Bespoke Circulating Tumor DNA (ctDNA) Analysis as a Predictive Biomarker in Solid Tumor Patients (pts) Treated with Single Agent Pembrolizumab (P)

Marco AJ Iafolla[1], Cindy Yang[2], Scott Dashner[3], Wei Xu[4], Aaron R Hansen[1], Philippe Bedard[1], Stephanie Lheureux[1], Anna Spreafico[1], Albiruni Abdul Razak[1], Hsin-Ta Wu[3], Svetlana Shchegrova[3], Amy Liu[4], Pamela S Ohashi[5], Dax Torti[6], Maggie Louie[3], Himanshu Sethi[3], Alexey Aleshin[3], Lillian L Siu[1]*, Scott Bratman[7], Trevor J Pugh[2]

[1]Department of Medical Oncology, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada; [2]Medical Biophysics, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada; [3]Natera Inc., San Carlos, CA, USA; [4]Biostatistics, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada; [5]Immunology, Campbell Family Institute for Cancer Research, University Health Network, Toronto, Ontario, Canada; [6]Ontario Institute for Cancer Research, Toronto, Ontario, Canada; [7]Department of Radiation Oncology, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada

CONQUER CANCER® THE ASCO FOUNDATION | MERIT AWARD RECIPIENT

Abstract number #2542

AMERICAN SOCIETY OF CLINICAL ONCOLOGY 2019
Chicago, IL, USA | May 31–June 4, 2019

Introduction

- The advent of ultra-sensitive and specific molecular assays has increased the feasibility of circulating tumor DNA (ctDNA) detection.[1]
- ctDNA is emerging as a viable tool to provide cancer diagnostic and prognostic information.[1]
- We seek to determine the clonal dynamics of serial ctDNA as a predictive biomarker in patients with advanced solid tumor receiving immune checkpoint blockade.

Methods

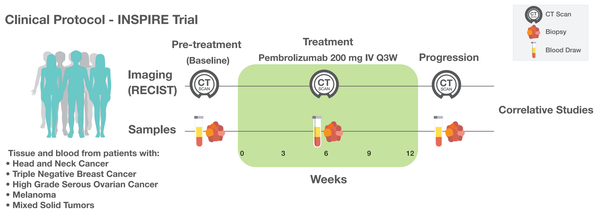
Patients and Treatment:

- Patients with various solid tumors in assigned cohorts were enrolled in a single center from March 21, 2016 to May 9, 2018 and treated with single agent P (anti-PD-1 antibody) 200 mg IV Q 3 weeks in the investigator-initiated Phase II trial (INSPIRE, NCT02644369).

Quantification of ctDNA:

- Illumina HiSeq2500 was used for whole exome sequencing (WES) of 70 pre-P tumor samples (mean coverage 121.5X) and matched germline controls from peripheral blood mononuclear cells (mean coverage 40.2X).
- Cell-free DNA (cfDNA) was extracted at baseline (B) and pre-cycle 3 (C3) using QIAamp Circulating Nucleic Acid kit (Qiagen, Hilden, Germany).
- Plasma samples were retrospectively analyzed for the presence of ctDNA with the bespoke16-plex assay targeting single nucleotide and indel variants using the Signatera™ workflow.

Figure 1. Schematic of Study Protocol



Results

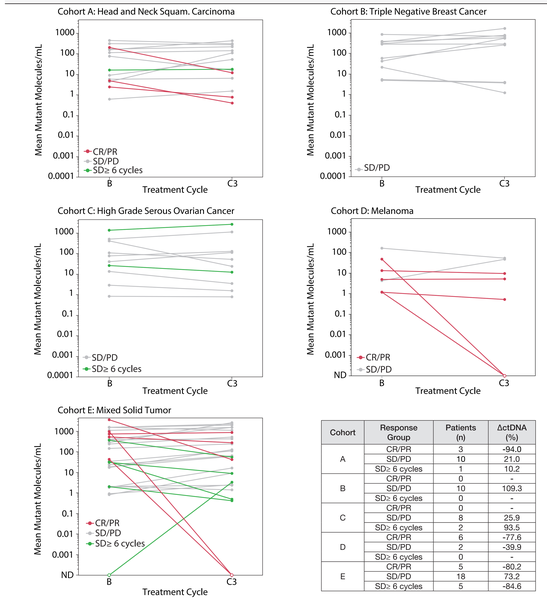
Table 1. Patient Demographics and Outcome[a]

Patients, N=70[b] P cycles, median (range)=4 (2–35)	Age[c] (years), median(range) = 60 (21–82) Follow up (months), median (range) = 14 (2–29)

Gender, n (%)		Ethnicity, n (%)	
Female	38 (54)	Asian	8 (11)
Male	32 (46)	African	2 (2.9)
		Caucasian	59 (84)
		Unknown or Not Reported	1 (1.4)
Cohort, n (%)		**Best response, n (%)**	
A: Head and neck squam. carcinoma	14 (20)	CR	2 (2.9)
B: Triple negative breast cancer	10 (14)	PR	12 (17)
C: High grade serous ovarian cancer	10 (14)	SD	21 (30)
D: Melanoma	8 (11)	PD	35 (50)
E: Mixed solid tumor	10 (40)	CBR[d]	22 (31)
Reason for coming off trial, n (%)			
1. RECIST progression	43 (65)	5. Investigator's decision	1 (1.5)
2. Clinical progression	10 (15)	6. Death	1 (1.5)
3. Intercurrent illness	4 (6.1)	7. Study completion	2 (3.0)
4. Toxicity	5 (7.6)		
Number of patients still receiving P=4 (5.7%)			

a, As of last clinical update on November 14, 2018.
b, Only 70 patients had B and C3 bloodwork for cfDNA analysis and tumor WES data.
c, At time of first P infusion.
d, Clinical benefit rate (CBR) is defined as complete response (CR), partial response (PR) or stable disease (SD) lasting ≥6 cycles (C6) of Pembrolizumab.

Figure 2. Overview of ctDNA Dynamics in Different Patient Cohorts



Cohort	Response Group	Patients (n)	ΔctDNA (%)
A	CR/PR	3	-94.0
	SD/PD	10	21.0
	SD≥ 6 cycles	1	10.2
B	CR/PR	0	-
	SD/PD	10	109.3
	SD≥ 6 cycles	0	-
C	CR/PR	0	-
	SD/PD	8	25.9
	SD≥ 6 cycles	2	93.5
D	CR/PR	6	-77.6
	SD/PD	2	-39.9
	SD≥ 6 cycles	0	-
E	CR/PR	5	-80.2
	SD/PD	18	73.2
	SD≥ 6 cycles	5	-84.6

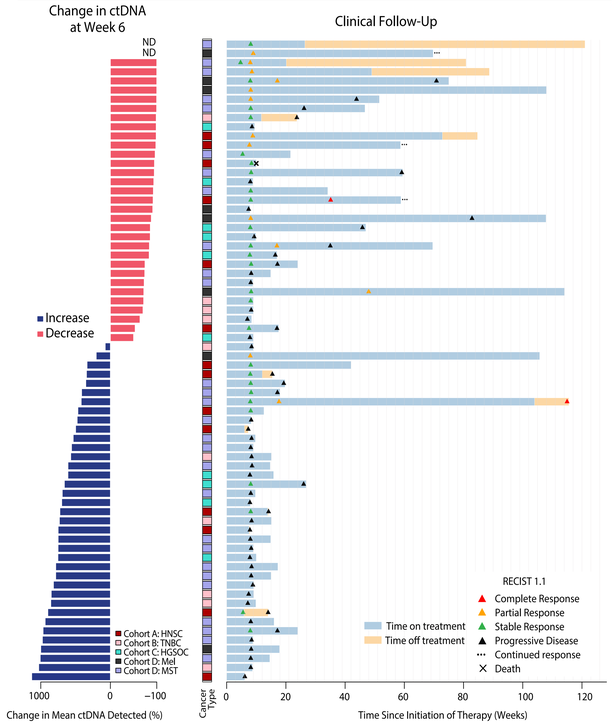
ΔctDNA, $ctDNA_{C3}$ compared to $ctDNA_B$; Closed circles indicate ctDNA positive samples and open circles indicate ctDNA negative samples; SD, stable disease; CR, complete response; PR, partial response; PD, progressive disease; B, baseline; C3, cycle 3; ND, not detected (below detection threshold).

Table 2. Correlation of ΔctDNA[a] with Clinical Response

Endpoint	Subgroup		ΔctDNA median (range)	P-value[b]
ORR; N = 68[c]	CR	n=13	-91.5% (-100–18.1%)	<0.001
	PR			
	SD	n=55	31.5% (-98.7–2,458.1%)	
	PD			
CBR; N = 68[c]	CR	n=20	-75% (-100–96.1%)	<0.001
	PR			
	SD ≥ C6			
	SD <C6	n=48	49% (-94.6–2,458.1%)	
	PD			

a, $ctDNA_{C3}$ compared to $ctDNA_B$; ctDNA units are expressed as mean mutant molecules per mL of plasma.
b, Calculated using Wilcoxon rank-sum test.
c, 2 patients with $ctDNA_B$=0 were excluded from ORR/CBR analyses.
ORR, overall response rate; CBR, clinical benefit rate; CR, complete response, PR, partial response, SD, stable disease.

Figure 3. Correlation of ΔctDNA with Clinical Response



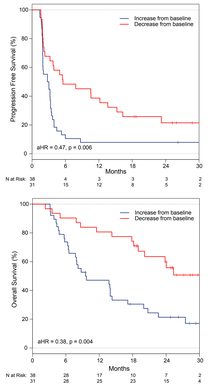
Swimmer plot depicting correlation between change in ctDNA and clinical responses in the cohort.
ΔctDNA, $ctDNA_{C3}$ compared to $ctDNA_B$; HNSC, Head and Neck Squamous Carcinoma; TNBC, Triple Negative Breast Cancer; HGSOC; High Grade Serous Ovarian Cancer; Mel, Melanoma; MST, Mixed Solid Tumors; ND, not detected (below detection threshold).

Table 3. Correlation of ΔctDNA with Patient Survival

Endpoint	Subgroup	Based on ΔctDNA	Adjusted HR[a] (95% CI)
Progression Free Survival N=69[b]	Decrease from baseline; n=31	Median=5.6 months 6 month=48.4%	0.47 (0.27–0.81) P-value 0.006
	Increase from baseline; n=38	Median=2.9 months 6 month=10.5%	
Overall Survival N=69[b]	Decrease from baseline; n=31	Median=24 months 6 month=90.3; 12 month=80.6%	0.38 (0.19–0.73) P-value 0.004
	Increase from baseline; n=38	Median=9.5 months 6 month=73.7%; 12 month=47.1%	

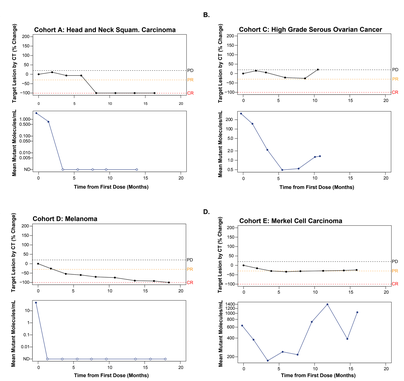
a, Calculated using univariate Cox proportional hazards model with adjustment on cohorts
b, 1 patient (with partial response) was excluded from PFS/OS analyses as both baseline and pre-cycle 3 ctDNA=0.

Figure 4. Correlation of ΔctDNA with Patient Survival



Kaplan-Meier analysis of **(A)** progression free survival and **(B)** overall survival by dichotomizing patients based on $ctDNA_{C3}$>$ctDNA_B$ versus $ctDNA_{C3}$<$ctDNA_B$. aHR, adjusted hazard ratio.

Figure 5. Correlation Between ctDNA Levels and CT Imaging



Correlation between ctDNA levels and CT imaging in response to treatment for four patients. Closed blue circles indicate ctDNA-positive samples and open blue circles idicate ctDNA-negative samples. PD, progressive disease; PR, partial response; CR, complete response; ND, not detected (below detection threshold).

Conclusions

- A strong correlation exists between ΔctDNA with OS, PFS, CBR and ORR in patients treated with pembrolizumab.
- ctDNA can predate radiological response.
- Limitations of study include small sample size and heterogeneous histologies
- Future plans include:
  - Complete additional ctDNA analysis between baseline and cycle 3
  - Complete all baseline ctDNA analysis to evaluate prognostic value
  - Conduct serial ctDNA analysis after cycle 3
  - Perform validation using a larger and more homogeneous cohort

References

1. Corcoran RB, Chabner BA. Application of Cell-free DNA Analysis to Cancer Treatment. N Engl J Med. 2018;379(18):1754-1765.

Acknowledgements

We would like to thank the patients and their families for their participation, the Princess Margaret Cancer Foundation and Terry Fox Research Institute for their support, and Merck for their contribution of pembrolizumab to this study.

Marco AJ Iafolla is supported in part by a fellowship through the BMO Chair in Precision Cancer Genomics.

UHN Toronto General Toronto Western Princess Margaret Toronto Rehab | UNIVERSITY OF TORONTO | signatera RESEARCH ONLY | natera Conceive. Deliver. Thrive.

***Corresponding Author Information:**
Lillian Siu, MD, FRCPC, Princess Margaret Cancer Centre, 700 University Avenue, Suite 7-624, Toronto, ON, M5G 1Z5, Canada
Telephone: 416-946-2911 | Fax: 416 946 4467 | Email: lillian.siu@uhn.ca

Copies of this poster obtained through Quick Response (QR) Code are for personal use only and may not be reproduced without permission from ASCO® and the author of this poster.