Clinical Applications of Molecular testing in Oncology and Hematology

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Molecular Diagnosis in Hematologic Malignancies.

Diagnostic Molecular Pathology

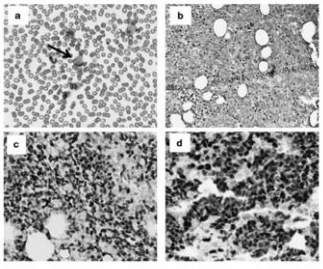
- USED AS STANDARD OF CARE FOR
- Risk identfication
  - Diagnosis
  - Prognosis
  - Prediction of response to therapy
  - Montoring therapeutic responses

- Histology/ Morphology
  - What the cells look like
- Immunohistochemistry (IHC)
  - Staining the cells to identify specific markers
- Flow cytometry
  - Looks at individual cells based on staining for specific markers
- Cytogenetics
  - Chromosome analysis
- FISH
  - Targeting specific chromosomes
- Molecular studies
  - Identifying abnormal gene products

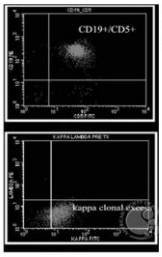
Morphology and IHC

ALL with blasts in the peripheral blood (a) and marrow (b).

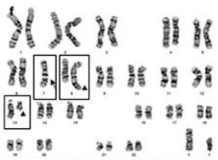
IHC documents the blasts are positive for TdT (c) and PAX-5 (d)



Flow and Cyto

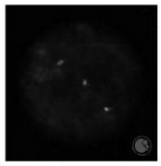


Clonal population of B- cells expressing CD19 and CD5 and kappa restriction

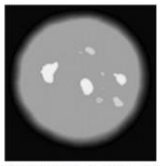


Conventional cytogenetics showing monosomy 7 and t(8;13)(q24.3;q14)

FISH



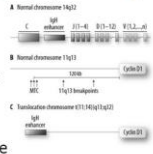
Red signal: ABL gene on a normal chromosome 9
Green signal: BCR on a normal chromosome 22
Yellow (combined): BCR/ABL fusion on the Philadelphia chromosome t(9;22)



Yellow signal: Trisomy 12 in a patient with CLL

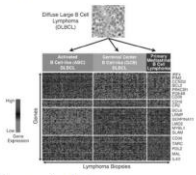
Polymerase Chain Reaction

- Method to rapidly and highly specifically amplify DNA fragments
- Advantages
  - Common, fairly inexpensive
  - Rapid, sensitive and specific
- Disadvantages
  - Requires knowledge of the specific nucleotide sequence
  - Sensitivity may result in false-positive results



Other Techniques

- Gene Expression Profiling
  - Microarray technology to identify a molecular signature of a tumor
- Proteomics
  - Microarray technology to identify protein expression profiles of tissue/cell type



Purpose of Molecular Tests

- Diagnostic accuracy
- Prognostic markers to predict outcomes
- Monitor for minimal residual disease

DIAGNOSTIC ACCURACY

Translocations w/o gene fusion

Tumor	Translocation	Activated Gene	Mechanism of Activation
B-All/Burkitt	t(8;14)(q24;q32)	MYC	Relocation to IgH locus
Large Cell Lymphoma	t(3;14)(q27;q32)	BCL6	Relocation to IgH locus
Mantle Cell Lymphoma	t(11;14)(q13;q32)	Cyclin D1	Relocation to IgH locus
Follicular B-cell lymphoma	t(14;18)(q32;q21)	BCL2	Relocation to IgH locus
T-cell ALL	t(8;14)(q24;q11)	MYC	Relocation to TCR α/δ locus
T-cell ALL	t(1;14)(p32;q11)	TAL1	Relocation to TCR α/δ locus

Translocations w/fusion product: hematologic tumors

TUMOR	Translocation	Gene fusion
Chronic myelogenous leukemia	t(9;22)	BCR-ABL(p210)

Acute promyelocytic leukemia	t(15;17);
	t(11;17)(q23;q21);
	t(5;17) (q35;q21);
	t(11;17)(q13;q21)
	der(17)
		PML-RAR
		PLZF-RAR
		NPM-RAR
		NUMA-RAR
		STAT5b-RAR

AML	t(8;21)(q22;q22)	AML1-ETO
AML and ALL (esp. infants and post-Rx)	11q23	MLL-(~30 partners)
Anaplastic large cell lymphoma (pediatric)	t(2;5)(p23;q35)	NPM-ALK
ALL	t(9;22)	BCR-ABL(p190)
MALT lymphoma	t(11;18)	API2-MLT

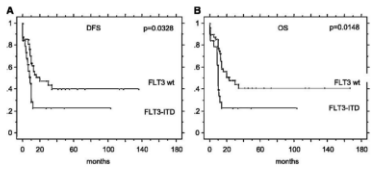
Translocations w/chimeric products: solid tumors

Tumor	Translocation	Product
Ewing's Sarcoma	t(11;22); t(21;22); t(7;22); t(12;22)	EWS/FLI1; EWS/ERG; EWS/ETV1; EWS/ETV4
Alveolar Rhabdomyosarc.	t(1;13); t(2;13)	PAX7/FKHR; PAX3/FKHR
Synovial sarcoma	t(X;18)	SYT/SSX1
DSRCT	t(11;22)	EWS/WT1
Myxoid/round cell liposarcoma	t(12;22)	CHOP/FUS
Clear cell sarcoma soft parts	t(12;22)	EWS.ATF-1
Extraskeletal myxoid chondrosarc	t(9;22)	EWS/TEC

PROGNOSTIC MARKERS TO PREDICT OUTCOMES

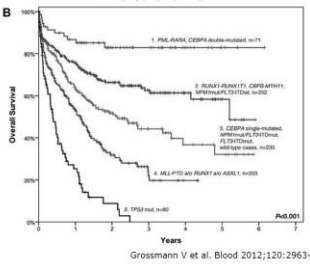
Prognostication

- Normal karyotype AML with or without Flt3-ITD mutation



Bienz M et al. Clin Cancer Res 2005;11:1416-1424

AML Model based on molecular mutations



Grossmann V et al. Blood 2012;120:2963-2972

CEBP-α

- On chromosome 19q
- Normal function: Transcription factor for maturation of granulocytes
- Mutated in 15 – 20% of patients with AML
- Improved outcomes for patients with this mutation, independent of other mutations

Flt3

- Chromosome 13q
- Normal function: tyrosine kinase that is important for proliferation and differentiation of hematopoietic progenitor cells
- Mutated in 30 – 40% of AML patients
  - ITD, D835 point mutation, overexpression without mutation
- Uncontrolled proliferation leads to inferior overall and disease-free survival

NPM1

- On chromosome 5q
- Normal function: controls genomic stability
- Mutation in 50 – 60% AML
  - Either insertion or deletion
  - Increased in women
- Sole mutation present, improved outcomes
  - Outweighed by other negative mutations like FLT3

MLL

- On chromosome 11q
- Normal function: encodes enzyme that regulates homeostasis
- Mutation in 7 – 8% of AML patients as a partial tandem duplication
- Decreases overall survival

IDH1 and IDH2

- IDH1 on Chromosome 2q
- IDH2 on Chromosome 15q
- Normal function: critical to the Krebs cycle
- Mutations in 15 – 30% AML patients
- Results in increased expansion of HSCs and impaired differentiation

BCL-2

- On chromosome 18q
- Normal function: inhibit apoptosis and modulates cell cycle progression
- In Burkitt's lymphoma, moves upstream of IgH t(14;18)
- Overexpression leads to prolonged cell survival

BCL-6

- On chromosome 3q
- Normal function: represses transcription
- Often overexpressed in DLCL
- Mutation leads to increased proliferation

TP53

- On chromosome 17p
- Tumor suppressor that prevents uncontrolled cell growth
- Mutation of 17p found in <u>many</u> cancers
  - CLL, DLCL, solid cancers

MONITOR FOR RESIDUAL DISEASE AFTER TREATENT

WAS TREATMENT SUCCESSFUL?

Routinely checked before and after treatment.

- AML: CEBP-α, FLT3-D835 point mutation, FLT3-ITD mutation, IDH1, IDH2, NPM1, MLL
- ALL: BCR/ABL, TEL-AML/AML1
- MDS: ASXL1, JAK2, ETV6, EZH2, P53, RUNX1
- Lymphoma: BCL-1 (CCND1), BCL-2, BCL-6, IgH, TCR

WHERE DOES THIS ALL FIT IN?

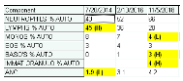
CASES FROM MY PRACTICE

CASE 1

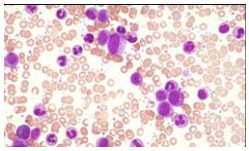
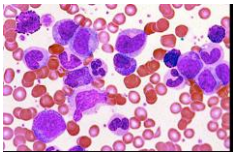
- 52 yr old male. H/O hyperlipidemia, HTN, bipolar disorder, back injury
- Presented in Sep 2018, with 3 wk h/o dizziness, fatigue, abdominal pain.
- Exam: T 98.4, BP 123/65, Pulse 73, BMI 22, Spleen tip palpable.

- Basename Value Date/Time
- • WBC COUNT 189.7 09/26/2018
- • HGB 11.1 09/26/2018
- • HEMATOCRIT 36.0 09/26/2018
- • PLATELET COUNT 179 09/26/2018
- • PLATELETS,BLD,QL, MAN CT CONFIRMD 09/26/2018

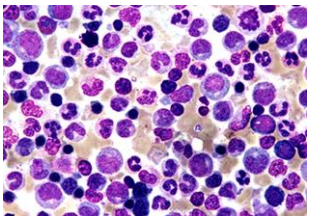
CASE 1



CASE 1 WORK UP

CASE 1 WORK UP



CASE 1 WORK UP; Bone marrow biopsy report

- The peripheral blood smear is not available for the evaluation.

The biopsy shows fragments of crushed bone with little bone marrow elements. The bone marrow demonstrates about 80% cellularity marrow
with marked myeloid proliferation. The reticulin stain is not contributory due to scant amount of marrow elements.

The clot sections demonstrate hypercellularity bone marrow (about 100%) with marked myeloid proliferation.

The aspirate is satisfactory for the evaluation. The myeloid blasts
are less than 1%. There is marked myeloid hyperplasia (M:E ratio about 39:1) and left shift. The megakaryocytes appear decreased in
number on the aspirate smears. Dwarf megakaryocytes are observed. The iron is present and no ring sideroblasts are identified

CASE 1 WORK UP; Bone marrow biopsy report

FLOW CYTOMETRY

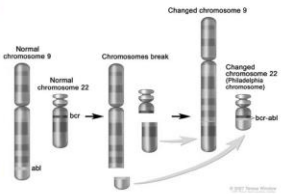
- A population of myeloid blasts accounts for approximately 0.7% of
- the acquired events. The blasts show equivocal partial expression
- of CD7, which if confirmed is an aberrant finding. Basophils
- account for approximately 2% of events. Correlation with the bone
- marrow and cytogenetic findings is suggested.

CYTOGENETICS and FISH

- Method: GTW FISH
- Number of cells/colonies: 20 Band Level: 350
- Karyotype: 45-46,XY,t(9;22)(q34;q11.2)[cp20].nuc
- ish(ABL1,BCR)x3(ABL1 con BCRx2)[89/100]
- FISH: Positive for Philadelphia chromosome
- translocation in 89% (89/100) of cells

BCR/Abl

- Fusion protein that results in increased activity of a tyrosine kinase
- Present in CML, ALL (30 -35% adult B-cell), and some AML



- Can be followed quantitatively with a Major Molecular Response (MMR) determined as ≤ 0.1% BCR-ABL (ratio of BCR-ABL/BCR)

CASE 1. Treatment

9/25/2018	9/28/2018	10/5/2018	10/12/2018	10/22/2018	10/29/2018	11/1/2018	11/5/2018	11/9/2018	11/20/2018	11/26/2018	11/30/2018	12/13/2018	12/26/2018
189.7 (L)	164.6 (LL)	150.0 (LL)	124.5 (LL)	110.1 (LL)	37.4 (LL)	9.2	6.4	4.9	3.6 (L)	2.8 (L)	3.4 (L)	4.0	6.2
3.55 (L)	3.37 (L)	3.47 (L)	3.23 (L)	3.00 (L)	3.95 (L)	3.34 (L)	3.45 (L)	3.23 (L)	3.54 (L)	3.83 (L)	3.56 (L)	4.23	4.34
11.1 (L)	10.1 (L)	10.7 (L)	10.0 (L)	11.6 (L)	11.9 (L)	10.2 (L)	10.8 (L)	10.0 (L)	11.1 (L)	11.7 (L)	10.9 (L)	12.6 (L)	13.4
36.0 (L)	31.5 (L)	35.2 (L)	31.6 (L)	37.5 (L)	38.5 (L)	32.5 (L)	33.7 (L)	31.7 (L)	34.8 (L)	37.0 (L)	34.4 (L)	40.8	41.2
101 (H)	99	103 (H)	98	99	98	97	98	98	98	97	97	96	95
16.8 (H)	17.0 (H)	17.3 (H)	17.1 (H)	17.5 (H)	17.3 (H)	17.2 (H)	17.2 (H)	17.1 (H)	16.0	15.3	14.8	14.3	14.3
179	158	196	255	252	196	153	117 (L)	145	178	150	134 (L)	126 (L)	143
1 (H)	0	0	0	0	0	0	1 (H)	0	0	0	0	0	0
	MAN DIFF			MAN DIFF	MAN DIFF	MAN DIFF	AUTO DIF	AUTO DIF	AUTO DIF	AUTO DIF		AUTO DIF	AUTO DIF

Case 1. Treatment Course.

6 mos Bone marrow and Cytogenetics

- The bone marrow appears relatively normocellular for age and shows
  trilineage hematopoiesis with a relative myeloid hyperplasia.
  Blasts are not increased. The patient's history of chronic myeloid leukemia is noted; correlation with cytogenetic and molecular studies is recommended for more sensitive markers of residual disease.
- Number of cells/colonies: 21 Band Level: 350

  Karyotype: 46,XY,t(9;22)(q34;q11.2)[2]/46,XY[17].nuc ish(ABL1,BCR)x3(ABL1 con BCRx2)[4/100]
  FISH: Positive for Philadelphia chromosome translocation in 4% (4/100) of cells

Quant PCR

- BCR/ABL GENE TRANSLOCATION, PCR, QUANTITATIVE

  Result
  Result IS%(p210) Breakpoint Reference Range
  Positive >50 e13a2/e14e2 LOD = 0.002 IS%
  LOQ = 0.002 IS%
  MMR = 0.10 IS%

  INTERPRETATION
  BCR-ABL1 fusion (e13a2/e14a2) is detected. %IS is greater than 50 and is outside of LOQ.
- Test
  BCR/ABL GENE TRANSLOCATION, PCR, QUANTITATIVE

  Result
  Result IS%(p210) Breakpoint Reference Range
  Positive 3.1719 e13a2/e14e2 LOD = 0.002 IS%
  LOQ = 0.002 IS%
  MMR = 0.10 IS%

CASE 2 . Benign Hematology

- Disorders of bleeding and clotting are commonly encountered in practice.
- Use of anti platelet agents including aspirin, Plavix is widespread so are anticoagulants such as Warfarin, LMWH, Direct thrombin inhibitors.
- The spectrum of bleeding and clotting disorders is wide and clinical manifestations are common to many of them.
- The accurate diagnosis is heavily dependent on laboratory tests and accurate interpretation.

Case 2: Benign Hematology

- 42 yr old female, H/o MI(STEMI) age 39. strong family h/o CAD, MI , stroke. S/P PTCA and Stents(2). Pt started on Aspirin and Plavix.
- Dec 2018: presented with left sided weakness and slurred speech.
- Was found to have right MCA thrombosis and she was administered tPA and she underwent thrombectomy on 12/15/18 with successful results and recanalization. Placed on heparin drip
- Following day MRI and MRA revealed re-thrombosis of MCA and occlusion of right internal carotid artery.
- Vascular surgery evaluation showed occlusion of right external iliac artery and right peroneal artery. She was taken to the OR and had percutaneous right external iliac and tibial AngioJet, she was taken back to the OR 3 days later for thrombectomy (open), at the end of the case there was no pulse in the right foot. She had been on heparin drip from 12/15-12/19 and switched to argatroban for concerns of HIT despite normal PLT levels and testing was ultimately negative for anti-PF4 antibodies. Switched to Argatroban for concerns of HIT. Testing –ve for PF4 antibodies.
- Additional work up: normal protein C and S, normal AT3 levels, negative LAC and APLS labs and she was found to be heterozygote only for Prothrombin Gene, normal factor V leiden. She also had a JAK2 V617F which was negative in 2015, also negative for PNH.

Platelet Function testing.

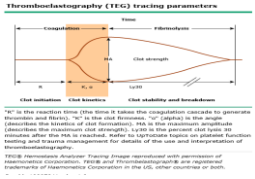
- Platelets play a key role in both hemostasis and thrombosis.
- Accurate measurement of platelet function critical for identifying patients with platelet dysfunction or hyperfunction, but it also is becoming increasingly important for the monitoring of modern antiplatelet therapy.
- A major problem concerning the testing of platelet function is the difficulty in simulating hemostasis in vitro. Platelets are also sensitive to manipulation, and are prone to artifactual in vitro activation.
- The ability to test platelet function in the routine laboratory improved with the introduction of platelet aggregometry.

List of established platelet function tests

Platelet function test	Aspects of platelet function measured	Advantages	Disadvantages
Bleeding Time	In vivo screening test	Physiological	Insensitive, Invasive & high inter-operator variability
Aggregometry - Turbidometric methods	Responsiveness to Panel of Agonists	Diagnostic	Labor intensive
			Non-Physiological
Aggregometry - impedance methods	Responsiveness to Panel of Agonists	Whole blood test	Insensitive
Aggregometry and luminescence	Combined aggregation and ADP release	More information	Semi-quantitative
Adenine Nucleotides	Stored and Released ADP	Sensitive	Specialized equipment
Thromboelastography (TEG)	Global Haemostasis	Predicts bleeding	Measures Clot properties only
			Insensitive to aspirin
Glass Filtrometer	High shear platelet function	Simple	Requires blood counter
Platelet Release Markers eg. BetaTG PF4	In vivo platelet activation markers	Simple, systemic measure of platelet	Prone to artifact

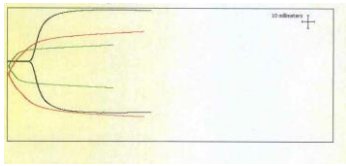
Thromboelastography (TEG) and ROTEM

- Thromboelastography (TEG) tests both platelet function and coagulation by assaying several parameters of clot formation dynamically in whole blood.



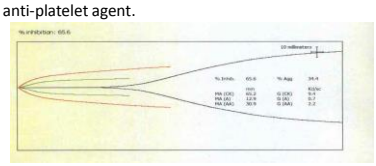
"R" is the reaction time (the time it takes the coagulation cascade to generate thrombin and fibrin). "K" is the clot firmness. "α" (alpha) is the angle (describes the kinetics of clot formation). MA is the maximum amplitude (describes the maximum clot strength). Ly30 is the percent clot lysis 30 minutes after the MA is reached. Refer to UpToDate topics on platelet function testing and trauma management for details of the use and interpretation of thromboelastography.

Case 2:
Platelet hyperreactivity noted.



Case 2.

- Added anti-platelet agent.



- Sucessful amputation with no further clotting

Molecular Diagnosis in Oncology- The age of Precision Medicine. Our goal for the Single patient.

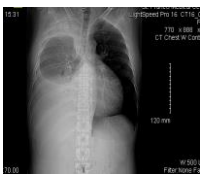
Connecting specific medical treatments to individual characteristics; The "Right patient with the Right Intervention"

- Pathology
- Genomic
- Transcriptomic
- Proteomic
- Metabolomic
- Pharmacogenomic
- Microbiomics

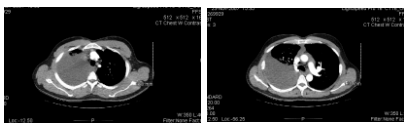
Clinical Applications of Comprehensive Genomic Profiling in Oncology

CASE

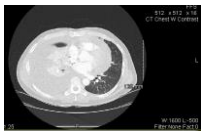
- 37 year old medical professional never smoker active, presented to ER 11/12 with worsening dyspnea of 3 weeks duration and decreased exercise capacity and back pain
- Initial Labs unremarkable
- Exam: Absent BS right lung field, distended neck veins
- Admitted to hospital



CASE



CASE



Course in hospital

- Tube thoracotomy
- Pericardiocenteces
- Pericardial window
- Cytology: Adenocarcinoma. IHC consistent with Pulmonary origin.

MRI Brain: Normal

Bone scan Extensive Mets involving spine, ribs and pelvis.

DIAGNOSIS: STAGE IV NCSLC**

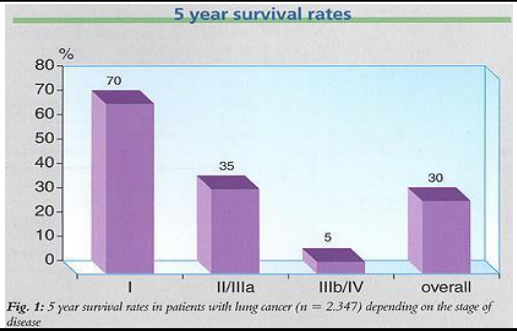
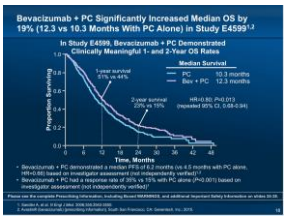


Fig. 1: 5 year survival rates in patients with lung cancer (n = 2.347) depending on the stage of disease

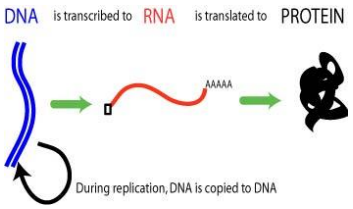
Lung Cancer Standard treatment for stage IV in 2011



The Evolution of Genomic Science.

1950-2016

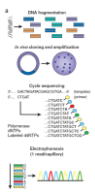
Central Dogma



History of Genomic Medicine:

- 1953: Discovery of DNA structure by Watson and Crick
- 1973: First sequence of 24 bp published
- 1977: Sanger Sequencing method published
- 1980: Nobel prize Wally Gilbert and Fred Sanger
- 1982: Gebank started
- 1983; Development of PCR
- 1987: 1st automated sequencer ABI 310
- 1989: C. elegans sequenced.
- 2000: Human Genome sequenced (COST approx. $3 billion).
- 2005: 1st 454 Life Sciences **NGS system**: GS20 system
- 2009: 1st Helicos single molecule sequencer
- 2011: 1st Ion Torrent NGS: PGM
- 2012: Oxford Nanopore technologies: Ultra long single molecule reads

Sanger sequencing



The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase.

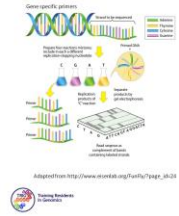
To each reaction is added only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP).

Following rounds of template DNA extension from the bound primer, the resulting DNA fragments are heat denature and separated by size using gel electrophoresis.

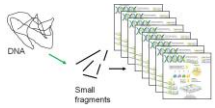
This is frequently performed using a denaturing polyacrylamide-urea gel with each of the four reactions run in one of four individual lanes (lanes A, T, G, C). The DNA bands may then be visualized by autoradiography or UV light and the DNA sequence can be directly read off the X-ray film or gel image.

Current strategies for sequencing

Single gene sequencing (Sanger):



Genomics: How can you sequence a whole genome?



Massively parallel

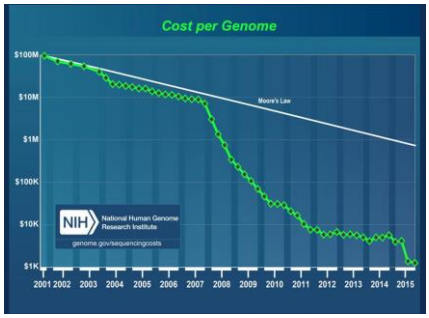
Presented By Richard Haspel at 2018 ASCO Annual Meeting

Basic NGS Workflow

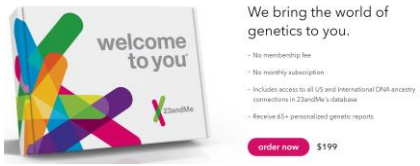
Sample Prep	Sequencing	Data Analysis
Isolation of material	Library QC	QC and pipeline analysis
PCR amplification	Cluster generation	Data interpretation
End repair, size selection	Instrument operation	

Platforms

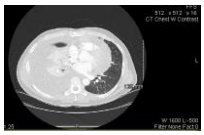
- Helicos Heliscope™ : recently available
- Pacific Biosciencies SMRT: launched 2010
- Ion Torrent: Thermo Fisher ; USED by STRATA (KPNC Collboration).



Commercialization:



CASE



Course in hospital

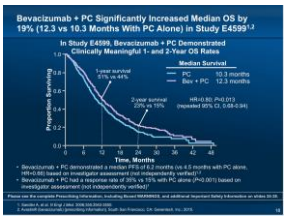
- Tube thoracotomy
- Pericardiocenteces
- Pericardial window
- Cytology: Adenocarcinoma. IHC consistent with Pulmonary origin.

MRI Brain: Normal

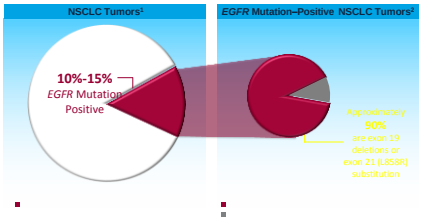
Bone scan Extensive Mets involving spine, ribs and pelvis.

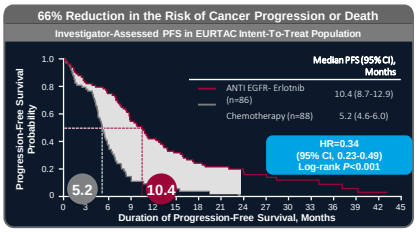
DIAGNOSIS: STAGE IV NCSLC**

Lung Cancer : treatment of advanced stage 2011.

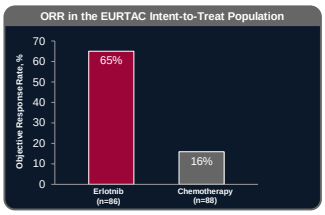


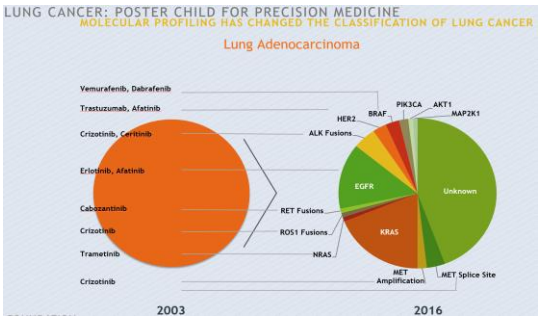
The Prevalence of *EGFR* Mutations NSCLC Tumors





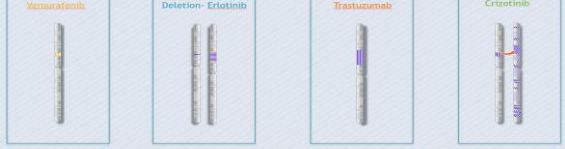
EURTAC: Objective Response Rate





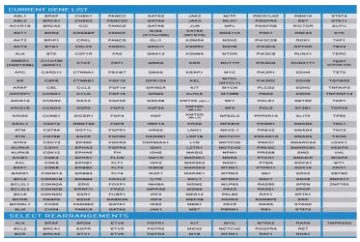
FOUR TYPES OF GENOMIC ALTERATIONS DRIVING TUMOR GROWT
LIMITATIONS OF TRADITIONAL TESTING

Base Substitutions	Insertions and Deletions	Copy Number Alterations	Rearrangements
BRAF V600E Vemurafenib	EGFR Exon 19 Deletion- Erlotinib	HER2 amplification Trastuzumab	ALK Fusion Crizotinib



Test	Detects	Can Miss
IHC	Protein expression	Any alteration not known of ahead of time
FISH	Copy number alterations, Rearrangements	Indels, Substitutions
Hot Spot Panels	Substitutions	Indels, Copy number alterations, Rearrangements

List of Current Genes & select Intronic Alterations with actionable mutations or Investigational agents.



CLINICAL RESEARCH KPNC ONCOLOGY

- **STRATA MATCHED trials** : 5 awaiting activation ; PARP Inhibitor, RET Mutation, ERBB2 overexpression , FGFR amplification *Her-2 Exon 20.*

- **NCI MATCH** : ALK, EGFR, CDK4/6 Amplification, C-kit, FGFR, GNAQ/GNA11, Her-2, MET, NTRK, PTEN los/Del Mutation, PIK3CA, ROS1, Smoothened (SMO) or patched 1 (PTCH1) mutations, TSC1or TSC2 mutations.

Common Techniques for oncologic mutation testing. 'HOT SPOT'.

1. Sequencing- Typical Sanger Sequencing
2. Gene Dosing: Measure presence or absence of a particular region of gene;
Multiplex Ligation Probe Analysis, Quatitative RT-PCR
3.FISH: Gene re-arrangement such as ALK
4. Transcriptome Profiling: Looking for RNA expression.

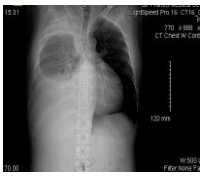
Approx cost to detect each alteration : $500-$2000.

ALL THESE TESTS DETECT JUST ONE SPECIFIC ALTERATION!

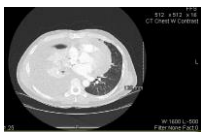
OR CAN DO THE SAME WITH GENOMIC SEQUENCING OR RNAseq(Reverse Transcribe)....

CASE

- 37 year old medical professional never smoker active, presented to ER 11/11 with worsening dyspnea of 3 weeks duration and decreased exercise capacity and back pain
- Initial Labs unremarkable
- Exam: Absent BS right lung field, distended neck veins
- Admitted to hospital



CASE



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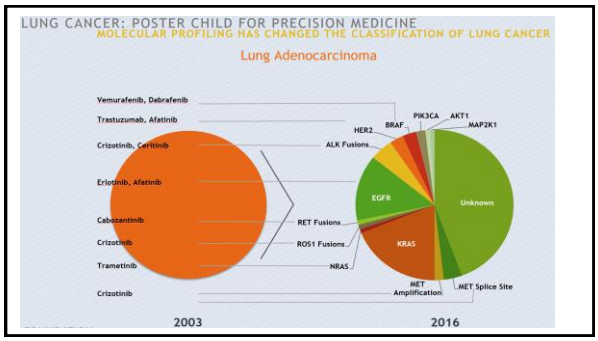
TESTED FOR EGFR MUTATION: POSITIVE FOR EGFR MUTATION AT EXON 20®

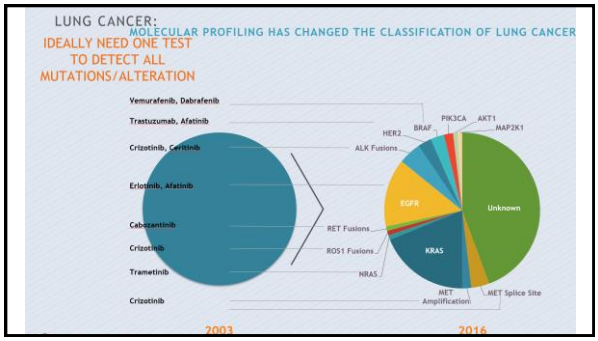
NEGATIVE FOR ALK REARRANGEMENT BY FISH

Started on Chemotherapy for Stage IV NSCLC.



LIMITATIONS OF SINGLE/LIMITED ALTERATION ANALYSIS

- Not comprehensive:
- Not a practical approach to assess several genetic alterations.
- COSTLY.
- Not consistent with precision medicine/patient centered care.
- Older technology/methods ; incapable of detecting all alterations.



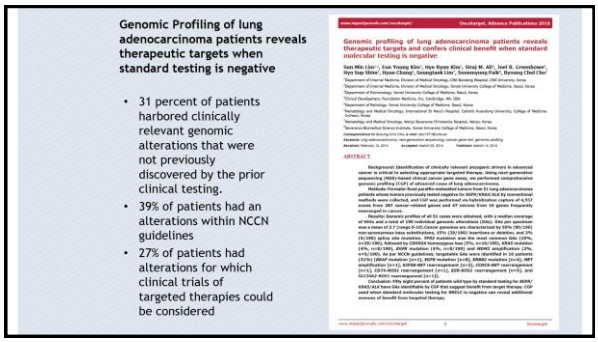
THE CASE FOR COMPREHENSIVE GENOMIC PROFILING IN CANCER THERAPY

1. RAPID IDENTIFICATION OF ACTIONABLE MUTATION; " DRUG-ABLE" TARGET
2. IDENTIFICATION OF PREVIOUSLY UNKNOWN CANCER GENES, DISCOVERY OF ADDITIONAL PATHWAYS FOR DRUG DEVELOPMENT


Clinical Cancer Research

Comprehensive Genomic Profiling Identifies Frequent Drug Sensitive EGFR Exon 19 Deletions in NSCLC Not Identified by Prior Molecular Testing

Abstract


Genomic Profiling of lung adenocarcinoma patients reveals therapeutic targets when standard testing is negative

- 31 percent of patients harbored clinically relevant genomic alterations that were not previously discovered by the prior clinical testing.
- 39% of patients had an alterations within NCCN guidelines
- 27% of patients had alterations for which clinical trials of targeted therapies could be considered

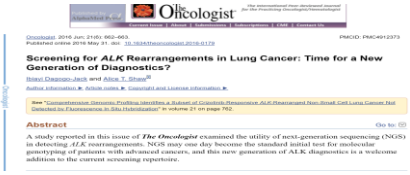
CGP Facilitates Implementations of the NCCN Guidelines for Lung Cancer Biomarker Testing and Identifies Patients Who May Benefit from Enrollment in Mechanism Driven Clinical Trials

- CGP performed on 6,832 cases of NSCLC from 2012-2015
- 71% (4, 876) harbored at least 1 genomic alteration involving 20% EGFR(20%), ALK (4.1%), BRAF(5.7%), (ERBB2)6.0%, MET (5.6%), ROS1 (1.5%0, RET (2.4%), KRAS (32%)
- In remaining cohort without these drivers 273 related genes were altered in at least .1% of cases. STK11(21%), NF1(13%), MYC (9.8%), RICTOR (6.4%) and more.
- CGP is practical and facilitates implementation of NCCN Guidelines and also identifies "pan neg." patients who may benefit from enrollment in mechanism driven clinical trials


Comprehensive Genomic Profiling Facilitates Implementation of the National Comprehensive Cancer Network Guidelines for Lung Cancer Biomarker Testing and Identifies Patients Who May Benefit From Enrollment in Mechanism-Driven Clinical Trials

CGP/NGS has higher Sensitivity.

- Ability to detect alterations that were not previously detected.


The Oncologist

Oncologist. 2016 Jun; 21(6): 662–663.
Published online 2016 May 31. doi: 10.1634/theoncologist.2016-0179
PMCID: PMC4912373

Screening for *ALK* Rearrangements in Lung Cancer: Time for a New Generation of Diagnostics?

Nikhil Chappidi-Jaeb and Alice T. Shaw[2]

Author information ▸, Article notes ▸, Copyright and License information ▸

See "Comprehensive Genomic Profiling Identifies a Subset of Crizotinib-Responsive ALK-Rearranged Non-Small Cell Lung Cancer Not Detected by Fluorescence In Situ Hybridization" in volume 21 on page 762.

Abstract Go to: ⊡

A study reported in this issue of ***The Oncologist*** examined the utility of next-generation sequencing (NGS) in detecting *ALK* rearrangements. NGS may one day become the standard initial test for molecular genotyping of patients with advanced cancers, and this new generation of ALK diagnostics is a welcome addition to the current screening repertoire.

ANALYTIC VALIDATION:
DEMONSTRATION OF HIGH ACCURACY AND REPRODUCIBILITY REQUIRED FOR CLINICAL USE

Base Substitutions
(MAF 5-100%)
Sensitivity: >99% PPV: >99%

Insertions/Deletions
(1-40bp, MAF 10-100%)
Sensitivity: 98% PPV: >99%

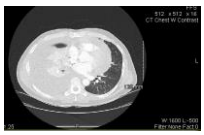
Copy Number Alterations
(zero or ≥8 copies)
Sensitivity: >95% PPV: >99%

Gene Fusions[1]
(select introns)
Sensitivity: >90% (>99% for ALK fusion[2])
PPV: >99%


Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing

1. Based on analysis of coverage and re-arrangement structure in the COSMIC database for solid tumor fusion genes where alteration prevalence could be established, complemented by detection of exemplar rearrangements in cell line titration experiments.
2. Yelensky et al. Presented at AACR 2014.

CASE



Course in hospital
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- Pericardial window
- Cytology: Adenocarcinoma

MRI Brain: Normal

Bone scan Extensive Mets involving spine, ribs and pelvis.

TESTED FOR EGFR MUTATION: POSITIVE FOR EGFR MUTATION AT EXON 20Ⓢ

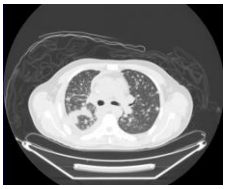
NEGATIVE FOR ALK REARRANGEMENT BY FISH

Started on Chemotherapy for Stage IV NSCLC.

CASE

Treated with Carbo/Taxol + Bevacizumab followed by Bevacizumab maintainence.

Progression 15 months later. Wt loss . New nodules + Brain mets.

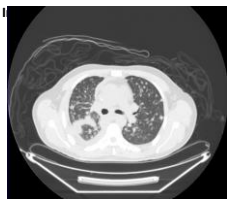


S/P Craniotomy + Gamma Knife. Salvage ChemotherApy Considered.

Biopsy sent for Next Gen Sequencing(Foundation One)

CASE

I



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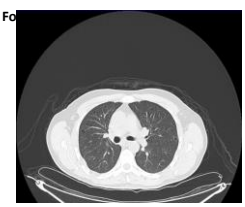

Mutually Exclusive Mutations- questionable.

Concurrent EGFR mutation and ALK translocation in non-small cell lung cancer

Randy F. Sweis, M.D.[1]

Sachdev Thomas, M.D.[2]

Ravi Salgia, M.D., PhD.[1*]

Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, Illinois, USA;

University of California San Francisco, San Francisco, California, USA


Cureus

Concurrent EGFR Mutation and ALK Translocation in Non-Small Cell Lung Cancer

Abstract

NGS in CLINICAL ONCOLOGY

- Comprehensive Genomic Profiling/Next Gen Sequencing of Cancers is rapidly gaining acceptance and represents a pivotal step towards precision medicine.
- Next Gen sequencing is comparable in price to routine imaging technologies
- It is expected to be as routine as special stains in the pathology lab
- As important as the X-RAY or Microscope in the diagnosis and management of cancers.
- Will lead to new targets for therapeutic exploitation
- Will require a new framework for the management of cancers in terms of regulatory issues, quality control and the conduct of efficient clinical trials.

Molecular Oncology Case Conference Launched Feb 2019.

GOALS and OBJECTIVES

NCI Community Oncology Research Program
A program of the National Cancer Institute at the National Institutes of Health

KAISER PERMANENTE

Conclusions

- The role of Laboratory medicine in management of cancers and blood disorders is invaluable and indispensable.
- Will have an expanding role in the era of precision medicine
- Close collaboration between physicians and laboratory scientists and technologists is critical in providing the best care for our members.
- Thank you.
- Questions?