 (0.57–0.93)</td>
<td>0.81 (0.64–1.04)</td>
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</tr>
</tbody>
</table>

*HR* <1 favours D (± T) + CT versus CT (unstratified analysis)

Assessed among mutation-evaluable patients with NSQ tumour histology; DCO, 12 Mar 2021

DCO, data cut-off; mo, months; mOS, median OS

Presented by Dr. Solange Peters, IASLC 2022 World Conference on Lung Cancer
OS by KEAP1 Mutation Status

**OS benefit observed for T+D+CT vs CT in KEAP1m with HR 0.43 (small sample size)**

### KEAP1m

<table>
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<tr>
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<th>T+D+CT</th>
<th>D+CT</th>
<th>CT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events, n/N</td>
<td>16/22</td>
<td>19/23</td>
<td>6/6</td>
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<tr>
<td>mOS, mo (95% CI)</td>
<td>13.7 (7.2–26.5)</td>
<td>8.1 (4.0–12.9)</td>
<td>8.7 (5.1–NE)</td>
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<tr>
<td>HR* (95% CI)</td>
<td>0.43 (0.16–1.25)</td>
<td>0.77 (0.31–2.15)</td>
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</table>

### KEAP1wt

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<thead>
<tr>
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<th>T+D+CT</th>
<th>D+CT</th>
<th>CT</th>
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</thead>
<tbody>
<tr>
<td>Events, n/N</td>
<td>226/303</td>
<td>241/307</td>
<td>262/312</td>
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<tr>
<td>mOS, mo (95% CI)</td>
<td>14.0 (11.8–16.1)</td>
<td>13.5 (11.7–14.9)</td>
<td>12.2 (10.6–13.9)</td>
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<tr>
<td>HR* (95% CI)</td>
<td>0.79 (0.66–0.94)</td>
<td>0.85 (0.71–1.01)</td>
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</table>

*HR < 1 favours D (± T) + CT versus CT (unstratified analysis)

Assessed among mutation-evaluable patients irrespective of tumour histology, due to small sample size; DCO, 12 Mar 2021
Conclusions

- In this exploratory analysis from POSEIDON, a trend for OS benefit was observed with 1L tremelimumab + durvalumab + CT in STK11m (HR vs CT 0.56 [95% CI 0.30–1.03]), KEAP1m* (HR 0.43 [0.16–1.25]) and KRASm (HR 0.56 [0.36–0.88]) NSQmNSCLC

- In these patient subgroups, addition of a limited course of tremelimumab to durvalumab (until PD) and 4 cycles of CT led to more frequent and deeper responses, including complete responses, with sustained disease control
  - The ORR was 45.2%, 45.5% and 55.0% in the STK11m, KEAP1m and KRASm subgroups, respectively

Presented by Dr. Solange Peters, IASLC 2022 World Conference on Lung Cancer
The NEW ENGLAND JOURNAL of MEDICINE

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VOL. 384 NO. 25

Sotorasib for Lung Cancers with KRAS p.G12C Mutation

BIOMARKERS THAT PREDICT RESPONSE TO IMMUNOTHERAPY
PD-L1 EXPRESSION
Four Categories of Tumors Based on Presence of PD-L1 and TILS (450 samples analyzed)

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<th>Subgroup B7-H1</th>
<th>TIL</th>
<th>Tumor Distribution</th>
<th>Possible Resistance Mechanism(s)</th>
<th>Analysis</th>
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<tr>
<td>-</td>
<td>-</td>
<td>I</td>
<td>Poor priming of general T cell responses to autologous tumor cells</td>
<td>Peripheral CD4+ and CD8+ T cell responses</td>
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<td></td>
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<td>Lack of inflammatory cell recruitment</td>
<td>Chemokine expression in biopsy or FFPE samples</td>
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<tr>
<td>+</td>
<td>+</td>
<td>II</td>
<td>Incomplete PD-1/B7-H1 pathway blockade and activation of alternate immune suppressive pathways</td>
<td>CD80 expression on TILs, expression of alternate suppressive pathways in TME</td>
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<tr>
<td>-</td>
<td>+</td>
<td>III</td>
<td>Alternate immune suppressive pathways</td>
<td>Expression of selected molecules in pathways with roles in evasion of NSCLC immnity</td>
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<tr>
<td>+</td>
<td>-</td>
<td>IV</td>
<td>Intrinsic induction of B7-H1 by oncogenes</td>
<td>Expression of molecules triggering aberrant signaling events</td>
</tr>
</tbody>
</table>

Table 3. Proposed mechanisms associated with NSCLC resistance to anti-PD-1/B7-H1 therapy

Velchetti et al (Rimm)
Dynamic Changes in PD-L1 Expression

- Among EGFR-mutant patients with paired, pre- and post-TKI resistant biopsies, PD-L1 expression status differed between biopsies in 13 (22%) patients.
- Among ALK-rearranged patients with paired, pre- and post-crizotinib biopsies, PD-L1 expression status differed between biopsies in 2 (25%) patients.

**Loss of PD-L1 Expression in an ALK-Positive Patient**

*Baseline*  
PD-L1 ≥ 50%

*Post-Crizotinib*  
PD-L1 Negative (No membranous staining)

*Post-Ceritinib (LDK378)*  
PD-L1 Negative

Gainor JF et al, J Clin Oncol 33, 2015 (abstr 8012)
TUMOR MUTATION BURDEN (TMB)
Tumor mutational load predicts survival after immunotherapy across multiple cancer types

![Graph showing overall survival by TMB within histology categories](image)

**Statistics for Non-small cell lung cancer**

<table>
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<th>Cancer type</th>
<th>No. of patients</th>
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<th>P-value</th>
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<td>All samples in cohort</td>
<td>1,662</td>
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<td>1.59 x 10^-6</td>
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<tr>
<td>Non-small cell lung</td>
<td>350</td>
<td>13.8</td>
<td>2.30 x 10^-3</td>
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<td>Renal cell carcinoma</td>
<td>151</td>
<td>5.9</td>
<td>0.569</td>
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**Drug class**

- Combo: 260
- CTLA4: 146
- PD-1/PDL-1: 1,256

---

Samstein et al. Nature Genetics, 2019
Oncogene-specific differences in tumor mutational burden

Marcelo V Negrao et al. J Immunother Cancer 2021
IMMUNE INFILTRATION
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- AML
- B-ALL
- CLL
- Burkitt's lymphoma
- DLBCL
- FL
- Multiple myeloma
- Astrocytoma
- Glioblastoma
- Meningioma
- Oligodendroglioma
- Bladder cancer
- Breast cancer
- Colon cancer
- Ewing sarcoma
- Gastric cancer
- Germ cell tumors
- Head and neck cancer
- Lung adenocarcinoma
- Lung squamous cell carcinoma
- Lung large cell carcinoma
- Melanoma primary
- Melanoma metastasis
- Osteosarcoma
- Ovarian cancer

Nature Medicine August 2015
The Immune Landscape of Cancer

Vésteinn Thorsson 1, 37, David L. Gibbs 1, 36, Scott D. Brown 2, Denise Wolf 3, Dante S. Bortone 4, Tai-Hsien Ou Yang 5, Eduard Porta-Pardo 6, 7, Galen F. Gao 8, Christopher L. Plaisier 1, 9, James A. Eddy 10, Elad Ziv 11, Aedin C. Culhane 12, Evan O. Paull 13, I.K. Ashok Sivakumar 14, Andrew J. Gentles 15, Raunaq Malhotra 16, Farshad Farshidfar 17, Antonio Colaprico 18, ... Ilya Shmulevich 1, 2
The immune landscape of cancer
# Six immune subtypes

<table>
<thead>
<tr>
<th></th>
<th>Macrophage: lymphocyte</th>
<th>Th1:Th2</th>
<th>Proliferation</th>
<th>Intratumoral heterogeneity</th>
<th>Other</th>
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<td>Wound healing</td>
<td>Balanced</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Highest M1 and highest CD8 T cells</td>
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<td>IFN-γ dominant</td>
<td>Lowest</td>
<td>Lowest</td>
<td>High</td>
<td>Highest</td>
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<td>Inflammatory</td>
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<td>Immunologically quiet</td>
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<td>Moderate</td>
<td>Moderate</td>
<td>Highest TGF-β signature</td>
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</table>

Thorrson et al. Immunity 2018
Immune infiltrate fraction in different tumor types

Thorsson et al. Immunity 2018
Survival according to immune subtype

Thorrson et al. Immunity 2018
Immunotherapy and targeted agents

The link
The immune response is determined by the collective states of *intracellular* molecular networks in tumor, immune, and other stromal cells and the *extracellular* network encompassing direct interaction among cells and communication via soluble proteins such as cytokines to mediate interactions among those cells.
Tumor-intrinsic signaling induces the exclusion and dysfunction of effective immunocytes

Yang et al., Journal of Hematology and Oncology 2019
Fast forward: near future
How to treat immune hot, cold or altered tumors

Galon and Bruni, Nature Reviews 2019
Thank you