Utility of ctDNA monitoring in metastatic melanoma disease surveillance

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Disclosures

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- Molecular MD, Corporation
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  - Licensed Intellectual Property
  - In-kind research support

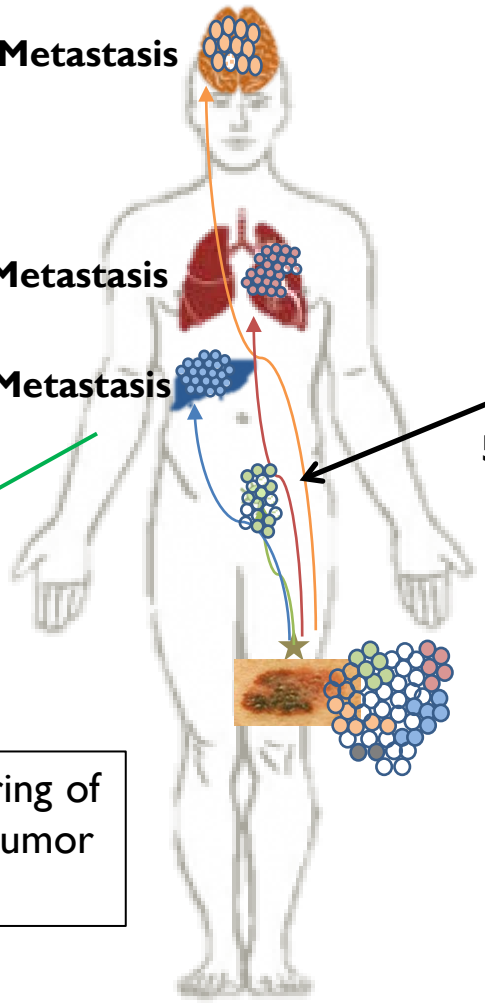
Melanoma is Highly Curable when Diagnosed and Treated at Early Stages

5-year survival = 15% - 30%

Brain Metastasis

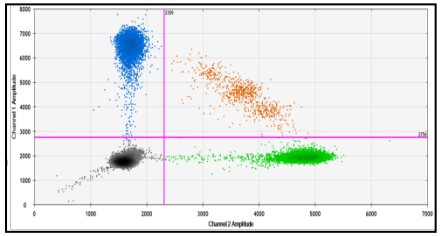
Lung Metastasis

Liver Metastasis



Lymph Node Metastasis
5-year survival = 63%

Primary melanoma
5-year survival = 98%



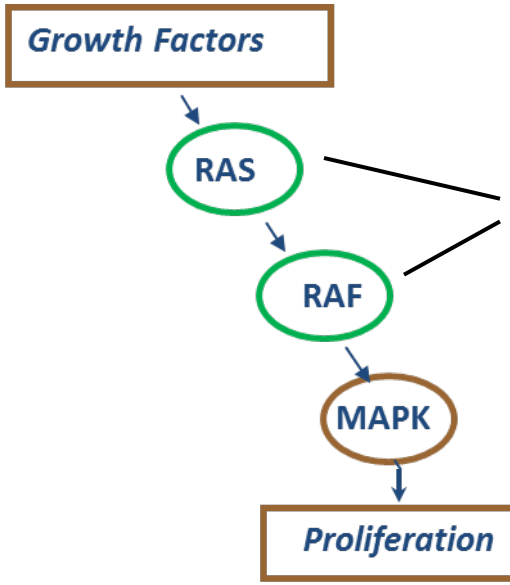
Blood-based monitoring of cell-free, circulating tumor DNA (ctDNA)

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

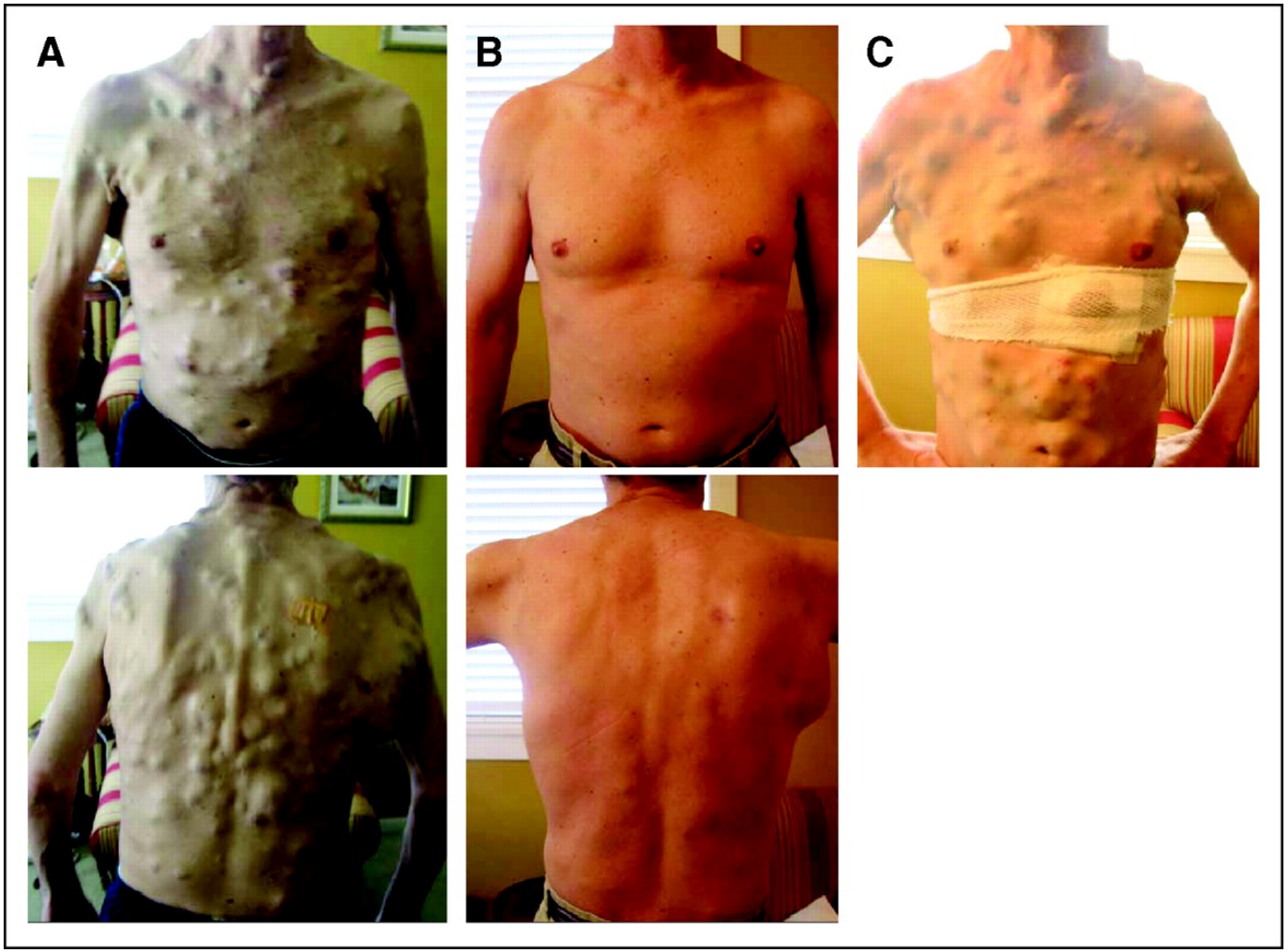
- Mutations in a normal cellular growth pathway cause it to be stuck in the 'on' position



- 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

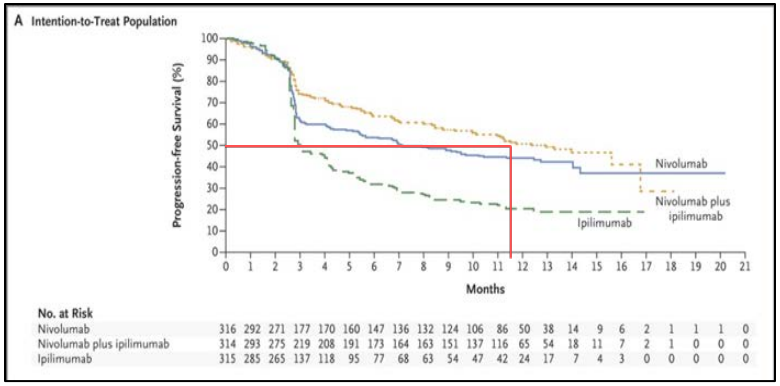
Dramatic Clinical Responses and Relapses with BRAF^{V600E} Inhibition



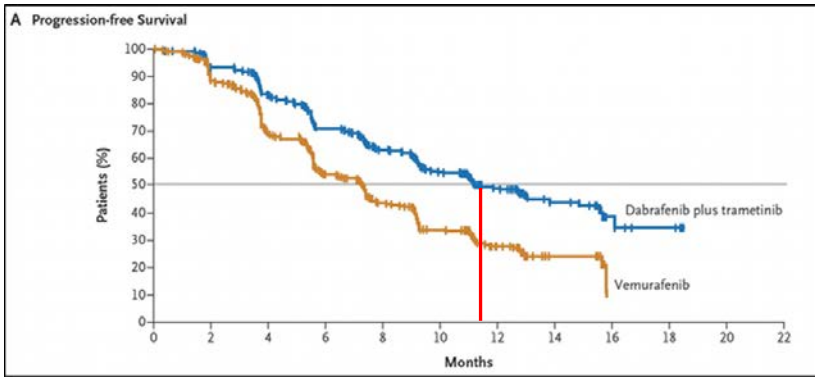
Baseline 15 weeks on Rx 23 weeks on Rx

Wagle et al. JCO 2011;29:3085-3096

Treatment failure remains common in metastatic melanoma



Immune checkpoint blockade

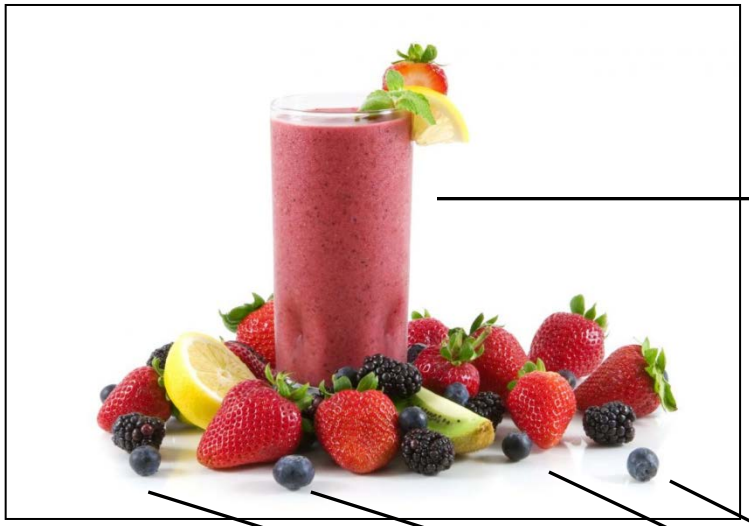


Dabrafenib + Trametinib
(anti-BRAF^{mt}) (anti-MEK)

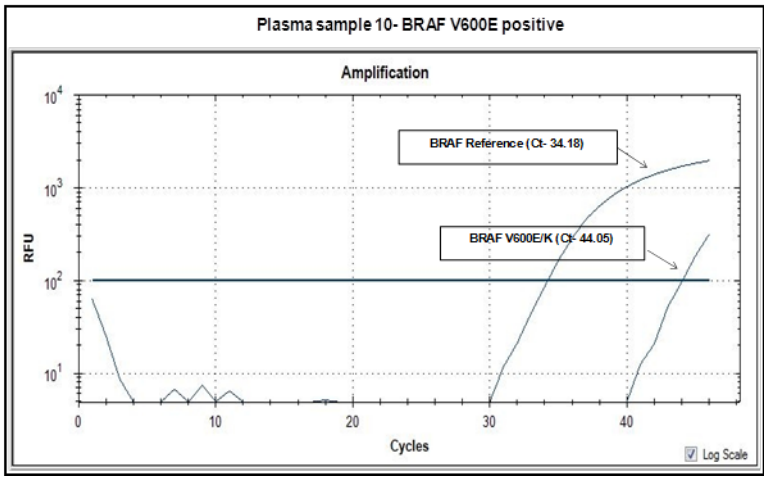
- Strategies to combine and/or switch treatments are under active investigation
- Recent studies suggest that patients with a lower disease burden may have improved survival outcomes
  - Normal LDH independently associated with longer median survival in BRAF or BRAF/MEK treated patients (24 months vs.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

Larkin J et al. (2015) *N Engl J Med* 373:23; Robert C et al. (2015) *N Engl J Med* 372:30; Menzies AM et al. (2015) Cancer 121:3826

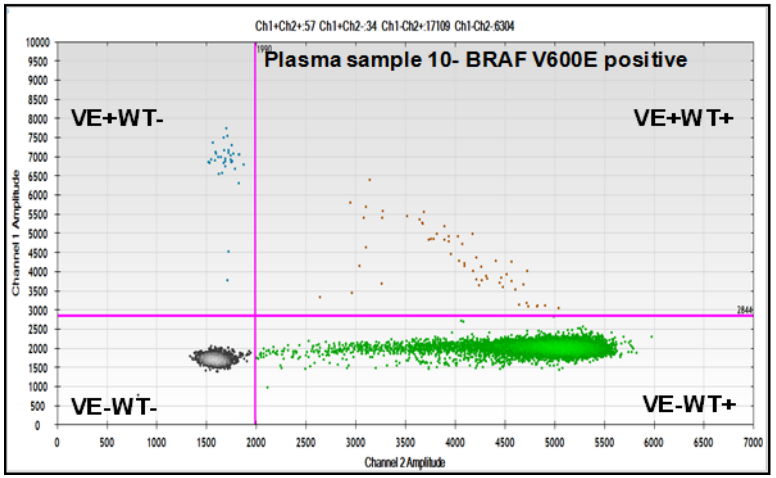
Advantages of Droplet Digital PCR



TAQMAN



ddPCR



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

Stock name	Total DNA (ng)/ rxn	% BRAF V600E DNA	BRAF V600E genomic DNA (ng)	BRAF V600E mutant copies			TAQMAN BRAF V600E Assay	
				EXPECTED		ddPCR	BRAF Reference Ct	BRAF V600E/K Specific Ct
D4	300	0.1	0.3	100		96.1	24.9	36.5
E5	300	0.01	0.03	10		10.5	24.7	38.4
F6	300	0	0	0		0	25.0	Not Detected
H8	30	10	3	1000		1065	28.0	31.5
I9	30	1	0.3	100		105	27.9	34.6
J10	30	0.1	0.03	10		10.6	28.1	38.8
K11	30	0.01	0.003	1		0.9	28.3	Not Detected
L12	30	0	0	0		0	28.1	Not Detected
N14	3	10	0.3	100		80	31.9	35.6
O15	3	1	0.03	10		7.6	31.7	38.7
P16	3	0.1	0.003	1		1.29	31.7	43.1
Q17	3	0.01	0.0003	0.1		0	31.7	Not Detected
R18	3	0	0	0		0	32.0	Not Detected

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

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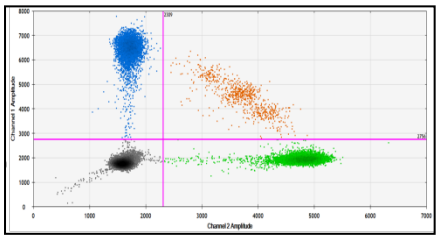
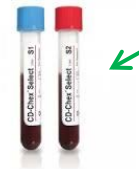
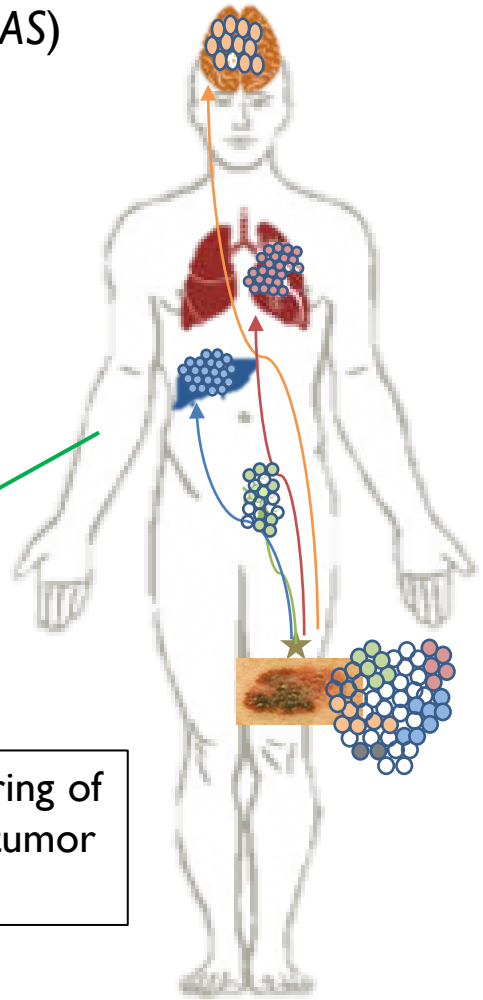
Study Design

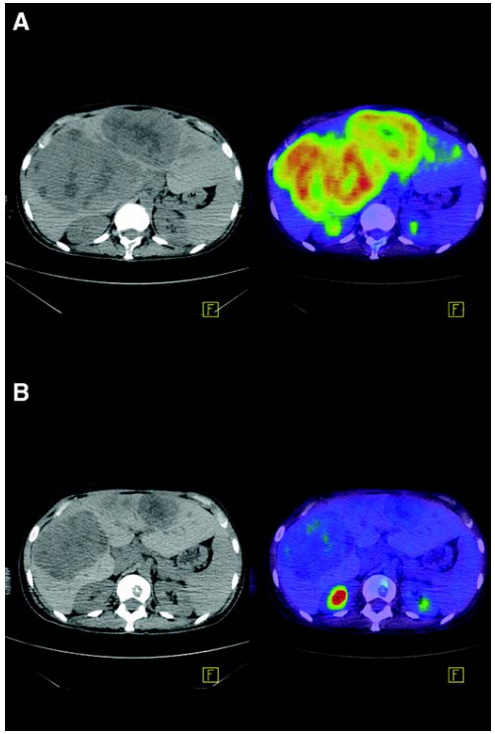
- Patients undergoing treatment for metastatic melanoma
- Determine tumor mutation type (*BRAF, NRAS*)
- Select 'personalized' blood test
- Measure tumor DNA in blood
- Compare to CT scans and blood LDH





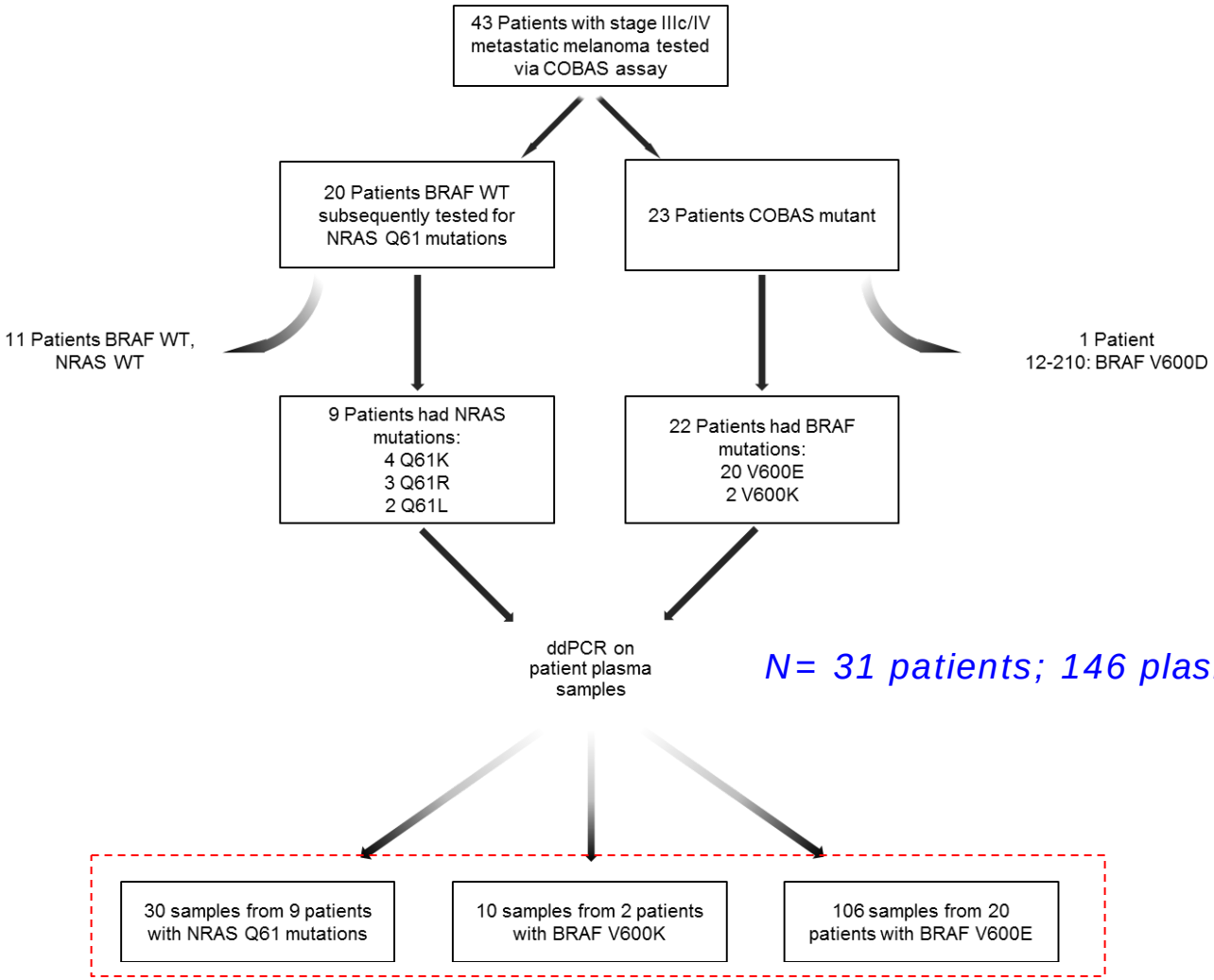


Blood-based monitoring of cell-free, circulating tumor DNA (ctDNA)



McArthur GA, et al. JCO (2012)

Patient Analysis Workflow



N= 31 patients; 146 plasma samples

Chang G, et al. (2016) Mol Onc 10:157-165

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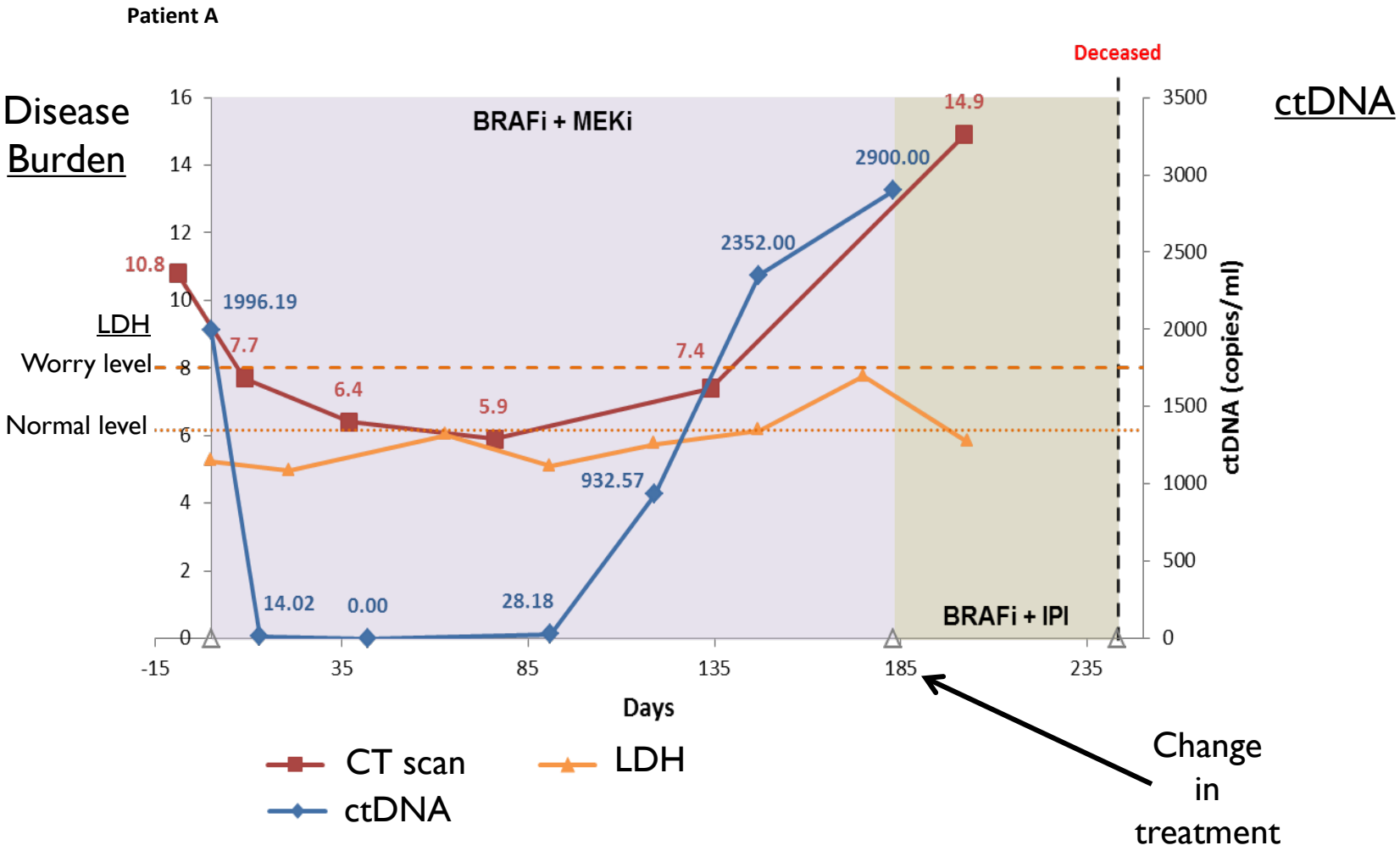
Circulating tumor DNA (ctDNA) is more sensitive than LDH in monitoring metastatic melanoma



Chang G, et al. (2016) Mol Onc 10:157-165

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

A

Pre-Treatment RECIST	ctDNA					LDH				
	Elevated Samples	Total Samples	% Elevated	Average copies/ml Elevated	Average copies/ml Total	Elevated Samples	Total Samples	% Elevated	Average IU/L Elevated	Average IU/L Total
<5 (RECIST Total cm)	5	7	71%	66.89	47.85	1	13	8%	658	477
5-10 (RECIST Total cm)	4	5	80%	2003.22	1602.58	3	5	60%	960	760
>10 (RECIST Total cm)	3	3	100%	9936.62	9936.62	3	5	60%	1015	808
Total	12	15	80%			7	23	30%		

ctDNA is more sensitive than LDH in detecting disease progression

(Overall sensitivity -- ctDNA = 82% vs. LDH 40%, p<0.001)

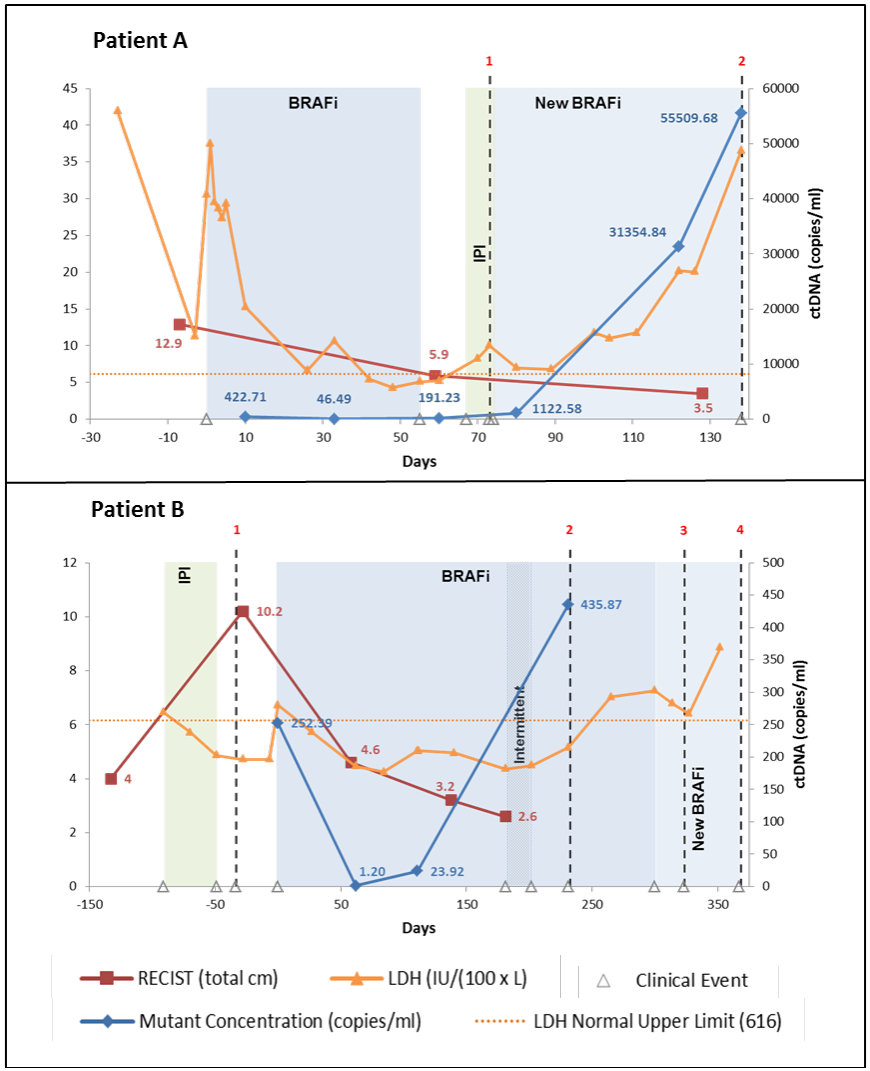
B

Progression Event	ctDNA					LDH				
	Elevated Samples	Total Samples	%	Average copies/ml for Elevated Samples	Average copies/ml for All Samples	Elevated Samples	Total Samples	%	Average IU/L for Elevated Samples	Average IU/L for All Samples
<5 (RECIST Total cm)	9	14	64%	177.03	113.9	4	14	29%	953	601
5-10 (RECIST Total cm)	9	9	100%	2232.86	2232.86	5	9	56%	974	770
>10 (RECIST Total cm)	4	5	80%	2574.8	2060.02	2	5	40%	763	625
Non-Target Lesions*	0	1	0%	-	0.71	0	1	0%	-	491
Bone Met*	1	1	100%	19.34	19.34	0	1	0%	-	511
Brain Met*	10	12	83%	1476.6	1230.56	6	12	50%	853	687
Death or Hospice*	2	2	100%	27756.88	27756.88	2	2	100%	2138	2138
Total	35	44	80%			19	44	43%		

*Progression event defined by non-RECIST criteria

Chang G, et al. (2016) Mol Onc 10:157-165

ctDNA monitoring can detect non-RECIST disease progression



Chang G, et al. (2016) Mol Onc 10:157-165

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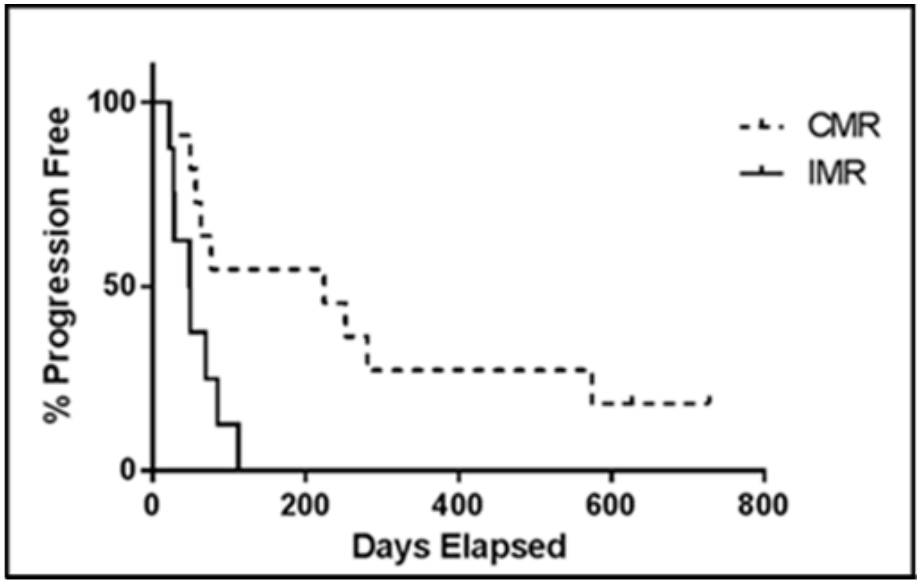
Decrease in ctDNA level in response to therapy may predict survival



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 $BRAF^{wild\text{-}type}/NRAS^{wild\text{-}type}$

TERT Promoter mutations are common in BRAF wt/NRAS wt melanoma

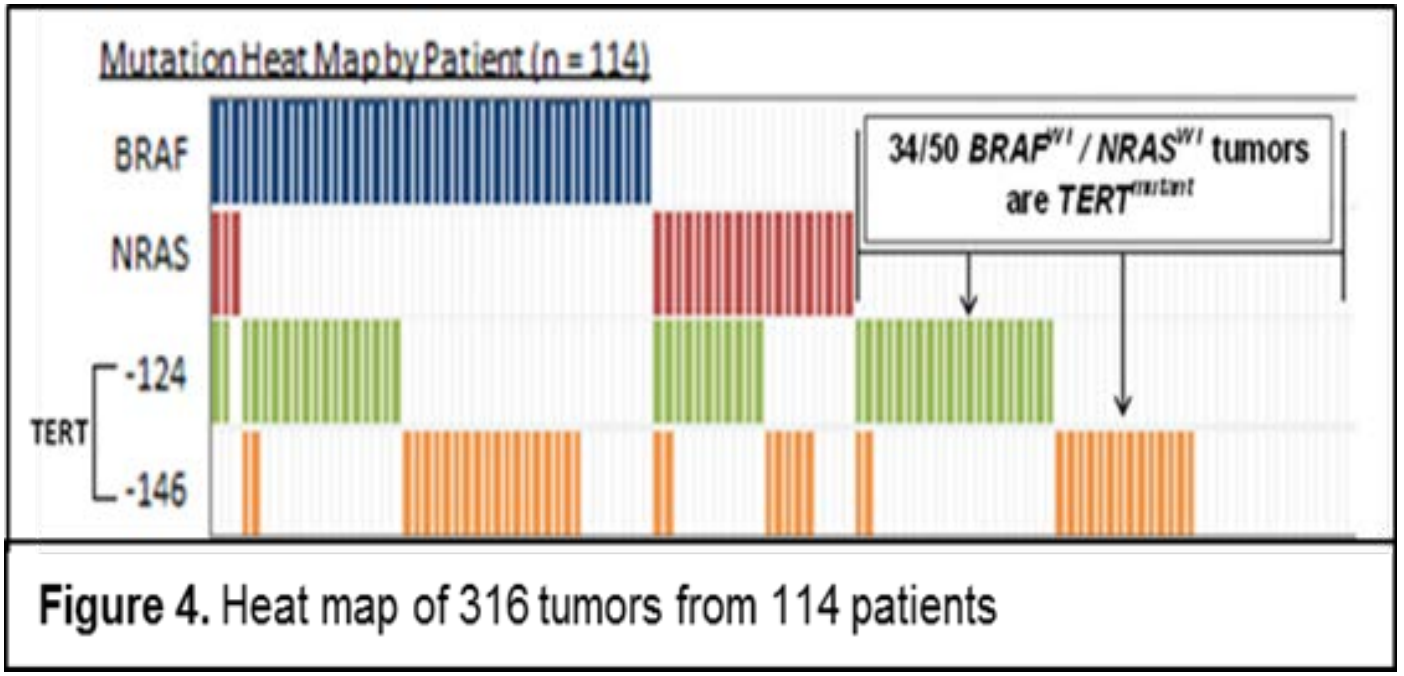


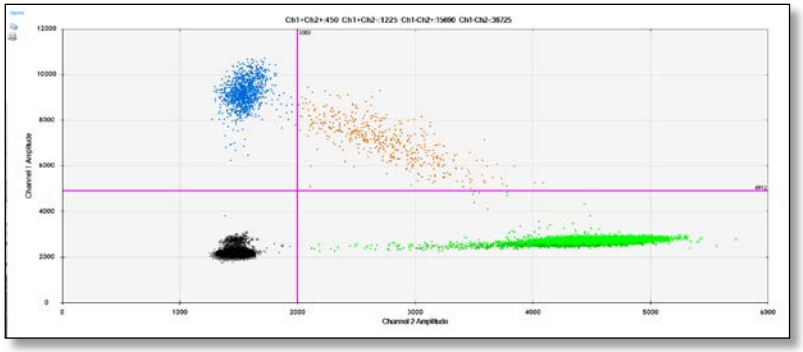
Figure 4. Heat map of 316 tumors from 114 patients

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

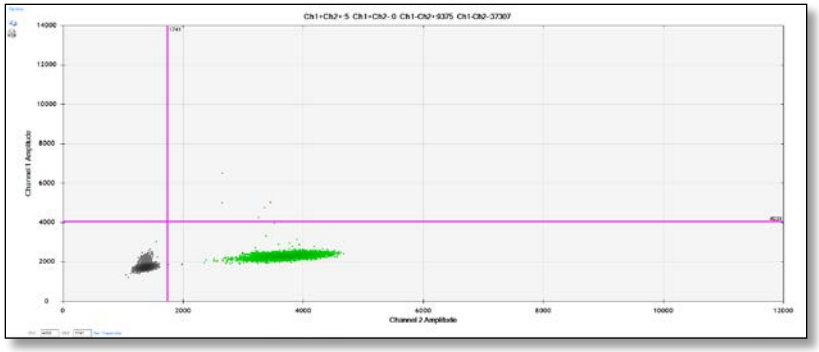
Chang G. et al. (2015) Proceedings of American Association for Cancer Research Special Conference on Advances in Melanoma: From Biology to Therapy, 2014 September 20-23; Philadelphia, PA. Abstract A31

ddPCR detection of TERT promoter mutations

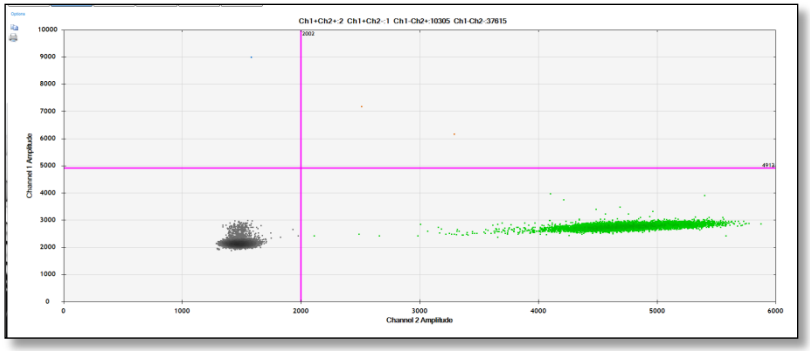
A172 - Glioblastoma



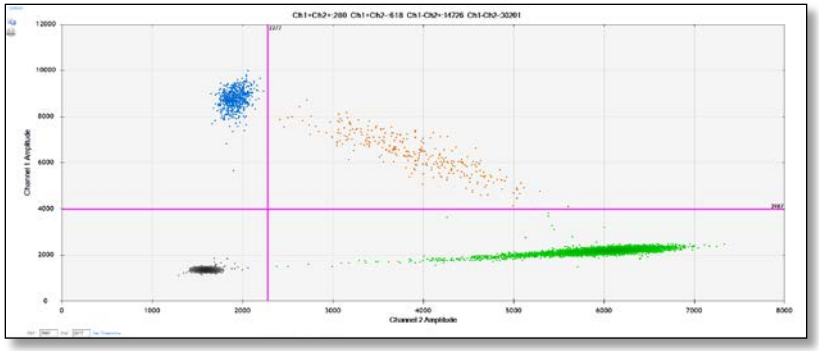
C228T



12-126 – Melanoma



C250T



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

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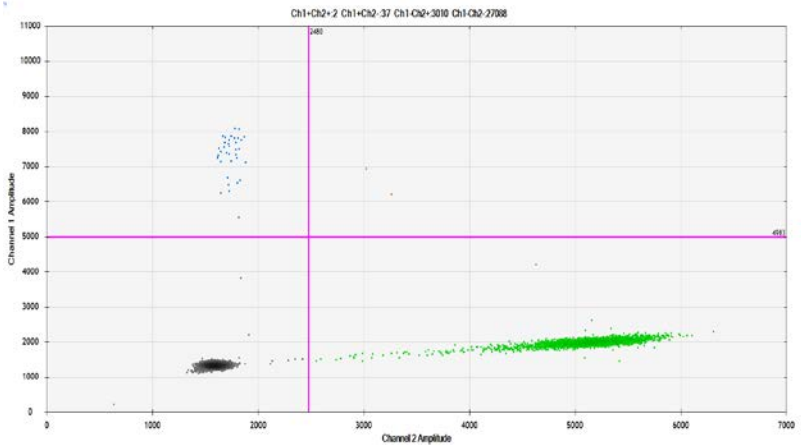
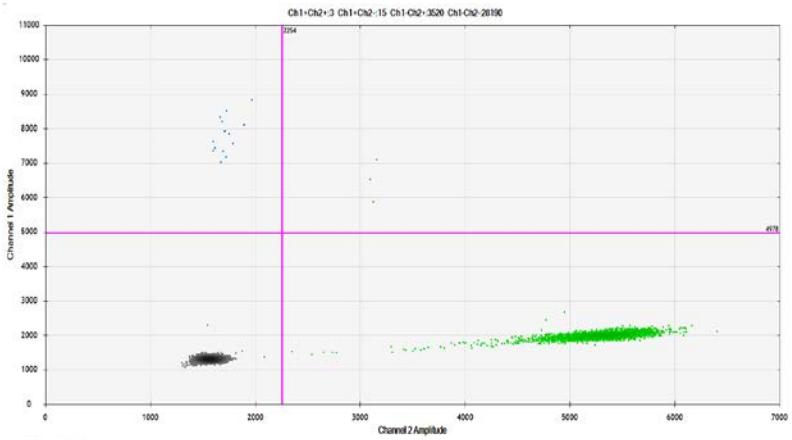
ddPCR detection of TERT C250T mutation in metastatic melanoma plasma

Patient #2 plasma

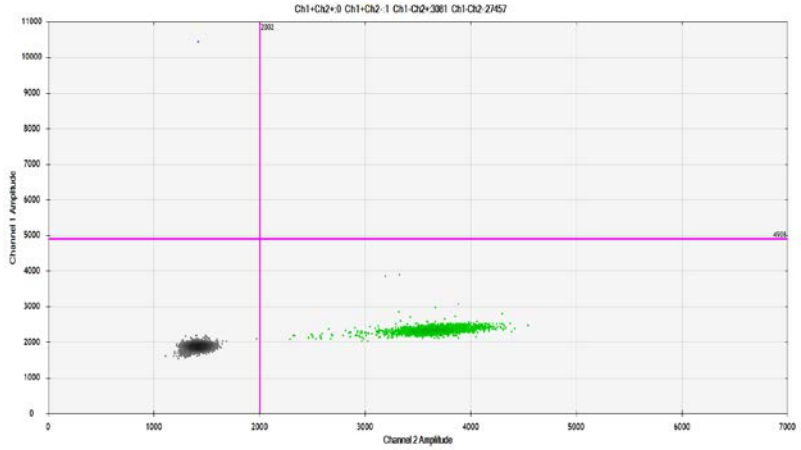


C250T detected in 2 plasma samples

Patient #4 plasma

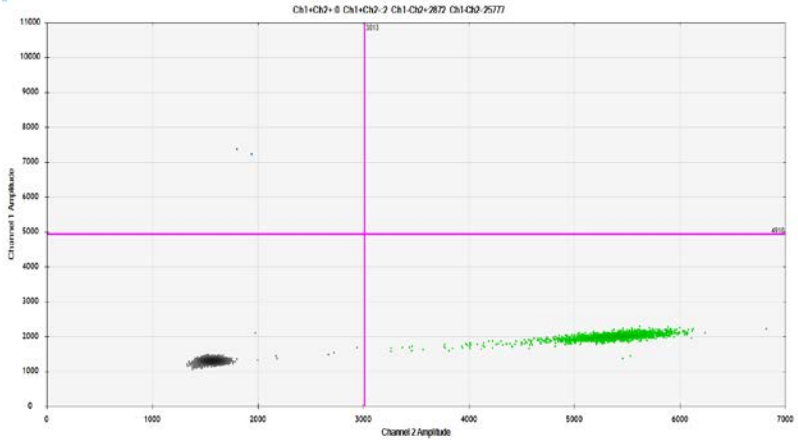


C228T



Plasma #6 No TERT detected

C250T



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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

Table 3		Follow Up Visit				
Scenario	Assessment	1	2	3	4	ctDNA
1	ctDNA	-	-	+	+	*TP*
	radiographic scan	-	-	+	+	
2	ctDNA	-	-	+	+	*TP*
	radiographic scan	-	-	-	+	
3	ctDNA	-	+	+	+	*TP*
	radiographic scan	-	-	-	+	
4	ctDNA	-	+	+	+	*FP*
	radiographic scan	-	-	-	-	
5	ctDNA	-	-	-	-	*TN*
	radiographic scan	-	-	-	-	
6	ctDNA	-	-	-	-	*FN*
	radiographic scan	-	-	-	+	

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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