Utility of ctDNA monitoring in metastatic melanoma disease surveillance

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Melanoma is Highly Curable when Diagnosed and Treated at Early Stages

- Primary melanoma
  - 5-year survival = 98%

- Brain Metastasis
  - 5-year survival = 15% - 30%

- Lung Metastasis

- Liver Metastasis

- Lymph Node Metastasis
  - 5-year survival = 63%

Blood-based monitoring of cell-free, circulating tumor DNA (ctDNA)
Disease Monitoring in Metastatic Melanoma

- Oncologists use frequent imaging studies to monitor disease
  - Typically CT scans as often as every 3-6 months
  - Expensive, time consuming
- No useful blood-based biomarker to monitor disease activity and guide decision-making as in other cancers
  - Prostate – Prostate Specific Antigen (PSA)
- Serum Lactate Dehydrogenase (LDH) is part of the AJCC Staging System, but has a low sensitivity and specificity to detect changes in tumor burden
- A sensitive and specific blood test for monitoring disease activity in metastatic melanoma could help clinicians detect treatment responses and failures more quickly and adjust therapies as needed
**Breakthrough Discoveries and New Treatments for Metastatic Melanoma**

- Mutations in a normal cellular growth pathway cause it to be stuck in the ‘on’ position

  - 5 ‘Hot Spot’ mutations in BRAF and NRAS in ~65% of melanomas
  - Drugs blocking the mutated BRAF proteins kill melanoma cells and improve survival

- Other drugs block a normal ‘off switch’ on immune cells -- tumors activate that switch to evade destruction -- blocking the switch results in immune cells destroying the cancer cells
Dramatic Clinical Responses and Relapses with BRAF$^{V600E}$ Inhibition

Baseline 15 weeks on Rx 23 weeks on Rx

Wagle et al. JCO 2011;29:3085-3096
Treatment failure remains common in metastatic melanoma

- Strategies to combine and/or switch treatments are under active investigation
- Recent studies suggest that patients with a lower disease burden may have improved survival outcomes
  - Normal LDH independently associated with longer median survival in BRAF or BRAF/MEK treated patients (24 months vs. 7 months, HR=0.31; p<0.001)

Advantages of Droplet Digital PCR

Digital PCR enables:
- Greater sensitivity to detect rare events
- Greater accuracy to measure quantities
- Greater precision in measurement
Cell Line Titration Series demonstrates excellent sensitivity and quantitation by ddPCR

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Presented at the 8th Circulating Nucleic Acids in Plasma and Serum conference, Baltimore, MD; November 2013. Session 1-Cancer
Study Design

- Patients undergoing treatment for metastatic melanoma
- Determine tumor mutation type (BRAF, NRAS)
- Select ‘personalized’ blood test
- Measure tumor DNA in blood
- Compare to CT scans and blood LDH

Patient Analysis Workflow

43 Patients with stage IIIc/IV metastatic melanoma tested via COBAS assay

20 Patients BRAF WT subsequently tested for NRAS Q61 mutations
11 Patients BRAF WT, NRAS WT
9 Patients had NRAS mutations:
4 Q61K
3 Q61R
2 Q61L

ddPCR on patient plasma samples

23 Patients COBAS mutant
1 Patient 12-210: BRAF V600D
22 Patients had BRAF mutations:
20 V600E
2 V600K

N= 31 patients; 146 plasma samples

30 samples from 9 patients with NRAS Q61 mutations
10 samples from 2 patients with BRAF V600K
106 samples from 20 patients with BRAF V600E

Circulating tumor DNA (ctDNA) is more sensitive than LDH in monitoring metastatic melanoma

### ctDNA is More Sensitive Than LDH in Detecting Metastatic Disease at Initiation of Systemic Therapy

<table>
<thead>
<tr>
<th>A</th>
<th>Pre-Treatment RECIST</th>
<th>ctDNA</th>
<th>LDH</th>
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<td>Elevated Samples</td>
<td>% Elevated</td>
<td>Average copies/ml Elevated</td>
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<tr>
<td>&lt;5 (RECIST Total cm)</td>
<td>5</td>
<td>7</td>
<td>71%</td>
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<td>5-10 (RECIST Total cm)</td>
<td>4</td>
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<tr>
<td>&gt;10 (RECIST Total cm)</td>
<td>3</td>
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<tr>
<td>Total</td>
<td>12</td>
<td>15</td>
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ctDNA is more sensitive than LDH in detecting disease progression  
*(Overall sensitivity -- ctDNA = 82% vs. LDH 40%, p<0.001)*

### B

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<th>Progression Event</th>
<th>ctDNA</th>
<th>LDH</th>
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<td>Brain Met*</td>
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<td>Death or Hospice*</td>
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<tr>
<td>Total</td>
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*Progression event defined by non-RECIST criteria

ctDNA monitoring can detect non-RECIST disease progression

Decrease in ctDNA level in response to therapy may predict survival

Significant association between ctDNA ‘molecular response’ and PFS (p<0.03)
Polsky lab - unpublished
Limitations and Future studies

• Sample collection
  – Convenience samples collected at irregular intervals
  – Representative of actual clinical practice
  – Need landmark time points with radiographic measures to properly evaluate metrics of sensitivity and specificity

• Eligible patients limited to BRAF or NRAS mutant
  – New mutation markers needed for $\text{BRAF}^{\text{wild-type}}/\text{NRAS}^{\text{wild-type}}$
TERT Promoter mutations are common in BRAF wt/NRAS wt melanoma

68% of patients lacking a BRAF or NRAS mutation had 1 of 2 TERT mutations

ddPCR detection of TERT promoter mutations

A172 - Glioblastoma

C228T

12-126 – Melanoma

C250T

Corless B, Chang C, et al (manuscript in preparation)
ddPCR detection of TERT C250T mutation in metastatic melanoma plasma

Patient #2 plasma

C250T detected in 2 plasma samples

Patient #4 plasma

Corless B, Chang C, et al (manuscript in preparation)
Current Plans

• **Analytical validation:** Develop standardized operating procedures for each ddPCR mutation-specific assay and determine each assay’s performance characteristics to enable adoption in CLIA-certified laboratories

• **Clinical validation:** Determine the sensitivity and specificity of ctDNA monitoring to detect disease recurrence in patients receiving adjuvant therapy for surgically resected, regionally metastatic disease
Clinical validation research plan

• Analysis of serial plasma samples from BMS CheckMate 238 adjuvant Ipilimumab vs. Nivolumab clinical trial
  – n=918 patients with resected stages IIIB, IIIC, or IV
  – ctDNA assessments
    • BRAF, NRAS, or TERT promoter mutations based on the patients’ tumor mutational genotype determined by Molecular MD Corp. (BRAF/NARS) / Polsky lab (TERT)
Clinical validation planned data analysis

• Determine the association between elevated ctDNA levels and the presence of melanoma relapse
• Assess the relationship between elevated ctDNA levels and relapse-free survival
• Define the sensitivity and specificity of the ctDNA assays with respect to the presence of melanoma relapse
• Develop a predictive model of relapse-free survival that incorporates ctDNA and other clinic-pathologic characteristics

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<td>6</td>
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Conclusions

• Serial monitoring of BRAF and NRAS ctDNA is superior to LDH in monitoring disease activity in metastatic melanoma

• ctDNA shows promise as a biomarker of metastatic disease activity in patients treated with systemic therapy

• ctDNA monitoring may help oncologists switch treatments when patient disease burden is lower than when it is detected radiographically

• Additional analytical and clinical validation studies are underway to help bring these assays to the clinic in the next 2-5 years
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