Abstract #: 2550

Evaluation of AXL expression on circulating tumor cells from EGFR mutated lung cancer patients who have relapsed after the EGFR TKI treatment

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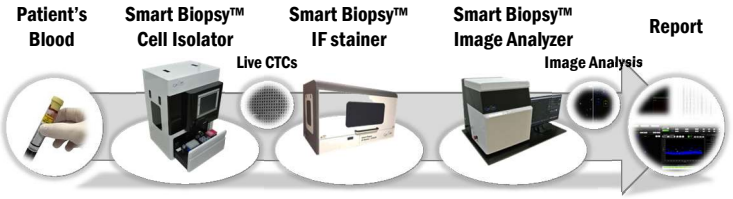
Background and Objective

Background: The EGFR (epidermal growth factor receptor) TKI (tyrosine kinase inhibitor) has become the standard treatment in lung cancer patients with EGFR mutations. When these patients are treated with EGFR TKI, 80~90% of the patients show responses to the drug. However the tumor begins to progress again following the development of resistance about 1 year later on average. About a half of resistance mechanisms are caused by the additional mutation of the EGFR gene (T790M), and the other half of the resistance mechanisms are caused by various mechanisms, with one of them being the overexpression of the AXL protein. Confirming AXL overexpression in circulating tumor cells (CTCs) can be an alternative method for tissue biopsy, but almost no research has been conducted on this so far.

Objective: To evaluate the AXL expression in CTC from NSCLC patinets in case resistance occurs after the EGFR inhibitor treatment.

SMART BIOPSY™ SYSTEM



- Automation system from CTC isolation to reporting.
- Size-based filtration (additional negative selection).
- Capture of Live CTCs.
- Quadruple IF staining & cell image analysis
- Expression levels of the target proteins in CTCs are reported.

METHODS

The blood samples (10 ml) were collected from 21 EGFR TKI treated and relapsed lung cancer patients (TKI group) and 10 non-treated patients (control group). The blood samples were divided into 2 parts and each samples were processed through Cytogen protocol to enrich CTCs. The enriched cells were immunofluorescent stained for CTC markers (EpCAM or Vimentin), AXL and WBC marker (CD45). The immunofluorescent stained cells were analyzed for each marker using Image Analysis program.

RESULTS

- CTC detection: all patients
- CTC counts range: 1 - 242 AXL
- AXL overexpression: 43% in TKI group and 20 % of control group

❖ **Control group** : EGFR TKI non-treated patients

	Sample	EGFR mutation	Additional T790M mutation	Cancer type	CTC count		AXL expression (fold change)
					Total	AXL+	≥3
1	Pt#N01	L858R	-	3 + 4	23	10	
2	Pt#N02	L858R	-	2	16	6	
3	Pt#N03	del19	-	2	4	3	
4	Pt#N04	L858R	-	2	12	11	
5	Pt#N05	del19	-	3 + 4	20	14	
6	Pt#N06	del19	-	2 + 3	4	3	+
7	Pt#N07	L858R	-	2	19	3	+
8	Pt#N09	L858R	-	1 + 2	6	4	
9	Pt#N10	L858R	-	5	17	2	
10	Pt#N11	del19	-	5	66	35	

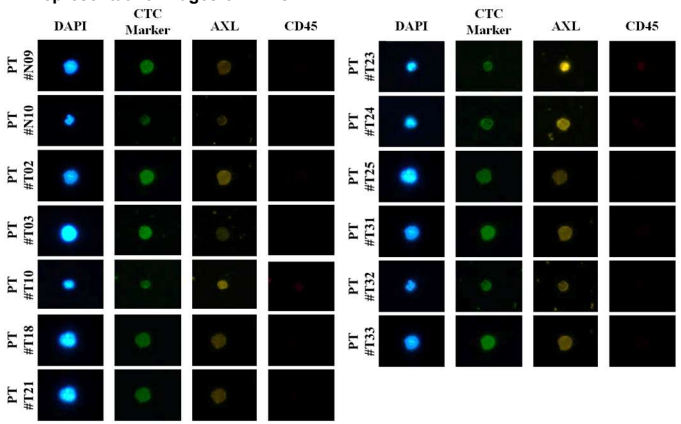
❖ **TKI group** : EGFR TKI treated and relapsed lung cancer patients

	Sample	EGFR mutation	Additional T790M mutation	Cancer Type*	CTC count		AXL expression (fold change)
					Total	AXL+	≥3
1	Pt#T02	Del 19	+	n.d.#	62	33	+
2	Pt#T03	Del 19	+	2	9	8	
3	Pt#T05	Del 19	+	2	1	1	
4	Pt#T07	L858R	-	4 + 5	242	51	+
5	Pt#T08	del 19	-	5	3	2	
6	Pt#T09	L858R	+	2 + 3	24	6	+
7	Pt#T10	L858R	+	4 + 5	14	3	+
8	Pt#T11	Del 19	+	4	23	5	+
9	Pt#T13	N/A	N/A	4 + 5	1	0	
10	Pt#T14	L858R	+	2	3	1	
11	Pt#T17	del19	+	2 + 3	11	5	+
12	Pt#T18	L858R	-	3 + 4	7	2	
13	Pt#T21	N/A	N/A	2	3	1	
14	Pt#T23	del19	+	2 + 4	24	15	+
15	Pt#T24	L858R	-	n.d.#	2	1	+
16	Pt#T25	del19	+	4	36	10	
17	Pt#T26	L858R	-	4 + 5	1	0	
18	Pt#T30	L858R	+	2	66	26	
19	Pt#T31	No mut.	-	4	221	16	+
20	Pt#T32	L858R	+	n.d.#	11	11	
21	Pt#T33	L858R	-	n.d.#	21	19	

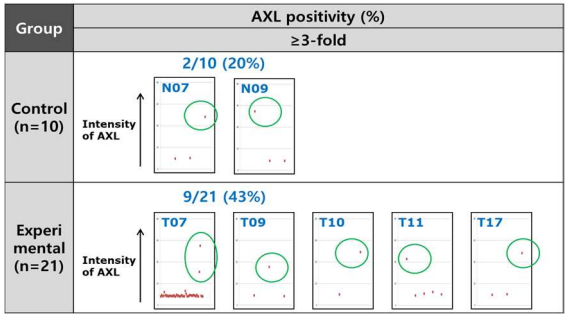
* Cancer types ; 1 Lepidic carcinoma, 2 Acinar carcinoma, 3 Micropapillary carcinoma, 4 Solid carcinoma, 5 Neuroendocrine carcinoma /

n.d. (not determined): poorly differentiated

❖ **Representative Images of CTCs**



❖ **Quantitative Analysis of AXL expression levels in CTCs**



CONCLUSIONS

We have tested a new approach to evaluate the AXL overexpression in CTCs from NSCLC patients with EGFR TKI resistance, and confirmed the feasibility of the method as an alternative tool to the tissue biopsy.

Here we suggested that the CTC based liquid biopsy can be a good alternative to the tissue biopsy in patients with tolerance after drug treatment.