The genetic testing of solid tumors has evolved rapidly. With an ever-increasing number of clinically significant and/or actionable gene alterations in addition to increasing biologic, gene, or mutation-specific therapies, single-target testing is no longer suitable for many modern oncology patients. This review explores panel-based testing, including its history and evolution from prior testing modalities. We also discuss its current usefulness, as best exemplified by lung cancer, and other special considerations including a summary of the pros and cons of panel implementation and use. Lastly, we discuss the successes and challenges of panel-based testing and explore future directions.
and treatment selection. Hence, assays and interpretation workflows must be designed thoughtfully to support molecular tumor profiling for children with cancer, including accommodation of small samples, detection of gene fusions, and consideration of potential germline associations.

Specimen Considerations in Molecular Oncology Testing 367

Qiong Gan and Sinchita Roy-Chowdhuri

Proper treatment of the patient with cancer depends on an accurate diagnosis of the tumor and is further directed by prognostic and more recently therapeutic molecular signatures in the era of precision medicine. Molecular oncology testing provides diagnostic, prognostic, and therapeutic information derived from the tumor genome. The aim of this review is to provide valuable information to laboratories for choosing optimal clinical specimens for molecular oncology testing by evaluating the strengths and weaknesses of different sample types from the procurement, processing, and pre-analytic selection matching to different test platforms.

Utility of Single-Gene Testing in Cancer Specimens 385

Mehenaz Hanbazazh, Diana Morlote, Alexander C. Mackinnon, and Shuko Harada

Molecular testing is now considered the standard of care to screen for disease, confirm the diagnosis, guide management, and use target therapy. Currently, several testing strategies are being used. One of the most common strategies is single-gene testing, which is often conducted for known mutations, such as BRAF in melanoma and EGFR in lung cancer. Subsequently, next-generation sequencing (NGS), which tests many genes simultaneously, was developed using targeted gene panels, whole-exome, or whole-genome sequencing. Ordering the best diagnostic tool and choosing between single-gene testing and NGS depends on several factors. In this review, we discuss different single-gene testing methodologies and the impact of using them in comparison to NGS/multigene panel.

Analytical Principles of Cancer Next Generation Sequencing 395

Tatyana Gindin and Susan J. Hsiao

This article covers analytical principles of cancer next generation sequencing (NGS). Cancer samples require special considerations due to the cancer-specific applications of testing, as well as cancer sample specific issues, including low input, low tumor purity, or fixation-related artifacts. Laboratories typically use a combination of approaches around specimen processing, assay design, and bioinformatics analysis to allow for successful detection of actionable biomarkers. Examples of these approaches for cancer NGS testing are discussed and reviewed here.
Principles and Validation of Bioinformatics Pipeline for Cancer Next-Generation Sequencing
Somak Roy

Clinical bioinformatics plays a key role in the implementation of clinical next-generation sequencing (NGS) testing infrastructure. Bioinformatics workflows in a clinical laboratory are complex and therefore need to be validated as part of an end-to-end NGS assay validation before clinical use. The validation cohort should be representative of the types of samples, types of variants, lower limits of detection of the assay, as well as sequence context of the panel. When validating an NGS bioinformatics pipeline, the pipeline validation lifecycle should be adhered to. Software containers and modern software automation tools can allow the building of a scalable and reliable clinical bioinformatics infrastructure and can be implemented in clinical bioinformatics operations in a phased way depending on the size and skillset of the bioinformatics team.

Best Practice for Clinical Somatic Variant Interpretation and Reporting
Jeffrey Schubert, Jinhua Wu, Marilyn M. Li, and Kajia Cao

Because the clinical impact of cancer genomics is being increasingly recognized, tumor sequencing will likely continue to expand in breadth and scope. Therefore, it is vital for laboratory professionals to adopt the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists guidelines and create a standardized system of classification and nomenclature for somatic variants. Combining robust bioinformatics pipelines with thorough data analysis is necessary to efficiently and reproducibly identify and assess the impact of clinically relevant variants.

Molecular Detection of Oncogenic Gene Rearrangements
Zehra Ordulu and Valentina Nardi

Oncogenic gene rearrangements have been exponentially significant for clinical management of cancer, from diagnosis to therapy and disease monitoring. Testing algorithms should be created with caution, and sample type, accessibility to testing method, turnaround time, and economic aspects should be taken into consideration. Herein, different molecular technologies for detecting these gene rearrangements are discussed and the benefits and limitations of each method are highlighted.

Copy Number Analysis in Cancer Diagnostic Testing
Tara Spence and Adrian M. Dubuc

Accurate detection of copy number alterations (CNAs) has become increasingly important in clinical oncology for the purpose of diagnosis, prognostication, and disease management. Cytogenetic approaches for the detection of CNAs, including karyotype, fluorescence in situ hybridization (FISH), and chromosomal microarray, remain mainstays in clinical laboratories. Yet, with rapidly decreasing costs and improved accuracy of CNA detection using emerging technologies such as next-generation sequencing and optical genome mapping, we are approaching a new era of cytogenomics and molecular oncology. The aim of this review is
to describe the benefits and limitations associated with the routine clinical application of available classic, emerging, and projected future technologies for the detection of CNAs in oncology.

Molecular Biomarkers of Response to Cancer Immunotherapy 469
Lauren L. Ritterhouse and Tasos Gogakos
Harnessing the immune system to advance cancer therapy has offered a new weapon in the quiver of clinical oncology. The lack of uniform, robust, or durable responses in many patients has necessitated the development of approaches for the accurate prediction of subgroups that are most likely to benefit from immunotherapy. This has led to the development and regulatory approval of predictive biomarkers, as well as associated companion diagnostics. Despite these strides, there still exists great heterogeneity in the choice of biomarkers, the laboratory assays that generate them, and their overall clinical utility. This article surveys broadly the predictive biomarkers of response to cancer immunotherapy, focusing on the biomarkers with current Food and Drug Administration (FDA) approval, and raising awareness of issues that may affect their broad applicability.

When Tissue Is the Issue: Expanding Cell-Free DNA “Liquid Biopsies” to Supernatants and Nonplasma Biofluids 485
Vera Paulson, Eric Q. Konnick, and Christina H. Lockwood
While tissue biopsy remains the gold standard for tumor biomarker testing, assays using plasma-derived cfDNA, aka circulating-tumor DNA (ctDNA), have recently demonstrated validity in the setting of limited tissue or recurrent disease. Tumor-derived cfDNA is also present in nonplasma biofluids and supernatants procured through interventional procedures. Evaluation of cfDNA extracted from these fluids may have benefits at nearly every stage of cancer patient management, from diagnosis and prognosis to monitoring disease progression and predicting therapeutic response. This review will focus on preanalytical, analytical, and postanalytical variables that must be considered when analyzing “liquid biopsies” outside the plasma compartment.

Germline Testing for the Evaluation of Hereditary Cancer Predisposition 497
Ozge Ceyhan-Birsoy
Germline testing for hereditary cancer predisposition has become increasingly important in the management of patients with cancer. Recent studies have demonstrated that hereditary cancer predisposition is more common than previously recognized and germline pathogenic variants may be actionable for patient treatment strategies. This article reviews the significance of hereditary cancer predisposition assessment and highlights the current practices in germline genetic testing approaches.