An Overview of Characteristics of Clinical Next-Generation Sequencing–Based Testing for Hematologic Malignancies

Bing M. Zhang, MD; Alissa Keegan, MD, PhD; Peng Li, MD, PhD; Neal I. Lindeman, MD; Rakesh Nagarajan, MD, PhD; Mark J. Routbort, MD, PhD; Patricia Vasalos, BS; Annette S. Kim, MD, PhD; Jason D. Merker, MD, PhD

Context.—With the increasing integration of molecular alterations into the evaluation of hematologic malignancies (HM), somatic mutation profiling by next-generation sequencing (NGS) has become a common clinical testing strategy. Limited data are available about the characteristics of these assays.

Objective.—To describe assay characteristics, specimen requirements, and reporting practices for NGS-based HM testing using College of American Pathologists proficiency testing survey data.

Design.—The College of American Pathologists NGS Hematologic Malignancies Survey (NGSHM) results from 78 laboratories were used to determine laboratory practices in NGS-based HM testing.

Results.—The majority of laboratories performed tumor-only (88.5% [69 of 78]), targeted sequencing of cancer genes or mutation hotspots (98.7% [77 of 78]); greater than 90% performed testing on fresh bone marrow and peripheral blood. The majority of laboratories reported a 5% lower limit of detection for single-nucleotide variants (73.1% [57 of 78]) and small insertions and deletions (50.6% [39 of 77]). A majority of laboratories used benchtop sequencers and custom enrichment approaches.

Conclusions.—This manuscript summarizes the characteristics of clinical NGS-based testing for the detection of somatic variants in HM. These data may be broadly useful to inform laboratory practice and quality management systems, regulation, and oversight of NGS testing, and precision medicine efforts using a data-driven approach.

Published online January 15, 2021. Accepted for publication October 23, 2020.

The clinical need for broader identification of somatic variants and the advancement in next-generation sequencing (NGS) technology together have driven the development and incorporation of NGS-based somatic variant testing in the clinical workup of many tumor types.1,2 Specifically, in hematologic malignancies (HM), the number of implicated genetic alterations has expanded rapidly in recent years, and several of these molecular markers are central to the diagnosis, classification, and clinical management of hematolymphoid neoplasms.3–6 Although there are available publications detailing the utilization and practices of NGS assays in solid tumors,7–12 data on the utilization of NGS in HM are lacking. To evaluate the current clinical laboratory practice in NGS-based testing of HM, the College of American Pathologists (CAP) Molecular Oncology Committee incorporated questions into the 2017 CAP Next-Generation Sequencing Hematologic Malignancies Survey (NGSHM-A 2017). The NGSHM Proficiency Testing (PT) Survey not only assessed the accuracy of the laboratory results but also included questions regarding the practice of the laboratories. The CAP Molecular Oncology Committee has compiled the laboratory responses from this survey to catalog assay characteristics, specimen requirements, and reporting practices for NGS-based testing of HM. A separate publication focuses specifically on testing performance.13 We herein report on the survey results of testing practice characteristics of 78 laboratories performing NGS-based
NGS-based Testing Characteristics for HMs—Zhang et al

Abbreviation: FFPE, formalin-fixed, paraffin-embedded.

Other includes: ascitic fluid, cell suspension, cerebrospinal fluid, FFPE bone marrow, FFPE bone marrow clots, flow processed specimens, methanol-acetic acid fixed cell pellets, pleural fluid, purified DNA, and saliva.

testing for somatic variant detection in HM. Taken together, this information will provide accurate data on the current state of testing practices for HM. These data may further inform a variety of topics related to NGS-based HM testing with an evidence-based approach, including ongoing discussion regarding clinical laboratory quality management systems, regulation, oversight, and ongoing precision oncology efforts.

METHODS

Data were derived from the first mailing of the CAP NGSHM-A 2017 Survey. This survey was a methods-based proficiency challenge for laboratories performing NGS of genes or mutational hotspots in HM from DNA specimens. Laboratories were asked to identify somatic single-nucleotide variants and small insertions or deletions in the following genes: ASXL1, ATM, BRAF, CALR, CEBPA, CREBBP, CSF3R, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KMT2D, MPL, MYD88, NOTCH1, NPM1, SF3B1, SRSF2, TET2, TP53, and U2AF1. The NGSHM-A 2017 Survey also included optional practice questions. Twenty-one of these questions were related to assay characteristics, specimen requirements, and reporting, to facilitate investigation of any differences in performance (see supplemental digital content at https://meridian.allenpress.com/aplm in the September 2021 table of contents). The Survey was sent to laboratories on February 21, 2017 and the results were due back on April 2, 2017. Only Survey results from laboratories that provided responses to these practice questions were analyzed.

RESULTS

Overall Profile of HM Clinical NGS Testing

Of 97 laboratories (including 25 international laboratories) that were enrolled in the NGSHM-A 2017 Survey, 78 laboratories provided answers to all or some of the optional survey questions related to assay characteristics, specimen requirements, and reporting. Of 78 laboratories, 75 (96.2%) laboratories performed targeted DNA sequencing of cancer genes or mutation hotspots; 1 laboratory performed targeted DNA and RNA sequencing; 1 laboratory performed exome sequencing; and 1 laboratory performed a combination of targeted DNA, exome, and genome sequencing.

Specimen Characteristics and Requirements for HM Clinical NGS Testing

Survey results for the use of tumor-only versus tumor-normal paired specimens, accepted specimen types, and genomic DNA input requirements are summarized in Table 1. The majority of the participating laboratories, 88.5% (69 of 78), used a tumor-only approach in their NGS-based somatic variant testing for HM. Of 9 laboratories that perform paired testing of tumor and normal specimens, a variety of control tissues were used, which included peripheral blood (6 laboratories), fresh “normal” tissue (eg, skin biopsy) (6), buccal swabs (5), fixed “normal” tissue (4), saliva (1), and nails (1). In addition, of 9 laboratories that tested paired tumor-normal specimens, 7 laboratories reported constitutional variants and 2 did not.

Labs reported assessment of a broad range of specimen types with their NGS-based assays for somatic variant detection in HM. More than 90% of laboratories tested fresh bone marrow and fresh peripheral blood specimens, and approximately a third or less tested formalin-fixed, paraffin-embedded specimen types, other fresh specimen types, or frozen tissues. DNA input requirements were assessed with 65.8% (50 of 76) of laboratories requiring 100 ng or less of genomic DNA input and 84.2% (64 of 76) requiring 200 ng or less of genomic DNA input.

Sequencing Platforms and Assay Methodology

The sequencing platforms, selection methods, and custom-enrichment approaches are summarized in Table 2. The benchtop Illumina MiSeq or MiSeqDx were used by just more than half of laboratories (51.3% [40 of 78]). One-third of laboratories (26/78) reported using a higher throughput platform such as the Illumina HiSeq, Illumina NextSeq, or Ion Torrent S5 series systems.

Of the target selection methods used by the participating laboratories, an approach using custom targeted content was used by 55.1% (43 of 78). The Illumina TruSight Myeloid Sequencing Panel was the most frequently used predesigned commercial panel with use by 30.8% of laboratories (24 of 78). A variety of other methods were used by 1 or 2 laboratories. Of the laboratories performing custom approaches (either amplicon or capture based), multiple vendor products were used as is shown in Table 2.

The NGS read lengths reported by laboratories ranged from 49 to 400 bp (Table 3). Approximately 37.2% of respondents (29 of 78) used 150-bp reads, which was the most common reported read length. A paired-end read configuration was used by 84.6% of laboratories (66 of 78), and the remaining 15.4% (12 of 78) used single-end reads.

Table 1. Survey Results Regarding Specimen Characteristics and Requirements

| Specimen Approach         | N (%) of Laboratories (n = 78) | Specimen Types            | N (%) of Laboratories (n = 77) | Genomic DNA Input Requirement, ng | N (%) of Laboratories (n = 76) |
|---------------------------|-------------------------------|---------------------------|-------------------------------|-----------------------------------|-------------------------------|
| Tumor only                | 69 (88.5)                    | Fresh bone marrow         | 74 (96.1)                     | 0–100                             | 50 (65.8)                     |
| Tumor-normal pair         | 9 (11.5)                     | Fresh peripheral blood    | 71 (92.2)                     | 101–200                           | 14 (18.4)                     |
| FFPE tissues              | 26 (33.8)                    | FFPE cell blocks          | 22 (28.6)                     | 501–1000                          | 4 (5.3)                       |
| Finite-needle aspirates   | 19 (24.7)                    | Fresh tissues             | 15 (19.5)                     | >2000                             | 1 (1.3)                       |
| Othera                    | 9 (11.7)                     | Frozen tissue             | 14 (18.2)                     |                                   |                               |

Abbreviation: FFPE, formalin-fixed, paraffin-embedded.

a Other includes: ascitic fluid, cell suspension, cerebrospinal fluid, FFPE bone marrow, FFPE bone marrow clots, flow processed specimens, methanol-acetic acid fixed cell pellets, pleural fluid, purified DNA, and saliva.
The reported lower limit of detection (LLOD) of the assays for single-nucleotide variants as well as small insertions and deletions less than 50 bp is summarized in Table 4. LLOD was reported in terms of somatic allele percentage, such that a 5% LLOD indicates the ability of a laboratory to detect 5% mutant alleles in the background of 95% wild-type alleles. Of 78 laboratories, 57 (73.1%) reported an LLOD of 5% for single-nucleotide variants, while 12 (15.4%) reported an LLOD between 1% and 4%, and the remaining 9 (11.5%) reported an LLOD between 10% and 15%. Of 77 laboratories that tested for small insertions and deletions, 39 (50.6%) reported an LLOD of 5% for insertions and deletions, while 8 (10.4%) reported an LLOD between 1% and 4%, and the remaining 30 (39.0%) reported an LLOD between 8% and 20%.

Laboratories were queried whether they included a sensitivity control at or near the LLOD of the assay in each run, and 44.2% (34 of 77) of laboratories included such a control.

The reported average number of reads covering targeted bases and minimum number of reads required for each targeted base are presented in Tables 5 and 6, respectively. Of the 78 laboratories, 65 (83.3%) reported achieving an

---

**Table 2. Survey Results Regarding Sequencing Platform and Assay Methodology**

| N (%) of Laboratories |
|-----------------------|
| Sequencing Platform (n = 78) |
| Illumina MiSeq | 29 (37.2) |
| Illumina HiSeq 2500 | 12 (15.4) |
| Illumina MiSeqDx | 11 (14.1) |
| Ion PGM System | 10 (12.8) |
| Illumina NextSeq 500 | 8 (10.3) |
| Ion Torrent S5/S5 XL | 3 (3.8) |
| Illumina HiSeq 3000/4000 | 3 (3.8) |
| Other, not specified | 2 (2.6) |

| Selection Method (n = 78) |
|---------------------------|
| Custom Enrichment Approacha | 43 (55.1) |
| Illumina TruSight Myeloid Sequencing Panel | 24 (30.8) |
| RainDance ThunderBolts Myeloid Panel | 2 (2.6) |
| Fluidigm Access Array | 2 (2.6) |
| Ion AmpliSeq Cancer Hotspot Panel | 2 (2.6) |
| Not applicableb | 1 (1.3) |
| Otherc | 4 (5.1) |

| Custom Enrichment Approach (n = 43) |
|------------------------------------|
| Agilent Custom SureSelect | 10 (23.3) |
| Ion AmpliSeq Custom DNA Panel | 9 (20.9) |
| Roche NimbleGen SeqCap EZ Designs | 5 (11.6) |
| Illumina TruSeq Custom Amplicon | 5 (11.6) |
| Agilent HaloPlex Custom Kit | 3 (7.0) |
| Nextera Rapid Capture Custom Enrichment Kit | 1 (2.3) |
| RainDance Custom Gene Panel | 1 (2.3) |
| Otherd | 7 (16.3) |
| Not specified | 2 (4.7) |

**Table 3. Sequencing Read Length**

| Read Length, bp | N (%) of Laboratories (n = 78) |
|----------------|--------------------------------|
| 100 | 12 (15.4) |
| 125 | 2 (2.6) |
| 150 | 29 (37.2) |
| 200 | 7 (9.0) |
| 250 | 10 (12.8) |
| 300 | 5 (6.4) |
| 400 | 2 (2.6) |
| Other | 11 (14.1) |

**Table 4. Reported Lower Limit of Detection for Single-Nucleotide Variants and Small Insertions and Deletions**

| LLOD (%) | N (%) of Laboratories |
|----------|-----------------------|
| Single-nucleotide variants (n = 78) |
| 1 | 1 (1.3) |
| 2 | 2 (2.6) |
| 3 | 4 (5.1) |
| 4 | 5 (6.4) |
| 5 | 57 (73.1) |
| 10 | 8 (10.3) |
| 15 | 1 (1.3) |

| Small insertions and deletions (n = 77) |
|---------------------------------------|
| 1 | 2 (2.6) |
| 2 | 2 (2.6) |
| 3 | 1 (1.3) |
| 4 | 3 (3.9) |
| 5 | 39 (50.6) |
| 8 | 1 (1.3) |
| 10 | 27 (35.1) |
| 15 | 1 (1.3) |
| 20 | 1 (1.3) |

**Table 5. Average Number of Reads Covering Targeted Bases**

| Metric not established | N (%) of Laboratories (n = 78) |
|------------------------|--------------------------------|
| 0–50 |
| 100–150 | 2 (2.6) |
| 151–250 | 2 (2.6) |
| 251–350 | 1 (1.3) |
| 351–500 | 3 (3.8) |
| 501–750 | 8 (10.3) |
| 751–1000 | 7 (9.0) |
| 1001–1500 | 8 (10.3) |
| 1501–2500 | 10 (12.8) |
| >2500 | 32 (41.0) |
| Metric not established | 5 (6.4) |

**Legend**

- **a** Includes both “custom enrichment approach” and “custom designed capture- or amplicon-based enrichment approach.”
- **b** Laboratory performs exome or genome sequencing for somatic variant detection.
- **c** Other includes Ion AmpliSeq Designer - AML Panel (2), Agilent ClearSeq Comprehensive Cancer Panel, and Illumina TruSeq Ampli-con Cancer Panel.
- **d** Other includes: Celomtics custom enrichment kit, custom DNA baits, custom LDT capture, IDT (Integrated DNA Technologies), IDT xGen custom panel, Roche - NimbleGen, QIAGen GeneRead DNAseq Targeted Panels V2.

Abbreviation: LLOD, lower limit of detection.
average coverage of more than 500 reads for each targeted base in the assay, while 8 (10.3%) laboratories reported between 51 and 500 reads and 5 (6.4%) had not established this metric (Table 5). Just less than half of laboratories (46.2% [36 of 78]) reported the minimum requirement of more than 250 reads for each targeted base in the assay (Table 6). The remaining laboratories reported a minimum read requirement of 250 or less reads (48.7% [38 of 78]) or did not have a minimum read requirement (5.1% [4 of 78]).

Confirmatory Testing and Reporting

Approximately 61.8% of laboratories (47 of 76 reporting laboratories) did not perform confirmatory testing of somatic variants (Table 7). The remaining 38.2% (29 of 76) of laboratories used one or more methods for confirmatory testing of some variants. These methods included Sanger sequencing (29 laboratories), other targeted mutation testing (eg, allele-specific polymerase chain reaction or real-time polymerase chain reaction) (17), fragment analysis (9), pyrosequencing (4), other NGS-based platform (2), Sequenom (1), droplet digital polymerase chain reaction (1), and primer extension (1).

Laboratories were queried about whether their clinical reports included variant allele fraction (VAF) and total coverage depth at the variant positions as well as whether they reported variants using a tiered approach (Table 8). In the clinical reports issued by the laboratories, 66.7% (52 of 78) of laboratories listed the VAF for all reported variants, 2.6% (2 of 78) of laboratories listed the VAF for variants when the allele fraction and tumor content suggest subclonality, and 30.8% (24 of 78) of laboratories did not list the variant allele fraction. In addition, approximately one-quarter of laboratories (24.4% [19 of 78]) included total coverage depth (variant and reference reads) at the variant position in the clinical report, while three-quarters of laboratories (75.6% [59 of 78]) did not include this information. Finally, more than half of laboratories (55.1% [43 of 78]) reported variants using a tiered approach, while 44.9% (35 of 78) did not report variants using a tiered approach.

Data about types of interpretation routinely provided by laboratories are summarized in Table 9. The vast majority of laboratories (92.2% [71 of 77]) provided some interpretive comment. The most common practices, performed by more than 70% of laboratories, were to report known clinical implications and to categorize variants into classes of medical significance. Approximately half of laboratories also routinely included known biological function (53.2%...
circumstances. Just half of laboratories (46.2% [36 of 78]) reported a minimum requirement of more than 250 reads for each targeted base in the assay, and an additional 19.2% (15 of 78) of laboratories reported a minimum requirement of between 151 and 250 reads. A key limitation of the coverage depth data is that coverage can be variably defined by laboratories. For example, coverage depth may be calculated using variable quality criteria at the base or alignment level and/or unique reads as determined during deduplication. The NGSHM-A 2017 Survey neither defined coverage nor had laboratories report how they determined coverage depth.

The AMP/ASCO/CAP working group made additional recommendations on variant reporting. This includes a recommendation that VAF and coverage be evaluated and reported when appropriate. At the time of this survey, two-thirds of laboratories (66.7% [52/78]) listed the VAF for all reported variants in the clinical report. Regarding coverage depth, approximately one-quarter of laboratories (24.4% [19 of 78]) included total coverage depth at the variant position in the clinical report. Because the AMP/ASCO/CAP recommendation indicated that VAF and coverage be evaluated and reported when appropriate, we cannot exclude that laboratories not reporting these metrics do so in a subset of cases when appropriate.

A central feature of the AMP/ASCO/CAP recommendations on the interpretation and reporting of sequence variants in cancer was the development of a 4-tiered classification system based on clinical significance as follows: tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants of unknown clinical significance; and tier IV, variants deemed benign or likely benign. The survey included a question about whether laboratories report variants using a tiered approach, but this question did not specifically ask about the use of the 4-tiered classification system proposed in the AMP/ASCO/CAP joint recommendations, and it is unclear if the joint recommendations are directly translatable to HM. Just more than half of laboratories (55.1% [43 of 78]) reported variants using a general tiered approach, and just less than half do not (44.9% [35 of 78]).

With respect to AMP/ASCO/CAP recommendations on the interpretation and reporting of sequence variants in cancer, there were several recommendations related to variant interpretation. The joint recommendations indicated, “It is useful to provide an interpretive comment on detected genetic alterations that puts the alteration in clinicopathologic context to inform management decisions.” At the time of the survey, the vast majority of laboratories (92.2% [71 of 77]) provided an interpretive comment, and more than 70% of laboratories reported known clinical implications and categorize variants into classes of medical significance. These results suggest significant adoption of the best practices included recommendations before publication of the AMP/ASCO/CAP joint recommendations.

There were also some recommendations that were not in routine practice at the time of the NGSHM-A 2017 Survey. Again, it is important to emphasize that these consensus recommendations were published around the time this survey was completed by the laboratories, so there was not sufficient time to expect laboratories to implement these consensus recommendations. Therefore, this survey likely reflects practices before the AMP/ASCO/CAP recommendations. These recommendations represent a potential opportunity for laboratory improvement. The AMP/ASCO/CAP joint recommendations indicated that cancer genes or mutation hotspots not meeting the minimal read requirement established by the laboratory should be indicated in the report. In early 2017, 40.3% (31 of 77) of laboratories reported undercovered or underperforming regions of the assay in a general or disease-specific manner. Likewise, the AMP/ASCO/CAP joint recommendations suggested pertinent negatives be reported in a disease-specific manner (eg, absence of an EGFR mutation in a patient with non-small cell lung cancer), and one-quarter (24.7% [19 of 77]) reported routinely providing this information in their reports. Both of these practices, reporting inadequately covered regions and pertinent negatives, represent laboratory best practice, and should be more broadly implemented.

The inclusion of sensitivity controls in NGS-based assays was inconsistent. The majority of laboratories (55.8% [43 of 77]) reported that they did not include a sensitivity control at or near the LLOD of the assay with each run. The CAP
Molecular Pathology Checklist Requirement MOL.34229 discusses controls for qualitative assays, and states, “For qualitative tests, positive, negative and sensitivity controls are included for each assay, when appropriate.” MOL.34229 further clarifies, “A sensitivity control may be required if the molecular assay is being used to detect low-level target sequences (e.g. pathogens, chimerism, mosaicism, tumor-normal admixtures).” The requirement provides laboratory directors the flexibility to determine whether a sensitivity control is necessary for their laboratory’s NGS-based assay for the detection of somatic variants in hematologic neoplasms. The AMP/CAP joint recommendations for the validation of NGS-based oncology panels recommended that the LLOD for each variant type be established with a minimum of 59 samples. If sufficient validation samples cannot be sourced with the targeted mutations and VAFs necessary to validate the LLOD, a sensitivity control should be used. The utility of this sensitivity control is enhanced when it monitors variants in poorly performing regions, such as cytosine-rich or guanine-rich regions of the CEBPA exon and the p.P95 hotspot in SRSF2.

The current manuscript has several limitations. First, the survey data were analyzed 2 years after data collection. Clinical NGS-based testing is a rapidly evolving area, so the data presented in this manuscript do not represent the most up-to-date practice. We do note that the major themes reported here are observed in the most recent NGSHM-A 2019 Survey results. That is (1) the majority of survey participants perform tumor-only, targeted sequencing of cancer genes or mutation hotspots; (2) the majority of survey participants performed testing on fresh bone marrow and peripheral blood with a reported 5% to 10% LLOD for single-nucleotide variants as well as small insertions and deletions; and (3) the majority of survey participants used benchtop sequencers and custom enrichment approaches. Second, the assay practice data were self-reported. The third limitation is that these data are only derived from laboratories participating in the CAP NGSHM proficiency testing program because, in the United States and internationally, there are other vendors of PT mechanisms to perform alternative PT. It is unclear to what extent these data represent specimen requirements, assay characteristics, and reporting practices of other laboratories.

The current manuscript provides practice data from 78 laboratories performing NGS-based testing in HM. We suggest these data can help inform (1) how laboratories implement NGS-based HM testing and design associated quality management systems; (2) how professional organizations can promote process improvement in this area; and (3) how to further promote broader precision medicine efforts. First, these practice data enable existing and laboratories that are new to this practice area to understand current practices in the field (eg, what are the average number of reads covering targeted bases and minimum number of required reads for each targeted base). Furthermore, in the above sections, we highlight practices that are incorporated by laboratories in their quality management systems (eg, inclusion of a sensitivity control). Second, these practice data will enable professional organizations, such as CAP, to further optimize the design of future HM PT and related programs in this area to support process improvement. As an example, the current data suggest PT programs should mimic fresh, tumor-only specimens that contain variants in mutation hotspots with variant allele fractions above the 5% to 10% LLOD of most assays. In addition, the frequent use of diverse sequencing platforms and assay methodologies in this NGS-based HM testing will likely require in silico PT materials to be customized to each laboratory. Third and finally, understanding current clinical practice is critical to inform local and national precision medicine efforts. Understanding the types of data that will likely be generated by this testing enables design of electronic medical record systems to better import and display these genetic data. Furthermore, understanding of the current state is critical to assess gaps when considering the desired future state in national and international precision medicine efforts.

Clinical molecular diagnostics laboratories are increasingly adopting NGS-based somatic variant detection for HM to meet the evolving clinical needs in this area. A data-driven understanding of the current status of clinical practice is required to assess opportunities for improvement and harmonization of practices. To our knowledge, these practice data, together with the concurrent study on testing performance, are the most comprehensive and detailed studies of practice and performance in NGS-based HM testing to date. As discussed above, we believe that these data will also be useful as evidence to support discussions involving clinical laboratory practice and quality management systems, regulation, and oversight of NGS-based oncology testing as well as ongoing precision oncology efforts.

References

1. Kanagal-Shamanna R, Singh RR, Routhout MJ, Patel KP, Medeiros LJ, Luthra R. Principles of analytical validation of next-generation sequencing based mutational analysis for hematologic neoplasms in a CLIA-certified laboratory. J Mol Diagn. 2016;18(4):473–477.

2. Kluk MJ, Lindsley RC, Aster JC, et al. Validation and implementation of a custom next-generation sequencing clinical assay for hematologic malignancies. J Mol Diagn. 2016;18(4):507–515.

3. Taylor J, Xiao W, Abdel-Wahab O. Diagnosis and classification of hematologic malignancies on the basis of genetics. Blood. 2017;130(4):410–423.

4. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, Paris: IARC Press; 2008.

5. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–2405.

6. Herzig R, Bartley AN, Bridge JA, et al. A window into clinical next-generation sequencing-based oncology testing practices. Arch Pathol Lab Med. 2017;141(12):1679–1685.

7. Li MM, Datto M, Duncavage EJ, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017;19(1):1–23.

8. Merker JD, Devereaux K, Iafriate AJ, et al. Proficiency testing of standardized samples shows very high interlaboratory agreement for clinical next-generation sequencing-based oncology assays. Arch Pathol Lab Med. 2019;143(4):463–471.

9. Kim AS, Bartley AN, Bridge JA, et al. Comparison of laboratory-developed tests and FDA-approved assays for BRAF, EGFR, and KRAS testing. JAMA Oncol. 2018;4(6):838–841.

10. Surrey LF, Oakley FD, Merker JD, et al. Next-generation sequencing (NGS) methods show superior or equivalent performance to non-NGS Methods on BRAF, EGFR, and KRAS proficiency testing samples. Arch Pathol Lab Med. 2019;143(8):980–984.

11. Moncur JT, Bartley AN, Bridge JA, et al. Performance comparison of different analytic methods in proficiency testing for mutations in the BRAF, EGFR, and KRAS genes: a study of the College of American Pathologists Molecular Oncology Committee. Arch Pathol Lab Med. 2019;143(10):1203–1211.

12. Keegan A, Bridge JA, Lindeman NI, et al. Proficiency testing of standardized samples shows high interlaboratory agreement for clinical next generation sequencing-based hematologic malignancy assays with survey material-specific differences in variant frequencies. Arch Pathol Lab Med. 2020;144(8):959–966.

13. Arora A, Ljundgren U, Arica ME, Corless C, et al. Guidelines for validation of next-generation sequencing-based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn. 2017;19(3):341–365.

Arch Pathol Lab Med—Vol 145, September 2021

NGS-based Testing Characteristics for HMs—Zhang et al 1115
15. Cottrell CE, Al-Kateb H, Bredemeyer AJ, et al. Validation of a next-generation sequencing assay for clinical molecular oncology. J Mol Diagn. 2014;16(1):89–105.
16. Gulley ML, Braziel RM, Halling KC, et al. Clinical laboratory reports in molecular pathology. Arch Pathol Lab Med. 2007;131(6):852–863.
17. Commission on Laboratory Accreditation, ed. Molecular Pathology Checklist. Northfield, IL: College of American Pathologists; 2017.
18. Litwack ED, Mansfield E, Shuren J. The FDA and genetic testing. N Engl J Med. 2015;372(23):2273–2274.
19. Evans BJ, Burke W, Jarvik GP. The FDA and genomic tests—getting regulation right. N Engl J Med. 2015;372(23):2258–2264.
20. Jaffe S. Planning for US Precision Medicine Initiative underway. Lancet. 2015;385(9986):2448–2449.
21. Lowy DR, Singer DS. Implementing the cancer moonshot and beyond. Lancet Oncol. 2017;18(11):e622–e623.