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ClariFind™ White Paper

INTRODUCTION

The correlation of clinical features with specific drug therapies and clinical trial options, customized to each patient, has become a crucial component of cancer care[1,2]. For this reason, our experts developed ClariFind to add clarity to molecular testing with personalized treatment options for cancer patients. ClariFind employs next-generation sequencing (NGS) for comprehensive genomic profiling of a patient's tumor sample to identify potential clinically actionable targets and associated therapies, which could have significant positive impacts on patient outcome.

WHAT IS THE CLINICAL UTILITY OF NGS IN CANCER STUDIES?

Multiple papers have reported on alterations that predict positive and negative responses to certain therapies[3-8] with the potential to improve outcomes including progression free survival.[9] In many cancers, mutations in more than one gene have been identified as predictive targets, as illustrated with the identification of EGFR, ALK, KRAS, ROS1, RET, MET, BRAF and ERBB2 (HER2) genes in lung cancer[10,11] and KRAS, NRAS, BRAF, PIK3CA and PTEN in colorectal cancer.[4] Other cancers such as breast cancer, exhibit even more heterogeneous variant profiles[12] and the list of targetable genes for a multitude of cancers is ever expanding.[13-19]

Cancer genes are more diverse than previously thought[19,20] and tumors from the same organs can exhibit quite different variant profiles.[12] Conversely, the same mutational profiles can be found across multiple cancers[21] and genetic studies should not be limited to genes that are historically characteristic for a single tumor type. Tumor location, which traditionally formed the basis for cancer treatment, is now eclipsed by genetic criteria, which has emerged as a defining prognostic and therapeutic indicator.[22,23]

Furthermore, since heterogeneity exists over the lifetime of a cancer, with differing patterns of genetic changes between primary and metastatic tumors,[24,25] it is essential in patients with metastatic disease to establish a baseline profile followed by sequential studies to follow the evolution of the tumor over time.[26,27]

A significant number of cancer patients do not achieve desired response with first-line/standard of care therapy or become resistant to therapy as the disease progresses.[28] Certain patients may better be served with alternate first tier therapies or clinical trial enrollment and, for some cancers, patient participation in a clinical trial is "unanimously endorsed"over first line therapies, particularly in advanced cases.[29] NCCN Guidelines recommend the best management for ANY patient with cancer is in a clinical trial and ASCO strongly encourages the use of NGS to determine eligibility.[30]

Leading organizations including the College of American Pathologists (CAP), the Association for Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO), and the National Comprehensive Cancer Network (NCCN) have published recommendations supporting mutational profiling,[10, 30, 31-38] which has become standard of care for a growing number of cancers; thus, moving NGS into the forefront of clinical cancer genomics.

The clinical utility of NGS has been well reported, and multiple and high throughput sequencing is now widely considered the gold standard for genetic diagnosis.[39] The benefits of this platform are numerous. Eliminating the need for multiple stepwise studies, NGS provides information beyond targeted mutation analysis and single gene-by-gene Sanger DNA sequencing.[40,41] Increased efficiency helps preserve specimens and avoid repeat biopsies.[42] This technology reduces costs both in the laboratory and clinically, by limiting the use of expensive therapies in patients that may be more inclined to respond poorly.[41]

HOW IS CLARIFIND DIFFERENT FROM OTHER TESTS?

ClariFind covers all DNA coding regions (+/-5-10bp flanking intronic sequences) of 277 key cancer genes. Single nucleotide changes, insertions, deletions and copy number alterations in 39 genes are detected. Tumor

mutational burden is also included in the analysis as a predictive biomarker to immunotherapy.[43] Additionally, our chemistry is designed for improved coverage of GC-rich regions in genes such as CEBPA and CCND1, which have poorer coverage with traditional approaches.[44] While many panels are appropriate for only a subset of tumors, ClariFind is appropriate in individuals with solid tumors and/or hematologic malignancies and can be completed on a variety of sample types. Using a unique molecular barcoding approach, our assay mitigates PCR duplicates and bias,and allows for confident low-level variant detection, from 5% down to 1%. ClariFind requires very little DNA — as low as 40 ng — enabling testing on small biopsies.

Utilizing robust data resources, analysis is performed by a team of in-house, board-certified molecular pathologists, curation scientists, and bioinformatics experts. Our proprietary reporting system includes a detailed interpretive summary that follows published guidelines set forth by AMP, ASCO and CAP.[2] With a focus on clinical significance, we provide patients with available personalized drug therapies and clinical trial options. Each case is thoroughly reviewed by board-certified clinical experts to aid in optimizing patient care and who remain available for further clinical consultation.

EXPERIENCE OF BAYLOR GENETICS

For nearly 40 years, Baylor Genetics has been the leading pioneer in genetic testing. Currently, we offer a full spectrum of cost-effective genetic testing and provide clinically relevant solutions. Our team's unmatched knowledge and experience deliver a combination of advanced technology and deep patient data sets that lead to more accurate interpretations. The team at Baylor Genetics is well versed in NGS technology and has reported extensively about our experience with this highly complex test.[39, 45-54]

WHO ARE WE?

Brian Y. Merritt, MD: Dr. Merritt is currently the Medical Director for the Cancer Genetics laboratory. He attended medical school at Baylor College of Medicine, where he also completed his residency and fellowship training in pathology. He obtained his certifications from the American Board of Pathology for Anatomic and Clinical Pathology in 2013, Hematology in 2014, and Molecular Genetic Pathology in 2015.

Pengfei Liu, Ph.D.: Dr. Liu earned his Ph.D. at Baylor College of Medicine, where he also went on to complete his fellowship in clinical molecular genetics. In 2015, Dr. Liu obtained his board certification from the American Board of Medical Genetics for Clinical Molecular Genetics. Currently, he is an Assistant Professor at Baylor College of Medicine for the Department of Molecular and Human Genetics, and a Lab Director for Exome Re-analysis at Baylor Genetics.

Shashikant Kulkarni, MS, Ph.D., FACMG: Dr. Kulkarni is the Chief Scientific Officer at Baylor Genetics and a Professor, Co-Vice Chair of Research, Co-Program Director of the Molecular and Human Genetics Program at Baylor College of Medicine. He earned his PhD. at All India Institute of Medical Sciences and completed fellowship and post graduate fellowships at Hammersmith HospitalImperial College, Harvard Medical School and Washington University School of Medicine.

Christine Eng, MD: Dr. Eng joined Baylor Genetics in 2000 and is Professor of Department of Molecular and Human Genetics at Baylor College of Medicine and Chief Medical Officer and Chief Quality Officer of BaylorGenetics Laboratories. She has been recognized for contributions to the implementation of genomics in clinical practice. She is senior author of articles in the NEJM and JAMA regarding exome sequencing and is principal investigator of the Genomic Sequencing Core for the NIH Undiagnosed Diseases Network.

CITATIONS

1. Chang, Fengqi, and Marilyn M. Li. "Clinical application of amplicon-based next-generation sequencing in cancer." *Cancer genetics* 206.12 (2013): 413-419.
2. Li, Marilyn M., 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." *The Journal of molecular diagnostics* 19.1 (2017): 4-23.

3. Hadoux, Julien, et al. “SDHB mutations are associated with response to temozolomide in patients with metastatic pheochromocytoma or paraganglioma.” *International journal of cancer* 135.11 (2014): 2711-2720.
4. Sepulveda, Antonia R., et al. “Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology.” *American journal of clinical pathology* 147.3 (2017): 221-260.
5. Slamon, Dennis J., et al. “Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2.” *New England Journal of Medicine* 344.11(2001): 783-792.
6. Shaw, Alice T., et al. “Crizotinib versus chemotherapy in advanced ALK-positive lung cancer.” *New England Journal of Medicine* 368.25 (2013): 2385-2394.
7. Douillard, Jean-Yves, et al. “Panitumumab–FOLFOX4 treatment and RAS mutations in colorectal cancer.” *New England Journal of Medicine* 369.11 (2013): 1023-1034.
8. Rosell, Rafael, et al. “Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial.” *The lancet oncology* 13.3 (2012): 239-246.
9. Haslem, Derrick S., et al. “Precision oncology in advanced cancer patients improves overall survival with lower weekly healthcare costs.” *Oncotarget* 9.15 (2018): 12316.
10. Lindeman, Neal I., et al. “Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association forthe Study of Lung Cancer, and the Association for Molecular Pathology.” *Journal of Thoracic Oncology* 13.3 (2018): 323-358.
11. De Roock, Wendy, et al. “KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectalcancer.” *The lancet oncology* 12.6 (2011): 594-603.
12. Roy-Chowdhuri, Sinchita, et al. “Multigene clinical mutational profiling of breast carcinoma using next-generation sequencing.” *American journal of clinical pathology* 144.5 (2015): 713-721.
13. Duncavage, Eric J., et al. “Targeted next generation sequencing of clinically significant gene mutations and translocations in leukemia.” *Modern pathology* 25.6 (2012): 795.
14. Ross, Jeffrey S., et al. “New routes to targeted therapy of intrahepatic cholangiocarcinomas revealed by next-generation sequencing.” *The oncologist* 19.3 (2014): 235-242.
15. Beltran, Himisha, et al. “Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity.” *European urology* 63.5 (2013): 920-926.
16. Amato, Eliana, et al. “Targeted next-generation sequencing of cancer genes dissects the molecular profiles of intraductal papillary neoplasms of the pancreas.” *The Journal of pathology* 233.3 (2014): 217-227.
17. Mulder, Babs G. Sibinga, et al. “Diagnostic value of targeted next-generation sequencing in patients with suspected pancreatic or periampullary cancer.” *Journal of clinical pathology* (2017): jclinpath-2017.
18. Zacher, Angela, et al. “Molecular Diagnostics of Gliomas Using NextGeneration Sequencing of a Glioma-Tailored Gene Panel.” *Brain Pathology* 27.2 (2017): 146-159.
19. McClure, Rebecca F., et al. “Clinical Significance of DNA Variants in Chronic Myeloid Neoplasms (CMNs): A Report of the Association for Molecular Pathology.” The Journal of Molecular Diagnostics (2018).
20. Greenman, Christopher, et al. “Patterns of somatic mutation in human cancer genomes.” *Nature* 446.7132 (2007): 153.
21. Kan, Zhengyan, et al. “Diverse somatic mutation patterns and pathway alterations in human cancers.” *Nature* 466.7308 (2010): 869.
22. Kandoth, Cyriac, et al. “Mutational landscape and significance across 12 major cancer types.” Nature502.7471 (2013): 333. Dienstmann, Rodrigo, et al. “Genomic medicine frontier in human solid tumors: prospects and challenges.” *Journal of Clinical Oncology* 31.15 (2013): 1874-1884.
23. Dienstmann, Rodrigo, et al. “Standardized decision support in next generation sequencing reports of somatic cancer variants.” *Molecular oncology* 8.5 (2014): 859-873.
24. MacConaill LE, Garraway LA. Clinical Implications of the Cancer Genome. *Journal of Clinical Oncology*. 2010;28:5219-5228
25. Gerlinger, Marco, et al. “Intratumor heterogeneity and branched evolution revealed by multiregion sequencing.” *New England journal of medicine* 366.10 (2012): 883-892.
26. Yachida, Shinichi, et al. “Distant metastasis occurs late during the genetic evolution of pancreatic cancer.” *Nature* 467.7319 (2010): 1114.
27. Ciardiello, Fortunato, et al. “Delivering precision medicine in oncology today and in future—the promise and challenges of personalised cancer medicine: a position paper by the European Society for Medical Oncology (ESMO).” (2014): 1673-1678.
28. Brastianos, Priscilla K., et al. “Genomic characterization of brain metastases reveals branched evolution and potential therapeutic targets.” *Cancer discovery* (2015).

29. Nagle, Peter W., et al. “Patient-derived tumor organoids for prediction of cancer treatment response.” Seminars in cancer biology. Academic Press, 2018.
30. Benson, Al B., et al. “Rectal Cancer, Version 2.2018, NCCN Clinical Practice Guidelines in Oncology.” *Journal of the National Comprehensive Cancer Network* 16.7 (2018): 874-901.
31. Mansfield, Aaron S., Ben Ho Park, and Michael P. Mullane. “Identification, Prioritization, and Treatment of Mutations Identified by Next-Generation Sequencing.” *American Society of Clinical Oncology Educational Book* 38 (2018): 873-880.
32. Arber, Daniel A., et al. “Initial diagnostic workup of acute leukemia: guideline from the College of American Pathologists and the American Society of Hematology.” *Archives of pathology & laboratory medicine* 141.10 (2017): 1342-1393.
33. Ettinger, David S., et al. “Non–small cell lung cancer, version 5.2017, NCCN clinical practice guidelines in oncology.” *Journal of the National Comprehensive Cancer Network* 15.4 (2017): 504-535.
34. Benson, Al B., et al. “NCCN guidelines insights: colon cancer, version 2.2018.” *Journal of the National Comprehensive Cancer Network* 16.4 (2018): 359-369.
35. Network, N. C. C. “Clinicalpractice guidelines in oncology: Colon Cancer Version 2 2016.” (2016).
36. Wood, Douglas E., et al. “Lung Cancer Screening, Version 3.2018, NCCN Clinical Practice Guidelines in Oncology.” *Journal of the National Comprehensive Cancer Network* 16.4 (2018): 412-441.
37. National Comprehensive Cancer Network. Clinical Practice Guidelines in Oncology. Thyroid Carcinoma. Version 2.2017 –May 17, 2017; NCCN.org. accessed 1/18/2018
38. National Comprehensive Cancer Network (NCCN) Clinical Practice Guideline in Oncology,Version 3.2017. www.nccn.org/professionals/physician_gls/PDF/breast.pdf. Accessed December 18, 2017.
39. Haugen BR, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016 Jan;26(1):1-133.
40. Yang, Yaping, et al. “Clinical whole-exome sequencing for the diagnosis of mendelian disorders.” *New England Journal of Medicine* 369.16 (2013): 1502-1511.
41. Aparicio, Samuel, and Elaine Mardis. “Tumor heterogeneity: next-generation sequencing enhances the view from the pathologist’s microscope.” (2014): 463.
42. Sabatini, Linda M., et al. “Genomic sequencing procedure microcosting analysis and health economic cost-impact analysis: a report of the association for molecular pathology.” *The Journal of Molecular Diagnostics* 18.3 (2016): 319-328.
43. Giardina, Tindaro, et al. “Implementation of next generation sequencing technology for somatic mutation detection in routine laboratory practice.” *Pathology* 50.4 (2018): 389-401.
44. Goodman, Aaron M., et al. “Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers.” *Molecular cancer therapeutics* (2017): molcanther-0386.
45. Grossmann, Vera, et al. “Strategy for robust detection of insertions, deletions, and point mutations in CEBPA, a GC-rich content gene, using 454 next-generation deep-sequencing technology.” *The Journal of Molecular Diagnostics* 13.2 (2011): 129-136.
46. Gargis, Amy S., et al. “Assuring the quality of next-generation sequencing in clinical laboratory practice.” *Nature biotechnology* 30.11 (2012): 1033.
47. Gargis, Amy S., et al. “Good laboratory practice for clinical next-generation sequencing informatics pipelines.” *Nature biotechnology* 33.7 (2015): 689.
48. Cottrell, Catherine E., et al. “Validation of a next-generation sequencing assay for clinical molecular oncology.” *The Journal of molecular diagnostics* 16.1 (2014): 89-105.
49. Lyon, Elaine, et al. “Next generation sequencing in clinical diagnostics: experiences of early adopters.” *Clinical chemistry* 61.1 (2015): 41-49.
50. Griffith, Malachi, et al. “Optimizing cancer genome sequencing and analysis.” *Cell systems* 1.3 (2015): 210-223.
51. Madhavan, Subha, et al. “ClinGen Cancer Somatic Working Group–standardizing and democratizing access to cancer molecular diagnostic data to drive translational research.” *bioRxiv* (2017): 212225.
52. Huang, Kuan-lin, et al. “Pathogenic germline variants in 10,389 adult cancers.” *Cell* 173.2 (2018): 355-370.
53. Adams, David R., and Christine M.Eng. “Next-generation sequencing to diagnose suspected genetic disorders.” *New England Journal of Medicine* 379.14 (2018): 1353-1362.
54. Walsh, Michael F., et al. “Integrating somatic variant data and biomarkers for germline variant classification in cancer predisposition genes.” *Human mutation* 39.11 (2018): 1542-1552.
55. Danos, Arpad M., et al. “Adapting crowdsourced clinical cancer curation in CIViC to the ClinGen minimum variant level data community-driven standards.” *Human mutation* 39.11 (2018): 1721-1732.