Ultrasensitive detection of circulating tumor DNA may predict recurrence in patients with early stages melanoma

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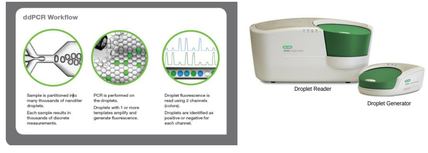
BACKGROUND

- Up to 70% of patients with resected high-risk melanoma develop disease recurrence within 5 years
- Adjuvant immunotherapy or targeted therapy can reduce the recurrence rate below approximately 60%; however, it is at the cost of possible toxicity including long-term side effects
- Cell-free (cf) DNA from the plasma of cancer patients contains a small amount of circulating tumor DNA (ctDNA) and offers an easily obtainable, low-risk, inexpensive and repeatedly applicable source of biologic material for *IDH* mutation analysis
- We hypothesize that detection of plasma-derived ctDNA from patients with resected melanoma can identify patients in high-risk of disease recurrence

METHODS

- We developed an ultrasensitive and specific droplet digital PCR – based method (Bio-Rad) to detect *BRAF*^{V600E}-mutated ctDNA in pre-amplified cell-free DNA with sensitivity up to 2 mutant copies in the wild-type background
- Plasma samples from patients with surgically resectable melanoma and *BRAF*^{V600E} mutation in tumor tissue were collected on the day of surgery and during follow-up visits for *BRAF*^{V600E} ctDNA detection (Figure 1). Results were correlated with clinical outcomes

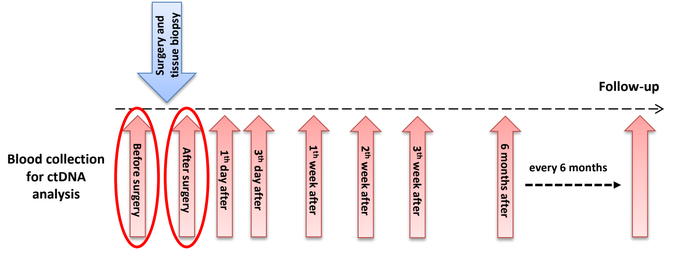


RESULTS

Table 1: Patients characteristics (N=23)

Characteristic	Total no. of patients (%)
All	23 (100)
Age, median in years	58 (range, 26-87)
Gender	
Male	11 (48)
Female	12 (52)
Race	
Caucasian	23 (100)
Stage TNM	
Stage 0	2 (9)
Stage IA	4 (17)
Stage IB	1 (4)
Stage IIA	3 (14)
Stage IIB	1 (4)
Stage IIC	5 (22)
Stage IIIA	2 (9)
Stage IIIB	1 (4)
Stage IIIC	4 (17)
***BRAF*^{V600E} status**	
***BRAF*^{V600E} mutation in the tissue**	23 (100)
***BRAF*^{V600E}-mutated ctDNA before surgery**	11 (48)
***BRAF*^{V600E}-mutated ctDNA after surgery**	8 (35)
Melanoma recurrence	4
Death of any cause	2

Figure 1: Study timeline



RESULTS

Figure 2: Patients with ctDNA in samples collected after surgery had shorter Disease-free survival than patients without ctDNA (P=0.03)

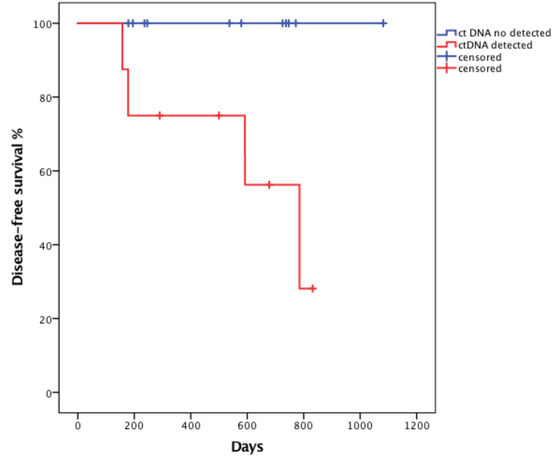


Table 2: ctDNA in pre- and post-surgical plasma samples and melanoma recurrence

***BRAF*^{V600E} mutations in ctDNA and melanoma recurrence in 19 patients with available data**

Category	Number	Disease Recurrence (%)	P value
***BRAF*^{V600E} in tissue**	23	4 (17)	-
***BRAF*^{V600E} in ctDNA before surgery**	11	2 (18)	NS
No *BRAF*^{V600E} in ctDNA before surgery	8	2 (25)	
***BRAF*^{V600E} in ctDNA after surgery**	8	4 (50)	**0.02**
No *BRAF*^{V600E} in ctDNA after surgery	11	0 (0)	

RESULTS

- Total of 23 patients with resectable melanoma (stage 0, n=2; stage 1, n=5; stage 2, n=9; stage 3, n=7) with BRAFV600E mutation in tumor tissue were enrolled (Table 1)
- *BRAF*^{V600E}-mutated ctDNA was detected in 11 (48%) patients before surgery and in 8 (35%) patients after surgery (Table 2)
- Patients with ctDNA in samples collected after surgery had more disease recurrences (4/8, 50% vs. 0/11, 0%; P=0.02) than patients without ctDNA in samples collected after surgery (Table 2)
- Patients with ctDNA in samples collected after surgery had shorter disease-free survival than patients without ctDNA in samples collected after surgery (P=0.03, Figure 2)

CONCLUSIONS

- Our preliminary data demonstrate that ultrasensitive droplet digital PCR method can detect ctDNA in patients with resectable melanoma
- Patients with detectable ctDNA in blood samples collected after surgery have higher frequency of melanoma recurrence
- Patients with detectable ctDNA in blood samples collected after surgery have shorter Disease-free survival

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