Detection of copy number alterations, ploidy and loss of heterozygosity across the genome in FFPE specimens – Utility for diagnosis and treatment with comparison to FISH-based and as a complement to sequencing assays

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Copy number and somatic mutations drive tumors

• Results from TCGA genomic profiling of 12 tumors published

• Ciriello et al (Nature Genetics, Oct 2013) reveal the existence of copy number (not mutation) driven “C” class tumors

1. Ovarian
2. Breast
3. Lung squamous
4. Head & neck
Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing

Garrett M Frampton1,2, Alex Fichtenholtz1,2, Geoff A Otto1, Kai Wang1, Sean R Downing1, Jie He1, Michael Schnall-Levin1, Jared White1, Eric M Sanford1, Peter Au1, James Sun1, Frank Juhn1, Kristina Brennan1, Kjell Iwani1, Ashley Maillet1, Jamie Bueh1, Emily White1, Mandy Zhao1, Sohail Balasubramanian1, Selmira Terzic1, Tina Richards1, Vera Banning1, Lazaro Garcia1, Kristen Mahoney1, Zac Zwikko1, Amy Donahue1, Himisha Beltran1,4, Juan Miguel Mosquera1,4, Mark A Rubin1,4, Sujezana Dogan2, Cyrus V Hedvat3, Michael F Berger3, Lajos Pusztai6, Matthias Lechner7, Chris Boshoff7, Mirna Jarosz1, Christine Vietz1, Alex Parker1, Vincent A Miller1, Jeffrey S Ross1,8, John Curran1, Maureen T Cronin1, Philip J Stephens1, Doron Lipson1 & Roman Yedensky1.
Molecular Inversion Probes (MIP)

- **No bias** - No genomic DNA amplification
- **Optimized for degraded DNA**: MIPs interrogate only 40bps footprint of the genomic DNA
- **Very clean data and high dynamic range**: Gets rid of unamplified DNA, only amplifies correct probe sequence
Added somatic mutations

Distribution of 74 mutations by gene

- **BRAF**, 4
- **TP53**, 20
- **PTEN**, 8
- **KRAS**, 11
- **EGFR**, 16
- **IDH1**, 1
- **IDH2**, 2
- **NRAS**, 7
- **PIK3CA**, 5

Sensitivity around 20%
Focus on SNPs allows for detection of gains and losses plus allelic confirmation of copy number.

**Gain**

**Loss**

log2ratio

B allele frequency
### B-Allele Frequency vs Allele Peaks

<table>
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<tr>
<th>% B</th>
<th>Del</th>
<th>Normal</th>
<th>Dup</th>
<th>cnROH</th>
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<td><img src="image" alt="B-dup" /></td>
<td><img src="image" alt="B-cnROH" /></td>
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</table>
Using allele track to determine ploidy

log2ratio

A - B

%B

Normal allele pattern Indicates higher level loss than log2ratio
Interpretation easier after correction for ploidy

hypodiploidy with -1, -2, -3, 5p loss, 5qLOH, -6, -9, 11p loss, 12p loss, -13, -17, 20q loss, -21
Likely in subclone: 12q partial loss, -19
Pattern similar to chromophobe renal tumor (-1,-2,-6,-10,-13,-17,-21)
Matching log2ratio with B-allele frequencies shows ploidy problem

Looking only at %B, can’t determine if 5p and 10p are diploid, or if 18 is diploid
Switching to allele peaks allows visualization if outer bands are moving in or out - can help determine if gain with allele balance, or truly diploid

Outer bands beyond 1 and -1
Gain involving both homologs
Allele balance maintained
Molecular Diagnostics of Gliomas

Value of single test to determine BRAF-KIAA1549 fusion, IDH1/2 mutation status, p53/17p status, 1p/19q status, 9p status, EGFR status, 10q status

Nikiforova and Hamilton, Arch Pathol Lab Med. 2011;135:558–568
Note whole arm 1p loss and 19q loss – no other copy number changes or LOH
IDH1 R132H mutation clearly identified in 1p/19q del cases

Filter Settings
- expt_signals
- Group (2): (ARUP, control, REF_EH)
- HotSpot: (IDH1_132)
Molecular Diagnostics of Gliomas

Due to 2 Mb gain

Nikiforova and Hamilton, Arch Pathol Lab Med. 2011;135:558–568
note 1p loss not whole arm, EGFR amplified, CDKN2 and chr10 loss
Molecular Diagnostics of Gliomas

Due to 2 Mb gain

Nikiforova and Hamilton, Arch Pathol Lab Med. 2011;135:558–568
Note 1p, 19q, 9p, chr10 and EGFR normal, but copy-neutral LOH of 17p
Mutation data with copy number data helpful in this case

Case with CN-LOH of 17p also shows mutation of TP53
LOH is selecting mutation
HER2 amplification positive cases
Very focal gain of HER2, can also determine involvement of nearby TOP2A

Case does not contain gain of TOP2A
Case does contain gain of TOP2A - greater levels than Her2
HER2 discordant cases – Example 1:

FISH positive by ratio for gain, but array does not show a gain
HER2 normal (as is all 17q)....but region around centromere (and all 17p) lost – skews the FISH ratio

Whole genome view identified this as a false positive FISH result
Example 2 for HER2 discordant:

FISH was equivocal, reflexed to qPCR and was called positive for gain
Mosaic monosomy 17 observed by array

Array identified this as a likely false positive PCR result
False positive FISH results can be due to relative loss around centromere rather than actual gain of HER2

Gunn et al. BMC Cancer 2010, 10:396
“ABCD” exam

- 20% of melanocytic lesions signed out as “atypical”
- 25% discordant rate among pathologists for these challenging lesions
- for every melanoma removed in the U.S., 30 benign nevi are removed
- most highly litigated area of pathology

Testing for melanoma

punch biopsy

benign nevus

melanoma

atypical

“rule out melanoma”
Characteristic chromosomal gains/losses associated with 95% of melanomas

- Frequent chromosomal gains: 1q, 6p, 7p, 7q, 8q, 17q, 20q
- Frequent chromosomal losses: 6q, 8p, 9p, 10p, 10q, 11q
- Benign nevi do not display these alterations
- Spitz nevi occasionally show isolated gains of 11p, 7q

Study on V1 of Oncoscan

- Sample set of 64 FFPE melanocytic lesions:
  - 23 benign nevi
  - 11 atypical melanocytic lesions of unknown potential (MLUMP)
  - 27 primary melanomas
  - 3 metastatic melanomas
- 98.5% of samples had passing MIP QC scores
- DNA yield 0.35-31 ng/µL (16-1395 ng total DNA)
- FFPE blocks ranged in age from 1-18 years

OncoScan showed 100% specificity in benign nevi (n=23) Chromosomal gains/losses consistent with melanoma in 89% of primary melanomas

- 24/27 primary melanomas

3/3 metastatic melanomas showed MIP patterns consistent with melanoma
Malignant melanoma FISH pattern: multiple copies of \textit{RREB1} (red signals)

Normal melanoma FISH pattern: 2 copies of \textit{RREB1} (red), \textit{MYB} (gold) \textit{CCND1} (green) 6 centromere (aqua)


In our series, 6/27 cases of primary melanoma would have been likely missed by this FISH panel
Deletion on 6q proximal to region covered by MelanoSITE FISH probe

15.5 Mb deletion proximal to MYB gene would not be detected using the commercially-available FISH probe set
New Case – indication Wilms tumor
Report Emphasis:
Gain of chr 8 and chr 12 is recurrent in Wilms tumor
Did NOT see 1p/16q loss/LOH
1q gain
11p LOH
22q LOH
17p loss
9q loss
7q loss

Cannot determine imprinting status of 11p15

VOUS:
942kb loss within 2q37.3
Involves 10 gene, including HDAC4

HDAC4 a critical gene for the 2q37 microdeletion syndrome

Incidental finding? Unrelated? Uncertain? Or pre-disposing?
From GeneReviews for 2q37 microdeletion syndrome:

**Diagnosis/testing.** Chromosome analysis confirms the diagnosis of 2q37 deletion syndrome in 80%-85% of affected individuals. In about 15%-20% of cases the small size of the deleted region can only be detected using deletion analysis (which relies on a variety of methods). In some individuals, 2q37 microdeletion syndrome results from chromosomal rearrangements involving 2q37 (e.g., chromosome 2 inversion, ring chromosome 2, or translocation between chromosome 2 and another chromosome). Mutation of HDAC4 has been proposed as causative for most of the features of the 2q37 microdeletion syndrome. Several affected individuals without microdeletions had inactivating mutation of HDAC4, a gene in the 2q37 deleted region, leading to the proposal that mutation of this gene may be causative for most of the features of the 2q37 microdeletion syndrome.

**Clinical characteristics.** 2q37 microdeletion syndrome is characterized by mild-moderate developmental delay/intellectual disability, brachymetaphalangy of digits 3-5 (often digit 4 alone) (>50%), short stature, obesity, hypotonia, characteristic facial appearance, autism or autism spectrum disorder (30%), joint hypermobility/dislocation, and scoliosis. Other findings include seizures (20%-35%), congenital heart disease, CNS abnormalities (hydrocephalus, dilated ventricles), umbilical/inguinal hernia, tracheomalacia, situs abnormalities, gastrointestinal abnormalities, and renal malformations. Wilms tumor has been reported in two individuals.

**Surveillance:** Ongoing routine primary care; periodic reevaluation by a medical geneticist to provide new recommendations and information about the syndrome; periodic neurodevelopmental and/or developmental/behavioral pediatric evaluation to assist in the management of cognitive and behavioral problems. Screening for renal cysts at age four years and again at puberty is suggested. For young children with a deletion that includes 2q37.1, screening for Wilms tumor can be considered.
Translational Oncology Core Pilot Study

Huntsman Cancer Institute

ARUP Laboratories
Translational Oncology Core
Pilot Study

- Enroll RELAPSED cancer patients
- Pre- and post-test survey to clinician
- Sequenom Panel (OncoCarta Custom)
  - 277 mutation in 25 genes
  - Return report to clinician
- OncoScan Array V3.0 (N=48)
  - Genome-wide Copy Number/LOH
  - 74 Mutations in 9 Genes
Huntsman Cancer Institute: Molecular Diagnostic/Translational Oncology Core (TOC) Pilot Study

- **Positive Mutation Frequency**
  48 out of 137 specimens were positive for ≥1 mutation (35%)
...So what did we find?
CCND1 amplification in BMP37

Metastatic melanoma – Sequenom, no mutations
Focal CDK6 gain (7 copies) in BMP-46

Metastatic CRC (right colon)
30% tumor content by Sequenom
KRAS G12C mutation
BMP16: Lung adenocarcinoma – Sequenom no mutations
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<tr>
<th>Rank</th>
<th>Status</th>
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<tr>
<td>1</td>
<td>Recruiting</td>
<td>Phase II Safety and Efficacy Study of Crizotinib in East Asian Patients With ROS1 Positive, ALK Negative Advanced NSCLC</td>
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<td>Conditions: Non Small Cell Lung Cancer; ROS1 Proto Oncogene; Crizotinib</td>
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<td>Intervention: Drug: Crizotinib</td>
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<td>A Phase 1/2a Study of Oral RXDX-101 in Adult Patients With Locally Advanced or Metastatic Cancer; Study Targeting ALK, ROS1, or TRKA/B/C</td>
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<td>Conditions: Locally Advanced Solid Tumors; Metastatic Solid Tumors</td>
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<td>Intervention: Drug: RXDX-101</td>
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<td>3</td>
<td>Recruiting</td>
<td>A Study Of PF-06463922 An ALK/ROS1 Inhibitor In Patients With Advanced Non Small Cell Lung Cancer With Specific Molecular Alterations</td>
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<td>Condition: ALK Positive Non Small Cell Lung Cancer</td>
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<td>Intervention: Drug: PF-06463922</td>
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<td>Phase 2 Study Assessing Efficacy and Safety of Crizotinib in Patients Harboring an Alteration on ALK, MET or ROS1</td>
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<td>Conditions: Hematologic Cancers; Solid Tumors; Metastatic Cancer</td>
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BMP3: Lung adenocarcinoma – Sequenom no mutations
FGFR1 amplification
BMP38 – lung carcinoma – Sequenom – no mutations, very abnormal for copy number and LOH

**MET amplification** (CN = 9.0)
**ERBB2 (Her2) amplification** (CN = 6.0)
Diagnosis: Undifferentiated pleomorphic sarcoma of heart localized to left atrium. Partial surgery with residual disease.

Treatment:
- 4 cycles of Gemcar and Taxotere (June 17 to September 6, 2011) - Neulasta part of each cycle.
- Recurrent disease not applicable for additional surgery. (Proton radiation October to December 2011)
- Since October 2013 on Votrient 800mg with three short interruptions.

ANY CLINICALLY RELEVANT INFORMATION FROM ONCOSCAN?

Gil Mor, MD PhD
Dept. of Obstetrics Gynecology & Reproductive Sciences
Reproductive Immunology Unit
Yale University School of Medicine

Sarcoma Sample (Yale)

- Imatinib ?
- Sorafenib ?
Triple negative relapsed breast cancer

Many amplifications, largest one is CDK6 at more than 40 copies
Acknowledgements

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