

## **SECTION 1: INTRODUCTION**

4 Structure of Chromosomes and Nucleic Acids  
Jeremy C. Wallentine, MD  
8 DNA Replication, Transcription, and Translation  
Jeremy C. Wallentine, MD  
12 Gene Mutations  
Jeremy C. Wallentine, MD

## **SECTION 2: COMMON TECHNIQUES FOR ANALYSIS OF ALTERATIONS IN CHROMOSOMES AND DNA**

18 Cytogenetics and FISH  
Mohammad A. Vasef, MD and David R. Czuchlewski, MD  
22 Chromosomal Microarray  
Mohammad A. Vasef, MD and Sibel Kantarci, PhD  
26 Amplification Methods  
Devon Chabot-Richards, MD  
30 DNA High-Resolution Melting Curve Analysis  
Amer Mahmoud, MD, FCAP  
32 Sequencing Technologies  
Eric Y. Loo, MD and Mohammad A. Vasef, MD  
36 High-Throughput Methods in Molecular Pathology  
Rama Gullapalli, MD, PhD  
38 DNA Methylation Analysis  
Devon Chabot-Richards, MD

## **SECTION 3: MOLECULAR GENETIC TESTS IN HEMATOPOIETIC TUMORS**

42 BCR::ABL1 Fusion  
Eric Y. Loo, MD and Mohammad A. Vasef, MD  
46 PML::RARA Fusion  
Devon Chabot-Richards, MD  
48 Reverse Transcription PCR for Myeloid Leukemia Transcripts  
Kristin Hunt Karner, MD  
50 FLT3, NPM1, and CEBPA Mutations  
Devon Chabot-Richards, MD  
54 JAK2 Mutations and Rearrangements  
Mohammad A. Vasef, MD  
56 MPL Mutations  
Eric Y. Loo, MD and David R. Czuchlewski, MD  
58 Calreticulin (CALR) Mutations  
Eric Y. Loo, MD and Mohammad A. Vasef, MD  
60 CSF3R Mutations  
Amer Mahmoud, MD, FCAP  
62 SETBP1 Mutations  
Amer Mahmoud, MD, FCAP  
64 KIT Mutations  
Eric Y. Loo, MD and Mohammad A. Vasef, MD  
68 IDH1 and IDH2 Mutations  
Eric Y. Loo, MD and David R. Czuchlewski, MD  
70 Other Mutations and Gene Panel Testing in Myeloid Neoplasms  
Mohammad A. Vasef, MD and David R. Czuchlewski, MD  
72 Immunoglobulin Heavy (IGH) Chain Gene Rearrangements  
Mohammad A. Vasef, MD and Anna P. Matynia, MD  
76 T-Cell Receptor Gamma (TRG) and Delta (TRD) Chain Rearrangements  
Mohammad A. Vasef, MD and Anna P. Matynia, MD  
80 T-Cell Receptor Beta (TRB) Chain Gene Rearrangements  
Mohammad A. Vasef, MD and Anna P. Matynia, MD  
84 Southern Blot Analysis of Antigen Receptor Genes  
Mohammad A. Vasef, MD  
86 EBV-Associated Human Neoplasms  
Mohammad A. Vasef, MD  
94 MYD88 Mutation  
Giovanni Insuasti-Beltran, MD  
96 NOTCH1 Mutation  
Giovanni Insuasti-Beltran, MD  
98 EZH2 Mutation  
Amer Mahmoud, MD, FCAP

## **SECTION 4: MOLECULAR GENETIC TESTS IN SOLID TUMORS, BONE AND SOFT TISSUE TUMORS, AND CNS TUMORS**

104 ALK Rearrangements and Mutations

Guanghua Wang, MD  
106 ROS1 Rearrangements  
Mohammad A. Vasef, MD and David R. Czuchlewski, MD  
108 RET Rearrangements  
Eric Y. Loo, MD and David R. Czuchlewski, MD  
110 FISH for MET Amplifications  
Devon Chabot-Richards, MD  
114 FISH for ERBB2 (HER2) Amplifications  
M. Carmen Frias Kletecka, MD and Kristin Hunt Karner, MD  
116 KRAS Mutations  
Guanghua Wang, MD  
118 BRAF Mutations  
Yongjie Zhou, MD, PhD and Guanghua Wang, MD  
120 EGFR Mutations  
Guanghua Wang, MD  
122 Targeted Hotspot Gene Panel Using Massively  
Parallel Sequencing (Next-Generation Sequencing)  
Mohammad A. Vasef, MD and David R. Czuchlewski, MD  
124 Targeted Hotspot Gene Panel Table  
Mohammad A. Vasef, MD and David R. Czuchlewski, MD  
130 MGMT Promoter Gene Methylation Assay  
Devon Chabot-Richards, MD

## **SECTION 5: MOLECULAR PATHOLOGY OF MYELOID NEOPLASMS**

134 Molecular Work-Up of Myeloid Neoplasms  
Mohammad A. Vasef, MD and David R. Czuchlewski, MD  
MYELOPROLIFERATIVE NEOPLASMS  
136 Chronic Myeloid Leukemia, BCR::ABL1-Positive  
Mohammad A. Vasef, MD, Kaaren K. Reichard, MD, and David R. Czuchlewski, MD  
144 Chronic Neutrophilic Leukemia  
Devon Chabot-Richards, MD and David R. Czuchlewski, MD  
148 Polycythemia Vera  
Mohammad A. Vasef, MD and M. Carmen Frias Kletecka, MD  
152 Primary Myelofibrosis  
Devon Chabot-Richards, MD, Kaaren K. Reichard, MD, and Daniel Babu, MD  
156 Essential Thrombocythemia  
Giovanni Insuasti-Beltran, MD  
160 Chronic Eosinophilic Leukemia, NOS  
Devon Chabot-Richards, MD, Kaaren K. Reichard, MD, and Daniel Babu, MD  
162 Myeloproliferative Neoplasm, Unclassifiable  
Devon Chabot-Richards, MD, Kaaren K. Reichard, MD, and Daniel Babu, MD

### **MASTOCYTOSIS**

166 Mastocytosis  
Mohammad A. Vasef, MD

### **MYELOID/LYMPHOID NEOPLASMS WITH EOSINOPHILIA AND GENE REARRANGEMENT**

174 Myeloid/Lymphoid Neoplasms With Eosinophilia and Tyrosine Kinase Gene Fusions  
Devon Chabot-Richards, MD and David R. Czuchlewski, MD

### **PREMALIGNANT CLONAL HEMATOPOIESIS AND CYTOPENIAS**

178 VEXAS Syndrome  
Mohammad A. Vasef, MD and Michael M. Franklin, DO  
182 Clonal Hematopoiesis and Premalignant Clonal Cytopenia  
Mohammad A. Vasef, MD

### **MYELODYSPLASTIC SYNDROME (MDS)**

184 Overview and Classification of Myelodysplastic Syndrome  
Devon Chabot-Richards, MD, David R. Czuchlewski, MD, and Carla S. Wilson, MD, PhD  
188 Myelodysplastic Syndrome With Mutated SF3B1  
Mohammad A. Vasef, MD and Carla S. Wilson, MD, PhD  
192 Myelodysplastic Syndrome, NOS  
Kristin Hunt Karner, MD  
200 Myelodysplastic Syndrome With del(5q)  
Giovanni Insuasti-Beltran, MD  
204 Myelodysplastic Syndrome With TP53 Multihit Mutations  
Kristin Hunt Karner, MD  
206 Myelodysplastic Syndrome/Acute Myeloid Leukemia  
Kristin Hunt Karner, MD  
208 Pediatric Myelodysplastic Syndrome and Refractory Cytopenia of Childhood

Devon Chabot-Richards, MD and Carla S. Wilson, MD, PhD

#### MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

214 Overview of Myelodysplastic/Myeloproliferative Neoplasms

Devon Chabot-Richards, MD

216 Chronic Myelomonocytic Leukemia

Kristin Hunt Karner, MD

220 Atypical Chronic Myeloid Leukemia

Devon Chabot-Richards, MD

224 Juvenile Myelomonocytic Leukemia

Devon Chabot-Richards, MD and Qian-Yun Zhang, MD, PhD

228 Myelodysplastic/Myeloproliferative Neoplasm, NOS

Devon Chabot-Richards, MD and Kathryn Foucar, MD

#### ACUTE MYELOID LEUKEMIA

232 Overview of Acute Myeloid Leukemia

Mohammad A. Vasef, MD and Kaaren K. Reichard, MD

240 Acute Myeloid Leukemia With t(8;21)/RUNX1::RUNX1T1

Devon Chabot-Richards, MD, Kaaren K. Reichard, MD, and David R. Czuchlewski, MD

244 Acute Myeloid Leukemia With inv(16) or t(16;16)/CBFB::MYH11

Devon Chabot-Richards, MD, Kaaren K. Reichard, MD, and David R. Czuchlewski, MD

248 Acute Promyelocytic Leukemia With t(15;17)/PML::RARA

Mohammad A. Vasef, MD

254 Acute Myeloid Leukemia With t(9;11)/MLL3::KMT2A

Devon Chabot-Richards, MD, Kaaren K. Reichard, MD, and David R. Czuchlewski, MD

258 Acute Myeloid Leukemia With t(6;9)/DEK::NUP214

Eric Y. Loo, MD and David R. Czuchlewski, MD

260 Acute Myeloid Leukemia With inv(3) or t(3;3)/GATA2; MECOM

Eric Y. Loo, MD and David R. Czuchlewski, MD

262 Acute Myeloid Leukemia (Megakaryoblastic) With t(1;22)/RBM15::MRTFA

Eric Y. Loo, MD and David R. Czuchlewski, MD

264 Acute Myeloid Leukemia With Myelodysplasia-Related Gene Mutations or Cytogenetic Abnormalities

Kathryn Foucar, MD and Mohammad A. Vasef, MD

270 Acute Myeloid Leukemia, NOS

Kristin Hunt Karner, MD

276 Myeloid Sarcoma

Devon Chabot-Richards, MD

280 Myeloid Proliferations Associated With Down Syndrome

Eric Y. Loo, MD and David R. Czuchlewski, MD

#### BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM

284 Blastic Plasmacytoid Dendritic Cell Neoplasm

Mohammad A. Vasef, MD

### **SECTION 6: MOLECULAR PATHOLOGY OF LYMPHOID NEOPLASMS**

292 Overview of Lymphoid Neoplasms

Nadine Aguilera, MD

296 Molecular Work-Up of Lymphoid Neoplasms

Mohammad A. Vasef, MD and David R. Czuchlewski, MD

#### DETERMINATION OF CLONALITY IN B-, T-, AND NK-CELL NEOPLASMS

298 Lymphoma-Associated Chromosomal Translocations

Kristin Hunt Karner, MD

#### PRECURSOR LYMPHOID NEOPLASMS

302 B-Lymphoblastic Leukemia/Lymphoma With Recurrent Genetic Abnormalities

Kristin Hunt Karner, MD

306 B-Lymphoblastic Leukemia/Lymphoma, BCR::ABL1-Like (Ph-Like ALL)

Joanna L. Conant, MD and Mohammad A. Vasef, MD

310 T-Lymphoblastic Leukemia/Lymphoma

Mohammad A. Vasef, MD

#### MATURE B-CELL NEOPLASMS

316 Small Lymphocytic Lymphoma/Chronic Lymphocytic Leukemia

Devon Chabot-Richards, MD

322 B-Cell Prolymphocytic Leukemia

Aaron Auerbach, MD, MPH

326 Splenic Marginal Zone Lymphoma

Aaron Auerbach, MD, MPH and Roberto N. Miranda, MD

332 Hairy Cell Leukemia

Mohammad A. Vasef, MD  
336 Splenic B-Cell Lymphoma/Leukemia, Unclassifiable  
Nadine Aguilera, MD  
344 Lymphoplasmacytic Lymphoma  
Aaron Auerbach, MD, MPH  
350 Monoclonal Gammopathy of Undetermined Significance  
Nadine Aguilera, MD  
354 Multiple Myeloma (Plasma Cell Myeloma)  
Nadine Aguilera, MD and Carla S. Wilson, MD, PhD  
362 Follicular Lymphoma  
Aaron Auerbach, MD, MPH, C. Cameron Yin, MD, PhD, and John Schmiege, MD, PhD  
370 Mantle Cell Lymphoma  
Aaron Auerbach, MD, MPH  
376 Diffuse Large B-Cell Lymphoma  
Nadine Aguilera, MD  
386 Burkitt Lymphoma  
Aaron Auerbach, MD, MPH and Carlos Bueso-Ramos, MD, PhD

#### MATURE T- AND NK-CELL NEOPLASMS

394 T-Cell Prolymphocytic Leukemia  
Nadine Aguilera, MD  
398 Chronic Lymphoproliferative Disorder of NK Cells  
Nadine Aguilera, MD  
402 Aggressive NK-Cell Leukemia  
Nadine Aguilera, MD  
406 Extranodal NK-/T-Cell Lymphoma  
Aaron Auerbach, MD, MPH and L. Jeffrey Medeiros, MD  
410 Adult T-Cell Leukemia/Lymphoma  
Nadine Aguilera, MD  
416 Intestinal T-Cell Lymphoma  
Aaron Auerbach, MD, MPH and Sa A. Wang, MD  
424 Hepatosplenic T-Cell Lymphoma  
Aaron Auerbach, MD, MPH and Roberto N. Miranda, MD  
428 Subcutaneous Panniculitis-Like T-Cell Lymphoma  
Aaron Auerbach, MD, MPH  
432 Mycosis Fungoides/Sézary Syndrome  
Aaron Auerbach, MD, MPH and Sa A. Wang, MD  
438 Primary Cutaneous CD30-Positive T-Cell  
Lymphoproliferative Disorders  
Aaron Auerbach, MD, MPH  
442 Peripheral T-Cell Lymphoma, NOS  
Aaron Auerbach, MD, MPH  
448 Nodal Follicular Helper T-Cell Lymphoma  
Aaron Auerbach, MD, MPH  
456 Anaplastic Large Cell Lymphoma, ALK-Negative  
Mohammad A. Vasef, MD  
462 Anaplastic Large Cell Lymphoma, ALK-Positive  
Mohammad A. Vasef, MD

#### **SECTION 7: MOLECULAR PATHOLOGY OF DENDRITIC CELL AND HISTIOCYTIC NEOPLASMS**

470 Histiocytic Sarcoma  
Nadine Aguilera, MD  
478 Langerhans Cell Histiocytosis  
Guanghua Wang, MD  
484 Interdigitating Dendritic Cell Sarcoma  
Aaron Auerbach, MD, MPH and Sa A. Wang, MD  
488 Follicular Dendritic Cell Sarcoma  
Aaron Auerbach, MD, MPH

#### **SECTION 8: MOLECULAR PATHOLOGY OF SOLID TUMORS**

##### HEAD AND NECK, INCLUDING SINONASAL TUMORS AND SALIVARY GLANDS

494 HPV-Associated Head and Neck Carcinomas  
Thérèse Bocklage, MD  
500 Head and Neck Mucosal Squamous Cell Carcinoma, Non-HPV Related  
Thérèse Bocklage, MD  
506 Important Alterations in Non-HPV-Related Head and Neck Squamous Cell Carcinoma  
Thérèse Bocklage, MD  
508 Translocation-Specific Salivary Gland Tumors  
Thérèse Bocklage, MD

518 Translocation-Specific Salivary Gland Tumors (Continued)

Th  rese Bocklage, MD

526 Nasopharyngeal EBV-Related Squamous Cell Carcinoma

Th  rese Bocklage, MD

530 Other Head and Neck Tumors

Th  rese Bocklage, MD

THYROID

538 Papillary Thyroid Carcinoma

Giovanni Insuasti-Beltran, MD

542 Follicular Thyroid Carcinoma

Giovanni Insuasti-Beltran, MD

546 Poorly Differentiated Thyroid Carcinoma

Von Samedy, MD, PhD

550 Anaplastic Thyroid Carcinoma

Von Samedy, MD, PhD

554 Medullary Thyroid Carcinoma

Giovanni Insuasti-Beltran, MD

LUNG

560 Adenocarcinoma, Lung

Jeremy C. Wallentine, MD

568 Squamous Cell Carcinoma, Lung

Jeremy C. Wallentine, MD

572 Small Cell Neuroendocrine Carcinoma

M. Carmen Frias Kletecka, MD and Samuel Reynolds, MD

576 Mesothelioma

Mohammad A. Vasef, MD and Khalil Sheibani, MD, FCAP

LUMINAL GASTROINTESTINAL TRACT

580 Gastric Adenocarcinoma

M. Carmen Frias Kletecka, MD and Joshua Anspach Hanson, MD

584 Colorectal Adenocarcinoma and Precancerous Lesions

M. Carmen Frias Kletecka, MD and Joshua Anspach Hanson, MD

590 Gastrointestinal Stromal Tumor

M. Carmen Frias Kletecka, MD and Joshua Anspach Hanson, MD

LIVER

598 HNF1A-Inactivated Hepatocellular Adenoma

Giovanni Insuasti-Beltran, MD and Joshua Anspach Hanson, MD

602  $\beta$ -Catenin-Activated Hepatocellular Adenoma

Giovanni Insuasti-Beltran, MD and Joshua Anspach Hanson, MD

606 Inflammatory Hepatocellular Adenoma

Giovanni Insuasti-Beltran, MD and Joshua Anspach Hanson, MD

610 Hepatocellular Carcinoma

Rama Gullapalli, MD, PhD

PANCREATICOBILIARY

614 Pancreatic Ductal Adenocarcinoma

Von Samedy, MD, PhD

618 Pancreatic Mucinous Cystic Neoplasm

Von Samedy, MD, PhD

620 Pancreatic Intraductal Papillary Mucinous Neoplasm

Von Samedy, MD, PhD

622 Cholangiocarcinoma

Rama Gullapalli, MD, PhD

TESTIS

624 Testicular Germ Cell Tumors

Edgar Fischer, MD, PhD

KIDNEY

630 Clear Cell Renal Cell Carcinoma

Edgar Fischer, MD, PhD

634 Chromophobe Renal Cell Carcinoma

Edgar Fischer, MD, PhD

636 Papillary Renal Cell Carcinoma

Edgar Fischer, MD, PhD

638 TFE3-Rearranged and TFEB-Altered Renal Cell Carcinomas

Edgar Fischer, MD, PhD

642 Wilms Tumor

Marc Barry, MD  
648 Clear Cell Sarcoma of Kidney  
Marc Barry, MD  
650 Oncocytoma  
Marc Barry, MD  
652 Rhabdoid Tumor of Kidney  
Marc Barry, MD

#### BLADDER

654 Urothelial Carcinoma  
Marc Barry, MD

#### ADRENAL

660 Pheochromocytoma/Paraganglioma  
M. Carmen Frias Kletecka, MD and Samuel Reynolds, MD  
666 Adrenal Cortical Carcinoma  
M. Carmen Frias Kletecka, MD and Samuel Reynolds, MD  
672 Neuroblastoma  
Giovanni Insuasti-Beltran, MD, Thèrèse Bocklage, MD, and Samuel Reynolds, MD  
680 Adrenal Cortical Adenoma  
M. Carmen Frias Kletecka, MD and Samuel Reynolds, MD

#### PROSTATE

684 Prostatic Adenocarcinoma, Acinar Type and High-Grade Prostatic Intraepithelial Neoplasia  
Edgar Fischer, MD, PhD

#### BREAST

688 ADH and DCIS (Dysplastic, Premalignant)  
David G. Hicks, MD  
694 Ductal Carcinomas  
David G. Hicks, MD  
700 Lobular Carcinoma  
David G. Hicks, MD  
706 Invasive Ductal Carcinoma of No Special Type With Medullary Features  
Rania Bakkar, MD  
710 Metaplastic Breast Carcinoma  
Rania Bakkar, MD  
716 Phyllodes Tumors  
Rania Bakkar, MD  
720 Basal-Like and Triple-Negative Breast Carcinomas  
David G. Hicks, MD and Susan C. Lester, MD, PhD

#### CERVIX/VULVA/VAGINA

730 Preneoplastic Conditions, Cervix/Vulva/Vagina  
Nancy Joste, MD  
734 Squamous Cell Carcinoma, Cervix/Vulva/Vagina  
Nancy Joste, MD  
738 Adenocarcinoma, Cervix/Vulva/Vagina  
Nancy Joste, MD

#### UTERUS

742 Endometrial Intraepithelial Neoplasia  
Lesley Lomo, MD  
744 Uterine Endometrioid Carcinoma  
Lesley Lomo, MD  
748 Uterine Serous Carcinoma  
Lesley Lomo, MD  
750 Clear Cell Carcinoma, Uterus  
Lesley Lomo, MD  
754 Uterine Sarcomas  
Thèrèse Bocklage, MD

#### OVARIES/FALLOPIAN TUBE

762 Serous Tumors of Ovary and Fallopian Tube  
Rania Bakkar, MD  
768 Other Surface Epithelial Tumors of Ovary  
Rania Bakkar, MD  
774 Sex Cord-Stromal Tumors of Ovary  
Rania Bakkar, MD  
780 Germ Cell Tumor, Ovary  
Rania Bakkar, MD and Aaron Auerbach, MD, MPH

## SKIN

784 Premalignant Conditions, Skin  
David Cassarino, MD, PhD  
788 Melanoma  
David Cassarino, MD, PhD  
792 Squamous Cell Carcinoma, Skin  
David Cassarino, MD, PhD  
796 Basal Cell Carcinoma, Skin  
David Cassarino, MD, PhD  
800 Sebaceous Tumors  
David Cassarino, MD, PhD  
802 Dermatofibroma and Dermatofibrosarcoma Protuberans  
David Cassarino, MD, PhD

## BONE

804 Overview of Molecular Pathology of Bone and Soft Tissue Tumors  
Thérèse Bocklage, MD  
810 Osteosarcomas  
Thérèse Bocklage, MD  
818 Ewing Sarcoma  
Thérèse Bocklage, MD  
824 Other Small Round Blue Cell Sarcomas  
Thérèse Bocklage, MD  
832 Giant Cell Tumor of Bone  
Thérèse Bocklage, MD  
838 Intermediate and Malignant Cartilaginous Tumors of Bone  
Thérèse Bocklage, MD

## SOFT TISSUE

848 Intermediate and Malignant Myofibroblastic/Fibroblastic Tumors  
Thérèse Bocklage, MD  
858 Liposarcomas  
Thérèse Bocklage, MD  
868 Muscle Sarcomas  
Thérèse Bocklage, MD  
878 Intermediate and Malignant Vascular Tumors  
Thérèse Bocklage, MD  
886 Malignant Peripheral Nerve Sheath Tumor  
Thérèse Bocklage, MD and David R. Lucas, MD  
892 Representative Genetic Findings in Bone and Soft Tissue Tumors  
Thérèse Bocklage, MD

## SARCOMAS OF UNCERTAIN DIFFERENTIATION

898 Rare Sarcomas of Uncertain Differentiation With Specific Molecular Alterations  
Thérèse Bocklage, MD  
908 Rare Sarcomas of Uncertain Differentiation With Specific Molecular Alterations (Continued)  
Thérèse Bocklage, MD

## CNS

916 Glioblastoma, IDH Wildtype  
Elisabeth Rushing, MD and Mohammad A. Vasef, MD  
920 Astrocytoma, IDH-Mutant  
Elisabeth Rushing, MD and Mohammad A. Vasef, MD  
924 Pilocytic Astrocytoma  
Aaron Auerbach, MD, MPH, Karen SantaCruz, MD, and Elisabeth Rushing, MD  
928 Oligodendroglioma, IDH Mutant, and 1p/19q Codeleted  
Aaron Auerbach, MD, MPH, Karen SantaCruz, MD, and Elisabeth Rushing, MD  
932 Ependymal Tumors  
Mohammad A. Vasef, MD and Fausto J. Rodríguez, MD  
938 Medulloblastoma  
Mohammad A. Vasef, MD and Fausto J. Rodríguez, MD  
944 Choroid Plexus Tumors  
Mohammad A. Vasef, MD and Fausto J. Rodríguez, MD  
948 Meningioma  
Mohammad A. Vasef, MD and Karen SantaCruz, MD  
952 Retinoblastoma  
Mohammad A. Vasef, MD and Fausto J. Rodríguez, MD

## **SECTION 9: QUALITY ASSURANCE AND REGULATORY ISSUES**

958 Federal Agencies and Regulation of Laboratories  
Amer Mahmoud, MD, FCAP  
960 FDA Regulations  
Yongjie Zhou, MD, PhD  
966 Proficiency Testing and Accreditation  
Amer Mahmoud, MD, FCAP