1. Q: What has changed from the draft recommendations posted for public comment in November/December 2011?

A: There have been several changes to the final recommendations and we encourage you to read the final document completely. Since the time that the draft recommendations were posted for comment, another literature search was performed and the evidence graded, resulting in changes to the strength of the recommendations.

2. Q: Why does the guideline recommend only the two biomarkers EGFR and ALK? And not next generation sequencing?

A: EGFR and ALK are the two biomarkers with the most compelling published evidence to support a role in determining therapy for patients with lung cancer. KRAS may have some value in excluding therapies, and this role is addressed in the guideline. Several other genetic changes may show promise as markers to guide therapy, but remain to be proven in larger studies before an evidence-based recommendation can be made; these are also discussed in the document. Similarly, the published evidence about the analytical performance of “next generation” sequencing is insufficient at this time to make a defensible statement either for or against. This is a guideline about what should be done today, not what we speculate about what should be done tomorrow, regardless of how confident we may feel about what the future will bring.

3. Q: The diagnosis of adenocarcinoma is usually established before the stage is known. In the majority of institutions, the pathologist will not know whether the patient is early or late stage. What should the pathologist do in that setting?

A: Communication, where practical, is always recommended. The pathologist should make a reasonable effort to determine stage, or an institutional decision should be made to determine, in advance, what the policy should be in this circumstance. In the absence of clinical evidence or an institutional policy to argue against testing, our opinion is that analysis of EGFR and ALK should be performed.

4. Q: How do I work with my oncologists to implement these guidelines?

A: Pathologist-oncologist communication is critical. Pathologists need to share with their oncologists the types of molecular tests that are available, along with the strengths and weaknesses of each test. Pathologists need to engage with oncologists as to which tests should be used and when to use them. The guideline makes recommendations when data was extremely clear about what should be done. There are a number of issues that have been left up to the institutions to make a decision in their patients' best interest. And that
should be done prospectively on an institution-wide base basis – not managed case by case.

5. Q: Why perform testing when such a small percentage of patients are EGFR and ALK positive?
   
   A: One of the important findings in recent years is that if you don’t test patients for these 2 abnormalities (EGFR and ALK) and you just give chemotherapy – the patients whose tumors contain EGFR do significantly worse with chemotherapy than they would with targeted therapy. So you’re actually doing harm by not testing patients with abnormalities and very likely the same thing will apply to patients with same subset ALK gene fusion. Also, 20-25% is not a small percentage, especially with such a common and lethal cancer. Approximately 20,000 patients per year in the US can receive life-prolonging therapy from these tests.

6. Q: Does the CAP offer proficiency testing for EGFR and ALK?
   
   A: CAP offers proficiency testing for EGFR in the Solid Tumors EGFR Survey. The CAP/ACMG FISH for Paraffin Embedded Tissue Survey offers occasional challenges for ALK testing (CYK). Visit the CAP Surveys Catalog here. Note that FISH proficiency testing (PT) is method-based; therefore, it is not necessary to specifically perform PT for the ALK probe twice per year if PT for other probes on paraffin-embedded solid tumors is performed. The exception is HER2 FISH for which PT is required twice per year.

7. Q: Are laboratories required to use an FDA-approved method for ALK testing?
   
   A: Our opinion is that a properly validated assay for ALK that shows equivalent performance to an FDA-approved test should be acceptable. How this relates to the administration of drugs whose labeling information mentions FDA-approved tests is outside our jurisdiction.

8. Q: When will we be required to be compliant with these guidelines?
   
   A: Laboratories are encouraged to adopt these international guideline recommendations to support best practices for EGFR and ALK testing. The CAP Accreditation Program will review and determine whether these recommendations will be incorporated into accreditation requirements.

9. Q: When testing multiple tumor samples from one patient, if one result is positive and others negative is the patient positive?
   
   A: 1.5: Expert Consensus Opinion.—For patients with multiple, apparently separate, primary lung adenocarcinomas, each tumor may be tested but testing of multiple different areas within a single tumor is not necessary. Separate primary tumors that harbor different mutations are not uncommon. If an EGFR mutation is discovered in any tumor, the patient may benefit from an EGFR TKI. Therefore, if a patient presents with apparently separate primary tumors (based on location and nonoverlapping histologic features), each primary tumor may be tested. However, the decision whether or not to test each of a patient’s multiple tumors depends on each patient’s clinical context and requires communication between the laboratory and the clinical care team.

10. Q: When testing multiple samples from one tumor, if one result is positive and others negative is the patient positive?
    
    A: 1.5: Expert Consensus Opinion.—For patients with multiple, apparently separate, primary lung adenocarcinomas, each tumor may be tested but testing of multiple different areas within a single tumor is not necessary.
First, let us assume that there are no technical issues with the testing (false negative or false positive) and no significant differences in the quality of the samples from different sites within the tumor (samples with necrosis, fibrosis or other non-neoplastic tissue, etc). Some data suggest that variation in EGFR copy number within a tumor may impact mutation detection rate in samples from different zones of the tumor and, if that is the case for your tumor, we would interpret your tumor is positive.

11. Q: In small samples where the differential is poorly differentiated adenocarcinoma versus squamous carcinoma, what should be prioritized? IHC for resolving differential or tissue for molecular testing?

A: As a general rule, one should diagnose adenocarcinoma and then prioritize molecular testing. There are some therapies, other than TKI therapies, which are dependent on a non-squamous histology. Minimal tissue should be used to establish the adenocarcinoma diagnosis. However, if there is very limited tissue and especially if a diagnosis of cell type cannot be readily established with the minimal tissue available, then best judgment can be done in the individual case, preferably in consultation with the patient’s oncologist. Flexibility may be called for. Among the issues to be considered with the oncologist are whether or not rebiopsy is an option and what treatment options are being considered (including whether or not a patient is otherwise a candidate for TKI therapy).

12. Q: Why two weeks as a turnaround time (TAT) for testing these molecular markers? Seems quite long.

A: It is our expert consensus opinion that a TAT goal of 1 week (5 working days) should be established for EGFR and ALK testing. It is our opinion that the maximum should be up to 2 weeks (10 working days).

13. Q: For practicing community pathologists, what is the recommendation when we have a non small cell carcinoma that is not SCC? Should we automatically do the EGFR, ALK and ROS1 in sequential order? (as we do ER/PR/HER2 for breast cancers) or should we continue to wait for the oncologists to request the test? From reviewing the webinar slides I believe the answer is yes and this is supported by the NCCN guidelines but I need a confirmation before we change our policy.

A: It would be ideal to institute reflex testing when the pathologist diagnoses a lung cancer as adenocarcinoma or cannot exclude adenocarcinoma. However, reflex testing generally requires Medical Staff approval. There are issues related to patients who may be excluded from TKI therapy for other reasons or issues of cost. You may need to educate your oncologists and have them agree to the reflex testing.

14. Q: Please expand on the suggestion to consider molecular testing in stage I-III patients.

A: The initial clinical trials for TKI therapy were done in patients with advanced lung cancer and the poorest prognosis (stage IV) which is a typical starting point in the development of new therapies. Therefore, the applicability of biomarker testing and TKI therapy were first established in patients with advanced cancers and recommendations can be made for advanced lung cancers. Lung cancer patients in stages I-IIIA are typically treated with surgery with varying protocols of adjuvant chemotherapy and radiation therapy with an intent to cure. However, the majority of these early stage lung cancers will progress and 5 year survival for even the earliest stage, stage IA, is only 50% and progressively less with advancing stage. Therefore, it makes sense to test these early stage patients for biomarkers either to treat while they are still apparently in early stage or to have the information for future treatment since the majority will recur. Resected specimens from early stage cancers provide more tissue for testing and only a small biopsy or cytology is likely to be obtained at recurrence. Clinical trials are currently underway to assess the outcomes of TKI therapy in early stage lung cancer patients, but until results are reported, we cannot make
an evidence-based recommendation about testing in these patients. Our expert consensus opinion is that testing of stage I-III is encouraged, but the decision to do so at the present time should be done locally in collaboration with the oncology team.

15. Q: How does one know cases with small adenocarcinoma components behave similarly to well differentiated adenocarcinomas?

A: The literature cited for recommendations 1.1 a, 1.1b, 1.2 and 1.3 provide the basis for testing lung cancers with an adenocarcinoma for EGFR and ALK and reports that these tumors may respond to TKI therapy.

16. Q: It would be wonderful if at the end of the webcast there was an algorithm for pathologist to use for ordering these tests.

A: Guidelines 10.1a and 10.1b provide a sequence of testing: EGFR testing is recommended first and ALK testing suggested second, followed then by other markers. If the laboratory in question does KRAS testing, recommendation 7.1 provides a detailed discussion of the role of KRAS testing.

17. Q: Should TKI mutations be performed upfront or after treatment in relapsed/ metastatic setting?

A: Both

18. Q: How do you see the need for doing biomarker analysis in large cell carcinoma?

A: This is discussed in detail in Recommendations 1.2 and 1.3. If large cell carcinomas are demonstrated to have markers of adenocarcinoma by IHC or histochemical stain, then they should be tested for EGFR and ALK. If one cannot exclude that the large cell carcinoma has an adenocarcinoma component, then they should be tested for EGFR and ALK.

19. Q: Can someone briefly mention the IHC used to assess for adenocarcinoma in a mixed or poorly differentiated tumor?

A: Nuclear stains are easiest to interpret. TTF-1 for adenocarcinoma and p40 for squamous cell carcinoma are best.

20. Q: What is the significance of EGFR mutation in exon 20?

A: EGFR exon 20 mutations are associated with resistance to erlotinib therapy. This is critically important, because patients with these mutations should not receive this treatment. The insertion/duplication mutations in exon 20 are associated with primary resistance - these are the only EGFR abnormality seen in these patients, and they never respond to therapy. The T790M point mutation is typically acquired as a secondary mutation in patients who initially respond to therapy, but then relapse; the acquisition of the T790M is the most common mechanism of acquired resistance to erlotinib. The T790M is also seen, rarely, in the germline of patients, where is suggests the presence of an as-yet unnamed hereditary cancer syndrome; families have been described with this mutation and onset of lung adenocarcinoma in childhood.

21. Q: What is the clinical evidence for low frequency EGFR mutations and how actionable are these results?

A: I'm not sure I completely understand this question, but I will respond about the significance of EGFR mutations that are seen in <10% of lung adenocarcinomas. After the two most common mutations, the exon 19 deletions and the exon 21 L858R point mutation, there are two additional tiers of mutations. The second tier includes mutations that are seen
in ~ 1-10% of lung adenocarcinomas: E709 and G719 mutations in exon 18, L861Q mutation in exon 21, insertion/duplications in exon 19 and 20. These mutations have been studied in small case series, or have been included with the more common mutations in large studies, but the numbers are too low that large prospective studies of these specific mutations have not been completed. We are left inferring significance from less powered studies, but the evidence suggests that these mutations, with the exception of the exon 20 insertion/duplication reported above, are also associated with response to targeted therapy. For some of these, the evidence is a bit mixed and the magnitude of the response may be a bit muted and more complicated to predict with confidence, especially when these mutations are seen in combination with other mutations. In particular the exon 18 mutations (G719, E709) are not uncommonly encountered with other mutations.

The third tier includes very rare mutations, seen in <1% of lung adenocarcinomas. A glimpse at the list of mutations in COSMIC shows many mutations that have been reported at a low frequency. For these, we have no clear evidence to support their significance, and we are left having to make inferences based on things like in vitro studies, predicted phenotype, and evolutionary conservation - shaky ground, indeed. For this reason, we do not recommend routinely testing for these mutations but, of course, they will be discovered, especially with newer technologies like next generation sequencing.

22. Q: Clearly there is clinical utility of EGFR mutation exon 19 deletion and exon 21 L858R response to EGFR inhibitors. So update us by finding a mis-sense mutation or variant administering costly medication is a good idea?

A: I do not understand this question. If the question is whether or not it is a good idea to give an expensive drug, I would say it is a good idea if the drug will work significantly better than the alternative, cheaper therapy. Erlotinib in EGFR mutated lung cancer promotes response and disease-free survival by about a year, with significantly fewer side effects and better quality of life. It does nothing for people who do not have EGFR mutations. The cost of the test will reduce the cost of the use of the drug for the wild type patients who will not benefit.

23. Q: How many tumor cells are needed to perform EGFR mutation?

A: It depends on the methodology and the purity of the sample (cancer cells/total cells). Some techniques are exquisitely sensitive and can detect sequence in a handful of tumor cells. Other techniques are less sensitive.

24. Q: It appears EGFR amplification is quite common in C American and European populations than Chinese (as most of the current results are coming from Chinese population?)

A: EGFR mutation is the relevant biologic finding. EGFR amplification is not useful clinically. EGFR mutations are more common in Asia than in Europe and North America.

25. Q: For patients who are EGFR mutation negative do you recommend KRAS testing on that patient to determine the treatment regimen and also prognosis?

A: IF EGFR mutation is negative, the next gene to consider for therapeutic targeting is ALK rearrangement. The lab can then either test for ALK rearrangement directly, or might consider performing a rapid KRAS test because if KRAS is mutated, then ALK will not be rearranged. The reason a lab might consider doing this is because KRAS mutations are common, ALK rearrangements are less common, and ALK testing by FISH can be costly and difficult to do in a high throughput fashion compared to KRAS. Doing KRAS up front would reduce the ALK testing volume by about 40% without missing any positive cases. However, the KRAS test will take some time, so this approach should only be considered if it can be
done quickly enough to still have time for ALK testing within 10 days of the sample reaching the lab.

26. Q: Do you have any recommendations on EGFR mutation-specific antibodies when specimen is too scant to perform EGFR mutation analysis by molecular assay?

A: This is a reasonable surrogate when no other test can be performed. The mutation-specific IHC will detect the L858R mutation and some of the exon 19 deletions, but not all of them. Overall, there will be a significant false negative rate with this approach, but it is better than not testing at all, especially if a rebiopsy cannot be performed.

27. Q: Please tell us about EGFR amplification and response to TKI therapy.

A: EGFR amplification does not independently predict response to TKI therapy in adenocarcinoma of the lung. Many cases with EGFR mutation also have increased EGFR copy numbers (i.e., polysomy or amplification), and these patients will respond to therapy. However, when amplification or polysomy of EGFR is present without mutations, response is not likely. When mutations are present without amplification or polysomy, response is predicted.