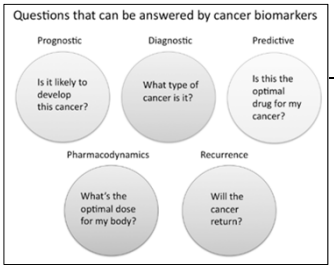
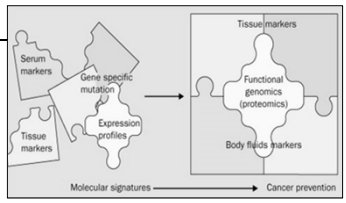
GENETIC BIOMARKERS & MULTI-GENE EXPRESSION PROFILES IN CANCER

2017-2018 FCDS Educational Webcast Series





December 14, 2017

Steven Peace, CTR

1

CDC & Florida DOH Attribution

"We acknowledge the Centers for Disease Control and Prevention, for its support of the Florida Cancer Data System, and the printing and distribution of the materials for the 2017-2018 FCDS Webcast Series under cooperative agreement 1NU58DP006350 awarded to the Florida Department of Health. The findings and conclusions in this series are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention".

CDC Centers for Disease Control and Prevention

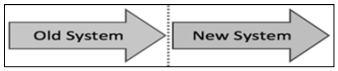
Florida HEALTH

FCDS would also like to acknowledge the Florida Department of Health for its support of the Florida Cancer Data System, including the development, printing and distribution of materials for the 2017-2018 FCDS Webcast Series under state contract CODJU. The findings and conclusions in this series are those of the author(s) and do not necessarily represent the official position of the Florida Department of Health.

2

FLccSC LMS – CEU Quiz –FCDS IDEA

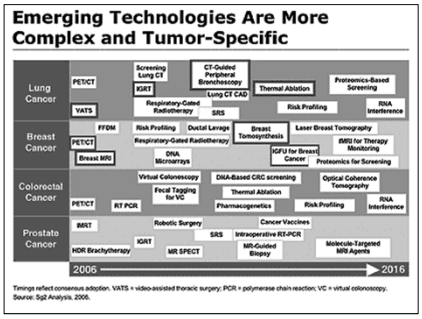
- Florida has changed how we track webcast attendance
- Florida has changed how we award CEUs for our webcast series
- Attendees must take and pass a 3-5 question CEU Quiz to be awarded CEUs
- Only registered FLccSC Users will be given access to the CEU Quiz
- Florida attendees must have a Florida FLccSC Account & pass the quiz to get CEUs
- South Carolina attendees must have a South Carolina FLccSC Account & pass the quiz to get CEUs
- Other Attendees can attend the live webcasts but cannot receive CEUs for attendance at this time
- Please remember this is a new system with new requirements - some still being worked out
- The CEU Quiz should be available about an hour after the webcast ends



3

Presentation Outline

- Introduction to Molecular Oncology
- Genomics for Imaging, Classification & Treatment
- What is this Test?
- Single Gene Expression Test
- Multi-Gene Expression Profiling
- Current Status of Cancer Biomarkers
- Next Generation Genomic Sequencing
- Next Generation Targeted Cancer Treatments
- CAP Biomarker Checklists & Other Resources
- Questions



4

Introduction to Molecular Oncology

- The human genome contains approximately 25,000 genes
- These genes work in concert to produce about 1,000,000 distinct proteins
- Proteomics is the knowledge of the precise proteins a cell makes
- Genomics is an area of genetics involved in sequencing/analysis of an organism's genome.
- The genome is the entire DNA content that is present within one cell of an organism.
- Genomic Medicine is a medical discipline that involves using genomic information about an individual as part of clinical care (e.g., for diagnostic or therapeutic decision-making) and the health outcomes and policy implications of clinical use.)
- Genomic/Molecular Oncology is the medical discipline that uses genomic information about an individual as a whole or specifically an individual's cancer as part of clinical care

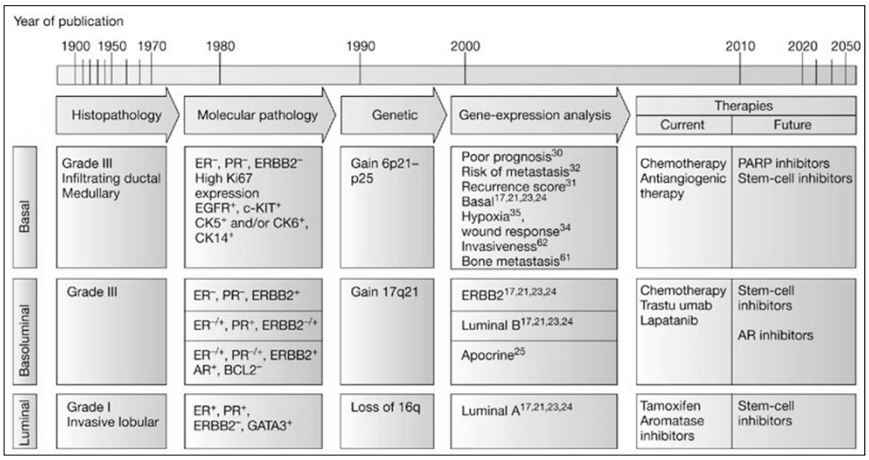
5

Importance of Cancer Genomics - NCI

- **Cancer is a genetic disease.**
- Cancer genomics research contributes to precision medicine by defining cancer types and subtypes based on their genetics and identify targets for new medicines
- "targeted therapies" specifically combat characteristics of cancer cells that are different from normal cells of the body. This makes them less likely to be toxic for patients compared to other treatments such as chemotherapy and radiation that can kill normal cells.
- How do "targeted therapies" work?
  - Inhibit enzymes that trigger the abnormal growth and survival of cancer cells
    - Imatinib (Gleevec) inhibits overactivity of protein Bcr-ABL tyrosine kinase in leukemia patients
  - Block aberrant gene expression characteristic of cancer cells
    - Trastuzumab (Herceptin) controls hyperactive signaling pathway (HER2 tyrosine kinase) - breast
  - Halt molecular signaling pathways that are in overdrive in cancer cells
    - Erlotinib (Tarceva) and gefitinib (Iressa) both restrict activation opf a protein (EGFR) in lung cancers

6

Comparison of the histopathology, molecular pathology, genetic, and gene-expression analysis methods used to delineate breast cancer tumor subtypes and suggested current and future therapies in a historical context

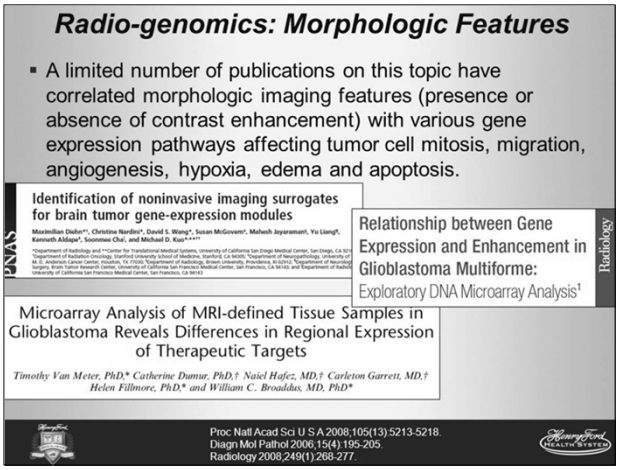


http://www.nature.com/article-assets/npg/nrclinonc/journal/v4/n9/images/ncponc0908-f1.jpg

7

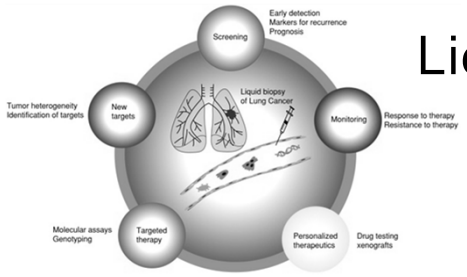
Genomics for Imaging, Classification & Treatment

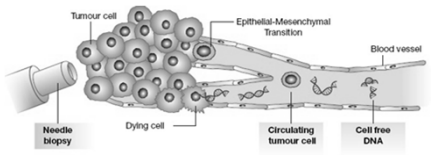
- Radiogenomics or Imaging Genomics – associates imaging features with genomic data in noninvasive techniques.
- Sometimes the imaging outputs are called imaging phenotype or radiophenotype of the tumor while the genomic outputs define molecular phenotype or genotype of disease
- Radiogenomics looks at the entire tumor not just a sample specimen of piece of the tumor.
- Provides extensive tumor information
  - Intra-tumor
  - Inter-Tumor
  - Peri-Tumor



8

Liquid Biopsy





	Biopsy	CTC	cfDNA
Invasive	+	–	–
All patients eligible	–	+	+
Instrumentation required	+	+	–
WGA required	–	+	+/–
RNA profiling	+	+	–
Research applicability	+++	++	+
Biomarker applicability	–	++	+++

- Liquid biopsy is a minimally invasive technology for detection of molecular biomarkers without the need for costly or invasive procedures.
- Circulating cancer cells or traces of the cancer's RNA or DNA in the blood can give clues about which treatments are likely to work for a patient.
- Circulating nucleic acids are protected by extracellular micro-vesicles, mainly exosomes.
- Exosomes are cell-derived vesicles that are present in many and perhaps all eukaryotic fluids, including blood, urine, and cultured medium of cell cultures.
- Exosomes maintain specified "compartments" of micro and macro molecules. Cancers create an expulsion of key proteins and microRNAs resulting in mis-expression of intracellular molecules which in turn interrupt cancer's intra and extra cellular communications pathways

9

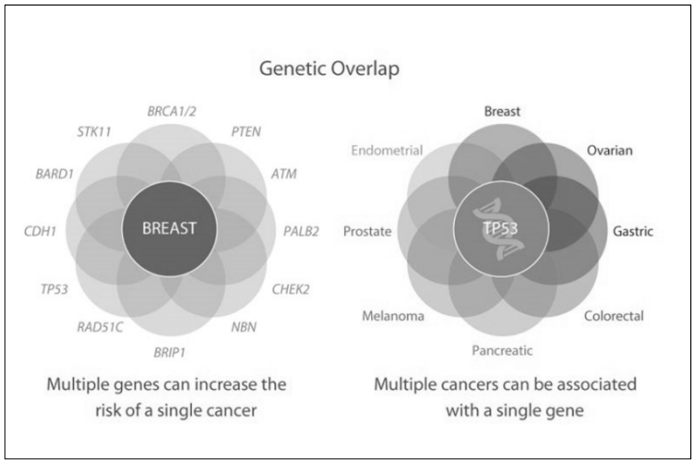
New Diagnostic Tools & Techniques

- Patterns of genetic changes detected in blood samples closely mirror those identified in traditional tumor biopsy. "Liquid Biopsy" provides an accurate snapshot of the genomic landscape of the tumor.
- "Liquid Biopsy" – Biofluids consist of circulating cell-free (ct)DNA and extracellular (ex)RNA from multiple tissues within the body.
- When circulating tumor DNA (ctDNA) was positive for key abnormalities in *EGFR*, *BRAF*, *KRAS*, *ALK*, *RET*, and *ROS1*, the same mutations were reported in tissue 94% to 100% of the time
- ctDNA testing revealed a treatment option for two-thirds of patients tested
- Next Generation Genome Sequencing allows researchers to build new biofluid-based DNA/RNA analytical methods.

10

Memorial Sloan Kettering Cancer Center and University of California San Francisco

New Diagnostic Tools & Techniques



11

Myriadgenetics.eu

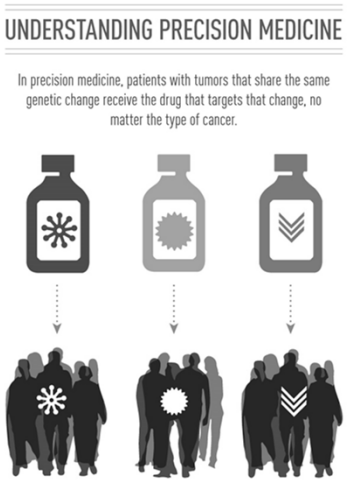
Genomics for Imaging, Classification & Treatment

- Breast cancer is classified based on molecular characteristics into distinct subgroups known as molecular subtypes. Molecular subtypes for breast cancer include – Luminal A, Luminal B, Triple-negative/basal-like, and HER2 type that vary in their aggressiveness and respond differently to therapies.
- Diffuse large B cell lymphoma can be subdivided into the ABC and GCB subtypes by genomic profiling, identifying patients that respond differently to current chemotherapy regimens and to molecularly targeted therapies.
- The Cancer Genome Atlas project identified four subtypes of endometrial cancer –OLE ultramutated, microsatellite instability (MSI) hypermutated, copy-number (CN) low, and CN high that correlate with patient survival. This research has already given rise to new clinical trials that investigate how subtyping can improve the future of endometrial cancer care.
- Lung cancer patients who have a gene fusion involving the ROS1 gene often respond well to treatment with a targeted therapy called crizotinib. In these cases, the disease is best defined and treated based on its unique genetic change.

12

Genomics for Imaging, Classification & Treatment

- Using the genetic changes in a patient's tumor to determine best target for treatment is known as precision medicine.
- Precision Medicine targets specific characteristics of the cancer by:
  - Inhibiting enzymes that trigger the abnormal growth and survival of cancer cells
  - Blocking aberrant gene expression characteristic of cancer cells
  - Halting molecular signaling pathways that are in overdrive in cancer cells



13

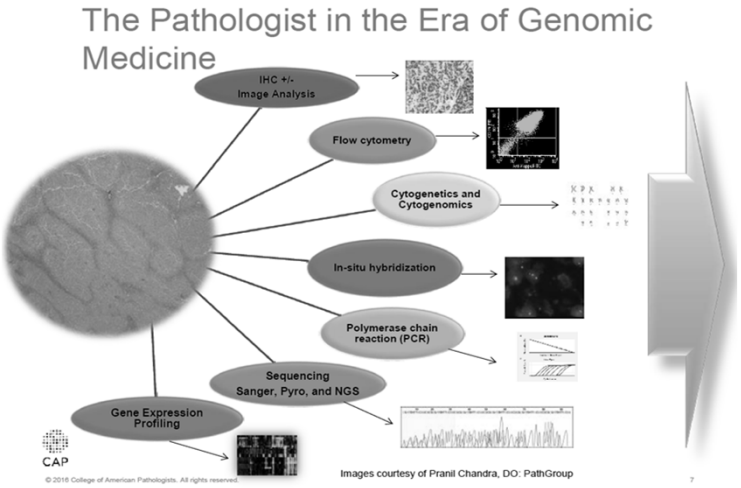
Targeted Therapies

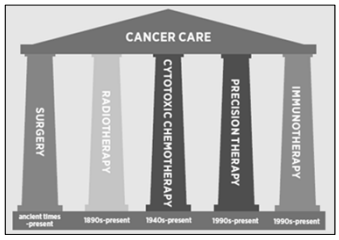
Ado-trastuzumab emtansine (Kadcyla)	HER2 (ERBB2/neu)
Afatinib (Gilotrif)	EGFR (HER1/ERBB1)
Aldesleukin (Proleukin)	
Alectinib (Alecensa)	ALK
Alemtuzumab (Campath)	CD52
Atezolizumab (Tecentriq)	PD-L1
Axitinib (Inlyta)	KIT, PDGFRβ, VEGFR
Belimumab (Benlysta)	BAFF
Belinostat (Beleodaq)	HDAC
Bevacizumab (Avastin)	VEGF ligand
Bortezomib (Velcade)	Proteasome
Bosutinib (Bosulif)	ABL
Brentuximab vedotin (Adcetris)	CD30
Cabozantinib (Cabometyx [tablet], Cometriq [capsule])	FLT3, KIT, MET, RET,
Canakinumab (Ilaris)	IL-1β
Carfilzomib (Kyprolis)	Proteasome
Ceritinib (Zykadia)	ALK
Cetuximab (Erbitux)	EGFR (HER1/ERBB1)
Cobimetinib (Cotellic)	MEK
Crizotinib (Xalkori)	ALK, MET, ROS1
Dabrafenib (Tafinlar)	BRAF
Daratumumab (Darzalex)	CD38
Dasatinib (Sprycel)	ABL
Denosumab (Xgeva)	RANKL
Dinutuximab (Unituxin)	B4GALNT1 (GD2)

Elotuzumab (Empliciti)	SLAMF7 (CS1/CD319/CRACC)
Erlotinib (Tarceva)	EGFR (HER1/ERBB1
Everolimus (Afinitor)	mTOR
Gefitinib (Iressa)	EGFR (HER1/ERBB1
Ibritumomab tiuxetan (Zevalin)	CD20
Ibrutinib (Imbruvica)	BTK
Idelalisib (Zydelig)	PI3Kδ
Imatinib (Gleevec)	KIT, PDGFR, ABL
Ipilimumab (Yervoy)	CTLA-4
Ixazomib (Ninlaro)	Proteasome
Lapatinib (Tykerb)	HER2 (ERBB2/neu)
Lenvatinib (Lenvima)	VEGFR2
Necitumumab (Portrazza)	EGFR (HER1/ERBB
Nilotinib (Tasigna)	ABL
Nivolumab (Opdivo)	PD-1
Obinutuzumab (Gazyva)	CD20
Ofatumumab (Arzerra, HuMax-CD20)	CD20
Olaparib (Lynparza)	PARP
Olaratumab (Lartruvo)	PDGFRα
Osimertinib (Tagrisso)	EGFR
Palbociclib (Ibrance)	CDK4, CDK6
Panitumumab (Vectibix)	EGFR (HER1/ERBB
Panobinostat (Farydak)	HDAC

Pembrolizumab (Keytruda)	PD-1
Pertuzumab (Perjeta)	HER2 (ERBB2/neu)
Ponatinib (Iclusig)	ABL, FGFR1-3, FLT3, VEGFR2
Ramucirumab (Cyramza)	VEGFR2
Regorafenib (Stivarga)	KIT, PDGFRβ, RAF, RET, VEGFR1/2/3
Rituximab (Rituxan, Mabthera)	CD20
Romidepsin (Istodax)	HDAC
Ruxolitinib (Jakafi)	JAK1/2
Siltuximab (Sylvant)	IL-6
Sipuleucel-T (Provenge)	
Sonidegib (Odomzo)	Smoothened
Sorafenib (Nexavar)	VEGFR, PDGFR, KIT, RAF
Temsirolimus (Torisel)	mTOR
Tocilizumab (Actemra)	IL-6R
Tofacitinib (Xeljanz)	JAK3
Tositumomab (Bexxar)	CD20
Trametinib (Mekinist)	MEK
Trastuzumab (Herceptin)	HER2 (ERBB2/neu)
Vandetanib (Caprelsa)	EGFR (HER1/ERBB1), RET, VEGFR2
Vemurafenib (Zelboraf)	BRAF
Venetoclax (Venclexta)	BCL2
Vismodegib (Erivedge)	PTCH, Smoothened
Vorinostat (Zolinza)	HDAC
Ziv-aflibercept (Zaltrap)	PlGF, VEGFA/B

Explosion of Data / Fragmented Data Sources



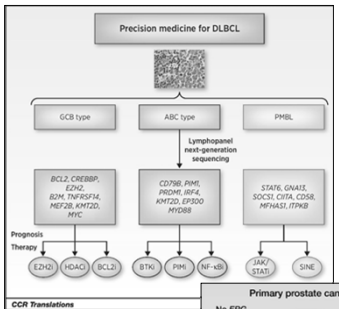


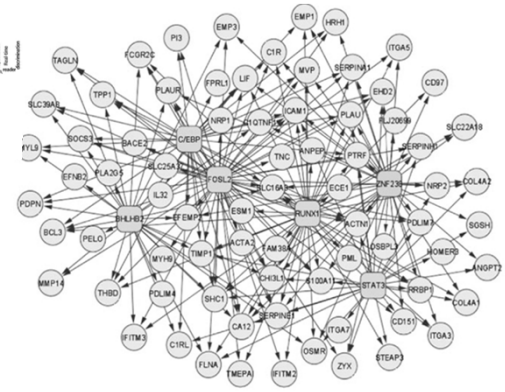
Multiplex Testing May Become Standard

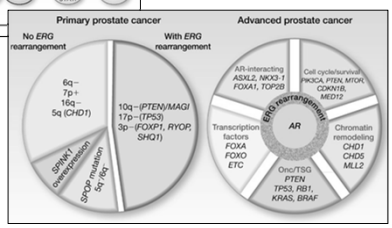
Combination Therapy Means Managing Multiple Treatment Data Sources

15

Today – Precision Cancer Medicine Workup

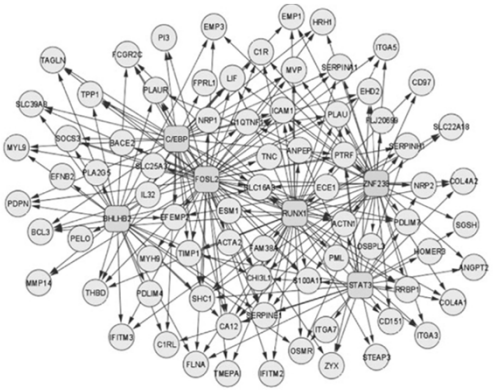






16

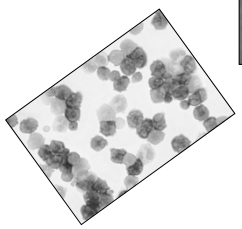
Today – Precision Cancer Medicine Workup

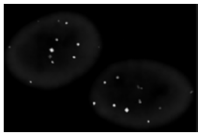


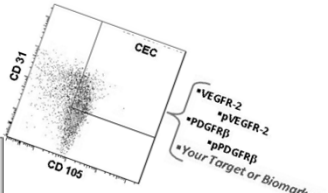
- Apoptosis
- β-catenin/Wnt signaling
- Cell cycle control
- Cellular architecture and microenvironment
- Chromatin remodeling/DNA methylation
- Cytotoxic chemotherapy mechanisms of action
- DNA damage/repair
- G-protein signaling
- Hedgehog signaling
- Hormone signaling
- Immune checkpoints
- JAK/STAT signaling
- Kinase fusions
- MAP kinase signaling
- Metabolic signaling
- PI3K/AKT1/MTOR
- Protein degradation/ubiquitination
- Receptor tyrosine kinase/growth factor signaling
- RNA splicing
- TGFβ signaling

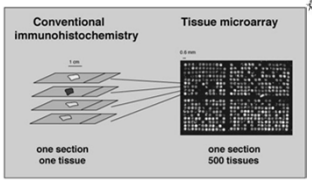
17

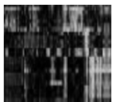
What is this test?

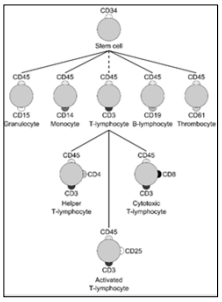


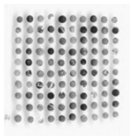


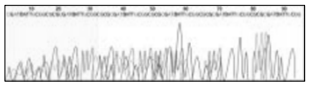


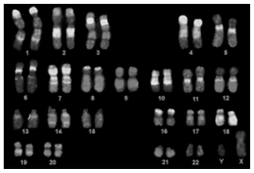


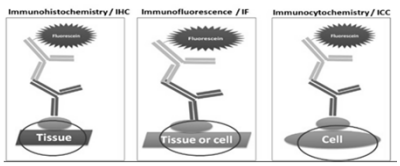












18

Tumor Marker or Genetic Alteration

Tumor Marker

- Tumor Markers are indicators of cellular, biochemical, molecular or genetic alterations by which neoplasia can be recognized.
- Tumor markers detect the presence of tumor based on quantitative and/or qualitative measurements in blood or secretions found in cells, tissues or body fluids.
- These surrogate measures of the biology of the cancer provide insight in the clinical behavior of the tumor.
- Biochemical or immunologic counterparts of differentiation states of tumor.

Genetic Alteration

- Cancer is a multigene disease that arises as a result of mutational and epigenetic changes coupled with activation of complex signaling intra and extra cellular networks.
- Alterations in 3 Classes of Genes
  - ProtoOncogenes
  - Tumor Suppressor Genes
  - DNA Repair Genes
- Types of Mutations
  - Gene Rearrangement
  - Point Mutations
  - Gene Amplification
- Resultant effects on death mechanisms embedded within cells coupled with dysregulation of cell proliferation events.

19

One Test Can Be Performed Multiple Ways

Test Description

ALK
- Immunohistochemistry (IHC) using ALK clone D5F3
- Fluorescence in situ hybridization (FISH)

EGFR
- Polymerase chain reaction (PCR) and pyrosequencing
  - Detects mutations at codons 719 (exon 18), 768 and 790 (exon 20), 858 and 861 (exon 21)
  - Detects deletions in exon 19

EGFR T790M (serum)
- Digital droplet PCR

KRAS
- PCR and pyrosequencing
  - Detects mutations at codons 12, 13 (exon 2), and 61 (exon 3)

c-MET
- IHC

MET
- FISH

PD-L1
- IHC

RET
- FISH – detects all *RET* gene fusions

ROS1
- IHC (using ROS1 clone D4D6) with FISH reflex if equivocal

FDA-approved PD-L1 companion tests

PD-L1 22C3 pharmDx by Immunohistochemistry with Interpretation, pembrolizumab (KEYTRUDA) 2013284
- Aids in prediction of response to pembrolizumab for patients with non-small cell lung cancer (NSCLC)
- Can be performed in conjunction with or instead of PD-L1 28-8

PD-L1 28-8 pharmDx by Immunohistochemistry with Interpretation, nivolumab (OPDIVO) 2013684
- Aids in prediction of response to nivolumab for patients with non-squamous non-small cell lung cancer (NSCLC) or melanoma
- Can be performed in conjunction with or instead of PD-L1 22C3

Monitor for EGFR T790M resistance

EGFR T790M Mutation Detection in Circulating Cell-Free DNA by Digital Droplet PCR 2012868
- Monitor for development of *EFGR* T790M drug-resistant mutation with *EGFR*-mutant non-small cell lung cancer in patients administered TKI therapy
- Monitor response to therapy and disease progression in patients treated with *EGFR* T790M-targeted drugs

Determine eligibility for TKI therapy (single tests)

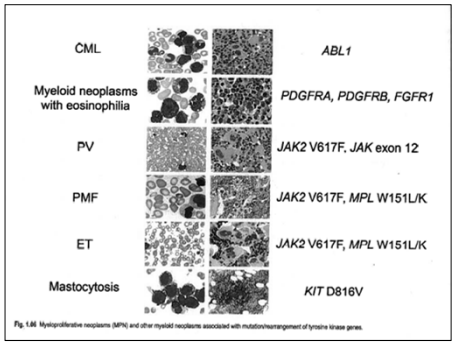
ALK (D5F3) with Interpretation by Immunohistochemistry 2007324
- Detects ALK fusion proteins

ALK (D5F3) by Immunohistochemistry with Reflex to ALK Gene Rearrangements by FISH 2011431
- Detects ALK fusion proteins (IHC) and *ALK* gene rearrangements (FISH)

20

What is this Test?

- Molecular Tumor Markers
  - Serum Protein Markers
  - PCR (Polymerase Chain Reaction)
    - Real Time PCR – Q PCR
    - Reverse Transcriptase PCR – RT PCR
  - DNA-based Tumor Markers
  - Tissue Microarrays
  - Micro RNAs

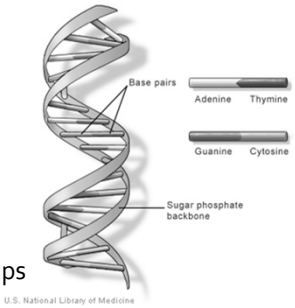


- Classification Group
  - Myeloid and Lymphoid Neoplasms with Eosinophilia and Abnormalities of PDGFRA, PDGFRB or FGFR1

21

What is this test?

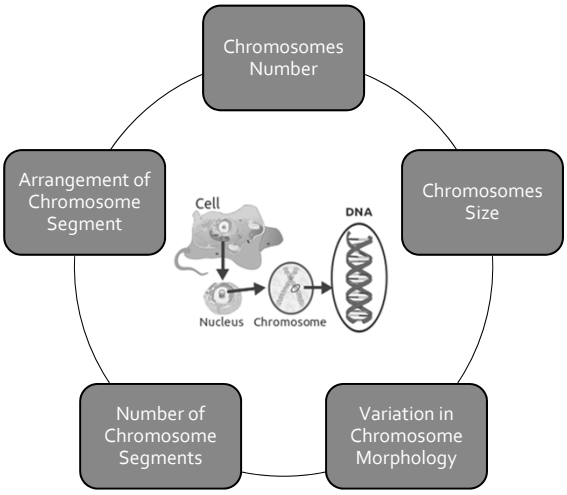
- Cytogenetics - Special studies that are used to identify genetic (usually chromosome-level) problems effecting the structure and function of the cell
- Cytogenetic Technique
  - Karyotyping – routine chromosome analysis
  - Spectral Karyotype Imaging (SKY) – 3-dye method
  - Polymerase Chain Reaction (PCR)
    - Real Time PCR
    - Reverse Transcriptase PCR
  - Fluorescent in-situ hybridization (FISH)
  - Chromogenic in-situ hybridization (CISH)
  - Microarray comparative genomic hybridization (CGH)
  - DNA Microarray – cDNA microarray and oligonucleotide/DNA chips



22

Cytogenetics Basics

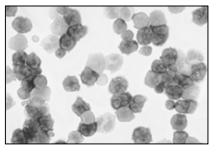
- Basic Chromosomal Abnormalities
  - Abnormal number of chromosomes
  - Abnormal structure of chromosome(s)
- Single chromosome mutations
  - Deletion
  - Inversion
  - Duplication
  - Rearrangement
- Two-chromosome mutations
  - Insertion
  - Translocation



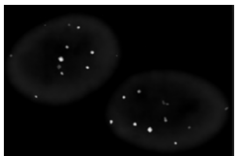
23

What is this test?

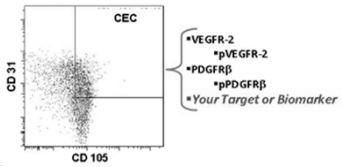
Cytochemistry: Staining cells. Stains cause color changes that identify certain leukemias or other cancers.



FISH: Identifies genetic changes and translocations.



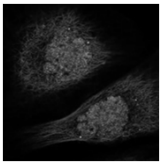
Flow cytometry: Cells from blood, BM, tissue are treated with antibodies and passed in front of a laser beam.



24

What is this test?

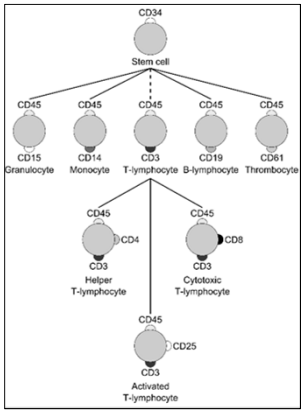
Immunocytochemistry: Cells from blood, BM, tissue are treated with special antibodies plus or minus fluorescence.



Immunophenotyping: Cells from blood, BM, tissue used to determine types of proteins, antigens or markers on surface of cell.

Referred to as CD – Cluster of Differentiation or Cluster Designation

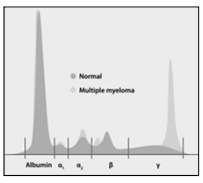
CD: The antigen found in the cell.

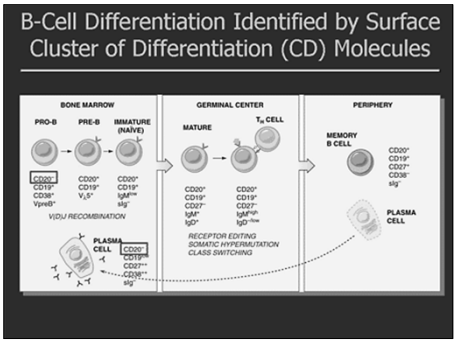


25

Immunophenotyping

- Immunophenotyping methods
  - Immunohistochemistry (IHC)
  - Immunofluorescence (FISH)
  - Electrophoresis (SEP or UEP)
  - Flow cytometry





- Specific Examples – New Preferred Terms
  - Primary cutaneous CD_4 positive small/medium cell T-cell lymphoma
  - Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma
  - Plasma cell myeloma CD20 positive subset

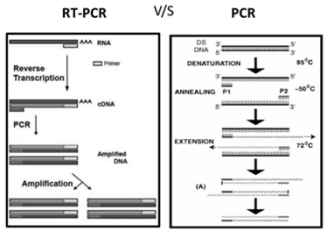
26

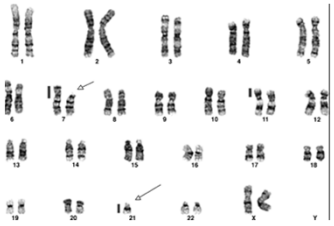
More Tests

Polymerase chain reaction (PCR): Measures blood cancer cells that cannot be detected by FISH.

RT-PCR: Based on PCR technology but allows the use of RNA as a template rather than DNA.

Karyotyping: To arrange and classify chromosomes based on number, size, shape, and other characteristics.





27

Single Gene Expression Test

- IDH2 (RT-PCR) for AML
- ALK receptor tyrosine kinase for lung, neuroblastoma,
- BCR-ABL1 for CML, ALL, AML
- JAK2 non-receptor tyrosine kinase for polycythemia vera, essential thrombocythemia, myelofibrosis and various myeloproliferative disorders
- EGFR (HEr1, HER2, HER3, HER4) for TKI (tyrosine kinase inhibitor) lung cancer
- PTK2 for breast cancer
- P53 (cellular tumor antigen) – "the guardian of the genome" conserves stability by preventing genome mutation. TP53 is tumor suppressor gene.

28

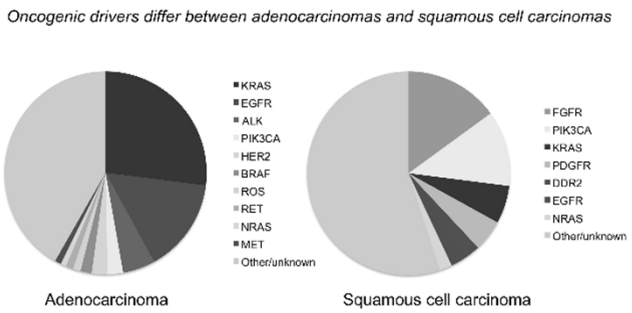
Single Gene Expression Test

Single Gene Testing			
Gene	**Testing method**	**Test result**	
ALK	IHC	Positive – cytoplasmic staining in tumor cells Equivocal – very weak cytoplasmic staining visible only on higher power by microscopy Negative – no cytoplasmic staining in tumor cells	May predict response to TKI therapy
	FISH	Positive – *ALK* gene rearrangements detected in ≥15% of nuclei • Does not identify translocation partner	May predict response to TKI therapy
EGFR	PCR/pyrosequencing	Positive – mutation detected	May predict response to TKI therapy
	EGFR T790M (serum) Digital droplet PCR	Positive – mutation detected • Expressed as percentage	Predicts resistance to TKI therapy
KRAS	PCR/pyrosequencing	Positive – mutation detected	May predict response to TKI therapy
MET	FISH	Positive – detects gene amplification	• May predict response to crizotinib TKI therapy • Associated with acquired resistance to EGFR inhibitors in 5-20% of patients with *EGFR*-mutated tumors
RET	FISH	Positive – gene rearrangements detected • Does not identify translocation partner	May predict response to TKI therapy
ROS1	IHC FISH reflex	Positive – any degree of membranous staining in tumor cells Equivocal – any degree of cytoplasmic staining in tumor cells • Reflexes to FISH for confirmation ○ Does not identify translocation partner	May predict response to TKI therapy

29

Mutation Panel Testing

- Non-Small Cell Lung Cancer (NSCLC)
  - EGFR
  - KRAS
  - ALK
  - ROS1
  - HER2
  - RET
  - MET
- Squamous Cell Lung Cancer
  - FGFR1
  - PDGFR
  - PIK3
- Small Cell Lung Cancer (SCLC)
  - NONE



30

Multi-Gene Expression Profiling

- Testing for Hereditary Cancer Syndromes
- Breast - Oncotype Dx Recurrence Score by RT-PCR (21 gene test)
- Breast - Oncotype DX Colon Recurrence Score (RT-PCR test of 12 genes)
- Breast - Mammaprint 70 Gene Recurrence Predictor
- Colon - ColoPrint (microarray) 18 gene test – risk of distant recurrence for stage II
- Colon - OncoDefender-CRC (risk of recurrence) 5 gene test –Stage I-II
- Colon - ColDx (microarray) 634 genes - risk of recurrence
- Colon - ColonPRS (163 genes) risk of recurrence for Stage II and Stage III Colon

31

Multi-Gene Expression Profiling

Table 1. Cancer-Related Genes Included in the *Oncotype* DX Breast Cancer Genomic Assay

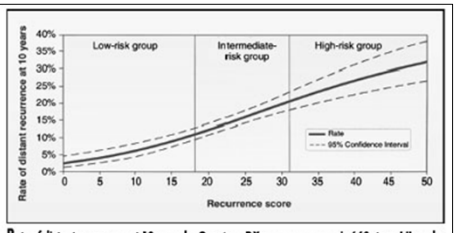
Proliferation genes
Cyclin B1
Survivin
STK 15
MYBL2
Ki-67

Hormonal influence
Estrogen receptor
Progesterone receptor
Bcl2
SCUBE2

Tumor invasion
Stromolysin 3
Cathepsin L2

HER-2
HER-2
Grb-7

Other
BAG-1
GSTM1
CD68



Rate of distant recurrence at 10 years by Oncotype DX recurrence score in 668 stage I-II, node-negative, ER-positive, tamoxifen-treated patients.

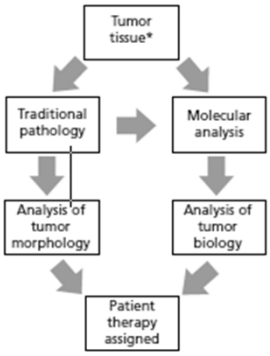
Table 2. Predictive Value of *Oncotype* DX Recurrence Score vs Standard Measures in Stage I-II, Node-Negative, ER-Positive, Tamoxifen-Treated Breast Cancer Patients

Measure	Significance excluding recurrence score	Significance including recurrence score
Age ≥ 50	$P = .004$	$P = .084$
Tumor size > 2 cm	$P = .058$	$P = .231$
Recurrence score	NA	$P < .00001$

32

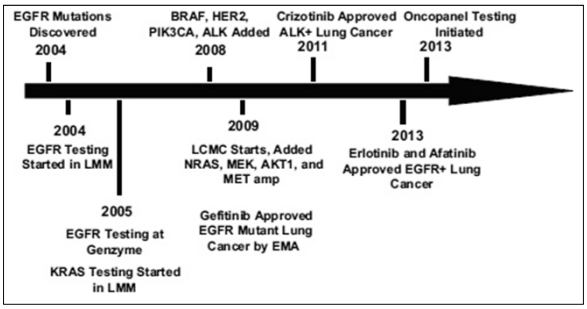
Current Status of Cancer Biomarkers

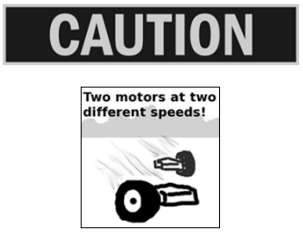
- Examples – no way can do a complete review of available cancer biomarkers



33

Site-Specific Data Items & Emerging Factors





Identification of and Testing for Next Generation Biomarkers, Genetic Tests and Multi-Gene Profiles and Establishing Data Collection Standards for Emerging SSFs

34

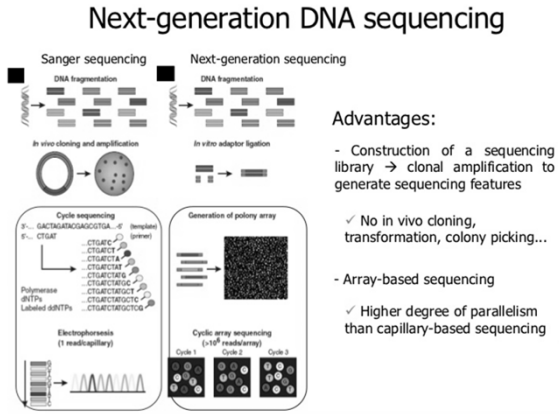
Next Generation Genomic Sequencing

- Advances in Genome Sequencing, Pharmacogenomics, Gene Editing, and Biometric Wearables Will Provide New Pathways to Improve Cancer Therapy Options – fast, reliable, standardized, portable, and beyond proof of concept
- Next-generation sequencing (NGS) is arguably one of the most significant technological advances in the biological sciences of the last 30 years. NGS has evolved to point where now in routine use when establishing cancer diagnosis.
- NGS in turn is translating to rapid development and approvals for ne diagnostic, prognostic & therapeutic targets
- Multi-Gene Assays cropping up everywhere – these are not standardized and are often proprietary and under study
- Example: Prostate - New three-in-one blood test could transform treatment of advanced prostate cancer through use of precision drugs such as PARP Inhibitor, olaparib, designed to target mutations in BRCA2 and PALB2 genes
  - Olaparib is good at killing cancer cells that have errors in genes that have a role in repairing damaged DNA.
  - Some patients respond to the drug for years
  - Other patients, the treatment either fails early, or the cancer evolves resistance.
  - Study also identified specific genetic mutations used to resist treatment with olaparib
  - The test is designed for use both before and after treatment
- Using the absolute amounts of cancer DNA in the bloodstream and also a readout of the specific mutations within that genetic material – researchers believe the test can usher in a new era of precision medicine for prostate cancer.

Source: *ICR, The Royal Marsden, UCL and Imperial College London*

35

Next Generation Genomic Sequencing



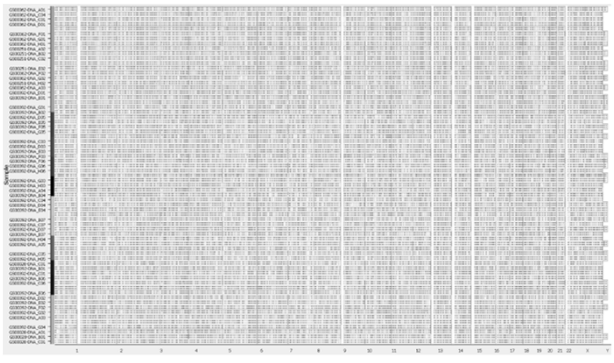
Advantages:

- Construction of a sequencing library → clonal amplification to generate sequencing features

  ✓ No in vivo cloning, transformation, colony picking...

- Array-based sequencing

  ✓ Higher degree of parallelism than capillary-based sequencing



36

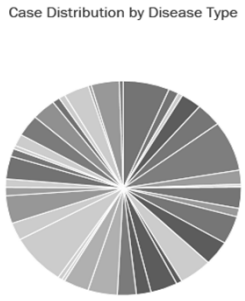
Next Generation Genomic Sequencing

- Next-generation genomic sequencing (NGS), also known as massively parallel sequencing, represents an effective way to capture a large amount of genomic information about a cancer.
- Thanks to advanced computing technology, speed, research collaborations, and cost improvements NGS can now be more readily implemented into a clinical workflow.
- Samples no longer need to be handled differently than standard diagnostic specimens
- Advances have enabled complex genomic data to be derived from peripheral blood.
- The concept of precision medicine goes hand in hand with an understanding of the cancer genome as determined by Next Generation Genomic Sequencing.

Next Generation Genomic Sequencing

- The Cancer Genome Atlas Project represents the largest effort to systematically characterize the molecular profiles of human cancers. Genetic sequencing has become faster and cheaper.
- The US National Cancer Institute's Genomic Data Commons (GDC) will bring together the two largest existing cancer datasets - The Cancer Genome Atlas and TARGET – with room to grow
- By profiling tumors—recording in detail the genetic sequence, structure, and differences from healthy cells—they will reveal clues about how to stop them – one huge unified database
- Development of advanced bioinformatics tools for analyzing and visualizing genomic data.
- Evaluation of genome atlas molecular data to predict patient survival.
- Evaluation of genome atlas molecular data and treatment targets to affect survival across types.
- Biomedical significance and clinical relevance of pseudo-genes and RNA editing in cancers.

Next Generation Genomic Sequencing



Data Availability Summary

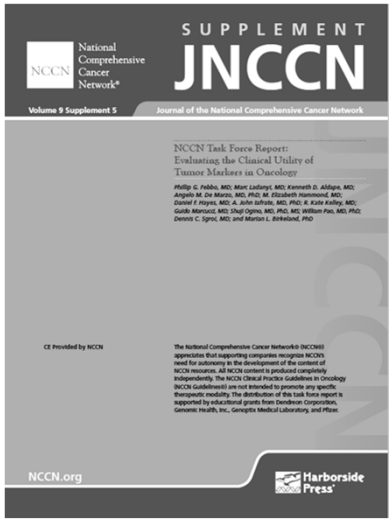
Programs	2
Projects	39
Disease Types	38
Cases	14531

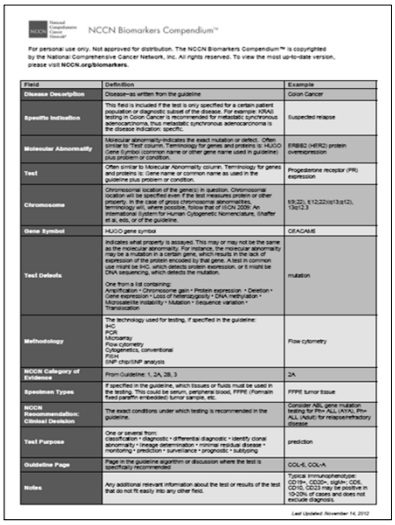
- The NCI's Genomic Data Commons (GDC) it is an expandable knowledge network supporting the import and standardization of genomic and clinical data from cancer research programs.
- The GDC provides the cancer research community with a unified data repository that enables data sharing across cancer genomic studies in support of precision medicine.
- The GDC supports several cancer genome programs at the NCI Center for Cancer Genomics (CCG), including The Cancer Genome Atlas (TCGA), Therapeutically Applicable Research to Generate Effective Treatments (TARGET), and the Cancer Genome Characterization Initiative (CGCI).

39

National Cancer Institute Genomic Data Commons

CAP Biomarker Checklists & Other Resources





CAP Solid Tumor Selected Tests by Tumor Type

COLLEGE of AMERICAN PATHOLOGISTS

Clinical Solid Tumor Molecular Oncology: Selected Tests by Tumor Type

This table is for quick reference only. Clinical decision making, including diagnosis and therapy, should not be based solely on this information. The information should be considered in conjunction with clinical information, imaging, and laboratory studies. Additional reading and investigation should be undertaken regarding the tabular entries before information is used in the clinical setting.

Tumor Type	Gene/Loci	Somatic Alteration	Clinical Use	References
Colorectal Adenocarcinoma				
	KRAS codons 12, 13, 59, 61, 117, 146	Mutation	Lack of response to EGFR monoclonal antibodies (p.G13D may be an exception)	2–3, 11–12, 14, 24, 31, 49, 52
	NRAS codons 12, 13, 59, 61, 117, 146	Mutation	Lack of response to EGFR monoclonal antibodies	11, 14, 52
	BRAF	p.V600E mutation	MSI stratification, prognostic factor, possible reduced response to EGFR monoclonal antibodies but insufficient evidence	3, 10–11, 31, 44, 46
	MLH1	Promoter methylation	Indicates sporadic MSI tumor	3, 10
	PIK3CA	Mutation	Possible improved survival with postoperative aspirin therapy	13, 29
Lung Adenocarcinoma				
	EGFR exons 18–21	Mutation	Response to EGFR inhibitors	5, 32, 35, 37, 38
	EGFR	p.T790M and some exon 20 insertion mutations	Resistance to EGFR inhibitors	5, 26, 30, 39, 53
	KRAS codons 12, 13, 61	Mutation	Exclusion of *EGFR* mutation	5, 8, 30, 42
	BRAF p.V600E	Mutation	Possible response to BRAF inhibitor	40
	ALK	Rearrangement	Response to TKI	5, 8, 28, 30
	RET	Rearrangement	Response to TKI	15, 17
	ROS1	Rearrangement	Response to TKI	4, 8
	MET	Amplification	Resistance to EGFR inhibitors	5, 8, 16

Source: College of American Pathologists (CAP) Allison M. Cushman-Vokoun, MD, PhD

41

CAP Solid Tumor Selected Tests by Tumor Type

Breast Carcinoma				
	HER2/ERBB2	Amplification	Response to HER2 monoclonal antibodies	18, 51
Gastric Adenocarcinoma				
	HER2/ERBB2	Amplification	Response to HER2 monoclonal antibodies	45
Thyroid Carcinoma				
Papillary Thyroid Carcinoma / Anaplastic Thyroid Cancer	*BRAF*	p.V600E mutation	Preoperative FNA diagnosis and prognosis, potential therapeutic target	9, 36, 43
	NRAS, HRAS, KRAS	Mutation	Preoperative FNA diagnosis	36
	RET-PTC	Rearrangement	Preoperative FNA diagnosis	36
Follicular Thyroid Carcinoma	*NRAS, HRAS, KRAS*	Mutation	Preoperative FNA diagnosis	36
	PAX8-PPAR$_\gamma$	Rearrangement	Preoperative FNA diagnosis	36
Melanoma				
Cutaneous & Mucosal	*BRAF* codon 600	Mutation	Response to BRAF inhibitors	19–20, 33
	KIT	Mutation	Response to TKI	7
Uveal	*GNAQ* or *GNA11*	Mutation	Diagnostic	50
	Chromosome 3	Loss (monosomy)	Unfavorable prognosis	23
GIST				
	KIT	Mutation	Response to TKI	41
	PDGFRA	Mutation	Response to TKI	41
	BRAF p.V600E	Mutation	Possible imatinib resistance	1, 34

Source: College of American Pathologists (CAP) Allison M. Cushman-Vokoun, MD, PhD

42

CAP Solid Tumor Selected Tests by Tumor Type

CNS Neoplasms				
Glioma	*MGMT*	Promoter methylation	Favorable response to alkylating agents	21
	IDH1 and *IDH2*	Mutation	Distinguishes reactive gliosis from glioma, favorable prognosis	27, 54
Oligodendroglioma	Chromosome 1p and 19q	Co-deletion	Favorable prognosis and response to therapy	6, 22
Pilocytic Astrocytoma	*BRAF*	Duplication/ fusion and p.V600E mutation (extracere-bellar)	Diagnostic	27, 47
Pleomorphic Xanthoastrocytoma and Ganglioglioma	*BRAF*	p.V600E mutation	Diagnostic	47
Cholangiocarcinoma/Pancreatic Carcinoma				
	KRAS codons 12, 13, 61	Mutation	Preoperative bile duct brushing diagnosis	25
Oropharyngeal Squamous Cell Carcinoma				
	HR HPV-related	Positive detection	Favorable response to chemoradiation therapy	48

Source: Allison M. Cushman-Vokoun, MD, PhD

MSI = Microsatellite Instability; TKI = Tyrosine-Kinase Inhibitors; HR HPV= High-Risk Human Papillomavirus
This table is meant to be a list of selected tests and is not a comprehensive resource.

August 2016

Source: College of American Pathologists (CAP) Allison M. Cushman-Vokoun, MD, PhD

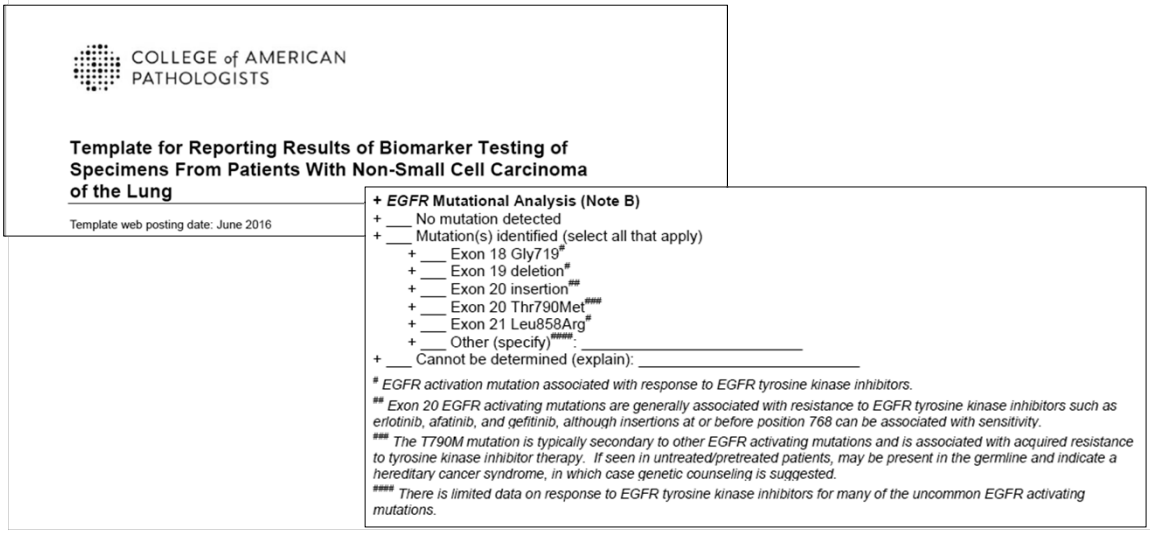
43

CAP Biomarker Checklists

CAP Checklist Available	CAP Checklist Available	CAP Checklist Available
Colon and Rectum	Breast	Chronic Lymphocytic Leukemia
Chronic Myelogenous Leukemia	Central Nervous System	Small Lymphocytic Lymphoma
Diffuse Large B Cell Lymphoma	GE Junction and Stomach	Endometrium
Lung	Melanoma	GIST – GI Stromal Tumor
Myeloproliferative Neoplasm	Myelodysplastic Syndrome	Thyroid

44

CAP Biomarker Checklists



COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Non-Small Cell Carcinoma of the Lung

Template web posting date: June 2016

+ *EGFR* Mutational Analysis (Note B)
+ ___ No mutation detected
+ ___ Mutation(s) identified (select all that apply)
  + ___ Exon 18 Gly719[#]
  + ___ Exon 19 deletion[#]
  + ___ Exon 20 insertion[##]
  + ___ Exon 20 Thr790Met[###]
  + ___ Exon 21 Leu858Arg[#]
  + ___ Other (specify)[####]: _____
+ ___ Cannot be determined (explain): _____

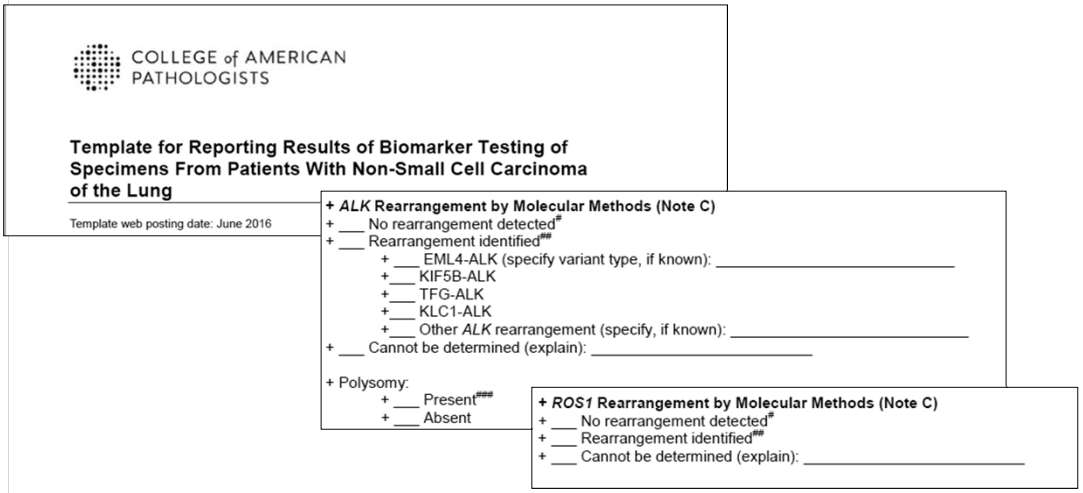
[#] *EGFR activation mutation associated with response to EGFR tyrosine kinase inhibitors.*

[##] *Exon 20 EGFR activating mutations are generally associated with resistance to EGFR tyrosine kinase inhibitors such as erlotinib, afatinib, and gefitinib, although insertions at or before position 768 can be associated with sensitivity.*

[###] *The T790M mutation is typically secondary to other EGFR activating mutations and is associated with acquired resistance to tyrosine kinase inhibitor therapy. If seen in untreated/pretreated patients, may be present in the germline and indicate a hereditary cancer syndrome, in which case genetic counseling is suggested.*

[####] *There is limited data on response to EGFR tyrosine kinase inhibitors for many of the uncommon EGFR activating mutations.*

CAP Biomarker Checklists



COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Non-Small Cell Carcinoma of the Lung

Template web posting date: June 2016

+ *ALK* Rearrangement by Molecular Methods (Note C)
+ ___ No rearrangement detected[#]
+ ___ Rearrangement identified[##]
  + ___ EML4-ALK (specify variant type, if known): _____
  + ___ KIF5B-ALK
  + ___ TFG-ALK
  + ___ KLC1-ALK
  + ___ Other *ALK* rearrangement (specify, if known): _____
+ ___ Cannot be determined (explain): _____

+ Polysomy:
  + ___ Present[###]
  + ___ Absent

+ *ROS1* Rearrangement by Molecular Methods (Note C)
+ ___ No rearrangement detected[#]
+ ___ Rearrangement identified[##]
+ ___ Cannot be determined (explain): _____

46

CAP Biomarker Checklists

COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Non-Small Cell Carcinoma of the Lung

Template web posting date: June 2016

+ *KRAS* Mutational Analysis
+ ___ No mutation detected
+ ___ Mutation(s) identified[#] (select all that apply)
  + Codon 12
  + ___ Gly12Cys (GGT>TGT)
  + ___ Gly12Asp (GGT>GAT)
  + ___ Gly12Val (GGT>GTT)
  + ___ Gly12Ser (GGT>AGT)
  + ___ Gly12Ala (GGT>GCT)
  + ___ Gly12Arg (GGT>CGT)
  + ___ Specific codon 12 mutation not stated
  + ___ Other codon 12 mutation (specify): _____
  + Codon 13
  + ___ Gly13Asp (GGC>GAC)
  + Gly13Arg (GGC>CGC)
  + ___ Gly13Cys (GGC>TGC)
  + ___ Gly13Ala (GGC>GCC)
  + ___ Gly13Val (GGC>GTC)
  + ___ Specific codon 13 mutation not stated
  + ___ Other codon 13 mutation (specify): _____
  + Codon 61
  + Gln61Leu (CAA>CTA)
  + ___ Specific codon 61 mutation not stated

47

CAP Biomarker Checklists

COLLEGE of AMERICAN PATHOLOGISTS

Template for Reporting Results of Biomarker Testing of Specimens From Patients With Non-Small Cell Carcinoma of the Lung

Template web posting date: June 2016

+ *BRAF* Mutational Analysis (Note A)
+ ___ No mutations detected
+ ___ *BRAF* V600E (c.1799T>A) mutation
+ ___ Other *BRAF* V600 mutation (specify): _____
+ ___ Cannot be determined (explain): _____

+ *ERBB2* Mutational Analysis (Note A)
+ ___ No mutations detected
+ ___ *ERBB2* 774_775insAYVM insertion mutation
+ ___ *ERBB2* 776_776G>VC insertion mutation
+ ___ Other *ERBB2* exon 20 mutation (specify):
+ ___ Other *ERBB2* mutation (non-exon 20) (specify): _____
+ Cannot be determined (explain):

+ *MET* Mutational Analysis (Note B)
+ ___ No mutation detected[#]
+ ___ *MET* D963_splice mutation detected[#]
+ ___ *MET* D1010N mutation detected[#]
+ ___ *MET* D1010_splice mutation detected[#]
+ ___ Other *MET* intron 13 mutation (specify)[#]: _____
+ ___ Other *MET* intron 14 mutation (specify)[#]: _____
+ ___ Other *MET* exon 14 mutation (specify)[#]: _____
+ ___ *MET* exon 14 deletion detected[##]
+ ___ Cannot be determined (explain): _____

[#] *MET mutation detection is typically based on DNA-based sequencing methods and may include coding (exon) or non-coding (intron) variants, most commonly located at or near the intron-exon junctions around exon 14.*

[##] *RNA-based reverse-transcriptase polymerase chain reaction or RNA sequencing may detect deletion of MET exon 14 without necessarily indicating the mechanism (DNA-based mutation) leading to the deletion.*

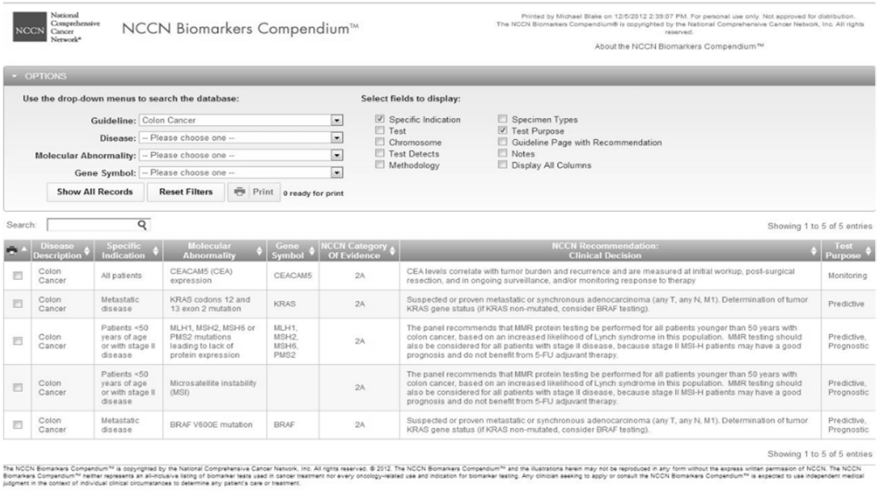
NCCN Biomarker Compendium

- Disease Description
- Specific Indication
- Molecular Abnormality
- Test – Name of Test
- Chromosome Location
- Gene Symbol (HUGO)
- Test Detects (property identified)
- Methodology (technique used)

Field	Definition	Example
Disease Description	Disease--as written from the guideline	Colon Cancer
Specific Indication	This field is included if the test is only specified for a certain patient population or diagnostic subset of the disease. For example: KRAS testing in Colon Cancer is recommended for metastatic synchronous adenocarcinoma, thus metastatic synchronous adenocarcinoma is the disease indication: specific.	Suspected relapse
Molecular Abnormality	Molecular abnormality-indicates the exact mutation or defect. Often similar to 'Test' column. Terminology for genes and proteins is: HUGO Gene Symbol (common name or other gene name used in guideline) plus problem or condition.	ERBB2 (HER2) protein overexpression
Test	Often similar to Molecular Abnormality column. Terminology for genes and proteins is: Gene name or common name as used in the guideline plus problem or condition.	Progesterone receptor (PR) expression
Chromosome	Chromosomal location of the gene(s) in question. Chromosomal location will be specified even if the test measures protein or other property. In the case of gross chromosomal abnormalities, terminology will, where possible, follow that of ISCN 2009: An International System for Human Cytogenetic Nomenclature, Shaffer et al, eds, or of the guideline.	t(9;22), t(12;22)(q13;q12), 13q12.3
Gene Symbol	HUGO gene symbol	CEACAM5
Test Detects	Indicates what property is assayed. This may or may not be the same as the molecular abnormality. For instance, the molecular abnormality may be a mutation in a certain gene, which results in the lack of expression of the protein encoded by that gene. A test in common use might be IHC, which detects protein expression, or it might be DNA sequencing, which detects the mutation. One from a list containing: Amplification • Chromosome gain • Protein expression • Deletion • Gene expression • Loss of heterozygosity • DNA methylation • Microsatellite instability • Mutation • Sequence variation • Translocation	mutation
Methodology	The technology used for testing, if specified in the guideline: IHC PCR Microarray Flow cytometry Cytogenetics, conventional FISH SNP chip/SNP analysis	Flow cytometry

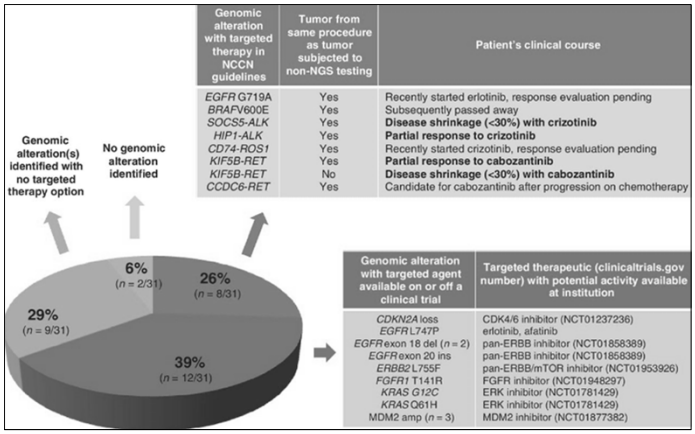
49

NCCN Biomarker Compendium



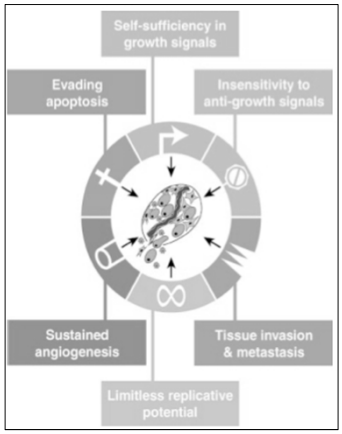
50

Next Generation Targeted Cancer Treatments



Genomic alteration with targeted therapy in NCCN guidelines	Tumor from same procedure as tumor subjected to non-NGS testing	Patient's clinical course
EGFR G719A	Yes	Recently started erlotinib, response evaluation pending
BRAF V600E	Yes	Subsequently passed away
SOCS5-ALK	Yes	**Disease shrinkage (<30%) with crizotinib**
HIP1-ALK	Yes	**Partial response to crizotinib**
CD74-ROS1	Yes	Recently started crizotinib, response evaluation pending
KIF5B-RET	Yes	**Partial response to cabozantinib**
KIF5B-RET	No	**Disease shrinkage (<30%) with cabozantinib**
CCDC6-RET	Yes	Candidate for cabozantinib after progression on chemotherapy

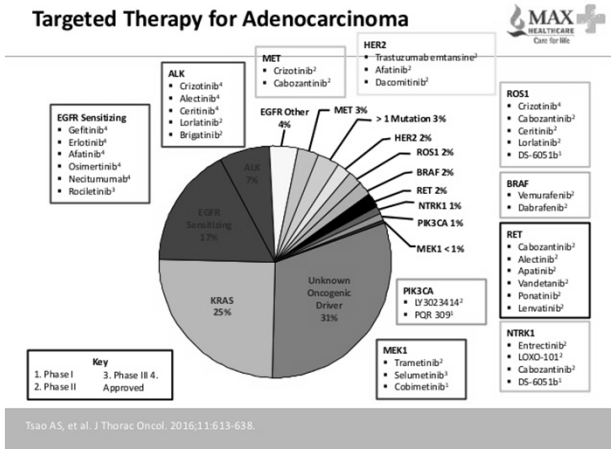
Genomic alteration with targeted agent available on or off a clinical trial	Targeted therapeutic (clinicaltrials.gov number) with potential activity available at institution
CDKN2A loss	CDK4/6 inhibitor (NCT01237236)
EGFR L747P	erlotinib, afatinib
EGFR exon 18 del (*n* = 2)	pan-ERBB inhibitor (NCT01858389)
EGFR exon 20 ins	pan-ERBB inhibitor (NCT01858389)
ERBB2 L755F	pan-ERBB/mTOR inhibitor (NCT01953926)
FGFR1 T141R	FGFR inhibitor (NCT01948297)
KRAS G12C	ERK inhibitor (NCT01781429)
KRAS Q61H	ERK inhibitor (NCT01781429)
MDM2 amp (*n* = 3)	MDM2 inhibitor (NCT01877382)



51

Next Generation Targeted Cancer Treatments

- Hormone Therapies
- Signal Transduction Inhibitors
- Gene Expression Modulators
- Apoptosis Inducers
- Angiogenesis Inhibitors
- Immunotherapies
- Monoclonal Antibodies
- Cancer Vaccines
- Gene Therapies



Tsao AS, et al. J Thorac Oncol. 2016;11:613-638.

52

Next Generation Targeted Cancer Treatments

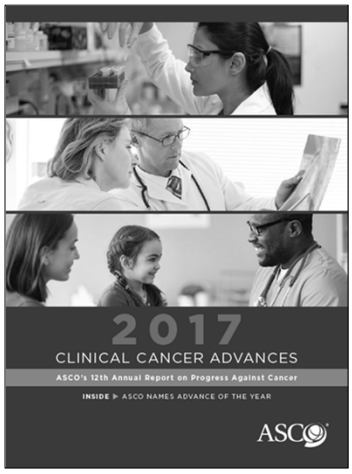



TABLE OF CONTENTS

To view this report online and learn more about progress against cancer, visit ASCO.org/CCA.
This report was also published in the *Journal of Clinical Oncology* at ascopubs.org/journal/jco on February 1, 2017.

Next Generation Targeted Cancer Treatments

- Neurosurgery at Maryland have genetically programmed a type of common cold virus (Adenovirus Delta-24-RGD) to attack glioblastoma multiforme.
- Targeting Cancer with Genetically Engineered Poliovirus (PVS-RIPO)
  - PVS-RIPO naturally infects almost all cancer cells – now genetically engineered version
  - PVS-RIPO naturally targets and destroys cancer cells from most common cancer types (pancreas, prostate, lung, colon, and many others)
  - PVS-RIPO kills cancer cells, but not normal cells, because its ability to grow (and kill) depends on biochemical abnormalities only present in cancer cells
  - The target for PVS-RIPO is also glioblastoma multiforme
- University of Pennsylvania using a deactivated HIV virus caused patients with acute lymphoblastic leukemia to go into remission. The T-cells become serial killer cells literally going from one tumor cell to the next to kill them.

54

Next Generation Targeted Cancer Treatments

- Checkpoint Inhibitors – Keytruda – "humanized" monoclonal antibodies
- PARP Inhibitors - PARP enzymes help repair damaged DNA. PARP inhibitors block this repair mechanism, causing some cancer cells to die.
- CAR-T Cells – immunotherapy using patient's own immune cells
- CRISPR/Cas9 – intentional gene sequence alteration – gene editing technique
- Cellular Immunotherapy – targeted immunotherapy
  - G12D is a Target for KRAS, NRAS, HRAS Mutant Cancers – mutations in KRAS gene
    - 95% of all pancreatic cancers
    - 50% of all colorectal cancers
  - Infusion of the patient's own Tumor Infiltrating Lymphocytes (TILs), called adoptive T cell transfer immunotherapy, to mediate effective antitumor immune responses against cancers that express the KRAS G12D mutation "presenting" the neoplasm as foreign to immune system
- Limitations: Managing Cytokine Release Syndrome (CRS) – CRS can be fatal

55

Next Generation Targeted Cancer Treatments

- Targeted Photoimmunotherapy - a novel type of cancer immunotherapy that uses infrared light to activate rapid and selective killing of cancer cells.
- Testing in head and neck cancers which overexpress EGFR.
- Near-infrared photoimmunotherapy uses an antibody–photoabsorber conjugate that binds to cancer cells.
- When near-infrared light is applied, the cells swell and then burst, causing the cancer cell to die.
- Limitation – deep tissue cannot be penetrated without light fiber implant or scope

56

Keytruda's Fast Track for Approvals

- **Pembrolizumab is approved to treat:**
  - **US Brand Name(s) – Keytruda**
  - **Type: Immune Checkpoint Inhibitor**
  - **FDA Approved - Yes**
- **FDA Approval for Pembrolizumab for Microsatellite Instability - High**
- **FDA Approval for Pembrolizumab for Urothelial Carcinoma**
- **FDA Approval for Pembrolizumab for Non-Small Cell Lung Cancer (2017)**
- **FDA Approval for Pembrolizumab for Hodgkin Lymphoma**
- **FDA Approval for Pembrolizumab for Non-Small Cell Lung Cancer (2016)**
- **FDA Approval for Pembrolizumab for Head and Neck Cancer**
- **FDA Approval for Pembrolizumab for Melanoma (2015)**
- **FDA Approval for Pembrolizumab for Non-Small Cell Lung Cancer (2015)**
- **FDA Approval for Pembrolizumab for Melanoma (2014)**

The process of mismatch repair enables cells to correct mistakes in their DNA code that sometimes occur during DNA replication. It's "like a spell-checker" for DNA, explained Dr. Gulley. Mismatch repair deficient (dMMR) cells, which lack this failsafe process, acquire multiple DNA mutations. Some dMMR cells acquire alterations in short, repetitive DNA sequences called microsatellites and are referred to as microsatellite instability-high (MSI-H).

57

Next Generation Precision Therapies Checkpoint Inhibitors

- Immune checkpoint inhibitors are drugs – often made of antibodies – that unleash an immune system attack on cancer cells.
- When immune system T-cells encounter another cell, they probe certain proteins on its surface, which serve as insignia of the cell's identity.
- If the proteins indicate the cell is normal and healthy, the T cell leaves it alone.
- If the proteins suggest the cell is infected or cancerous, the T cell will lead an attack against it.
- Once T cells initiate an attack, the immune system increases a series of additional molecules to prevent the attack from damaging normal tissues in the body.
- These molecules are known as immune checkpoints.
- Cancer cells use immune checkpoint molecules to trick the immune system such that the immune system identifies the cancer cells as normal cells with normal proteins on its surface, and leaves the cancer cell alone to reproduce and proliferate unfettered.
- Example: genomic alterations in 196 tumors related to development of Hepatocellular Carcinoma include mutations in the *TERT* gene promotor, mutations in the *TP53* and *CTNNB1* (β-catenin) genes, and elevated expression of several immune checkpoint genes. These are now therapies for therapeutic intervention using Checkpoint Inhibitors targeting TERT, TP53 and CTNNB1.

58

Checkpoint Inhibitors

ADVANCE OF THE YEAR:
IMMUNOTHERAPY 2.0
EXPANDING USE AND REFINING PATIENT SELECTION

This year, ASCO has named Immunotherapy 2.0 as the advance of the year. This selection recognizes the growing wave of progress using cancer immunotherapy, which has extended and improved the lives of patients, many of whom had few other effective treatment options.

It has taken scientists more than a century to learn how to harness the immune system to fight cancer. A number of strategies to achieve this have been tried, but one approach—blocking immune checkpoints—has been particularly effective against a range of different cancers. Immune checkpoints are specialized proteins that act as brakes on the immune system, ensuring that immune defenses are engaged only when they are needed and for as long as they are needed. They prevent the immune system from becoming overactive, which can lead to excessive inflammation or autoimmune disease.

CLINICAL CANCER ADVANCES 2017 9

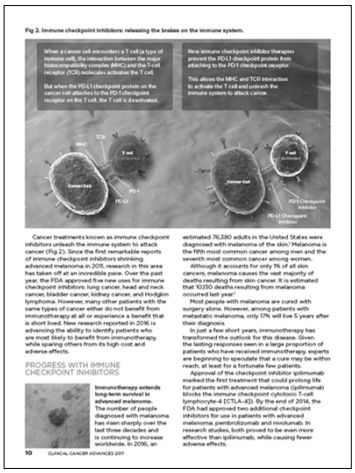


Fig 2. Immune checkpoint inhibitors: releasing the brakes on the immune system.

When a cancer cell encounters a T cell (a type of immune cell), the interaction between the major histocompatibility complex (MHC) and the T-cell receptor (TCR) molecules activates the T cell.

But when the PD-L1 checkpoint protein on the cancer cell attaches to the PD-1 checkpoint receptor on the T cell, the T cell is deactivated.

New immune checkpoint inhibitor therapies prevent the PD-L1 checkpoint protein from attaching to the PD-1 checkpoint receptor.

This allows the MHC and TCR interaction to activate the T cell and unleash the immune system to attack cancer.

MHC
TCR
T cell
Cancer Cell
PD-1
PD-L1
T cell activated
Cancer Cell
PD-1 Checkpoint Inhibitor
PD-L1 Checkpoint Inhibitor

Cancer treatments known as immune checkpoint inhibitors unleash the immune system to attack cancer (Fig 2). Since the first remarkable reports of immune checkpoint inhibitors shrinking advanced melanoma in 2011, research in this area has taken off at an incredible pace. Over the past year, the FDA approved five new uses for immune checkpoint inhibitors: lung cancer, head and neck cancer, bladder cancer, kidney cancer, and Hodgkin lymphoma. However, many other patients with the same types of cancer either do not benefit from immunotherapy at all or experience a benefit that is short lived. New research reported in 2016 is advancing the ability to identify patients who are most likely to benefit from immunotherapy, while sparing others from its high cost and adverse effects.

PROGRESS WITH IMMUNE CHECKPOINT INHIBITORS

Immunotherapy extends long-term survival in advanced melanoma. The number of people diagnosed with melanoma has risen sharply over the last three decades and is continuing to increase worldwide. In 2016, an estimated 76,380 adults in the United States were diagnosed with melanoma of the skin.[1] Melanoma is the fifth most common cancer among men and the seventh most common cancer among women.

Although it accounts for only 1% of all skin cancers, melanoma causes the vast majority of deaths resulting from skin cancer. It is estimated that 10,130 deaths resulting from melanoma occurred last year.[1]

Most people with melanoma are cured with surgery alone. However, among patients with metastatic melanoma, only 17% will live 5 years after their diagnosis.

In just a few short years, immunotherapy has transformed the outlook for this disease. Given the lasting responses seen in a large proportion of patients who have received immunotherapy, experts are beginning to speculate that a cure may be within reach, at least for a fortunate few patients.

Approval of the checkpoint inhibitor ipilimumab marked the first treatment that could prolong life for patients with advanced melanoma (ipilimumab blocks the immune checkpoint cytotoxic T-cell lymphocyte-4 [CTLA-4]). By the end of 2016, the FDA had approved two additional checkpoint inhibitors for use in patients with advanced melanoma: pembrolizumab and nivolumab. In research studies, both proved to be even more effective than ipilimumab, while causing fewer adverse effects.

10 CLINICAL CANCER ADVANCES 2017

59

Next Generation Precision Therapies CAR-T and CRISPR/CAS9

- **CAR-T = Chimeric Antigen Receptor T Cells**
  - Aim is to "reprogram" the immune system to target specific proteins on cancer cell
  - New Therapy Based on Anti-CD19 CAR-T cells
  - BCMA-targeted CAR T-Cells for Plasma Cell Myeloma
  - KTE-C19 for Diffuse Large B-Cell Lymphoma
  - CTL019 for Acute Lymphoblastic Leukemia
  - JCAR015 for Leukemia

- **CRISPR/CAS9 – Gene Editing Approach**
  - Specific Manipulation of the Defective Gene Sequence Targets Errant DNA in Cancer Cells
  - Intentional Break in Gene Sequence Used to Trigger Self-Repair Mechanism in Cellular DNA
  - Inject Genome-Edited Immune Cells into Patient – NSCLC
  - Gene Editing Approach more promising for solid tumors

60

Next Generation Precision Therapies PARP Inhibitors

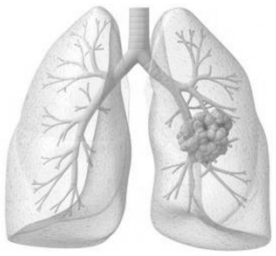
- PARP enzymes help repair damaged DNA. Below example is olaparib (Lynparaza) for glioma.
- PARP inhibitors block this repair mechanism, causing some cancer cells to die.
- How It Works - some cancer therapies, like chemotherapy and radiation, damage DNA. If the damage is not extensive, cells can often repair it and carry on. But if cells have defects in their ability to repair DNA, they may not be able to recover and die. Ex: BRCA1 & BRCA2 genes have reduced DNA-repair abilities.
- Cancer cells with DNA repair mechanism mutations, which in low grade glioma affect a gene called IDH, similar to BRCA1 or BRCA2 gene mutation, have an impaired ability to repair DNA damage. Treating these cells with PARP inhibitors, drugs that further disrupt DNA repair, effectively killed the cancer cells.
- IDH-mutant gliomas are more vulnerable to chemotherapy
- Amplifying this vulnerability enhanced toxic effects resulting in kill of more glioma cells.
- PARP inhibitors killed glioma cells with IDH mutations but not cells with normal IDH.
- PARP inhibitor enhanced the toxic effects of chemotherapy treatment on IDH-mutant cells
- Combining olaparib with temozolomide (alkylating chemo agent) enhanced DNA-damage and killing effects

61

Biomarkers & Genetic Abnormalities

Table 1.07 Major genetic changes in lung cancer

Alterations	Small cell carcinoma (%)	Adenocarcinoma (%)	Squamous cell carcinoma (%)
Mutation			
BRAF	0	< 5	0
EGFR Caucasian	< 1	10–20	< 1
Asian	< 5	35–45	< 5
ERBB2/HER2	0	< 5	0
KRAS Caucasian	< 1	15–35	< 5
Asian	< 1	5–10	< 5
PIK3CA	< 5	< 5	5–15
RB	> 90	5–15	5–15
TP53	> 90	30–40	50–80
Amplification			
EGFR	< 1	5–10	10
ERBB2/HER2	< 1	< 5	< 1
MET	< 1	< 5	< 5
MYC	20–30	5–10	5–10
FGFR1	< 1	< 5	15–25
Gene rearrangement			
ALK	0	5	< 1
RET	0	1–2	0
ROS1	0	1–2	0
NTRK1	0	< 1	0
NRG1	0	< 1	0



EMERGING TARGETED AGENTS FOR PATIENTS WITH GENETIC ALTERATIONS

Genetic Alteration (ie, Driver event)	Available Targeted Agents with Activity Against Driver Event in Lung Cancer
BRAF V600E mutation*	vemurafenib[1] dabrafenib[2]
MET amplification	crizotinib[3,4]
ROS1 rearrangements	crizotinib[5]
HER2 mutations	trastuzumab[6] (category 2B) afatinib[7] (category 2B)
RET rearrangements	cabozantinib[8] (category 2B)

*Non-V600E mutations have variable kinase activity and response to these agents.

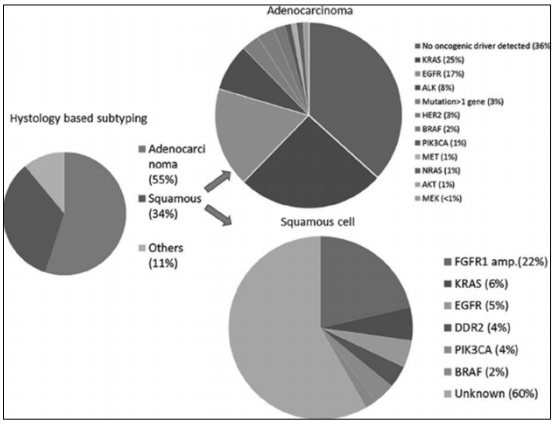
Source: WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart, 2015 and NCCN Guidelines NSCLCv7.2015

62

Biomarkers & Genetic Abnormalities

- Class of Antineoplastic Agents for NSCLC – Target Gene Therapy
  - EGFR – Opdivo/Nivolumab
  - EGFR – Tarceva/Erlotinib
  - EGFR – Gilotrif/Afatinib
  - EGFR – Iressa/Gefitinib
  - EGFR – Portrazza/Necitumumab
  - EGFR T790M – Tagrisso/Osimertinib

  - ALK – Opdivo/Nivolumab
  - ALK – Xalkori/Crizotinib
  - ALK – Zykadia/Ceritinib
  - ALK – Alecensa/Alectinib
  - ALK – Alunbrig/Brigatinib



63

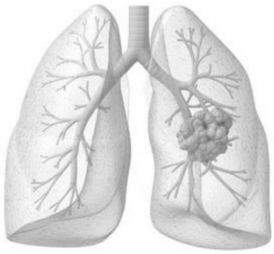
Biomarkers & Genetic Abnormalities

- Class of Antineoplastic Agents for NSCLC – Target Gene Therapy
  - BRAF V600E – Tafinlar/Dabrafenib)
  - BRAF V600E – Mekinist (Trametinib)
  - ROS1 – Xalkori (Crizotinib)

- Class of Antineoplastic Agents for NSCLC – Immunotherapy
  - PD-1 – Keytruda/Pembrolizumab
  - PD-L1 – Tecentriq/Atezolizumab

- Treatment Targets for NSCLC – Angiogenesis Inhibitors & Targets
  - Bevacizumab (Avastin)
  - VEGF Receptor Ramucirumab (Cyramza)

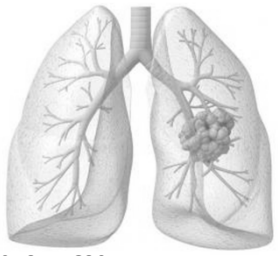
- Maintenance Therapy for NSCLC – Chemotherapy
  - Alimta/Pemetrexed - stable disease, partial/complete response s/p Platinum



64

Biomarkers & Genetic Abnormalities

- Class of Antineoplastic Agents for NSCLC – Target Gene Therapy – Future
  - HER2/ERBB2 – Trastuzumab – This is a protein not a mutant gene
  - MET – Crizotinib
  - MET – Cabozantinib
  - RET – Cabazantinib
  - RET – Vandetanib
  - RET – Alectinib



- Class of Antineoplastic Agents for NSCLC – Future
  - Molecular Testing – Next Generation Sequencing – Multiple Muta
  - FISH and IHC Improvements
  - Liquid Biopsy
  - Combination Trials

65

SEER*Rx

https://seer.cancer.gov/seertools/seerrx/

SEER*Rx Interactive Antineoplastic Drugs Database

immuno ✖ Search

Download Drugs | Download Regimens

Drugs (484) | Regimen (0)

Showing results 1–25 << < Prev Next > >>

▲ Relevance	Name	Category	Primary Site	Code?
	HIV-1 *Immuno*gen	AIDS drug		No
	Idiotype *immuno*.	Biologic therapy (BRM, *immuno*therapy)	Lymphoma	Yes
	LMB-7 *Immuno*toxin	Chemotherapy	Colorectal cancer	Yes
	Moxetumomab pasudotox	Biologic therapy (BRM, *immuno*therapy)		See Remarks
	LMB-1 *Immuno*toxin	Chemotherapy	Colorectal cancer	Yes
	LMB-9 *Immuno*toxin	Chemotherapy	Colorectal cancer	Yes
	*Immuno*toxin BL22	Chemotherapy	Leukemia, lymphoma	Yes
	LHRH *Immuno*therapeutic	Biologic therapy (BRM, *immuno*therapy)	Prostate cancer	Yes
	anti-Tac(Fv)-PE38 (*immuno*toxin)	Biologic therapy (BRM, *immuno*therapy)	Leukemia	Yes
	Daclizumab	Biologic therapy (BRM, *immuno*therapy)		Yes
	Lentinan	Biologic therapy (BRM, *immuno*therapy), AIDS drug	AIDS, Opportunistic infection	Yes
	Biostim	Biologic therapy (BRM, *immuno*therapy)		Yes

66

SEER*Rx

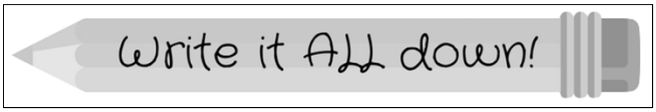
https://seer.cancer.gov/seertools/seerrx/

	*Immuno*toxin BL22	Chemotherapy	Leukemia, lymphoma	Yes
	LHRH *Immuno*therapeutic	Biologic therapy (BRM, *immuno*therapy)	Prostate cancer	Yes
	anti-Tac(Fv)-PE38 (*immuno*toxin)	Biologic therapy (BRM, *immuno*therapy)	Leukemia	Yes
	Daclizumab	Biologic therapy (BRM, *immuno*therapy)		Yes
	Lentinan	Biologic therapy (BRM, *immuno*therapy), AIDS drug	AIDS, Opportunistic infection	Yes
	Biostim	Biologic therapy (BRM, *immuno*therapy)		Yes
	ICT-107	Biologic therapy (BRM, *immuno*therapy)	Brain	Yes
	Panitumumab	Biologic therapy (BRM, *immuno*therapy)	Colorectal, kidney, lung, prostate cancer	Yes
	Bovine *Immuno*globulin Concentrate, Cryptosporidium parvum	AIDS drug		No
	Ubenimex	Biologic therapy (BRM, *immuno*therapy)		Yes
	Multikine (Leukocyte Interleukin Injection)	Biologic therapy (BRM, *immuno*therapy)	head & neck cancer, Prostate	Yes
	TP OKT 3 Lymphocytes	Ancillary Agent, Biologic therapy (BRM, *immuno*therapy)		No
	Trastuzumab	Biologic therapy (BRM, *immuno*therapy)	Breast, colorectal, lung, ovarian, pancreatic, prostate cancer	Yes
	DCVax-Lung	Biologic therapy (BRM, *immuno*therapy)	Lung cancer	Yes
	Levamisole	Biologic therapy (BRM, *immuno*therapy)		Yes
	Pertuzumab	Biologic therapy (BRM, *immuno*therapy)	Breast	Yes
	BCG	Biologic therapy (BRM, *immuno*therapy)		Yes
	Bectumomab	Biologic therapy (BRM, *immuno*therapy)		Yes
	Alemtuzumab	Biologic therapy (BRM, *immuno*therapy)	Leukemia, lymphoma	Yes

67

Text Documentation Reminder

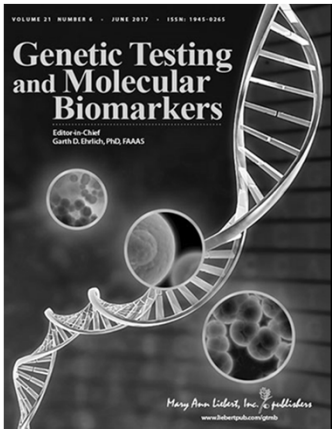
- Dates
- CT Scans
- Screening
- Tumor Size – clinical and pathological
- Nodal Status – clinical and pathological
- All Metastatic Sites
- Results of Genetic Profile – what is positive and what marker studies were performed
- Specific Agents for Chemotherapy
- Specific Agents for Targeted Therapies
- Radiation Fields and Dosage



- ALL Surgical Procedures to Primary Site
- ALL Surgical Procedures to Lymph Nodes
- Caution: Do not code Surgery to Other Regional or Distant Sites unless cancer-related.
- When assigning post-treatment stage be very cautious that patient meets criteria for *yp.*
- *This year we do not collect yc – perhaps next yr*

68

Resources



Genetic Testing and Molecular Biomarkers is the leading peer-reviewed journal covering all aspects of human genetic testing including molecular biomarkers. The Journal provides a forum for the development of new technology; the application of testing to decision making in an increasingly varied set of clinical situations; ethical, legal, social, and economic aspects of genetic testing; and issues concerning effective genetic counseling. This is the definitive resource for researchers, clinicians, and scientists who develop, perform, and interpret genetic tests and their results.

Genetic Testing and Molecular Biomarkers **coverage includes:**

- Diagnosis across the life span
- Risk assessment
- Carrier detection in individuals, couples, and populations
- Novel methods and new instrumentation for genetic testing
- Results of molecular, biochemical, and cytogenetic testing
- Genetic counseling

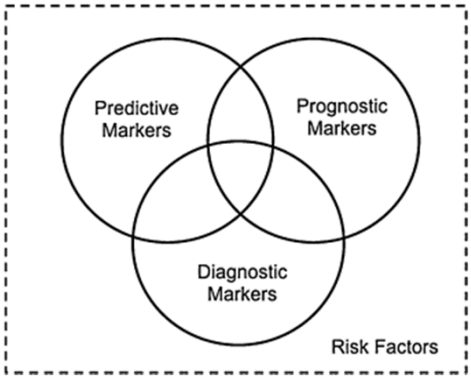
69

Resources

- College of American Pathologists – CAP Biomarker Checklists
- NCCN Biomarker Compendium
- CAP Solid Tumor Selected Tests by Tumor Type - College of American Pathologists (CAP) A. M. Cushman-Vokoun, MD, PhD
- The Genome Atlas Project and The NCI Cancer Genomic Data Commons
- Types of Molecular Tumor Testing/Immunotherapy/cDNA – mycancergenome.org
- A "Fourth-Generation" DNA Base Editor Could Replace CRISPR; 9/4/2017; https://futurism.com/a-fourth-generation-dna-base-editor-could-replace-crispr/
- Collecting Genomic Data in Cancer Surveillance: Rationale and Results – Standford
- The Pathologist in the Era of Genomic Medicine – PathGroup - College of American Pathology (CAP)
- ASCO 2017 Clinical Cancer Advances
- **American Association for Cancer Research (AACR), June 5, 2017 - doi: 10.1158/0008-5472.CAN-17-0758**
- College of American Pathologists – Dr Richard Moldwin – Explosion in Cancer Data: What to do?
- WHO/IARC Classification of Neoplasms Series Updates and Revisions (2012-2016)

70

Questions



71