The 'Perfect Pairing':
ICE COLD-PCR with cfDNA for Highly Sensitive Mutation Detection

Transgenomic develops and commercializes biomarkers and personalized diagnostics with the goal of improving medical diagnoses and patient outcomes.

Personalized Oncology Solutions for the Global Community
Agenda

- History of Transgenomic
- Technology and History ICE COLD-PCR
- Project 1 (Transgenomic)
- Project 2 (MD Anderson, Dr. Filip Janku)
- Conclusions
- Next Phase (Transgenomic)
- Future Direction and Advances
- Long Term Vision
Transgenomic
Leverage Proprietary Technology and Molecular Genetics Expertise to Provide a Fully Integrated Molecular Diagnostics Solution

**Biomarker Identification**
Identifies new genetic biomarkers for pharma sector and develops assays for patient testing

**Genetic Assays and Platforms**
Provides genetic assays, testing platforms and equipment to labs worldwide

**Patient Testing**
Provides genetic testing services
- Oncology
- Cardiology
- Neurology
ICE COLD-PCR Methodology

Selectively Amplifies Mutant Sequences = Enriches for All Mutations

Denature DNA

95°C

MT

WT

95°C

MT

WT

Selectively Denature Mismatched Sequences at The Critical Temperature (Tc)

WT

MT

RS

Tc

WT

MT

RS

Reduce Temperature: Cross-Hybridize

MT

RS

MT

WT

RS

WT

WT

References Sequence Oligonucleotide Enables Improved Hybridization Kinetics

Reduce Temperature for Primer Annealing to Both Strands of the Mutant Sequences

WT

RS

WT

MT

RS

WT

MT

Exclusive license from Dr. Mike Makrigiorgos, DFCI
ICE COLD-PCR Selectively Amplifies Mutant DNA “PCR on Steroids”

Enriching the Mutant Population = Increasing the Sensitivity -- For Use in ANY Downstream Analysis --
ICE COLD-PCR Selectively Amplifies Mutant DNA “PCR on Steroids”

Enriching the Mutant Population = Increasing the Sensitivity -- For Use in ANY Downstream Analysis --

Exclusive license from Mike Makrigiorgos, DFCI
ICE COLD-PCR: Enhanced Mutation Detection: Multiple Sample Types – Multiple Platforms

ICE COLD-PCR works with DNA from any sample type:
- Tissue (Biopsy)
- FFPE
- Blood
- cfDNA
- CTCs
- FNAs
- Urine
- Sputum
ICE COLD-PCR: Enhanced Mutation Detection: Multiple Sample Types – Multiple Platforms

ICE COLD-PCR works with DNA from any sample type:
- Tissue (Biopsy)
- FFPE
- Blood
- cfDNA
- CTCs
- FNAs
- Urine
- Sputum
Enhanced Mutation Detection Assays for DNA from FFPE, cfDNA or Other Body Fluids

Increasing level of Mutation Detection

Limit of Detection (Percent)

- Sanger: 15% to 0.1%: ~150-fold enrichment in LOD
- NGS: 2.5% to 0.01%: ~250-fold enrichment in LOD
ICE COLD-PCR: One Assay – Multiple Mutations

Sanger Sequence Detection of Any Mutation in the Sample

Limit of Sanger Sequencing Detection of KRAS Mutations Following ICE COLD-PCR

- 42 Point mutations in KRAS codons 12 & 13
  - THEREFORE:
  - 42 Allele-Specific Probes Required
  - OR
  - 1 ICE COLD-PCR Assay
ICE COLD-PCR: One Assay – Multiple Mutations

**EGFR Deletions**

- **NGS Only:** 1% and 5% Deletion Sample
- **ICE COLD-PCR + NGS:** 1% Deletion Sample
- **ICE COLD-PCR + NGS:** 5% Deletion Sample

<table>
<thead>
<tr>
<th>Deletion</th>
<th>Variant Frequency</th>
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<tbody>
<tr>
<td>p.E746_T751&gt;1</td>
<td>6</td>
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<tr>
<td>p.E746_A750delE751</td>
<td>14</td>
</tr>
<tr>
<td>p.E746_T751&gt;A</td>
<td>9</td>
</tr>
<tr>
<td>p.E746_S752&gt;A</td>
<td>13</td>
</tr>
<tr>
<td>p.E746_S752&gt;V</td>
<td>7</td>
</tr>
<tr>
<td>p.E746_S752&gt;D</td>
<td>6</td>
</tr>
<tr>
<td>p.L747_T751&gt;Q</td>
<td>7</td>
</tr>
<tr>
<td>p.L747_T751&gt;P</td>
<td>4</td>
</tr>
<tr>
<td>p.L747_P753&gt;S</td>
<td>15</td>
</tr>
<tr>
<td>p.L747_P753&gt;VA</td>
<td>4</td>
</tr>
<tr>
<td>p.S752_I759delE759P</td>
<td>8</td>
</tr>
<tr>
<td>p.S752_I759delSPKANKE1</td>
<td>11</td>
</tr>
<tr>
<td>p.P753_I759delSPKANKE1</td>
<td>13</td>
</tr>
</tbody>
</table>

- Deletions are difficult to detect on NGS platforms
- Over 60 Relevant *EGFR* Exon 19 Deletions in NSCLC Patient Tumors

**THEREFORE:**

- 60 Allele-specific probes required
- **OR**
- **1** ICE COLD-PCR Assay for all Exon 19 Deletions
ICE COLD-PCR Detects Somatic Mutations in cfDNA from Plasma (Merck: 53 NSCLC Patients)

Historical Published Results:
KRAS mutation data for DNA from FFPE sections
- Standard PCR: ~25-30% mutant

Study Results 53 NSCLC Patients:
KRAS mutation data from plasma cfDNA
- Standard PCR: 11% (6/53)
- ICE COLD-PCR: 25% (13/53)

ICE COLD-PCR detects low level mutations in cfDNA ("Liquid Biopsy")
ICE COLD-PCR Detection of Mutations in cfDNA: Impact on Cancer Management

- Progressive Disease
  - No Monitoring
  - ICE COLD-PCR Analysis
  - Tumor Growth
  - Tumor Detected
  - Stable Disease
  - Treatment Started
  - Monitoring
  - Surveillance
  - Time
Overall Project Goals

- Prove ICE COLD-PCR as a relevant tool to assist in improving patient outcome
- Concordance studies between mutations detected in tumor and in cfDNA
- Partnering with world-class oncology organizations for clinical expertise
- All “Liquid Biopsies”
  - First target cfDNA from plasma
ICE COLD-PCR Research Collaborations

Research collaborations are ongoing in various types of cancer at the most prestigious institutions worldwide.

Provide blood/plasma samples for proof that ICE COLD-PCR can be a relevant tool to assist in improving patient outcome.

This validation project provides the data to show the clinical importance and correlation for blood samples compared to tissue samples as a proof of concept for the mutation detection in “Liquid Biopsies.”

Dana-Farber/Brigham and Women’s Cancer Center
University of Nebraska Medical Center
UPMC Life Changing Medicine
The University of Texas MD Anderson Cancer Center
Perelman School of Medicine University of Pennsylvania
Paediatric Hospital Università Cattolica
Princess Margaret Hospital
Transgenomic
Advancing Personalized Medicine
Project 1: Application of ICE-COLD-PCR to the Detection of Rare NRAS Mutations in Myelodisplastic Syndrome (MDS) (Mike Makrigiorgos, DFCI)

International Prognostic Scoring System (IPSS): basis for treatment guidelines

- Patients in same IPSS risk group have varied clinical outcomes
  - Additional prognostic indicators needed
  - Previous studies suggested NRAS mutations could be such an indicator

Current Study:

- 295 Patients in the low/intermediate IPSS risk group
- Sanger Sequence analysis of
  - Conventional Methods (LOD >5%)
  - Fast-COLD-PCR (LOD 2-5% or 0.5-2%)
  - ICE COLD-PCR (LOD 0.1-0.5%)

What level of mutations is clinically significant?

Murphy et.al. 2013. *Leukemia* 27:2067-2111
ICE COLD-PCR Analysis of NRAS Mutations: Impact on Overall Survival Lower IPSS Risk MDS (Mike Makrigiorgos, DFCI)

Overall Survival in Lower IPSS Risk MDS

- NRAS Mutant >5% MAF (n=8) – Conventional Methods
- NRAS Mutant 2-5% MAF (n=3) – fast COLD-PCR
- NRAS Mutant 0.5-2% MAF (n=7) – fast COLD-PCR
- NRAS Mutant 0.1-0.5% MAF (n=6) – ICE COLD-PCR
- NRAS Wild-Type (n=271)

“…….Multivariable analysis that considered IPSS risk group, sex, age and the mutation status of 16 other recurrently mutated genes demonstrates that the prognostic significance of NRAS mutations detected by fast-COLD-PCR is independent of other known risk factors …… As the technical limit of sensitivity should preferably be lower than the clinically relevant threshold, detection of NRAS with ICE COLD-PCR followed by determination of whether the mutation is clinically relevant (higher or lower than 0.5%) would be appropriate.”

Murphy et.al. 2013. Leukemia 27:2067-2111

Ability to differentiate mutation load enables patient treatment decisions which in turn means better patient outcome.
Project 1 Conclusions

ICE COLD-PCR Analysis:

- Helps determine subgroups of MDS patients with differing responses.
- Enables patient stratification for treatment options.
- Provides critical information from tissues of MDS patients for clinical treatment plans.
## MD ANDERSON: FAST FACTS

<table>
<thead>
<tr>
<th>FY 2014</th>
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<tbody>
<tr>
<td>Total patients served (2012)</td>
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<tr>
<td>New patients served (2012)</td>
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<tr>
<td>Admissions</td>
</tr>
<tr>
<td>Total visits</td>
</tr>
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</table>

### Department of Investigational Cancer Therapeutics: Mission

To translate laboratory discoveries and clinical observations into hypothesis-driven clinical trials leading to targeted, tailored and personalized cancer treatments
Liquid Biopsies

- Cell-free (cf) DNA
  - Plasma
  - Serum
  - Other liquid material
- Circulating tumor cells (CTC)
- Exosomes

Liquid Biopsies: Sensitivity Requirements

- Human cell contains 6pg of DNA
- “Average” advanced cancer has about 15-20ng of DNA per ml of plasma
- “Average” advanced cancer has less than 10 CTCs per 7.5 ml of blood
- Plasma has less DNA than serum; however, a lot of DNA in serum samples is from leukocytes
- Because only proportion of cfDNA originates from the tumor sensitivity requirements are from 1 mutant in allele in 1,000 to 10,000 of wild-type
MD Anderson and Transgenomic Project:
ICE COLD-PCR and plasma cfDNA testing for mutations in *KRAS* and *BRAF*

- **Objectives** (75 patients with advanced cancers and known tumor tissue *BRAF* or *KRAS* mutation status)
  - To measure the degree of concordance between results of ICE COLD-PCR mutation analysis of *BRAF* and *KRAS* mutations from cfDNA and tumor tissue (obtained and tested as standard of care) in patients with advanced cancers.
  - To assess *BRAF* and *KRAS* mutations in cfDNA at multiple time-points, at baseline before treatment, then up to monthly, and at the time of disease progression in patients with advanced cancers treated with anticancer therapy.
  - To assess treatment outcomes in analyzed patients with advanced cancers

- **Assay Protocol:**
  - Receipt of 3 – 4 mL frozen plasma
  - Isolation of cfDNA
  - Perform ICE COLD-PCR Assays for *KRAS* and *BRAF*
  - Sanger Sequence Analysis
Concordance Analysis: cfDNA vs. tissue

### BRAF

<table>
<thead>
<tr>
<th>Tested (N=31)</th>
<th>BRAF Mutation CLIA</th>
<th>BRAF Wild-Type CLIA</th>
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<tr>
<td>BRAF Mutation Plasma</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>BRAF Wild-Type Plasma</td>
<td>10</td>
<td>1</td>
</tr>
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<td>Observed agreements</td>
<td>21 (68%)</td>
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### KRAS

<table>
<thead>
<tr>
<th>Tested (N=29)</th>
<th>KRAS Mutation CLIA</th>
<th>KRAS Wild-Type CLIA</th>
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<tr>
<td>KRAS Mutation Plasma</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>KRAS Wild-Type Plasma</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Observed agreements</td>
<td>23 (79%)</td>
<td></td>
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</table>
# Longitudinal Assessment of cfDNA BRAF Mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>1 (Melanoma)</th>
<th>2 (Melanoma)</th>
<th>3 (Melanoma)</th>
<th>4 (Melanoma)</th>
<th>5 (Erdheim-Chester disease)</th>
<th>6 (Papillary thyroid cancer)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
<td>BRAF inhibitor (response, intolerance)</td>
<td>BRAF inhibitor (response→ PD)</td>
<td>BRAF inhibitor → BRAF and MET inhibitors (PD)</td>
<td>Ipilimumab and temodar</td>
<td>BRAF inhibitor (intolerance)</td>
<td>Sorafenib</td>
</tr>
<tr>
<td><strong>Tissue (CLIA)</strong></td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
</tr>
<tr>
<td><strong>Baseline cfDNA</strong></td>
<td>Wild-type</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Sirolimus + HCQ</td>
<td>Vemurafenib + chemotherapy</td>
<td>Vemurafenib + chemotherapy</td>
<td>Vemurafenib + chemotherapy</td>
<td>Peg-interferon</td>
<td>Vemurafenib + chemotherapy</td>
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<tr>
<td><strong>Response</strong></td>
<td>PD</td>
<td>SD (minor response)</td>
<td>SD (minor response)</td>
<td>SD (minor response)</td>
<td>SD (minor response)</td>
<td>SD</td>
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<tr>
<td><strong>Follow up cfDNA</strong></td>
<td>V600E</td>
<td>Wild-type</td>
<td>Wild-type</td>
<td>Wild-type</td>
<td>Wild-type</td>
<td>Wild-type</td>
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</tbody>
</table>
# Longitudinal Assessment of cfDNA KRAS Mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
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</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
<td>Appendiceal carcinoma</td>
<td>Colorectal carcinoma</td>
</tr>
<tr>
<td><strong>Prior therapy</strong></td>
<td>Chemotherapy</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td><strong>Tissue baseline</strong></td>
<td>KRAS G13D</td>
<td>KRAS G12D</td>
</tr>
<tr>
<td><strong>cfDNA baseline</strong></td>
<td>Wild-type</td>
<td>Wild-type</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Anti-VEGF</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td><strong>Response</strong></td>
<td>PD</td>
<td>PD</td>
</tr>
<tr>
<td><strong>cfDNA follow-up</strong></td>
<td>KRAS G13D</td>
<td>KRAS G12D</td>
</tr>
</tbody>
</table>
Quantification of Mutation in cfDNA

74 year old female with appendiceal carcinoma and *BRAF* V600E mutation

46 year old female with melanoma and *BRAF* V600E mutation
Project 2 CONCLUSIONS

- Liquid biopsies have a potential to further advance personalized medicine by offering a source of easily obtainable material for mutation analysis.

- Liquid biopsies can help to determine prognosis, detect early recurrence and disease progression.

- Liquid biopsies can be used for monitoring of molecular profile in patients undergoing cancer therapy to detect specific mechanisms of resistance.

- Novel multiplex technologies capable of testing for multiple mutations need to be developed.

- Barriers to implementation of next-generation sequencing need to be overcome.

- Novel sources of DNA (urine, exosomes, CTCs) need to be investigated.
Overall Survival in Lower IPSS Risk MDS

NRAS Mutant >5% MAF (n=8) – Conventional Methods
NRAS Mutant 2-5% MAF (n=3) – Fast COLD-PCR
NRAS Mutant 0.5-2% MAF (n=7) – Fast COLD-PCR
NRAS Mutant 0.1-0.5% MAF (n=6) – ICE COLD-PCR
NRAS Wild-Type (n=271)

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Disease</th>
<th>Tissue Mutation</th>
<th>Treatment prior to baseline cfDNA</th>
<th>Baseline cfDNA Mutations</th>
<th>Follow up cfDNA Mutation</th>
<th>Treatment Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Erdheim-Chester</td>
<td>BRAF V600E</td>
<td>No</td>
<td>BRAF V600E</td>
<td>Wild-Type</td>
<td>SD</td>
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<td>6</td>
<td>Papillary Thyroid Cancer</td>
<td>BRAF V600E</td>
<td>No</td>
<td>BRAF V600E</td>
<td>Wild-Type</td>
<td>SD</td>
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<td>2</td>
<td>Melanoma</td>
<td>BRAF V600E</td>
<td>No</td>
<td>BRAF V600E</td>
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<td>SD</td>
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<td>3</td>
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<td>BRAF V600E</td>
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<td>BRAF V600E</td>
<td>Wild-Type</td>
<td>SD</td>
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<td>1</td>
<td>Melanoma</td>
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<td>Wild-Type</td>
<td>BRAF V600E</td>
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<tr>
<td>7</td>
<td>Appendiceal Carcinoma</td>
<td>KRAS G13D</td>
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<td>Wild-Type</td>
<td>KRAS G13D</td>
<td>PD</td>
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<tr>
<td>8</td>
<td>Colorectal Carcinoma</td>
<td>KRAS G12D</td>
<td>Yes</td>
<td>Wild-Type</td>
<td>KRAS G12D</td>
<td>PD</td>
</tr>
</tbody>
</table>
Projects 1 and 2 Summary

- ICE COLD-PCR can be used for the analysis of mutations in any tissue.
- ICE COLD-PCR is especially suited for analysis of mutations in any “Liquid Biopsy” sample:
  - As a **Tissue “Surrogate”** by offering a source of easily obtainable material for mutation analysis and treatment determination.
  - As a **Surveillance Method** by helping to determine prognosis, detect early recurrence and disease progression.
  - As a **Monitoring Method** for assessing the molecular profile of mutations in patients undergoing cancer therapy to detect:
    - Specific mechanisms of resistance
    - Occurrences of new mutations

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ICE COLD-PCR Paired with NGS: Future Directions
ICE COLD-PCR: Impact on Cancer Management

![Graph showing ICE COLD-PCR analysis impact on cancer management]

- Tumor Growth
- Progressive Disease
- No Monitoring
- Stable Disease
- Monitoring
- Surveillance
- Treatment Started
- Time
Phase II Goal

A novel multiplex technology capable of the simultaneously detecting Multiple Mutations in Multiple Genes for Multiple Samples at the level of sensitivity needed for use in a “Liquid Biopsy”
**Rapid & Focused Development Plan:**
Multiplexed ICE COLD-PCR Paired with NGS

**Development**
- Multiplex ICE COLD-PCR assays for lung cancer, CRC, melanoma, etc.
- Analyze using Ion Torrent or MiSeq (FDA approved)
- Partnering with MD Anderson and others for clinical validation studies
- Partnering with Pharma for clinical trials to companion diagnostics

**Performance Target**
- Focus on cfDNA from lung cancer, CRC and melanoma
- Increased sensitivity over current NGS detection
- Compatible with any current NGS instrument
- Correlation with patient treatment decisions
- Correlate with patient outcome (biopsies)

**Benefits**
- Better diagnosis & personalized drug therapy
- Faster diagnosis turnaround
- Achieve optimal therapeutic benefits
- No need for tissue biopsies
- Continual patient monitoring
- **Personalized Cancer Management**

“LIQUID BIOPSY”
Non-invasive, Rapid and Easy Detection of Low Level Mutations
### Technology Comparison Summary

<table>
<thead>
<tr>
<th>Assay/Probe</th>
<th>Mutants Detected</th>
<th>Mutants Detected in 20 Exons</th>
<th># Assays for Detecting 20 Mutations</th>
<th># Probes to detect 20 Mutations</th>
<th>Combination with Sequencing</th>
<th>Average LOD of Assay</th>
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<tbody>
<tr>
<td>Sanger</td>
<td>No</td>
<td>Unlimited</td>
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<td>N/A</td>
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<tr>
<td>NGS</td>
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<td>+++</td>
<td>1</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>Surveyor</td>
<td>No</td>
<td>Unlimited</td>
<td>N/A</td>
<td>1</td>
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<tr>
<td>ICP (NGS)</td>
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<td>+++</td>
<td>+++</td>
<td>1</td>
<td>0</td>
<td>Yes</td>
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<tr>
<td>MX ICP (NGS)</td>
<td>No</td>
<td>+++</td>
<td>++++</td>
<td>1</td>
<td>0</td>
<td>Yes</td>
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<td>Emulsion PCR + Flow Cytometry</td>
<td>Yes</td>
<td>Probe Specific</td>
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<td>20</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Digital PCR</td>
<td>Yes</td>
<td>Probe Specific</td>
<td>1</td>
<td>20</td>
<td>20</td>
<td>20</td>
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</tbody>
</table>

- **Multiplexed ICE COLD-PCR:**
  - One Assay; All Mutations with LODs ~0.01%
  - Supercharges Any NGS or Other Downstream Analysis
Multiple Samples, Multiple Amplicons – 1 NGS Analysis for Standard PCR or ICE COLD-PCR of cfDNA from Plasma

- Analysis of 3 Genes/5 Exons/12 Plasma Samples
- NGS: only 3 Mutations detected in 2/12 Samples
- ICE COLD-PCR paired with NGS: 15 Mutations Detected in 12 Samples
MX ICE COLD-PCR + NGS: Ideally Suited for Mutation Detection in both Oncogenes and TumorSuppressor Genes

**Oncogenes:**
- Few Mutations
- Usually Localized Sites

**Tumor Suppressor Genes:**
- Large Numbers of Mutations
- Diffuse sites

**KRAS** (COSMIC Database)

**TP53** (COSMIC Database)
MX ICE COLD-PCR + NGS: Ideally for Mutation Detection in Oncogenes and TumorSuppressor Genes

- **Oncogenes (eg. KRAS)**
  - Few Mutations
  - Usually Localized Sites
  - High Percentage of Mutations in Limited Set of Cancers

- **Tumor Suppressor Genes (eg. TP53)**
  - Large Numbers of Mutations
  - Diffuse Sites
  - High Percentage of Mutations in Many Cancers

- ICE COLD-PCR Assays require no further development as new relevant mutations are validated
- All mutations detected, broad applicability for oncogenes and tumor suppressor genes
Custom *TP53* NGS Assay: Complete Exon Coverage

- **One NGS Assay**: All *TP53* Mutations; LOD 3% (currently)
- With Addition of ICE COLD-PCR; Projected LOD 0.01%
ICE COLD-PCR Assay for \( TP53 \) Exon 5

ICE COLD-PCR paired with Sanger

- **Cell Line K562**
  - WT for \( TP53 \) at R175

- **Wild-Type**

ICE COLD-PCR Paired with NGS:
- Increased Sensitivity for Detection of Low Level Mutations in \( TP53 \)

### ICE COLD-PCR Paired with NGS

<table>
<thead>
<tr>
<th>Chrom</th>
<th>Position</th>
<th>Gene Sym</th>
<th>Target ID</th>
<th>Type</th>
<th>Zyg</th>
<th>Ref</th>
<th>Var</th>
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<th>P-value</th>
<th>Coverage</th>
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<th>Var Cov</th>
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<td>chr17</td>
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<td>TP53</td>
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<td>C</td>
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<td>31537</td>
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Utility of MX ICE COLD-PCR Paired with NGS:

- There are more than 15 tumor types that have druggable mutation targets amenable to MX ICE COLD-PCR.
- There are more than 250 early stage clinical trials examining druggable mutation targets amenable to MX ICE COLD-PCR.
- Collection of sera/plasma is becoming commonplace in clinical trial protocols. These “Liquid Biopsy” samples will be used for:
  - Mutation characterization of clinically actionable and resistance mutations
  - Identification of mutations that may become important as future biomarkers
  - Longitudinal assessment of treatment regimes
  - Surveillance of disease progressions
- Clinical results would validate the utility of MX ICE COLD-PCR in Cancer Management for patients.
# MX ICE COLD-PCR: Clinical Product Concepts

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Biopsy Test **</td>
<td>Targeted to cancers and patients where tissue samples are unavailable or can’t be taken easily or efficiently</td>
</tr>
<tr>
<td>Screening Test</td>
<td>Multiple amplicons from different genes and different cancer indications</td>
</tr>
<tr>
<td>Targeted Drug Test</td>
<td>Multiple amplicons from different genes and targeted towards specific cancer drug</td>
</tr>
<tr>
<td>Monitoring Test</td>
<td>Replica of above tests but looking at monitoring treatment and or progression</td>
</tr>
<tr>
<td>Surveillance Test</td>
<td>Replica of above tests but looking at on-going level of mutations over long periods at even lower levels</td>
</tr>
</tbody>
</table>

** First target test, which will drive adoption and will be accepted rapidly by Oncologists/Pathologist
Clinical Applications

- Detection of oncogenic mutations and molecular targets in plasma cfDNA when tissue is in short supply (approximately 10-20% of patients with metastatic cancer)

- Monitoring of pertinent oncogene mutant allele fraction in serial plasma cfDNA samples as a surrogate marker for response to anticancer therapy

- Detection of emerging mutations in serial plasma cfDNA samples leading to secondary resistance to anticancer therapy

- Detection of low frequency mutations in the tumor tissue to determine possible mechanisms of future resistance
ICE COLD-PCR Conclusions

Compelling data
- cfDNA as a sample source for ICE COLD-PCR
- Platform independent
- Mutation independent
- LOD of ICE COLD-PCR paired with NGS ~0.01%

Development of MX ICE COLD-PCR allows sensitive mutation analysis for:
- Multiple Mutations
- Multiple Genes
- Multiple Samples

Ongoing clinical validations
- EGFR, KRAS, BRAF, NRAS, TP53…

The 'Perfect Pairing': ICE COLD-PCR with cfDNA for Highly Sensitive Mutation Detection
ICE COLD-PCR: A Valid Assay for Patient Monitoring and Surveillance
Phase I: Utility as a tumor tissue surrogate

1. “Liquid Biopsy” Surrogate for Cancers without Available Tissue

2. “Monitoring” for Treatment Response

3. “Monitoring” & “Surveillance” to assist in patient treatment plan

Thank You
ICE COLD-PCR: One Assay – Multiple Mutations

**EGFR Deletions**

- **NGS Only:**
  - 1% and 5% Deletion Sample
- **ICE COLD-PCR + NGS:**
  - 1% Deletion Sample
  - 5% Deletion Sample

Variants and frequencies for different deletion samples:

- p.E746_T751>1
  - NGS: 20
  - ICE COLD-PCR + NGS: 27
- p.E746_A750delELREA
  - NGS: 14
  - ICE COLD-PCR + NGS: 9
- p.E746_T751>A
  - NGS: 7
  - ICE COLD-PCR + NGS: 6
- p.E746_S752>A
  - NGS: 6
  - ICE COLD-PCR + NGS: 7
- p.E746_S752>V
  - NGS: 4
  - ICE COLD-PCR + NGS: 7
- p.L747_T751>Q
  - NGS: 8
  - ICE COLD-PCR + NGS: 15
- p.L747_P751>P
  - NGS: 10
  - ICE COLD-PCR + NGS: 41
- p.L747_P755>S
  - NGS: 8
  - ICE COLD-PCR + NGS: 24
- p.S752_I759delISPKANKEI
  - NGS: 13
  - ICE COLD-PCR + NGS: 29
- p.S752_I759delISPKANKEI
  - NGS: 11
  - ICE COLD-PCR + NGS: 47
- p.P753_I759delPKANKEI
  - NGS: 6
  - ICE COLD-PCR + NGS: 6

**Transgenomic**
Advancing Personalized Medicine
Multiple Samples, Multiple Amplicons – 1 NGS Analysis for Standard PCR or ICE COLD-PCR of cfDNA from Plasma

- Analysis of 3 Genes/5 Exons/12 Plasma Samples
- NGS: only 3 Mutations detected in 2/12 Samples
- ICE COLD-PCR paired with NGS: 15 Mutations Detected in 12 Samples

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**ICE COLD-PCR**

**STEP 1:**
- Denature
- 5' T A 5'
- 5' C G 5'
- 5' C PO4

**STEP 2:**
- Hybridize
- 5' T 5'

**STEP 3:**
- Differentially Denature at Tc
- Final Extension
- 5' C 5'

**Mutation Confirmation**
- Sanger
- NGS
- Any other platform

**STEP 4:**
- Anneal Amplification Primers
- 5' T 5'

**STEP 5:**
- Extension
- 5' C 5'

**KEY**
- Amp Primer 1
- Amp Primer 2
- 5' C PO4
- RS-oligo

**Linear Amp Exp. Amp**

**Exclusive license from Dr. Mike Makrigiorgos, DFCI**

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# Longitudinal Assessment of cfDNA BRAF Mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
<td>Melanoma</td>
<td>Melanoma</td>
<td>Melanoma</td>
<td>Melanoma</td>
<td>Erdheim-Chester disease</td>
<td>Papillary thyroid cancer</td>
</tr>
<tr>
<td><strong>Prior therapy</strong></td>
<td>BRAF inhibitor (response, intolerance)</td>
<td>BRAF inhibitor (response-&gt; PD)</td>
<td>BRAF inhibitor -&gt; BRAF and MET inhibitors (PD)</td>
<td>Ipilimumab and temodar</td>
<td>BRAF inhibitor (intolerance)</td>
<td>Sorafenib</td>
</tr>
<tr>
<td><strong>Tissue (CLIA)</strong></td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
</tr>
<tr>
<td><strong>Baseline cfDNA</strong></td>
<td>Wild-type</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
<td>BRAF V600E</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>Sirolimus + HCQ</td>
<td>Vemurafenib + chemotherapy</td>
<td>Vemurafenib + chemotherapy</td>
<td>Vemurafenib + chemotherapy</td>
<td>Peg-interferon</td>
<td>Vemurafenib + chemotherapy</td>
</tr>
<tr>
<td><strong>Response</strong></td>
<td>PD</td>
<td>SD (minor response)</td>
<td>SD (minor response)</td>
<td>SD (minor response)</td>
<td>SD (minor response)</td>
<td>SD</td>
</tr>
<tr>
<td><strong>Follow up cfDNA</strong></td>
<td>V600E</td>
<td>Wild-type</td>
<td>Wild-type</td>
<td>Wild-type</td>
<td>Wild-type</td>
<td>Wild-type</td>
</tr>
</tbody>
</table>
## Longitudinal Assessment of cfDNA KRAS Mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology</td>
<td>Appendiceal carcinoma</td>
<td>Colorectal carcinoma</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>Chemotherapy</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>Tissue baseline</td>
<td>KRAS G13D</td>
<td>KRAS G12D</td>
</tr>
<tr>
<td>cfDNA baseline</td>
<td>Wild-type</td>
<td>Wild-type</td>
</tr>
<tr>
<td>Treatment</td>
<td>Anti-VEGF</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>Response</td>
<td>PD</td>
<td>PD</td>
</tr>
<tr>
<td>cfDNA follow-up</td>
<td>KRAS G13D</td>
<td>KRAS G12D</td>
</tr>
</tbody>
</table>
Quantification of Mutation in cfDNA

**74 year old female with appendiceal carcinoma and BRAF V600E mutation**

**46 year old female with melanoma and BRAF V600E mutation**