Molecular Profiling

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Objectives

- Defining molecular profiling
- Technologies
- Why do we profile tumors?
- Current testing & limitations
- Future directions

What is Molecular Profiling?

- "The classification of tissue or other specimens for diagnostic, prognostic, and predictive purposes based on multiple gene expression….., is a technology that holds major promise for optimizing the management of patients with cancer"
- Utilizes biomarkers
  - a measurable indicator of the severity or presence of some disease state
- Utilizes multiple testing modalities

http://theoncologist.alphamedpress.org/content/12/3/301.full

Biomarker utility can be context dependent (or not)

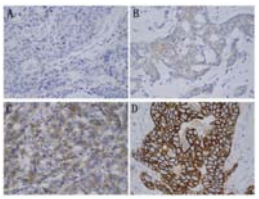
- Traditional paradigm of specific biomarkers within diseases/conditions that can help diagnose, select treatment, or provide prognostic information
  - Lung CA: *EGFR, ALK, ROS1, etc.*
  - *Biomarkers for one disease can be useless in another*
- Paradigm being challenged by biomarkers that may be more generally predictive of therapeutic response
  - *NTRK1,2,3*
  - MSI/MMR
  - PD-1/PD-L1

Why do we need to use multiple testing modalities?

- Biomarker detection testing: nucleic acids, proteins, epigenetic changes
- Different tests can have variations in
  - Sensitivity
  - Specificity
  - Clinically relevant limits of detection
  - Specimen types
  - Specimen needs
- Understanding what information each specific testing modality and individual test can and cannot detect is essential

What are Molecular Profiling technologies?

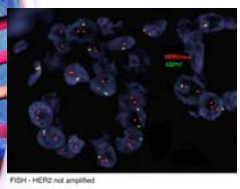
- Immunohistochemistry (IHC)

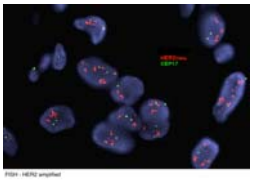


Ji H, Xuan Q, Nanding A, Zhang H, Zhang Q. The Clinicopathologic and Prognostic Value of Altered Chromosome 17 Centromere Copy Number in Breast Cancer: Fish Equivocal Breast Carcinomas. Wikman H, ed. *PLoS ONE*. 2015;10(7):e0133924. doi:10.1371/journal.pone.0133924.

What are Molecular Profiling technologies?

- In-situ Hybridization (CISH/FISH): detects gene deletions, amplifications, translocations and fusions





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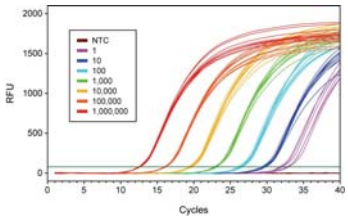
What are Molecular Profiling technologies?

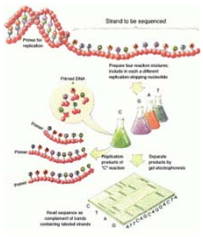
- Quantitative Polymerase Chain Reaction (qPCR): amplifies and quantifies a targeted DNA molecule



http://couragene.com/products/real-time-pcr/

What are Molecular Profiling technologies?

- Sanger Sequencing
  - Sequencing by incorporation of dideoxynucleotides



Wikipedia.org

What are Molecular Profiling technologies?

- Pyro Sequencing (PyroSeq)
  - Sequence small DNA sequences
  - DNA methylation- epigenomics

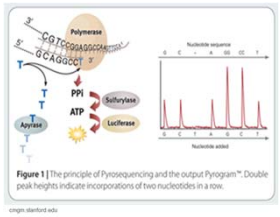


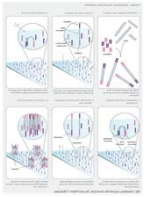
Figure 1 | The principle of Pyrosequencing and the output Pyrogram™. Double peak heights indicate incorporations of two nucleotides in a row.

cmgm.stanford.edu

What are Molecular Profiling technologies?

- Next-Generation Sequencing (NGS)
- Rapidly examines and more broadly detects DNA mutations, copy number variations and gene fusions across the genome
  - Informatics is key

Illumina



Variations of advanced sequencing

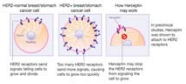
- Targeted sequencing
  - Most common NGS testing
  - Can be one gene or a panel of genes
  - 1000's of amplicons
- Whole exome sequencing (WES)
  - Targeted sequencing- hundreds of thousands of probes
  - 1.5% of genome
- Whole genome sequencing (WGS)

Ok, enough pathology…why do we care about molecular profiling?

- Multiple reasons
- Not possible to do a deep dive on each of these, but will use a specific example to illustrate utility

Initially to guide treatment selection

- Breast Cancer
  - Her2/neu (ERBB2) Transtuzumab
  - The first field in which molecular profiling has been approved and reimbursed for clinical use
  - FDA approved to treat her2/neu expressing metastatic breast cancers in September 1998
  - ER/PR
- Lung
  - EGFR
  - IHC vs molecular



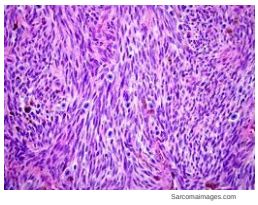
https://www.accessdata.fda.gov/drugsatfda_docs/appletter/1998/trasgen092598L.pdf

Still used to guide treatment selection

- *EGFR* resistance
  - EGFR resistance develops frequently
  - EGFR T790M detection can help clinician decide
    - *When to switch therapy*
    - *Which therapy may be best choice*
  - Multiple testing options
    - *FFPE*
    - *Cell-free circulating DNA*
    - *FNA*
    - *Others*

Diagnose a specific neoplastic process

- MPN - *JAK2*, *CALR*
- Synovial sarcoma
  - t(X;18)(p11;q11)
  - SS18-SSX1
  - SS18-SSX2
  - SS18-SSX4



Sarcomaimages.com

Identify clinically relevant mutations

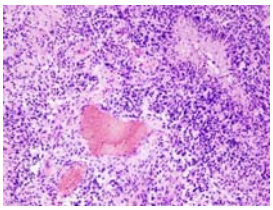
Biomarkers Significant for Study and Treatment of Hematologic Cancers

Chromosome and Gene Abbreviations	Associated Cancer	Treatment Correlation
Philadelphia chromosome t(9;22) (translocation between chromosomes 9+22)	Chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL)	Responds to imatinib (Gleevec®), dasatinib (Sprycel®), nilotinib (Tasigna®)
IDH2 *(R140 or R172)*	Acute myeloid leukemia (AML)	Responds to enasidenib (Idhifa®)
JAK2 V617F	Myeloproliferative neoplasms (MPNs): polycythemia vera (PV), myelofibrosis (MF), essential thrombocythemia (ET)**	Responds to ruxolitinib (Jakafi®)
PML-RARA	Acute promyelocytic leukemia (APL)	Responds to *all-trans* retinoic acid (ATRA), arsenic trioxide (Trisenox®)
FLT3-ITD	Acute myeloid leukemia (AML)	Responds to midostaurin (Rydapt®)
ALK rearrangement	Anaplastic large-cell lymphoma (ALCL)	Responds to crizotinib (Xalkori®)*
BRAF V600E	Hairy cell leukemia	Responds to vemurafenib (Zelboraf®)*

Leukemia and Lymphoma society. Cancer Molecular Profiling #31. https://www.lls.org/sites/default/files/National/USA/Pdf/Publications/FS31_Cancer_Molecular_Profiling.pdf

Provide predictive and/or prognostic information

- IDH1 mutations
  - Strong predictor of a better prognosis in glioblastoma
  - Specific marker of secondary glioblastomas



Pathologyoutlines.com

Nobusawa S, Watanabe T, Kleihues P, Ohgaki H. IDH1 mutations as molecular signature and predictive factor of secondary glioblastomas. Clin Cancer Res. 2009 Oct 1;15(19):6002-7.

Follow progression of disease

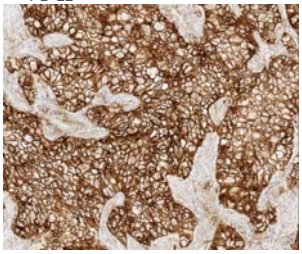
- Chronic myeloid leukemia (*BCR-ABL*) resistance
  - Patient is non-responsive to TKI therapy
  - Change in hematologic or cytogenetic remission
  - Change in BCR-ABL transcript, loss of major molecular remission
  - Progression to accelerated or blast phase

National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: Chronic myeloid leukemia.

Cortes J, Jabbour E, Kantarjian H, et all. Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with Multiple tyrosine kinase inhibitors. Blood 2007;110: 4005-4011

Determine eligibility for immuno-oncology drugs

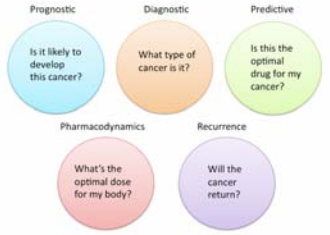
PD-L1



https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013S009a.pdf

Discover new biomarkers

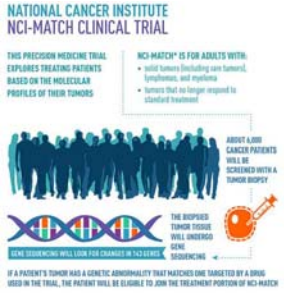
Questions that can be answered by cancer biomarkers



Liu H, Li F, Zhu Y, Li T, Huang H, Lin T, Hu Y, Qi X, Yu J, Li G. Whole-exome sequencing to identify somatic mutations in peritoneal metastatic gastric adenocarcinoma: A preliminary study. Oncotarget. 2016 Jul 12;7(28).
Wikipedia.org

Identify eligible patients for clinical trials

Foundation Medicine, Caris Assays Identify Patients for NCI-MATCH
JUNE 9, 2017



https://www.clinicalomics.com/articles/foundation-medicine-caris-assays-identify-patients-for-nci-match/1125

Biomarker-driven clinical guidelines

Clinical Significance of DNA Variants in Chronic Myeloid Neoplasms

A Report of the Association for Molecular Pathology

Rebecca F. McClure,[*†] Mark D. Ewalt,[*‡] Jennifer Crow,[*‡] Robyn L. Temple-Smolkin,[¶] Mrudula Pullambhatla,[¶] Rachel Sargent,[*‡] and Annette S. Kim[*;**]

sequencing remains critical for patient management. The following genes are a minimum recommended list to provide relevant clinical information for the management of most CMNs: *ASXL1, BCOR, BCORL1, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PHF6, PPM1D, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1,* and *ZRSR2*. This list is not comprehensive for all myeloid neoplasms and will evolve as insights into

And many more (CRC, Lung, Hemeonc…)

McClure RF, Ewalt MD, Crow J, Temple-Smolkin RL, Pullambhatla M, Sargent R, Kim AS.Clinical Significance of DNA Variants in Chronic Myeloid Neoplasms: A Report of the Association for Molecular Pathology. J Mol Diagn. 2018 Aug 20.

Common current testing – traditional biomarker concept

- Esophageal/ Gastric adenocarcinoma
  - Her2
- Lung Cancer
  - EGFR, BRAF, ALK, ROS1, etc..
- Colorectal Cancer
  - KRAS, Extended RAS
- Brain Cancer
- Head and Neck Squamous cell carcinomas
  - PDL-1
- Examples, not all inclusive list

Tumor type agnostic biomarkers

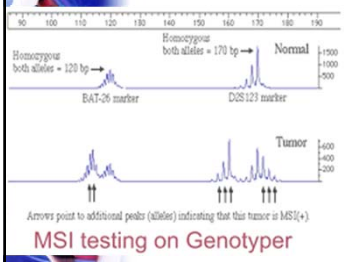
- Biomarkers that may be more generally predictive of poor prognosis or therapeutic response are being discovered / utilized
  - MMR / MSI
  - Tumor mutational burden
  - *NTRK1,2,3*
  - PD-1 / PD-L1

MSI / MMR

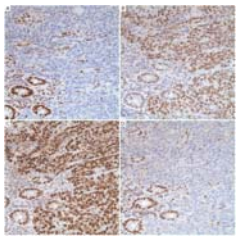
- "Pembrolizumab (Keytruda®), Merck's anti-programmed cell death-1 (PD-1) monoclonal antibody (mAb), received accelerated approval in May 2017 by the US Food and Drug Administration for the treatment of adult and pediatric patients with unresectable or metastatic solid tumors that have been identified as having microsatellite instability-high (MSI-H) or deficient DNA mismatch repair (dMMR)".

Yan L, Zhang W. Precision medicine becomes reality—tumor type-agnostic therapy. *Cancer Communications*. 2018;38:6. doi:10.1186/s40880-018-0274-3

MSI / MMR



MSI testing on Genotyper



lynchscreening.net
Pathologyoutlines.com

Tumor mutation burden

- Quantitative biomarker used to predict sensitivity to checkpoint inhibitor therapy
  - PD-L1 expression does not always predict response to immunotherapy agents
  - Increased number of gene mutations may incite a stronger anti-tumor immune response to immunotherapy
  - Low, intermediate, and high compared with reference median genomic TMB
    - *Currently no consensus on reporting*

Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Medicine*. 2018;7(3):746-756. doi:10.1002/cam4.1372.

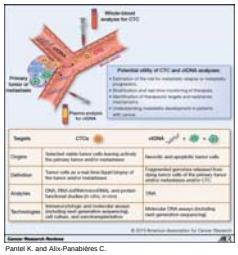
Fabrizio DA, George TJ, Dunne RF, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. *Journal of Gastrointestinal Oncology*. 2018;9(4):610-617. doi:10.21037/jgo.2018.05.06.

Rapidly advancing area

- Technology advancing literally every day
- Many new biomarkers transitioning into clinical use with
  - Emerging / limited published literature
  - Non-standardized detection methods
  - Bioinformatics challenges
- Important to understand that there are challenges to existing technologies that can impact this clinical service

Limitations on current molecular profiling tests

- Liquid biopsies
  - Guardant360 - Guardant Health Inc
  - PlasmaSELECT-R64 from Personal Genome Diagnostics



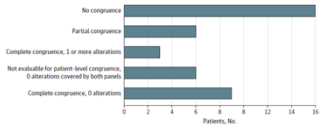
Pantel K. and Alix-Panabières C.
Real-time Liquid Biopsy in Cancer Patients: Fact or Fiction? Cancer Research. November 2013

Limitations on current molecular profiling tests

RESEARCH LETTER

Patient-Paired Sample Congruence Between 2 Commercial Liquid Biopsy Tests

Figure 2. Congruence Analysis of cfDNA-Targeted Next-Generation Sequencing in 2 Independent Commercial Platforms



Patients negative for cell-free DNA (cfDNA) alterations in both tests were classified as complete congruence for 0 alterations (9/40 [22.5%]). For congruence analysis, patients who had 1 or more alterations reported, but none was covered by both tests, were excluded and classified as not evaluable for patient-level congruence (6/40 [15%]). The proportion of patients with complete congruence for 1 or more alterations, partial, and no congruence was 3 of 40 (7.5%), 6 of 40 (15%), and 16 of 40 (40%), respectively, among the 2 platforms.

Pantel K. and Alix-Panabières C.
Real-time Liquid Biopsy in Cancer Patients: Fact or Fiction? Cancer Research. November 2013

ASCO/CAP Liquid Biopsy Tests in People with Cancer: An Expert Review
More Evidence Needed to Establish Effective and Appropriate Use in the Clinic
March 2018

Much more to this story that we still have not discovered

Clinical impact of extensive molecular profiling in advanced cancer patients

Sophie Cousin[1,2], Thomas Grellety[2], Maud Toulmonde[1,2], Céline Auzanneau[3], Emmanuel Khalifa[3], Yechan Laizet[4], Kevin Tran[4], Sylvestre Le Moulec[1,2], Anne Floquet[2], Delphine Garbay[2], Jacques Robert[3], Isabelle Hostein[3], Isabelle Soubeyran[3] and Antoine Italiano[1,2*]

phase trials. The treatment was matched with a tumour profile in 86 cases (15%). T
non-inclusion were non-progressive disease (31.5%) and general status deteriorati

Cousin *et al. Journal of Hematology & Oncology* (2017) 10:45
DOI 10.1186/s13045-017-0411-5

Not all challenges are technical

- N of 1
- Failed studies not published
- Standardization of testing, reporting and informatics among providing laboratories
- Integrative reporting

Future directions

- Constant improvement on providing evidence-based panels
- Improving technologies
- In house testing
- Better informatics and decision support tools
- Combination immunotherapy profiles
- Further subcategorization of tumors
- Additional clinical utility establishment
- New technologies (multiple more –omics)

My office

"just meet me at the pylons"