

Gregory A. Hosler · Kathleen M. Murphy

# Molecular Diagnostics for Dermatology

Practical Applications of  
Molecular Testing for the  
Diagnosis and Management of  
the Dermatology Patient

 Springer

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Practical Applications of Molecular  
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## Abbreviations

A	Adenine
ACGH	Array-based comparative genomic hybridization
AD	Autosomal dominant
ADCC	Antibody-dependent cell cytotoxicity
ADE	Adverse drug event
AFB	Acid-fast bacilli
AFH	Angiomatoid fibrous histiocytoma
AIN	Anal intraepithelial neoplasia
AJCC	American Joint Committee on Cancer
AKT1	v-akt murine thymoma viral oncogene homologue 1
ALCL	Anaplastic large cell lymphoma
ALL	Acute lymphoblastic leukemia
AMA	American Medical Association
AML	Acute myeloid leukemia
AMP	Association for Molecular Pathology
APL	Acute promyelocytic leukemia
AR	Autosomal recessive
ARMS	Amplification refractory mutation system
ATRA	All-trans retinoic acid
AVL	Atypical vascular lesion
BA	Bacillary angiomatosis
BAC	Bacterial artificial chromosomes
BAP1	BRCA1-associated protein 1
BCL-2	B-cell lymphoma 2
BCL-6	B-cell lymphoma 6
bDNA	Branched deoxyribonucleic acid amplification
BP	Base pair
BRAF	v-raf murine sarcoma viral oncogene homologue B1
BRIM	BRAF-in-melanoma
C	Cytosine <i>or</i> constant (domain)
CADMA	Competitive amplification of differentially melting amplicons
CAMTA1	Calmodulin-binding transcription activator 1
CAP	College of American Pathologists
CCS	Clear cell sarcoma
CD	Cluster of differentiation
CDC	Centers for Disease Control and Prevention <i>or</i> complement-dependent cytotoxicity



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CDK4	Cyclin-dependent kinase 4
CDKN2A	Cyclin-dependent kinase N2A
CE	Capillary electrophoresis
CEA	Carcinoembryonic antigen
CF	Cystic fibrosis
CGH	Comparative genomic hybridization
CISH	Chromogenic in situ hybridization
CLIA	Clinical Lab Improvement Act
CLL	Chronic lymphocytic leukemia
CML	Chronic myelogenous leukemia
CMML	Chronic myelomonocytic leukemia
COSMIC	Catalogue of Somatic Mutations in Cancer
CPE	Cytopathic effect
CPT	Current procedural terminology
CR	Conserved region (domain)
CREB	cAMP response element binding protein
CSD	Cat scratch disease
CSF	Cerebrospinal fluid
CTCL	Cutaneous T-cell lymphoma
CTLA-4	Cytotoxic T-lymphocyte antigen 4
CVS	Chorionic villus sampling
CYP	Cytochrome p450
D	Diversity (as in V-D-J)
DAPI	4',6-Diamidino-2-phenylindole
ddNTP	dideoxynucleotide triphosphate
DFA	Direct fluorescent antibody
DFSP	Dermatofibrosarcoma protuberans
DGGE	Denaturing gradient gel electrophoresis
DIHS	Drug-induced hypersensitivity syndrome
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DOE	Department of Energy
DRESS	Drug rash with eosinophilia and systemic symptoms
DTIC	Dacarbazine
EBV	Epstein-Barr virus
EDV	Epidermodysplasia verruciformis
EGFR	Epidermal growth factor receptor
EHE	Epithelioid hemangioendothelioma
EHK	Epidermolytic hyperkeratosis
EORTC	European Organization for Research and Treatment of Cancer
EPCAM	Epithelial cell adhesion molecule
ERK	(aka MAPK) mitogen-activated protein kinase
ETS	E-twenty-six (gene family)
EWS	Ewing sarcoma
FAMM	Familial atypical mole melanoma (syndrome)
FDA	United States Food and Drug Administration
FET	Fus-Ewsr1-Taf15 (gene family)
FFPE	Formalin fixed and paraffin embedded

FISH	Fluorescence in situ hybridization
FR	Framework region (domain)
FRET	Fluorescence resonance energy transfer
G	Guanine
GCF	Giant cell fibroblastoma
GIST	Gastrointestinal stromal tumor
GMS	Gömöri methenamine silver
GNA11	Guanine nucleotide-binding protein subunit $\alpha$ -11
GNAQ	Guanine nucleotide-binding protein G(q) subunit $\alpha$
GWAS	Genome-wide association studies
H&E	Hematoxylin and eosin
HCCC	Hyalinizing clear cell carcinoma
HCV	Hepatitis C virus
HHV-8	Human herpesvirus 8
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HNPPC	Hereditary nonpolyposis colon cancer
HPV	Human papillomavirus
HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homologue
HRSA	Health Resources and Services Administration (US Department of Health)
HSP	Heat shock protein
HSV	Herpes simplex virus
HTLV-1	Human T-cell leukemia virus type 1
ICD	International Statistical Classification of Diseases and Related Health Problems (codes)
Ig	Immunoglobulin
IGH	Immunoglobulin heavy chain
IGK	Immunoglobulin light chain kappa
IGL	Immunoglobulin light chain lambda
IHC	Immunohistochemistry
ISCL	International Society for Cutaneous Lymphoma
ISCN	International System for Human Cytogenetic Nomenclature
ISH	In situ hybridization
IVD	In vitro diagnostic
J	Joining (as in V-D-J)
JBAIDS	Joint Biological Agent Identification and Diagnostic System (anthrax detection)
JM	Juxtamembrane (domain)
JMML	Juvenile myelomonocytic leukemia
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue
KOH	Potassium hydroxide
KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue
KS	Kaposi sarcoma
KSHV	Kaposi sarcoma herpesvirus
LANA-1	Latency-associated nuclear antigen 1
LCA	Leukocyte common antigen

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LCH	Langerhans cell histiocytosis
LCR	Ligase chain reaction
LDT	Lab-developed test
LGFMS	Low-grade fibromyxoid sarcoma
LYP	Lymphomatoid papulosis
MALT	Mucosa-associated lymphoid tissue (lymphoma)
MAP	MUTYH-associated polyposis
MAPK	Mitogen-activated protein kinase (pathway)
MART-1	Melanoma antigen recognized by T cells 1
MC1R	Melanocortin-1 receptor
MCC	Merkel cell carcinoma
MCV	Merkel cell polyomavirus (or MCPyV)
MDM2	Mouse double minute 2 (gene/protein)
MEK	(aka MAP2K) mitogen-activated protein kinase kinase
MET	(aka HGFR) hepatocyte growth factor receptor
MF	Mycosis fungoides
MFH	Malignant fibrous histiocytoma
MGMT	O(6)-methylguanine DNA methyltransferase
miRNA	microribonucleic acid
MiTF	Microphthalmia transcription factor
MLH1	Human homologue of <i>E. coli</i> MutL 1
MLPA	Multiplex ligation-dependent probe amplification
MMR	Mismatch repair
MOTT	Mycobacteria other than tuberculosis
mRNA	messenger ribonucleic acid
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSH	Melanocyte-stimulating hormone
MSH2	Human homologue of <i>E. coli</i> MutS 2
MSH6	Human homologue of <i>E. coli</i> MutS 6
MSI	Microsatellite instability
MSMD	Mendelian susceptibility to mycobacterial diseases
MSS	Microsatellite stable
mtDNA	Mitochondrial deoxyribonucleic acid
MTOR	Mechanistic target of rapamycin (gene/protein)
MTS	Muir-Torre syndrome
MUTYH	mutY homologue (gene/protein)
N	Nucleotide
NCI	National Cancer Institute
NER	Nucleotide-excision repair
NGS	Next-generation sequencing
NIH	National Institutes of Health
NK	Natural killer (cells)
NPV	Negative predictive value
NRAS	Neuroblastoma rat sarcoma viral oncogene homologue
NSCLC	Non-small cell lung cancer
NSE	Neuron-specific enolase
NTM	Nontuberculous mycobacteria
OMIM	Online Mendelian Inheritance in Man

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PAS	Periodic acid-Schiff
PBP	Penicillin binding protein
PCFCL	Primary cutaneous follicle center cell lymphoma
PCMZL	Primary cutaneous marginal zone B-cell lymphoma
PCR	Polymerase chain reaction
PD-1	Programmed cell death 1
PEL	Primary effusion lymphoma
PET-FISH	Paraffin-embedded tissue fluorescence in situ hybridization
PGDFR	Platelet-derived growth factor receptor
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase
PLC	Pityriasis lichenoides chronica
PLEVA	Pityriasis lichenoides et varioliformis acuta
PMS-2	Postmeiotic segregation increased, <i>S. cerevisiae</i> , 2 (gene/protein)
PNET	Primitive neuroectodermal tumor
PPK	Palmoplantar keratoderma
PPV	Positive predictive value
PTEN	Phosphatase and tensin homologue
RAF	Rapidly accelerated fibrosarcoma (gene family)
RAPID	Ruggedized advanced pathogen identification device
RAS	Rat sarcoma (gene family)
RB	Retinoblastoma (gene/protein)
RFLP	Restriction fragment length polymorphism
RMSF	Rocky Mountain spotted fever
RNA	Ribonucleic acid
ROC	Receiver operating characteristic (curve)
ROS	Reactive oxygen species
RR	Relative risk
RSS	Recombination signal sequences
RSV	Respiratory syncytial virus
RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcription polymerase chain reaction
SALT	Skin-associated lymphoid tissue (lymphoma)
SCC	Squamous cell carcinoma
SCC <sub>mec</sub>	Staphylococcal cassette chromosome
SCF	Stem cell factor
SCLC	Small cell lung carcinoma
SCPLTCL	Subcutaneous panniculitis-like T-cell lymphoma
SDA	Strand displacement amplification
siRNA	Small interfering ribonucleic acids
SJS	Stevens-Johnson syndrome
SLL	Small lymphocytic lymphoma
SMRT	Single molecule real time
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SPA	Staphylococcal protein A
SS	Sézary syndrome
SSCP	Single-strand conformation polymorphism
T	Thymine

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TB	Tuberculosis
TCR	T-cell receptor
TEN	Toxic epidermal necrolysis
TERT	Telomerase reverse transcriptase
TM	Transmembrane (domain)
TMA	Transcription-mediated amplification
TMZ	Temozolomide
TNF	Tumor necrosis factor
TNM	Tumor-node-metastasis (staging)
TNMB	Tumor-node-metastasis-blood (staging)
tRNA	Transfer ribonucleic acid
TTF-1	Thyroid transcription factor 1
Tyrp-1	Tyrosinase-related protein 1
U	Uracil
V	Variable (as in V-D-J)
VEGFR	Vascular endothelial growth factor receptor
VIN	Vulvar intraepithelial neoplasia
VZV	Varicella zoster virus
WGS	Whole-genome sequencing
WHO	World Health Organization
XLD	X-linked dominant
XLR	X-linked recessive
XP	Xeroderma pigmentosum
YAC	Yeast artificial chromosomes

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# Contents

<b>1</b>	<b>Introduction</b> . . . . .	1
	Reference . . . . .	4
<b>2</b>	<b>Basics of Nucleic Acids and Molecular Biology</b> . . . . .	5
2.1	Introduction . . . . .	6
2.2	DNA (Deoxyribonucleic Acid) . . . . .	7
2.2.1	Structure . . . . .	7
2.2.2	Genes . . . . .	8
2.2.3	Replication . . . . .	8
2.2.4	The Human Genome . . . . .	9
2.3	The Human Genome Project . . . . .	11
2.4	RNA (Ribonucleic Acid) . . . . .	12
2.4.1	Structure . . . . .	12
2.4.2	Function . . . . .	13
2.5	Transcription and Translation . . . . .	13
2.5.1	Gene Expression . . . . .	15
2.5.2	Reverse Transcription . . . . .	15
2.6	Nucleic Acid Alterations . . . . .	16
2.6.1	Types of DNA Alterations . . . . .	16
2.6.2	Causes of DNA Alterations . . . . .	18
2.6.3	Repair of DNA Alterations . . . . .	18
2.7	Nucleic Alterations and Disease . . . . .	20
2.7.1	Germline Alterations . . . . .	20
2.7.2	Benign Genetic Variants . . . . .	20
2.7.3	Somatic Alterations and Neoplasia . . . . .	23
2.8	Genomes of Infectious Agents . . . . .	25
2.9	Summary . . . . .	25
	References . . . . .	26
<b>3</b>	<b>Molecular Methods</b> . . . . .	27
3.1	Introduction . . . . .	28
3.2	General Considerations for Assay Design and Implementation . . . . .	30
3.2.1	Types of Genetic Alterations and Performance Requirements . . . . .	30
3.2.2	Specimen Type and Composition . . . . .	30
3.2.3	Lab-Developed Tests (LDT) Versus FDA-Approved In Vitro Diagnostic (IVD) Tests . . . . .	33

3.3	The Basics of a Molecular Test . . . . .	34
3.3.1	Hybridization: Virtually All Molecular Tests Are Based on the Principle of Hybridization. . . . .	34
3.3.2	Enzymes. . . . .	35
3.4	Non-amplification Nucleic Acid Analysis Methods . . . . .	36
3.4.1	Karyotyping (Cytogenetic Analysis) . . . . .	36
3.4.2	In Situ Hybridization (ISH): Chromogenic In Situ Hybridization (CISH) and Fluorescent In Situ Hybridization (FISH) . . . . .	38
3.4.3	Southern Blot . . . . .	40
3.4.4	Microarrays and Comparative Genomic Hybridization (CGH) . . . . .	41
3.5	Amplification Methods . . . . .	43
3.5.1	Polymerase Chain Reaction (PCR) . . . . .	44
3.5.2	Microsatellite Instability Analysis (MSI) . . . . .	47
3.5.3	T-Cell and B-Cell Gene Rearrangement Analysis . . . . .	47
3.5.4	Real-Time PCR . . . . .	49
3.5.5	Other Amplification Methods . . . . .	53
3.6	Sequencing . . . . .	53
3.6.1	Sanger Sequencing . . . . .	53
3.6.2	Pyrosequencing . . . . .	54
3.6.3	Next-Generation Sequencing . . . . .	55
3.7	Practical Considerations . . . . .	58
3.7.1	What to Look for in a Laboratory and/or Test Result . . . . .	58
3.7.2	Costs and Reimbursement . . . . .	59
3.8	Summary and Looking Ahead . . . . .	60
	References . . . . .	60
<b>4</b>	<b>Melanoma. Part I. Risk Assessment, Diagnosis, and Prognosis: Using Molecular Tools to Diagnose Melanoma, Predict Its Behavior, and Evaluate for Inheritable Forms . . . . .</b>	<b>63</b>
4.1	Introduction . . . . .	64
4.2	The Genetics of Melanoma: Assessing Risk . . . . .	66
4.2.1	Loci Associated with Melanoma Risk . . . . .	67
4.2.2	Testing for Germline Mutations . . . . .	70
4.3	Diagnosis . . . . .	71
4.3.1	Comparative Genomic Hybridization (CGH) . . . . .	75
4.3.2	Fluorescence In Situ Hybridization (FISH) . . . . .	77
4.3.3	Mutational Analysis . . . . .	81
4.3.4	Gene Expression Profiling . . . . .	82
4.4	Prognosis . . . . .	82
4.4.1	Molecular Evaluation of the Sentinel Lymph Node . . . . .	84
4.4.2	Chromosomal Aberrations by FISH . . . . .	84
4.4.3	Ocular Melanoma . . . . .	85
4.4.4	Other Molecular Prognostic Biomarkers . . . . .	85
4.5	Practical Considerations for Ordering and Performing Molecular Tests . . . . .	85

4.5.1	Genetic Testing for Familial Melanoma . . . . .	85
4.5.2	CGH Versus FISH . . . . .	86
4.5.3	Mutational Analysis of Melanoma Signaling Molecules and Gene Expression Profiling . . . . .	89
4.5.4	Prognostic Testing . . . . .	89
4.6	Classification of Melanoma: Current and Near-Future Perspectives . . . . .	90
	References. . . . .	92
<b>5</b>	<b>Melanoma. Part II. Personalized Medicine: Using Molecular Tools to Guide Targeted Therapy . . . . .</b>	<b>97</b>
5.1	Introduction . . . . .	98
5.2	Melanoma Tumor Progression. . . . .	99
5.3	Melanoma Signaling Pathways and the Biology of Melanoma . . . . .	100
5.3.1	MAP Kinase Pathway . . . . .	102
5.3.2	KIT. . . . .	104
5.3.3	PI3K/AKT/mTOR Pathway. . . . .	105
5.3.4	Others. . . . .	106
5.4	Clinical Trials and Therapeutic Strategies. . . . .	106
5.4.1	Signaling Molecule and Pathway Inhibition . . . . .	107
5.4.2	Immunotherapy . . . . .	113
5.4.3	Resistance to Therapy and Clinical Relapse . . . . .	114
5.4.4	Combination Therapy and Emerging Therapeutic Strategies . . . . .	115
5.5	Practical Considerations for Ordering and Performing Molecular Tests . . . . .	117
5.5.1	Targeted Mutation-Specific Molecular Assays. . . . .	117
5.5.2	Immunohistochemistry . . . . .	124
5.5.3	Companion Testing: The New Reality? . . . . .	125
5.6	Summary . . . . .	126
	References. . . . .	127
<b>6</b>	<b>Leukemia and Lymphoma. Part I. Mycosis Fungoides and Sézary Syndrome: Using Molecular Tools to Aid in the Diagnosis, Staging, and Therapy for Mycosis Fungoides and Sézary Syndrome . . . . .</b>	<b>133</b>
6.1	Introduction . . . . .	134
6.2	Diagnosis . . . . .	135
6.2.1	Clinical Features . . . . .	135
6.2.2	Histology . . . . .	135
6.2.3	Immunohistochemistry . . . . .	135
6.2.4	The Need for Molecular Testing . . . . .	138
6.2.5	Molecular Studies . . . . .	138
6.2.6	Diagnostic Algorithms for MF/ SS . . . . .	147
6.3	Staging and Prognosis . . . . .	148
6.3.1	Assessing Prognosis by PCR. . . . .	148
6.3.2	Assessing Prognosis by FISH and aCGH . . . . .	151
6.4	Therapy . . . . .	151



6.5	Practical Considerations for Ordering, Performing, and Interpreting Molecular Tests . . . . .	151
6.5.1	Assay Selection and Design. . . . .	152
6.5.2	Interpretation of the PCR TCR Gene Rearrangement Assay . . . . .	156
6.6	Summary . . . . .	161
	References. . . . .	162
<b>7</b>	<b>Leukemia and Lymphoma. Part II: Primary Cutaneous B-Cell Lymphoma and Other Non-MF/SS Hematopoietic Tumors . . . . .</b>	<b>167</b>
7.1	Introduction . . . . .	168
7.2	Determination of Clonality in B-Cell Infiltrates . . . . .	169
7.2.1	Immunohistochemistry and Flow Cytometry . . . . .	169
7.2.2	Molecular Studies . . . . .	169
7.3	Diagnostic Applications for Molecular Testing. . . . .	173
7.3.1	Primary Cutaneous B-Cell Lymphomas . . . . .	174
7.3.2	Non-MF/SS Primary Cutaneous T-Cell Lymphomas . . . . .	179
7.3.3	B-Cell Versus T-Cell Lymphoma. . . . .	181
7.3.4	Other Hematopoietic Tumors Primarily and Secondarily Involving the Skin. . . . .	181
7.4	Other Applications for Molecular Testing. . . . .	184
7.4.1	Prognosis . . . . .	184
7.4.2	Therapy . . . . .	186
7.5	Practical Considerations for Ordering, Performing, and Interpreting Molecular Tests . . . . .	186
7.5.1	Gene Rearrangement Assays. . . . .	186
7.5.2	Other Molecular Methods for the Diagnosis and Management of the Cutaneous Leukemia/Lymphoma Patient . . . . .	193
7.6	Summary . . . . .	195
	References. . . . .	195
<b>8</b>	<b>Tumors of the Soft Tissue: Using Molecular Tools to Aid in the Diagnosis of Soft Tissue Tumors and the Management of the Sarcoma Patient . . . . .</b>	<b>199</b>
8.1	Introduction . . . . .	200
8.2	Diagnosis . . . . .	200
8.2.1	Genetic Aberrations in Soft Tissue Pathology. . . . .	202
8.2.2	Examples of Soft Tissue Tumors with Characteristic Molecular Defects . . . . .	204
8.3	Prognosis . . . . .	215
8.3.1	Translocations and Fusion Genes . . . . .	216
8.3.2	Gene Amplification . . . . .	216
8.4	Therapy . . . . .	216
8.4.1	Fusion-Gene Targeted Therapy . . . . .	217
8.4.2	Mutation-Specific and Other Signaling Pathway-Directed Therapies . . . . .	217

8.5	Molecular Tests Performed on Soft Tissue Tumors and Practical Considerations . . . . .	218
8.5.1	FISH . . . . .	218
8.5.2	RT-PCR . . . . .	220
8.5.3	Others . . . . .	222
8.6	Summary . . . . .	224
	References . . . . .	224
<b>9</b>	<b>Genodermatoses. Part I: Muir-Torre Syndrome . . . . .</b>	<b>231</b>
9.1	Introduction . . . . .	232
9.2	Pathophysiology of MMR-Defective MTS . . . . .	233
9.3	Clinical Features . . . . .	236
9.4	Histologic Features . . . . .	237
9.5	Immunohistochemical Features . . . . .	238
9.6	Assessing MMR Defects: Immunohistochemistry and PCR-Based Assays . . . . .	239
9.6.1	Immunohistochemistry for MMR . . . . .	239
9.6.2	Molecular MSI Testing . . . . .	239
9.6.3	IHC Versus MSI . . . . .	241
9.6.4	Genetic Testing . . . . .	243
9.7	Approach to the Suspected MTS Patient . . . . .	245
9.7.1	Defining MTS . . . . .	245
9.7.2	An Algorithmic Approach to the Diagnosis of MTS . . . . .	246
9.8	Summary . . . . .	250
	References . . . . .	250
<b>10</b>	<b>Genodermatoses. Part II: Other Hereditary Dermatologic Disease . . . . .</b>	<b>253</b>
10.1	Introduction . . . . .	254
10.2	Genodermatoses Associated with Cutaneous and/or Visceral Tumors (Inheritable Tumor Disorders) . . . . .	257
10.3	Inheritable Vascular Disorders . . . . .	263
10.4	Inheritable Bullous Disorders . . . . .	263
10.5	Inheritable Keratinization Disorders . . . . .	270
10.6	Ectodermal Dysplasias and Other Inheritable Disorders of the Sweat Glands, Hair, Nails, and/or Teeth . . . . .	278
10.7	Inheritable Connective Tissue Disorders . . . . .	278
10.8	Inheritable Disorders of Pigmentation . . . . .	278
10.9	Inheritable Metabolic Disorders . . . . .	278
10.10	Miscellaneous Disorders . . . . .	301
10.11	Practical Issues of Testing . . . . .	301
10.11.1	Testing Strategy . . . . .	301
10.11.2	Interpretation . . . . .	309
10.11.3	Cost and CPT Coding . . . . .	309
10.12	Summary . . . . .	311
	References . . . . .	312

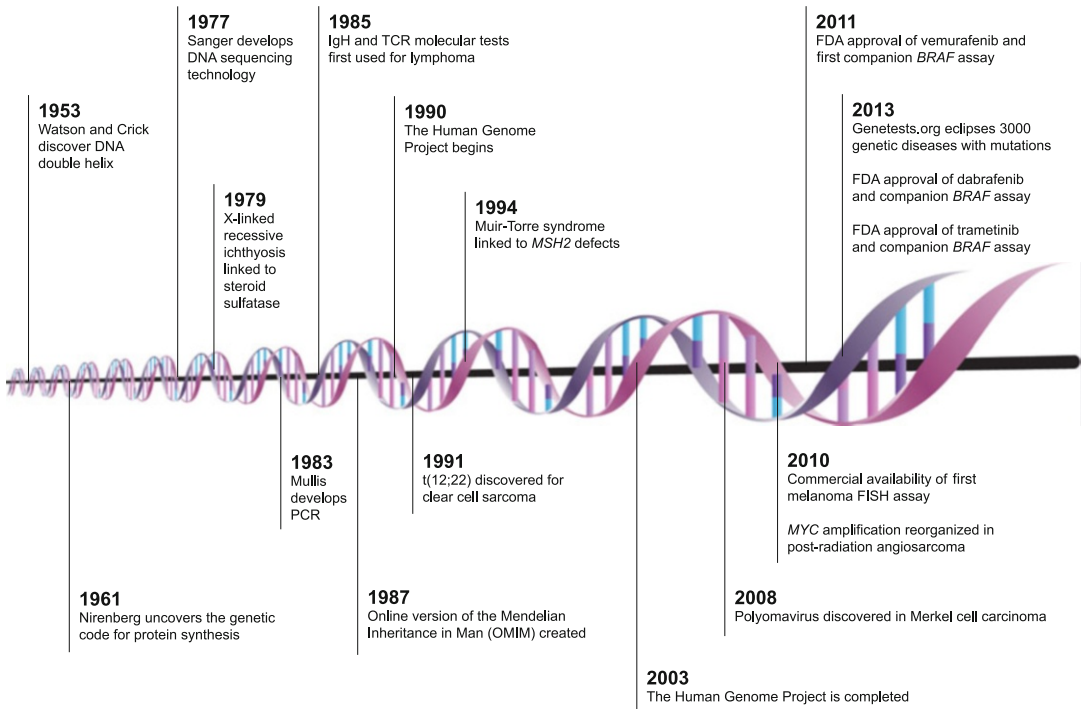
<b>11 Infectious Disease Testing</b> . . . . .	313
11.1 Introduction . . . . .	314
11.2 Assay Design and Testing Strategies . . . . .	316
11.3 Clinical Molecular Infectious Disease Testing . . . . .	318
11.4 Viruses . . . . .	319
11.5 Viral Infections Associated with Neoplasia . . . . .	320
11.5.1 Human Papillomavirus (HPV) . . . . .	320
11.5.2 Human Herpesvirus 8 (HHV-8) . . . . .	322
11.5.3 Merkel Cell Polyomavirus (MCV or MCPyV) . . . . .	323
11.6 Herpesvirus . . . . .	324
11.7 Fungi . . . . .	324
11.8 Parasites . . . . .	326
11.8.1 Leishmania . . . . .	326
11.9 Bacteria . . . . .	327
11.9.1 Mycobacteria . . . . .	327
11.9.2 Rickettsia . . . . .	330
11.9.3 Lyme Disease . . . . .	331
11.9.4 Syphilis . . . . .	331
11.9.5 Bartonella . . . . .	332
11.9.6 Cutaneous Anthrax . . . . .	333
11.10 Drug Resistance Testing . . . . .	334
11.10.1 Methicillin-Resistant Staphylococcus aureus . . . . .	334
11.11 Genetic Factors That Influence Susceptibility/ Resistance to Infectious Agents . . . . .	335
11.12 Practical Considerations . . . . .	335
11.12.1 External Controls (Positive, Negative, and No-Template) . . . . .	336
11.12.2 Sensitivity Control . . . . .	337
11.12.3 Internal Control . . . . .	337
11.12.4 Inhibition Control . . . . .	337
11.13 Summary . . . . .	337
References . . . . .	338
<b>12 Emerging Molecular Applications and Summary</b> . . . . .	341
12.1 Molecular Testing in Current Clinical Practice . . . . .	342
12.1.1 Clinically Significant Targets . . . . .	344
12.1.2 New Technologies . . . . .	345
12.2 Looking Ahead . . . . .	346
12.2.1 Theranostics . . . . .	346
12.2.2 Pharmacogenetics . . . . .	347
12.3 Summary . . . . .	352
References . . . . .	353
<b>Appendix</b> . . . . .	355

## Content

Reference ..... 4

For many, understanding molecular medicine is like standing at the tip of a long oceanic pier, gazing out. This vast, boundless body of information is enticing to some, overwhelming to most. If we choose to ignore it, at the very least, we will be lesser providers of care. We can choose to accept it or, better, embrace it, and we will not only benefit our patients but elevate the quality of modern medicine, entering new diagnostic and treatment frontiers.

Over a century of research on nucleic acids has led to step-by-step advancements in the understanding of their role in inheritance and disease. The uncovering of the double helix structure of DNA by James Watson and Francis Crick in 1953 was instrumental, beginning an era of manipulating these genetic building blocks to predict, diagnose, and manage disease, spawning the discipline of molecular diagnostics (Fig. 1.1). The completion of the Human Genome Project in 2003 was another notable leap. As part of this project, the entire 3.2 gigabase human genome was sequenced [1]. Since then, more genomes have been sequenced, including those from research organisms such as *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (roundworm), pathogens such as *Haemophilus influenzae*, and, of course, more humans, including James Watson himself. Out of the Human Genome Project, we learned of the approximately 25,000 human genes, a surprisingly low total capable of orchestrating our development and every menial and complex task. We confirmed that all humans are >99.9 % genetically alike,



**Fig. 1.1** Timeline of significant events in molecular diagnostics. There have been innumerable impactful events in the history of molecular diagnostics over the past half

century. Several, including some in the field of dermatology, are highlighted here

even at the base pair level, with the other <0.1 % holding the mystery to all of our individual differences and genetic sources of disease. And, perhaps most importantly, the human genome became accessible to the entire investigative world, providing an unprecedented template for molecular research. The field of molecular medicine became poised to explode. Molecular diagnostics has captivated medicine in a “Gangnam Style” fashion—fresh, new, and unavoidable. But unlike the popular song, molecular diagnostics has staying power.

In contrast to more conventional diagnostic tools such as histology, cultures, and biochemical assays, molecular diagnostics is traditionally defined by the use of DNA-based (or RNA-based) tests for the diagnosis of human disease. The field has evolved, however. Molecular diagnostics is no longer limited to mere *diagnostics*, separating itself from other ancillary tests in its ability to

predict disease behavior and a patient’s response to therapeutic targets. In colon cancer, for example, the diagnosis is usually not in question, but molecular testing—*KRAS* mutational analysis, for example—is ordered to predict whether or not the tumor will respond to a specific therapy—cetuximab. Now, the trifecta of “molecular diagnostics” includes *diagnostics* (identifying and classifying disease), *prognostics* (predicting disease course), and *theranostics* (predicting response to therapy), with the latter arguably the most rapidly growing area. And the field refuses to stay stagnant, as applications continue to reach new areas, such as risk assessment and therapeutic monitoring.

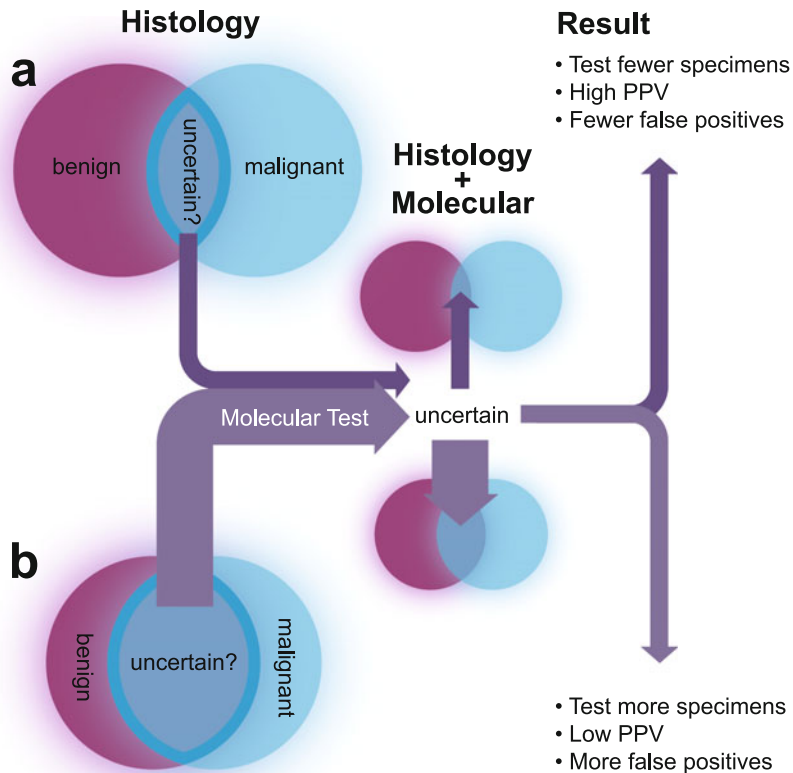
In dermatology, the incorporation of molecular diagnostics has admittedly lagged behind other disciplines, with only few and focused practical applications. This narrative is beginning to change, however, with recent important advancements and exciting new applications, touching all

areas of the above italicized trifecta. As examples, molecular tests are now used to help identify germline mutations in the genodermatoses, somatic mutations in tumors such as melanoma and various sarcomas, and the presence of certain cutaneous infectious agents, just to name a few. For melanoma and lymphoma, testing can potentially predict tumor behavior and modify patient staging. And, regarding theranostics, there is no better impactful example in dermatology than the recent observation that targeted therapy to the mutated B-Raf<sup>V600E</sup> in a subset of melanoma patients dramatically reduces tumor burden and, in rare cases, leads to apparent cure. The entire treatment paradigm for melanoma and other cancers is evolving. “Excision and pray” approaches are being replaced by personalized medicine. Treatment regimens are now being tailored to the individual based on their genome and their tumor’s genome. In cases of relapse, second and third rounds of targeted therapy may induce second and third rounds of remission, respectively. Ultimately, in patients unable to achieve a cure, therapy may evolve to constant tumor genome surveillance with molecularly based fine-tuning of treatments, transforming cancer, as we currently know it, into a chronic illness not unlike HIV and diabetes.

With every new test comes hope for revolutionizing applications. In their wake, however, we often struggle with how to implement them. For example, there is a great tendency to overuse new diagnostic tests, supplanting conventional means. Molecular diagnostic tests are like any other ancillary test, dependent on the prevalence of the disease in the population tested. Testing a large number of samples in a population of low disease prevalence will increase the number of false positives and result in a poor predictive value for the assay. Molecular testing is designed to shape a diagnosis for the pathologist, not be a crutch for the “parapathologist” (see Fig. 1.2 for further development of this concept). New tests may also introduce unanticipated practical or ethical problems. We are now able to generate immense patient and/or tumor genetic data,

most of which we do not understand. We must resist the temptation of testing just because we can, without an evidence-based infrastructure. A recent Supreme Court decision on gene patenting and the new practice of linking specific molecular tests to the FDA approval of therapy have opened avenues and introduced new wrinkles, respectively, for laboratories interested in test development.

Indeed, this is an exciting time in dermatology, and our goal as authors is to present this current (and near-future) state of affairs of molecular testing as it pertains to the dermatology patient, recognizing that this is in constant flux. In the following chapters, we begin with a basic introduction to molecular biology and commonly used methods for molecular diagnostics. We continue by covering practical applications of molecular diagnostics over a cross section of dermatologic disease, including melanoma, lymphoma, soft tissue tumors, genodermatoses, and infectious disease. Throughout the text, we emphasize the role of the dermatopathologist in test selection, preparing the sample, and interpreting results. And as molecular assays trend toward the generation of thousands of data points in a single reaction, we underline the importance of critically evaluating data in the context of the individual patient, often requiring input by the entire care team. We offer some practical advice, to those ordering molecular tests as well as to those considering performing such tests, with the following chapters serving as a potential template for a comprehensive dermatologic molecular diagnostic test menu. Our focus is on current, practical applications, but we also take several opportunities to look ahead, exploring the future of molecular diagnostics in dermatology and its potential impact on later generations. So as we pull off the fresh seal of the molecular peanut butter jar, exposing its contents with that initial scoop, we hope that all readers—clinicians, pathologists, laboratorians, or other inquisitive minds—independent of their level of molecular expertise, can find some nugget that will provoke thought or perhaps even change their practice.



**Fig. 1.2** Conceptual schematic of the role of a new diagnostic test. With every new diagnostic test, there is a positive or negative result. The power of the test, or its ability to distinguish the presence or absence of disease, is dependent on its performance characteristics, including but not limited to sensitivity and specificity. This concept can be applied to a biochemical assay, a molecular test, or even looking through the microscope. Using melanoma as an example, the experienced pathologist may look at an H&E section through the microscope and be able to distinguish melanoma from nevus in most cases, with a small but significant overlapping area corresponding to ambiguous lesions or lesions with indeterminate biology (**a**). The “parapathologist” will have a different starting point, less

able to distinguish benign from malignant, with virtually overlapping circles (**b**). With the use of a molecular or other ancillary test, the goal is to pull those circles apart, minimizing the overlapping area. The blue bold lines along the edges of the overlapping circles represent a narrow population of cases with the highest (positive and negative) pretest probability. In (**b**), there is overutilization (more area in intersection of circles leading to additional testing) with many of the tested cases having a low pretest probability and, thus, higher numbers of false-positive and false-negative results. Ancillary tests are designed to supplement conventional tests and rarely completely eliminate interpretive overlap. *PPV* positive predictive value

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## Contents

2.1	<b>Introduction</b> .....	6
2.2	<b>DNA (Deoxyribonucleic Acid)</b> .....	7
2.2.1	Structure.....	7
2.2.2	Genes.....	8
2.2.3	Replication.....	8
2.2.4	The Human Genome.....	9
2.3	<b>The Human Genome Project</b> .....	11
2.4	<b>RNA (Ribonucleic Acid)</b> .....	12
2.4.1	Structure.....	12
2.4.2	Function.....	13
2.5	<b>Transcription and Translation</b> .....	13
2.5.1	Gene Expression.....	15
2.5.2	Reverse Transcription.....	15
2.6	<b>Nucleic Acid Alterations</b> .....	16
2.6.1	Types of DNA Alterations.....	16
2.6.2	Causes of DNA Alterations.....	18
2.6.3	Repair of DNA Alterations.....	18
2.7	<b>Nucleic Alterations and Disease</b> .....	20
2.7.1	Germline Alterations.....	20
2.7.2	Benign Genetic Variants.....	20
2.7.3	Somatic Alterations and Neoplasia.....	23
2.8	<b>Genomes of Infectious Agents</b> .....	25
2.9	<b>Summary</b> .....	25
	<b>References</b> .....	26

## Key Points

- Nucleic acids are essential for all forms of life.
- The human genome is composed of approximately three billion base pairs of DNA, which are organized into two copies of each of 22 autosomes (non-sex chromosomes) and one pair of sex chromosomes (either XX or XY), for a total of 46 chromosomes.
- In humans, DNA stores the genetic code of life. It is the blueprint, or recipe, for producing all of the proteins needed to carry out cellular functions.
- RNA carries out many diverse and highly specialized cellular functions. These functions primarily involve the processes of transcription and translation, which lead to the production of proteins. RNA functions not only to produce proteins but also to regulate the production process.
- The term “gene expression” is used to indicate the production of RNA and/or protein from a gene. A gene may be silent (no expression) or may be highly expressed. The expression level of genes results in the phenotype of a cell.
- DNA can be altered from normal (wild type) in a wide variety of ways including chromosomal number alterations,



structural alterations, and sequence alterations.

- DNA alterations are either inherited (germline) or acquired (somatic). Germline mutations result in inherited diseases. Somatic mutations are important drivers of neoplasia.
- The structural and chemical properties of nucleic acids can be exploited to develop molecular diagnostic tests with an array of clinical utilities.

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## 2.1 Introduction

Many think of Watson and Crick's description of the double-stranded helix as the beginning of nucleic acid research, while in fact, nucleic acids were first discovered almost 100 years prior (1869) by Swiss scientist Friedrich Miescher. As indicated by the name nucleic acid, initial work discovered these molecules in the nucleus of cells and determined that they had acidic properties. Early work also determined that there are two basic types of nucleic acids, *deoxyribonucleic acid* (DNA) and *ribonucleic acid* (RNA). Although these basic properties were understood, it would take decades to reveal the structure and function of these molecules. Around the same time (1865), the Austrian monk Gregor Mendel established the idea that physical characteristics are passed from one generation to the next by discrete units, later to be called genes. Over the next several decades, the parallel research into the function of nucleic acids and the mechanism of inheritance started to converge. The microbiologist Oswald Avery and his colleagues at the Rockefeller Institute in New York are largely credited with the collision of these two areas, establishing that DNA, not proteins as many had hypothesized, was the carrier of genetic information [1].

James Watson and Francis Crick, along with significant contributions from Rosalind Franklin, determined the structure of DNA in 1953. This historic discovery is considered the beginning of

the development of modern genetics. Understanding the structure of DNA provided an almost immediate understanding of how DNA was replicated and how it might be passed from one generation to the next. In their landmark publication, Watson and Crick wrote "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" [2]. The discovery of the double-helix structure of DNA also laid the foundation for the development of molecular biology methods and tools, further accelerating research and discovery.

It is now well established that nucleic acids are found in all living cells and in viruses and are essential for all forms of life. Also well established is the concept that while the structures of DNA and RNA are similar, their function and some important chemical characteristics are very different. The sequencing of the entire human genome and the rapid advances in the fields of genetics and molecular biology have set the stage for a much greater understanding of human disease. Application of this knowledge is leading to improvements in making diagnoses and identifying effective treatments. The concept that nucleic acid alterations resulted in inherited diseases was obvious early on. It was not until the early 1990s, however, that researchers began to appreciate the genetic nature of cancer [3].

This discussion relates specifically to the structure and function of human DNA and RNA. It is not the purpose of this chapter to provide a comprehensive review of nucleic acids and molecular biology. There are entire textbooks devoted to these topics. Rather, the purpose of this chapter is to review basic concepts in molecular biology and nucleic acid chemistry to provide an understanding of the nomenclature and vocabulary required to comprehend molecular testing and its impact on patient care. The normal structure and cellular functions of nucleic acids are reviewed, providing a foundation for the discussion of molecular methods used in the clinical molecular laboratory (Chap. 3). In addition, this chapter begins the discussion of how deviations from normal structure and function result in disease, with special attention given to dermatologic disease.