Exploitation of Treatment Induced Tumor Lysis to Enhance Sensitivity of ctDNA Analysis: A First-In-Human Pilot Study

Schulich MEDICINE & DENTISTRY

London Health Sciences Centre
London Regional Cancer Program

Inivata

Breadner, D.[1], Vincent[1], M., Correa, R.[2], Black, M.[1], Warner, A.[2], Qu, M.[2], Logan, D.[1], Sanatani, M.[1], Younus, J.[1], Yaremko, B.[2], Rodrigues, G.[2], Blanchette, P.[1], Bhat, V.[3], Morris, C.[4], Green, E.[4], Jones, G.[4], Allan, A.[3], Palma, D.[2], Raphael, J.[1]

[1]Division of Medical Oncology; [2]Division of Radiation Oncology; [3]Division of Experimental Oncology at the London Regional Cancer Program, London, Ontario, Canada, At the Schulich School of Medicine and Dentistry; [4]Invivata Inc. Research Triangle Park, North Carolina, United States

Background

- The treatment of metastatic NSCLC (mNSCLC) is evolving and becoming increasingly directed by histology, immunotherapy markers (PD-L1 and TMB) and genetic alterations, such as EGFR, ALK, ROS1, BRAF, NTRK, RET, MET, and HER2.
- Genetic analysis is typically done on tissue samples which requires adequate tissue for testing or repeat biopsies if more dynamic information is needed.
- Historically many patients undergo repeat biopsies to achieve adequate tissue.
- Circulating tumor DNA (ctDNA) are fragments of tumor-derived DNA circulating in the bloodstream.
- ctDNA testing is used commercially and recommended by guidelines to avoid repeat biopsies in patients with inadequate tissue for biomarker testing, with a histological diagnosis of NSCLC, and in patients with EGFR mutated (EGFR+) NSCLC progressing on early generation EGFR-TKIs for assessment of a T790M mutation, conferring sensitivity to osimertinib and other third-generation EGFR-TKIs.
- ctDNA sensitivity is around 70% in mNSCLC, and lower in earlier stage disease.
- Considering the high false negative rates, patients often have to undergo repeat biopsies following a negative ctDNA test result.
- Kageyama (Oncotarget 2018) and Boniface (2020 AACR Advances in Liquid Biopsy) have reported small series of patients with increases in ctDNA levels in the days following radiation therapy (RT), and even less is known about the effects of cytotoxic chemotherapy on ctDNA levels acutely following treatment.

Hypothesis

- Patients with NSCLC starting a new and effective therapy have an acute period of tumor lysis initially after starting therapy.
- Analysis of ctDNA at routine clinic appointments, often before starting treatment or before the next cycle of chemotherapy is not the optimal time for ctDNA detection.

Measuring ctDNA in the hours to days after starting a new therapy will improve ctDNA analysis sensitivity because of the acutely higher levels of ctDNA.

Methods

- We consented patients with NSCLC fitting the criteria below:

Eligibility Criteria
Histologically proven NSCLC
Age 18 or older
ECOG PS ≤ 3
Willing and able to undergo phlebotomy at the planned timepoints

And fitting into a planned cohort:

Cohort 1 (n=10)	Treatment naïve disease starting concurrent chemoradiation with platinum doublet chemotherapy
Cohort 2 (n=10)	Systemic treatment naïve mNSCLC starting cytotoxic platinum doublet chemotherapy
Cohort 3 (n=20)	mNSCLC starting palliative radiation to thoracic or brain disease

- Cohort 3 thoracic RT 2.5 – 4.0 Gy per fraction and brain RT 6 – 9 Gy per fraction

Methods

- Each patient had two baseline ctDNA samples and multiple post-treatment samples collected, as shown in Figure 1.

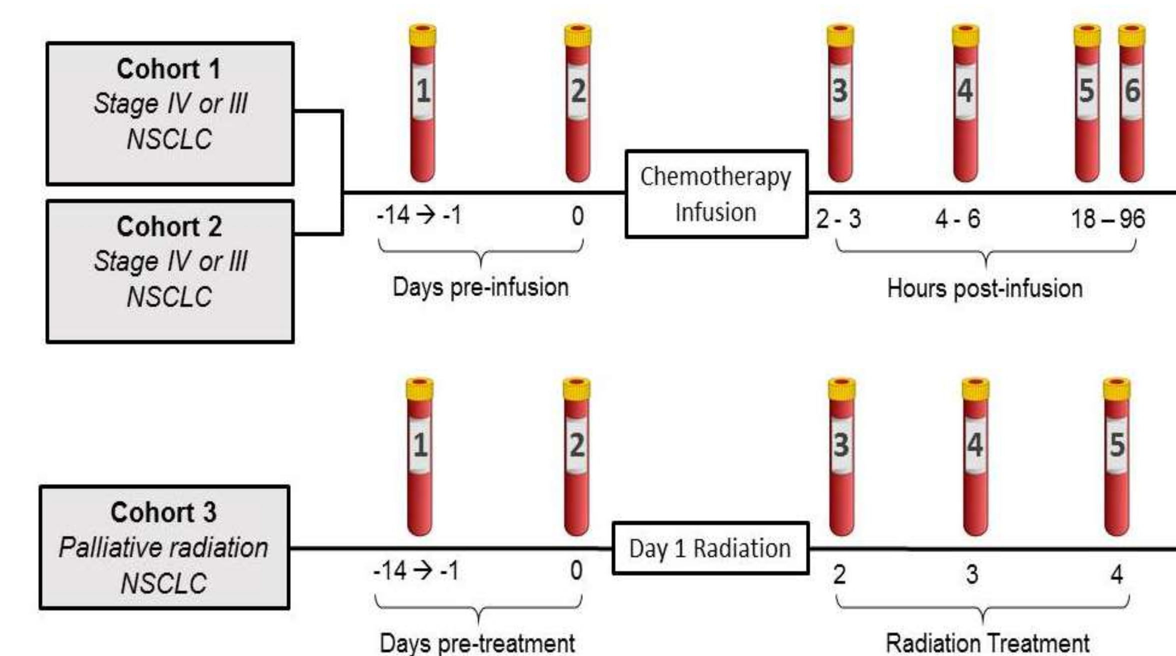
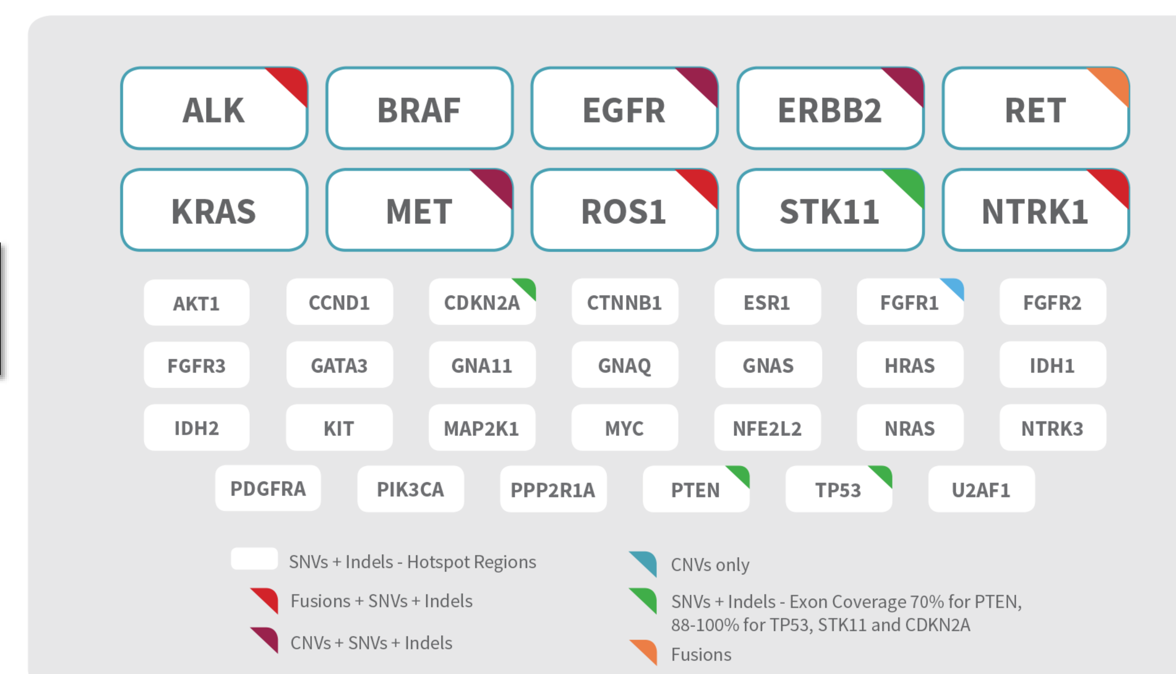


Figure 1: All patients had a baseline sample collected immediately prior to starting treatment and a preceding sample within 14 days. Patients in cohort 1 and 2 had samples collected 2-3 and 4-6 hours after the start of cytotoxic chemotherapy, as well as on the following two business days. Patients in cohort 3 had samples collected prior to their day 2,3,4 radiation treatments.

- Blood samples were collected in Streck BCT and centrifuged within 24 hours.
- Samples were analyzed using the 37-gene amplicon-based NGS Inivata InVision-First® Lung ctDNA Assay.
- Quantified ctDNA levels were calculated as mutant molecules/ ml of plasma.

Inivata's InVision-First® Lung ctDNA Assay



Objectives

Primary Objective

- To determine when ctDNA levels peak after the initiation of treatment

Secondary Objectives

- To detect genetic alterations at the time-point of maximal ctDNA levels that were not evident in baseline testing
- To determine the percentage of patients who are non-secretors of ctDNA at baseline but have detectable ctDNA acutely post-treatment

Patient Characteristics

	Total	Cohort 1+2	Cohort 1 CRT	Cohort 2 Chemo	Cohort 3 RT
n=	40	20	10	10	20
Age, median (range)	66.5 (41 – 83)	67.2 (41 – 80)	61.0 (41 – 80)	70.8 (63 – 78)	65.6 (56 – 83)
Female	42.5%	35%	40%	30%	50%
Pathology:					
Adenocarcinoma	67.5%	70%	60%	80%	65%
Large cell	2.5%	0	0	0	5%
Squamous	15%	20%	30%	10%	10%
NSCLC - NOS	15%	10%	10%	10%	20%
ctDNA absent at baseline	8 (20%)	4 (20%)	3 (30%)	1 (10%)	4 (20%)

- 1 patient only had baseline samples and is excluded from the analysis (RT plan changed to a single fraction after accrual).

Chemotherapy Regimen	n=
Carboplatin with paclitaxel (weekly)	7
Carboplatin with vinblastine (d1-3)	2
Cisplatin with etoposide*	2
Cisplatin with vinblastine (d1-3)	1
Carboplatin with pemetrexed	2
Carboplatin, pemetrexed and pembrolizumab	3
Carboplatin with gemcitabine	1
Cisplatin with pemetrexed	1
Carboplatin with vinorelbine	1

* Includes one patient treated with cisplatin 33 mg/m^2 and etoposide 100 mg/m^2 d1-3 and one patient treated with cisplatin 50mg/m^2 d1 and etoposide 50 mg/m^2 d1-5.

RT dose and fractionation	n=
9 Gy alternate days (brain only)	2
6 Gy daily (brain only)	2
4 Gy daily (thoracic)	4
3 Gy daily (thoracic)	10
2.67 Gy daily (thoracic)	1

Excludes one patient that had a change in planned RT to only a single fraction

Results – ctDNA Levels

- ctDNA levels for patients treated with chemotherapy doubled on average with a maximum observed 7 hours after the initiation of chemotherapy, with interquartile range between 2 and 26 hours.
- Patients treated with RT experienced an average 73% increase in ctDNA levels with maximum occurring (median) at day 2 after starting RT.

		Cohort 1+2 (Chemo + CRT)	Cohort 1	Cohort 2	Cohort 3
n=		20	10	10	19
Peak ctDNA level – median (IQR)*		7 hours (2 – 26)	3.9 hours (2.0 – 21)	23.3 hours (4.9 – 38.5)	2 days (1 – 3)
Percentage change in ctDNA level*	Mean	108%	111%	105%	73%
	Median	43%	32%	43%	12%
	IQR	-20 to 125%	-10 to 186%	-25 to 120%	-12 to 104%

* Excludes 4 patients that had no detectable ctDNA at baseline that produced detectable ctDNA upon treatment. Patients that never produced detected ctDNA are included and considered to have a change in ctDNA levels of 0.

Results – ctDNA Levels cont.

Brain Only Radiation

- Four patients had brain radiation.
- One patient did not have detectable ctDNA on any sample, baseline or post-treatment.
- The change in ctDNA levels for the other three patients were increases of 7%, 17% and 50%.
- These changes are in keeping with the changes noted in patients getting thoracic radiation.

Results – New Alterations

- 40% of patients treated with chemotherapy had new genetic alterations detected acutely post-treatment.
- 4 of 8 (50%) of patients that did not produce ctDNA at baseline had ctDNA become evident acutely post-treatment.

	Cohort 1+2 (Chemo + CRT)	Cohort 1	Cohort 2	Cohort 3
Non-secretors n=	4	3	1	4
New alterations in non-secretors at baseline	3 (75%)	2 (67%)	1 (100%)	1 (25%)
ctDNA present at baseline n=	16	7	9	15
New alterations in those with other alterations at baseline	5 (31%)	2 (29%)	3 (33%)	2 (13%)
All patients n=	20	10	10	19
New alterations	8 (40%)	4 (40%)	4 (40%)	3 (16%)

Discussion

- ctDNA levels increase acutely after the initiation of cytotoxic chemotherapy or radiation.
- Analysis of ctDNA in the acute post-treatment phase appears to improve ctDNA sensitivity compared to samples taken at routine clinic visits.
- Half of all patients without detectable ctDNA at baseline have ctDNA detected on acute post-treatment samples.
- This was a proof of concept study done in patients with different histologies with a heterogenous group of therapies.
- The technique of taking ctDNA acutely after treatment in patients that may benefit clinically from this data can be considered.
- For example, patients with EGFR+ mNSCLC progressing on early-generation EGFR-TKIs without a detectable T790M mutation in routine ctDNA testing OR patients with mNSCLC starting palliative RT without adequate tissue for molecular testing for EGFR, ALK, ROS1, NTRK, RET, etc.

Conclusion

- Treatment induced tumor lysis increases ctDNA sensitivity in this proof of concept study, the implications of this knowledge warrant further exploration.