CTC and ctDNA Profiling to Detect 6 NCCN-Guideline Recommended Classes of Alterations For Immunotherapy and Targeted Therapy Selection Using Sample From a Single Blood Draw

AACR American Association for Cancer Research
Abstract 3635 / 13
AACR Annual Meeting 2018
Chicago, IL

CellMax Life

Huang-Pin B. Hsieh[1], Jen-Chia Wu[1], Feng-Ming Lin[1], Julian Lucas[1], Alex Atkins[1], Pratyush Gupta[1], Hung-Jen Shao[1], Yen-Lin Chen[2], Wen-Jie Huang[3], Chia-Hsun Hsieh[4], Ruey-Kuen Hsieh[5], Kuo-Wei Chen[6], Ming-Hong Yen[7], Mana Javey[1], Shih-En Chang[1], Twinkal Marfatia[1], Drew Watson[8], Mahul Amin[9], Ashish Nimgaonkar[10], Oscar Segurado[11], Rui Mei[1]*

[1]CellMax Life, Sunnyvale, CA; [2]Cardinal Tien Hosp., New Taipei, Taiwan; [3]Mackay Mem. Hosp., Taipei, CA; [4]Chang Gung Mem. Hosp., Taoyuan, Taiwan; [5]Mackay Mem. Hosp., Taipei, Taiwan; [6]Cheng Hsin Gen. Hosp., Taipei, Taiwan; [7]Cathay Gen. Hosp., Taipei, Taiwan; [8]Kit Bio Inc, Los Altos, CA; [9]Univ. of Tennessee Hlth.Sci. Ctr., Memphis, TN; [10]Johns Hopkins Hosp., Baltimore, MD; [11]MedicAffairs Consulting, San Jose, CA; * Corresponding author: rui@cellmaxlife.com

BACKGROUND AND PURPOSE

The availability of targeted and immunotherapies has provided NSCLC patients with more effective treatment options. However, this has resulted in an increase in the number and modality of tests required for treatment selection. Given **30-50%** of advanced lung cancer patients have insufficient or unavailable tissue for comprehensive genomic profiling, there is a need for a non-invasive assay that can accurately detect all guideline-recommended markers for NSCLC treatment selection. To meet this need, we have developed a **blood test that detects six classes of alterations (SNV, indels, fusions, CNA, microsatellite instability and CTC PD-L1 expression) for therapy selection**.

METHODS AND STUDY DESIGN

The CellMax circulating tumor cells (CTCs) PD-L1 assays are performed on the CMx™ microfluidic platform.

Brief description of the workflow [Fig.1]:

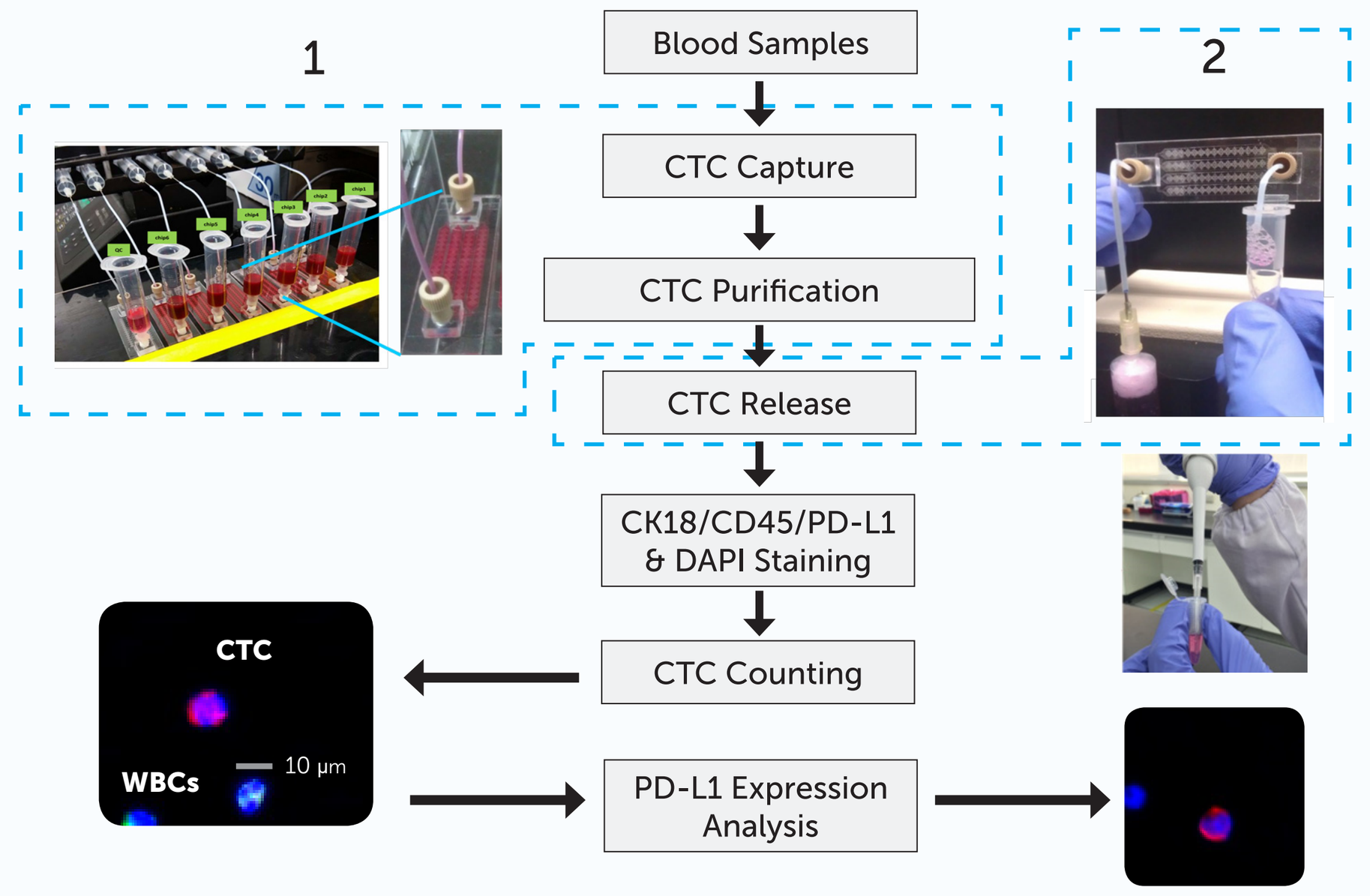
1. 2mL peripheral blood per patient were run through a microfluidic chip and CTCs were captured with a proprietary EpCAM antibody implanted on an anti-fouling lipid bilayer coating which promotes capture sensitivity and specificity. Gentle purification is accomplished by a PBS wash in chip
2. Captured cells were released from the chip by a sweep of property-matched air foams that dislocated the lipid bilayer from the chip surface, avoiding harsh breaking of cell-antibody bonding
3. Released cells were stained with antibodies against CK18, CD45, PD-L1 and DAPI for CTC enumeration and PD-L1 analysis
4. PD-L1 protein analysis performed by quantifying fluorescent intensity of PD-L1 antibody (BioINK)

Criteria for positive PD-L1 expression on a CTC are as follows [Fig.2]:

1. Cell-size ≥9μm with good morphology	2. CK18 intensity: >cutoff	3. CD45 intensity: <cutoff
4. No typical WBC nuclear shapes	5. PD-L1 intensity: > cutoff (established by PD-L1 negative control cells)	6. PD-L1 staining is complete circumferential or partial linear plasma membrane staining[1]

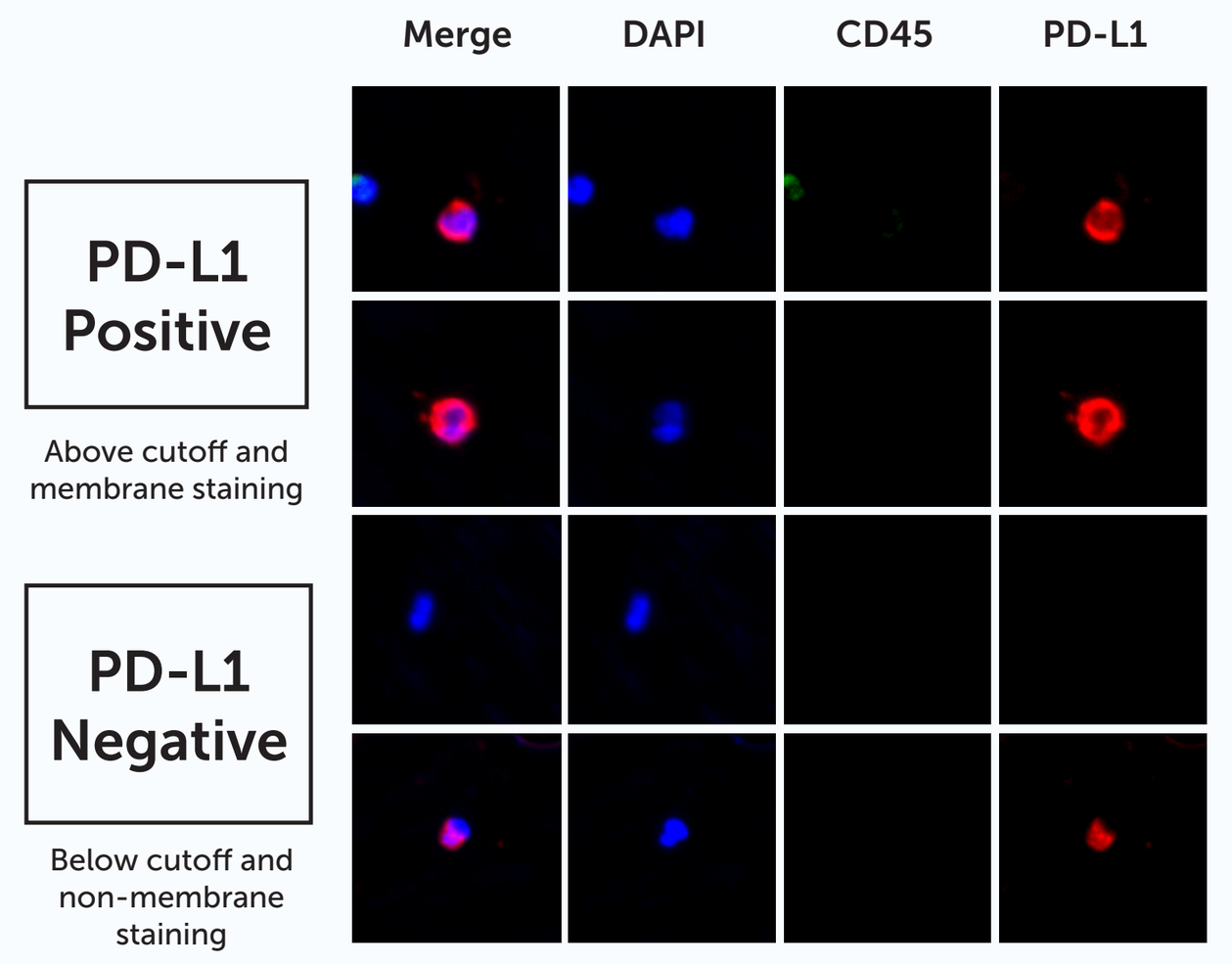
For the detection of 5 genomic variant classes (SNVs, indels, fusions, CNVs, MSI), the SMSEQ™ liquid biopsy platform was used. It was validated in accordance with the latest ACMG/AMP guidelines to accurately identify a wide range of clinically relevant variant types from cfDNA[2]. **Please refer to AACR 2018 abstract 3652 "Analytical validation of a novel circulating tumor DNA detection platform for targeted and immunotherapy selection" for more information.**

Method: CTC PD-L1 Assay Workflow



[Fig.1] A visual workflow of the CellMax CTC PD-L1 assay being performed on the CMx™ microfluidic platform.

PD-L1 Protein Expression on CTC



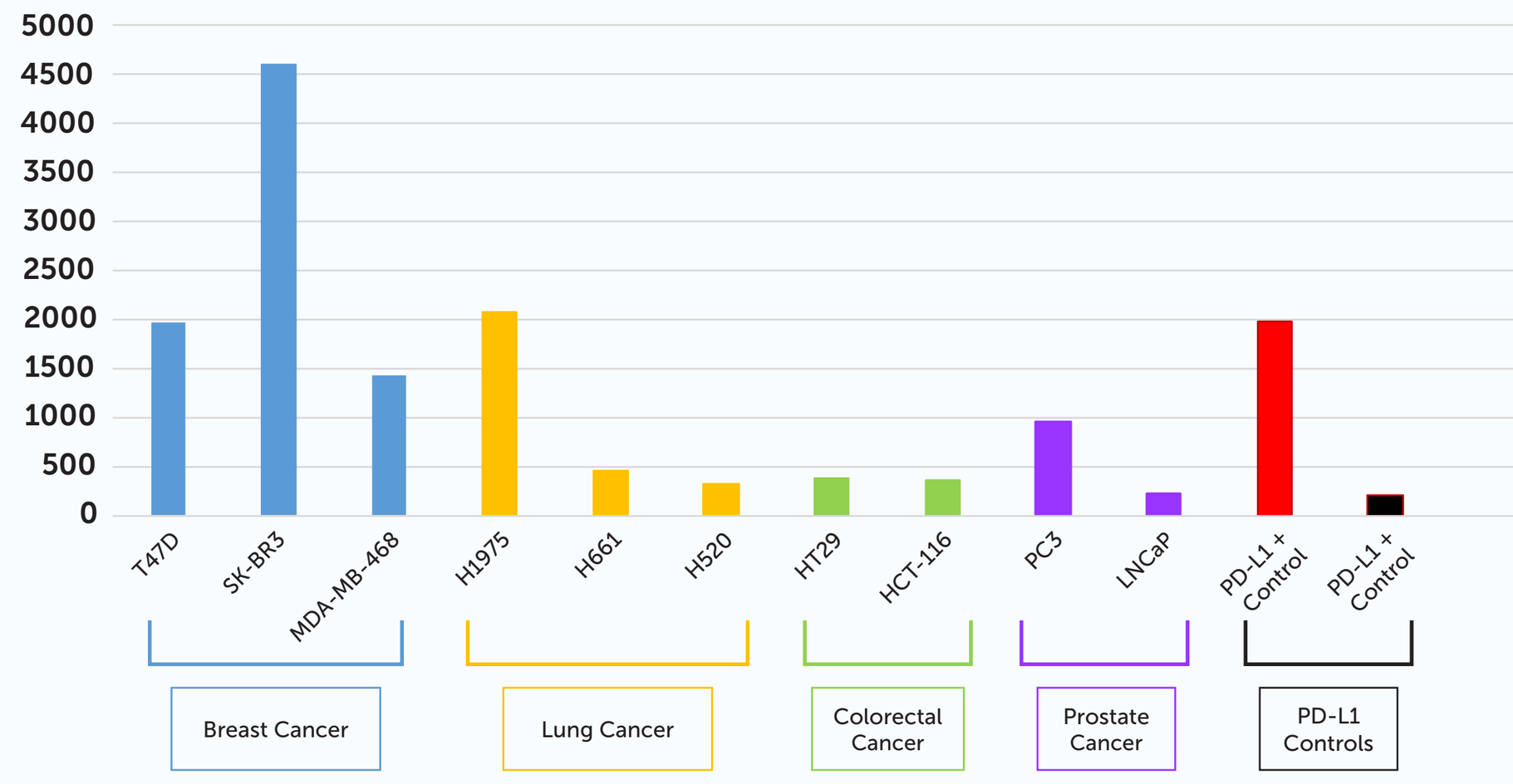
[Fig.2] CTC showing criteria for positive PD-L1 expression under a microscope

RESULTS

PD-L1 Expression in Cancer and Control Cell Lines

To establish the CTC PD-L1 assay, 10 cancer cell lines (3 breast, 3 lung cancer, 2 colorectal, 2 prostate) as well as PD-L1 positive and negative control cells were processed on the CMx platform and immunofluorescently stained with CK18, CD45 and PD-L1 antibodies plus DAPI nuclear counter-stain as used in patient samples to identify cancer cells and measure PD-L1 protein expression levels. The PD-L1 high expresser cell line (SK-BR3) and PD-L1 negative control cells showed a greater than 20-fold difference in relative fluorescent intensity **[Fig.3]**. The cutoff for PD-L1 positivity is established based on the negative control cells and fluorescent background.

PD-L1 Relative Fluorescent Intensity



[Fig.3] In the CellMax DNA Genetic Cancer Risk Test workflow, genomic DNA is converted to sequence-ready libraries, enriched for target regions, sequenced, and variants are called.

Clinical Study Cohort

51 NSCLC patients were included in the study and received CTC PD-L1 blood tests **[Fig.4]**. **Nearly two-thirds (32/51, 63%) of these patients were early stages (0~3)** and 15/51 (31%) had confirmed Stage 4 cancer; 4 had unknown staging. For the cohort, gender is even with 24 male and 27 female. Age distribution ranges from 37 to 84, with a median at 64. Tissue IHC were available for 35 of the tested patients.

CTC PD-L1 Clinical Study Cohort

Total Patients = 51		Number	Percent
Staging	Early Stages	32	63%
	Late Stage	15	29%
	Unknown Stage	4	8%
Gender	Male	24	47%
	Female	27	53%
Age	Range	37-84	
	Median	64	
PD-L1 Tissue IHC	IHC available	35	69%
	IHC unavailable	16	31%

[Fig.4] A summary of the 51 treatment naive NSCLC patients who participated in the CellMax CTC PD-L1 clinical study

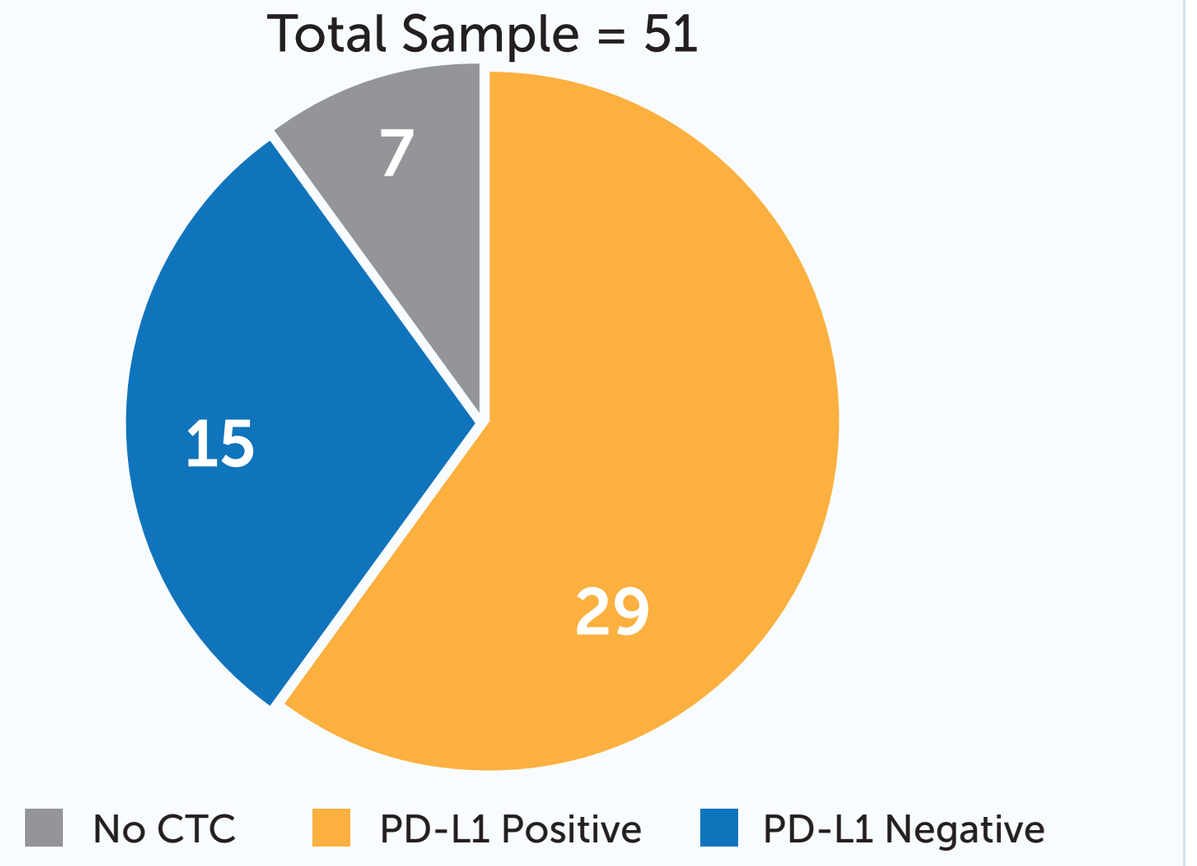
RESULTS

CTC Detection Rate and PD-L1 +

- CTCs were detected in 44/51 (86%) of all patients, range 1-47 (median 3)
- CTCs were detected in 87% (28/32) of early and 86% (13/15) of late stage patients
- **66% (29/44) of patients with detectable CTCs were found to have at least one PD-L1 expressing CTC (PD-L1 positive), consistent with PD-L1 clinical trial (66%) on tissue[3]**

	CTC Detection Rate		
	All Patients (Stages 0-4)	Early Stages (Stages 0-3)	Late Stage (Stage 4)
# of Subjects	51	32	15
CTC Detection (≥1 CTC/2mL)	86%	87%	86%
Range	1-47	1-47	1-32
Median	5	5	5

PD-L1 CTC Positive Rate
Total Sample = 51



PD-L1 High Expression in Patients

- 9/35 (25%) IHC PD-L1 were high (≥50% PD-L1+ cells), consistent with literature[4]
- **These patients are eligible for first-line immunotherapy**
  - 8 of these 9 high expressers had detectable CTCs (range 1-24, mean 9, median 8.5)
  - 7 of these 9 (78%) patients were also PD-L1 CTC positive
  - **Multiple PD-L1+ CTCs were detected in 6 out of these 7 patients**

PD-L1+ CTCs Among IHC PD-L1 High Expressors

Patient ID	Age	Gender	Stage	CTC Count	PD-L1+	PD-L1 %	IHC
2	57	Male	1B	3	2	67%	80%
5	69	Female	3	10	7	70%	75%
34	52	Male	4	9	3	33%	70%
8	64	Female	3A	24	20	83%	90%
12	82	Male	4	1	1	100%	60%
13	43	Female	4	15	8	53%	90%
27	67	Male	4	4	3	75%	60%

PD-L1 Positive Rates in Patients

- IHC results available for 35 patients; 31 (89%) of these patients had detectable CTCs
- 22 (63%) patients were PD-L1+ per IHC
- 23 (66%) patients were PD-L1+ per CTC
- **These tissue and blood PD-L1 positive percentages are consistent with what has been reported in clinical trial (66%) for NSCLC patients[3]**

	IHC Results	CTC Results
Number of Patients	35	35
PD-L1 +	22	23
% PD-L1 +	63%	66%
% PD-L1 + IHC in trial	66%*	

SMSEQ™ Performance

Detection of 5 NCCN Guidelines Recommended Alterations[2]

For details, see: **Abstract 3652, Poster Board Number: 10, Session Date and Time: Tuesday Apr 17, 2018 8:00 AM - 12:00 PM**
Title: **Analytical validation of a novel circulating tumor DNA detection platform for targeted and immunotherapy selection**

	Detection Range	Sensitivity	Specificity
Single nucleotide variants (SNVs)	0.10% - 0.20%	>80%	>99.999%
	>0.20%	>99%	>99.999%
Small Insertions and Deletions	0.10% - 0.20%	>70%	>99.999%
	>0.20%	>99%	>99.999%
Fusions	>1.0%	>95%	>99.999%
Copy Number Alterations	>4.5 copies	>95%	>99%
MSI	2.0 - 10.0%	>80%	>99%
	>10%	>99%	>99.999%

PD-L1 Positive CTCs Detected in IHC Negative Patients

- 37% (13/35) of the patients tested negative by tissue IHC – these patients were not eligible for immunotherapy
- **However, all of the 13 IHC PD-L1- patients had detectable CTCs (range 1-12, median 5) and 9 of the them are PD-L1 positive (~50% of the CTC PD-L1 positive patients had multiple PD-L1 positive CTCs) - treatment efficacy follow up on these patients would be very meaningful.**

Sensitive Detection of CTCs in Early-Stage NSCLC

- **CellMax PD-L1 assay has demonstrated sensitivity of detecting CTCs of all stages in NSCLC, including 87% sensitivity of detecting CTCs in early stages**
- Of the 7 (out of 9) IHC PD-L1 High Expressers confirmed by CTCs, 3 are early stages
- Of the 9 (out of 13) IHC PD-L1 negative but CTC PD-L1 positive patients, 4 are early stages

CONCLUSIONS

Tissue insufficiency and procurement challenges are the primary reasons why ~90% of patients diagnosed with advanced NSCLC are not comprehensively tested per NCCN-guidelines in the community setting where most cancer is treated, leading to suboptimal treatment selection.

In this poster, we present the CellMax liquid biopsy, an accurate blood test that enables the detection of all 6 NCCN-recommended markers (SNV, indels, fusions, CNA, microsatellite instability and CTC PD-L1 expression) for immunotherapy and targeted therapy selection. It has the potential to significantly improve adherence to NCCN testing guidelines and enable optimal treatment selection.

REFERENCES:

1. Copyright: Interpretation Manual Non-Squamous Non-Small Cell Lung cancer by DAKO, An Agilent Company, for PD-L1 IHC 28-8 pharmaDx
2. AACR 2018 Abstract 3652 / 10 - Analytical validation of a novel circulating tumor DNA detection platform for targeted and immunotherapy selection
3. https://www.keytruda.com/hcp/nsclc/pd-l1-expression-testing
4. Sorensen, S.F., et al., PD-L1 Expression and Survival among Patients with Advanced Non–Small Cell Lung Cancer Treated with Chemotherapy. Translational Oncology, 2016. 9(1): p. 64-69