COLLEGE of AMERICAN PATHOLOGISTS

March 28, 2016

Robert M. Califf, MD
Commissioner
U.S. Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Re: Docket No. FDA-2015-N-4990 for “Next Generation Sequencing-Based Oncology Panels";

Dear Dr. Califf:

The College of American Pathologists (CAP) appreciates this opportunity to comment on the Food and Drug Administration (FDA) discussion paper for the FDA Public Workshop on “*Next Generation Sequencing-Based Oncology Panels*”. The CAP is a medical society serving more than 18,000 physician members and the global laboratory community. It is the world's largest association composed exclusively of board-certified pathologists and is the worldwide leader in laboratory quality assurance. The College advocates accountable, high-quality, and cost-effective patient care. The CAP Laboratory Accreditation Program (LAP) is responsible for accrediting more than 7,000 clinical laboratories worldwide. Our members have extensive expertise in providing and directing laboratory services and also serve as inspectors in the Centers for Medicare & Medicaid Services (CMS)-deemed CAP accreditation program. The CAP welcomes the opportunity to work with the FDA to address standards for next-generation sequencing-based oncology panels.

The CAP Accreditation Program improves patient safety by advancing the quality of pathology and laboratory services through education and standard setting, and ensuring laboratories meet or exceed regulatory requirements. The CAP also provides laboratories with a wide variety of proficiency testing (PT) programs and has the responsibility to evaluate the accuracy of test performance and interpretation in more than 23,000 laboratories worldwide. The program allows laboratories to evaluate their performance regularly and improve the accuracy of the patient results they provide. Through these programs, the CAP provides individual laboratories with unknown specimens for testing. Pertinent to this workshop, the College launched in 2015 PT for next generation sequencing where laboratories have the ability to test up to 200 variants in a method-based challenge using either gene panels, exome, and/or genome sequencing. The CAP is following the initial NGS PT program for germline variants with NGS PT for the detection of somatic variants and other NGS clinical testing applications. The participants analyze the specimens and return the results to the CAP for evaluation. In turn, each participating laboratory receives a report of its performance as well as a report summarizing the results of all participating laboratories.

The CAP’s accreditation and proficiency testing programs address assessment of analytical performance, and CAP has developed a specific checklist to address Next Generation Sequencing

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technologies. FDA should not require a parallel approval process, and we encourage FDA to consider the existing quality control mechanisms in place.

Many of the general principles and issues surrounding clinical NGS of cancer specimens are well known, and CAP's response will not attempt to address all of them. Instead, CAP's response will focus on those additional issues that are unique to the "Pre-analytical and Quality Metric Approaches" that were the topic of the workshop in February 25, 2016." In response to the specific questions posed in the discussion paper, the CAP offers the following:

Pre-analytical and Quality Metric Approaches

Questions for discussion regarding pre-analytical and quality metric approaches to validate specific sample claims:

A. Are there pre-analytical steps that are most critical for NGS-based oncology panel performance?

Yes, those issues include sampling, library complexity and depth of coverage. Pathologist review of the tissue to select areas of highest tumor cellularity and viability is critical. There also needs to be an understanding that intrinsic tumor heterogeneity (both of the primary tumor itself and of metastases) means that ANY tumor sample submitted to NGS testing will not necessarily be representative of the tumor as a whole[1]. When considering library complexity, the starting material is a critical variable (i.e., 10 ng of DNA achieved via multiple rounds of PCR from 10 cells has far less information than 10 ng of DNA achieved directly from 1400 cells).

B. Are there tumor types that are more challenging for NGS-based oncology panels (e.g., brain, pancreas, etc.) and in what processing contexts (e.g., fresh frozen vs. FFPE)?

There are no generally consistent differences between tumor types given the same cellularity and viability. Also, it is important to keep the significance of formalin fixation in perspective, and not overly focus on this. Though formalin fixation leads to sequence artifacts, the rate is well below 1%. In addition, there is more variability in NGS sequence metrics between different genes in fresh tissue (due largely to GC content) than there is between paired fresh-formalin fixed tissue[2]. NGS will also work from cytology specimens.

C. What could be the appropriate level of validation needed to support both FFPE and fresh frozen tissue claims? For instance, should performance of the NGS-based oncology panel be validated with matched clinical samples, differently prepared cell cultures (e.g., cell cultures frozen or embedded to closely mimic how clinical samples are treated), or some other way?

Yes, we agree that NGS-based oncology panels should be validated to matched clinical samples, differently prepared cell cultures (e.g., cell cultures frozen or embedded to closely mimic how clinical samples are treated).

D. Are there differences in pre-analytical validation that should be expected when specimens are RNA versus DNA?

[1] Yachida S, et al. *Nature* 2010; 467:1114-1117
[2] Spencer D, et al*. J Mol Diagn* 2013;15:623-633

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The validation principles are the same. It is true that different extractions, different bioinformatic pipelines, etc. are required, but the pre-analytic principles are the same.

E. Should differences in tumor cellularity be accounted for in pre-analytical quality control parameters? If so, how should these differences be addressed?

Yes, differences should be accounted for in pre-analytical quality control. One significant part of assay validation is the lower limit of variant frequency that can be detected, this in turn determines the minimum tumor cellularity required. For example, if an NGS assay has a lower limit of variant detection of 10%, if a mutation is expected to be heterozygous in a malignancy, the tumor cellularity must be at least 20%. In addition, tissue cellularity determines the amount of DNA that is recovered, which impacts library complexity.

F. Are there specific concerns that should be addressed for FFPE-based specimens (consider variables such as fixation times, types of formalin, fixative pH, likelihood of deamination due to variant genomic context)?

These variables should be considered when specimen requirements are outlined for an NGS test, however, 10% Neutral Buffered Formalin (NBF) is the standard fixative (as opposed to other formalins) and certainly that factors like decalcification prior to formalin fixation might be significant.

G. When there are modifications in pre-analytical parameters (e.g., specimen processing, etc.) are there conditions under which there should be a comparison of assay output (e.g., run the assay from start to finish with samples representing the new pre-analytical parameters) versus conditions under which demonstration that critical quality control metrics are met (e.g., ensure the critical controls metrics such as nucleic acid purity, quantity, and amplifiability are still acceptable) would be sufficient?

Yes, there are conditions where a comparison of assay output is optimal versus demonstrating critical quality control metrics. Depending on the modification, either comprehensive or limited re-validation is appropriate.

H. Is there a specific level of validation that would be appropriate in order to add or modify specimen types (tissue source and/or tissue sample processing) for an already legally marketed NGS-based oncology panel?

It is impossible to state a general rule. Depending on the modification, either comprehensive or limited re-validation is appropriate.

I. Should critical quality metrics be established that apply to all NGS-based oncopanels and, if so, how can it be ensured that the proposed critical quality metrics are suitable to preserve assay performance?

Yes, quality metrics are required for example, sensitivity, specificity, and confidence range.

Analytical Validation and Bioinformatics

Questions for discussion regarding analytical validation approaches to NGS-based oncology panels.

A. Is there a level of clinical importance for a variant that should warrant individual analytical validation of a variant reported by a NGS-based oncology panel?

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No, we believe this should be avoided. Either an NGS assay is validated for the entire target region sequenced or not. To propose "individual analytical validation" is to say that every mutation in every gene must be a separate test, since nobody will ever say "That mutation in that gene isn't important enough that we need to make sure we're getting the correct answer; we don't care if we get that mutation wrong."

B. Should the number of variants being reported by an NGS-based oncology panel determine whether a representative variant approach to analytical validation is acceptable? If not, are there other validation approaches that should be considered?

See the answer immediately above.

C. Are there parameters (e.g., variant type, variant size, local sequence context, global sequence context, other) that are most important to capture in a representative variant set? Are there differences in sequencing platform that would impact selection of a representative variant set?

Yes, differences in assay design (amplification-based vs hybrid capture-based); platform (Ion Torrent vs Illumina); types of variants targeted (SNVs, indels, CNVs, and SVs); etc. all critically impact assay performance. They will determine what the validation set must look like.

D. In addition to validating individual assay steps, are there “best methods” to demonstrate the analytical validity of the complete assay system, starting with sample acquisition through the report generation?

Yes, there are publications on this topic, for different in assay designs (amplification-based vs hybrid capture-based); platforms (Ion Torrent vs Illumina); types of variants targeted (SNVs, indels, CNVs, and SVs); etc.

E. Once analytical validity has been satisfactorily established for a specific set of variants, are there requirements or controls that should be in place to add, subtract, or substitute variants from the panel? For example, if an NGS-based oncology panel included a 3 base pair deletion, and a 3 base pair deletion was included in the representative variant panel, would it be appropriate for the manufacturer swap in a new 3 base pair deletion variant without any additional analytical validation?

It is impossible to state a general rule. Depending on the modification, local sequence context, platform, etc., either comprehensive or limited re-validation is appropriate.

F. Should the types of variants (single nucleotide variants (SNVs), indels, translocations, amplifications, etc.), size of variants (small indels vs large deletions, etc.), local sequence contexts (GC-rich, homopolymeric, etc.) and global sequence contexts (pseudogenes, etc.) included in an NGS-based oncology panel inform the appropriate analytical validation strategy? If so, are there particular validation strategies that are best-suited to establishing analytical performance of the NGS-based oncopanel?

Yes, the types of variants (single nucleotide variants (SNVs), indels, translocations, amplifications, etc.), size of variants (small indels vs large deletions, etc.), local sequence contexts (GC-rich, homopolymeric, etc.) and global sequence contexts (pseudogenes, etc.) should be included in an NGS-based oncology panel analytic validation. There are publications to support this process.

G. Should commutability studies be conducted in order to infer the performance of the assay on clinical samples from data obtained in cell lines or plasmids? Yes.

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H. Are there valid approaches to distinguish somatic versus germline variants? For example, would software approaches based on allelic frequency difference be sufficient or would matched tumor/normal tissue comparisons be needed?

Software approaches are not sufficient. Most experts with experience doing clinical NGS recognize that, for oncology specimens, the measured variant allele frequency (VAF) is NOT a reliable indicator of the percentage of tumor cells that harbor the mutation, and thus not a reliable indicator of whether the mutation is acquired or inherited. Given the intrinsic genetic instability of most tumors (which often manifests itself as indels, loss of heterozygosity, CNVs, and so on) this is not surprising.

Tumor/normal comparisons are essential (for logistical reasons) when the NGS target region gets to be large (i.e., thousands of genes or so) to filter out inherited variants in order to highlight the acquired variants. However, tumor/normal comparisons add unnecessary expense for assays with smaller target regions (hot-spot panels, up to many hundreds of genes). For NGS assays with smaller target regions, reflex testing when a variant is suspected to be inherited is much more cost effective.

Tumor/normal comparisons ignore the hundreds/thousands/millions of variants found in the "normal specimen". Even though these variants can be filtered out of the "tumor specimen" to simplify analysis of the cancer tissue, the question remains as to the ethical obligation to evaluate the "normal specimen" variants themselves.

I. Are there best approaches for defining assay sensitivity and specificity in a way that accurately reflects assay performance for NGS-based oncology panels?

In addition to the existing evidence, the CAP is working on additional guidelines that will include metrics. These papers will include "wet specimens" as well as "in silico" approaches.

J. Is it useful or practical to consider establishing an approved modification protocol to add or subtract variants from the panel without additional FDA premarket review? If so, are there key criteria that should be included in this modification protocol?

Yes, it would be useful for the field if an established modification protocol existed. One element of such a protocol could be a demonstration of acceptable metrics. We also recommend further discussion among the field.

K. Are there risk-based strategies can be employed by FDA and manufacturers to determine when bioinformatics pipeline changes have significant potential to impact assay performance?

While "wet lab" specimens could be used as standards, the best way to do this is via "in silico" approaches.

Clinical and Follow-on Companion Diagnostic Claims

Questions for discussion regarding clinical evidence and follow-on companion diagnostic claims:

A. Are there key considerations for evidence that would or would not be sufficient for providing a reasonable assurance of safety and effectiveness for a follow-on companion diagnostic claim? Please consider the evaluation of differences in assay performance using procured specimens from the original therapeutic trial, procured specimens to mirror the therapeutic patient population, or other specimen types (e.g., the companion diagnostic used FFPE but the NGS panel utilizes fresh frozen tissue).

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No, all patient specimens have are the presence or absence of a mutation/mutations, and all an NGS test or a companion diagnostic test has is the ability to find the mutation if present. Hence, either an assay finds the mutation or not, where the accuracy of the answer is determined by comparison to an external gold standard.

B. Are there appropriate expectations for routine reporting of variants without established companion diagnostic claims? Please consider variants with comparable analytical performance to similar variants with established companion diagnostic claims, the availability of targeted agents to patients, and other means of establishing assay clinical performance.

Professional societies (like AMP, CAP, and ACMGG) have proposed guidelines. This area is within the realm of medical practice.

C. Are there disclaimers that should be considered around issues of panel comprehensiveness? Please consider cases of absent or inadequate coverage of genes/variants with associated therapeutics or disease states, absent or inadequate coverage of known hotspots, and other variations in panel composition that could potentially impact assay interpretation.

Yes, providing information on the comprehensiveness of the test should be important elements of a report.

Please contact Helena Duncan, CAP Assistant Director, Economic and Regulatory Affairs at hduncan@cap.org or Fay Shamanski, PhD, CAP Assistant Director, Economic and Regulatory Affairs at fshaman@cap.org if you have any questions on these comments.

Closing,

The College of American Pathologists

Sent via www.regulations.gov

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