Title: Immunohistochemical Evaluation of STEAP1 Expression in Thoracic Malignancies

Authors:
1. Ujjwal Karki, Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, USA
2. Suman Gaire, Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, USA
3. Hyein Jeon, MedStar Georgetown Cancer Institute, Washington, DC, USA
4. Cristina Merkhofer, MedStar Georgetown Cancer Institute, Washington, DC, USA
5. Joshua Reuss, Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, USA
6. Stephen Liu, Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, USA
7. Anju Duttargi, Department of Pathology, Georgetown University, Washington, DC, USA
8. Chul Kim, Georgetown Lombardi Comprehensive Cancer Center, Washington, DC, USA

Background:
Six-Transmembrane Epithelial Antigen of Prostate 1 (STEAP1) is a cell surface protein highly expressed in prostate cancer that represents an emerging therapeutic target. A Phase 3 trial with STEAP1-targeted T-cell engager, Xaluritamig, is currently ongoing in prostate cancer. While few studies have described STEAP1 expression in lung adenocarcinoma, only one utilized immunohistochemistry (IHC), and expression across broader lung cancer histologic and genomic subgroups, as well as thymic carcinoma, remains unknown.

Methods:
Patients with thoracic malignancies at MedStar Georgetown University Hospital were retrospectively identified. Pre-treatment formalin-fixed, paraffin-embedded tumor samples were retrieved. STEAP1 expression was

evaluated by IHC using a commercial antibody (ab207914, Abcam) utilizing a composite immunoreactivity score (IRS, 0-7) calculated as the sum of the staining extent (0%=0, 1–25%=1, 26–50%=2, 51–75%=3, 76–100%=4) and intensity (0=absent, 1=weak, 2=moderate, 3=strong) as previously described (Liu et al, *World J Surg Oncology*, 2022). Statistical comparisons were performed using Mann-Whitney U test ($p<0.05$).

Results:
A total of 37 treatment-naive tumor samples were analyzed: thymic carcinoma (TC; n=3), small cell lung cancer (SCLC; n=3), and non-small cell lung cancer (NSCLC; n=31). Among patients with NSCLC, the median age at diagnosis was 72 years (range 42-88); 68% were female, and 52% had no prior smoking history. Most (90%) had Stage IV disease, and 10% had Stage III. 54% of samples were from the primary site and 46% from a metastatic site. Histologic subtypes included adenocarcinoma (LUAD; 83%) and squamous cell carcinoma (LUSC; 17%). Driver alterations were identified in 61% (n=19): *EGFR* (n=4), *ALK* (n=4), *ROS1* (n=4), *MET* exon 14 skipping (n=5), and *RET* (n=2). STEAP1 expression per histology and oncogene driver, as well as comparisons, are summarized in **Table**. STEAP1 IRS was significantly higher in driver-positive versus driver-negative NSCLC. Subgroup analysis demonstrated higher STEAP1 IRS in *ALK*, *MET* exon 14, and *RET* tumors compared with driver-negative NSCLC, whereas *EGFR* and *ROS1* did not differ significantly from driver-negative NSCLC. No significant difference in IRS was observed between LUAD and LUSC. All TC samples exhibited IRS 6. Among SCLC samples, STEAP1 IRS were 3, 4, and 5.

Conclusion:
STEAP1 is consistently expressed in NSCLC with significantly higher expression in tumors harboring driver mutations, especially in *MET* exon 14, *ALK*, and *RET* alterations. Uniformly strong STEAP1 expression was observed in thymic carcinoma, while expression was modest to high in SCLC. Expansion of these cohorts is planned to validate these findings and inform development of STEAP1-targeted therapies in thoracic malignancies.

Table. STEAP1 expression Immunoreactivity scores (IRS) in treatment-naive thoracic malignancies

Cancer type	IRS, Median (range)	IRS=4, n (%)	IRS=5, n (%)	IRS=6, n (%)	Intensity 2 or 3, n (%)	>50% cells stained, n (%)
All (n=37)	4 (2-6)	15 (40.5)	7 (19)	8 (21.6)	30 (81)	15 (40.5)
Thymic carcinoma (n=3)	6 (6-6)	0 (0)	0 (0)	3 (100)	3 (100)	3 (100)
SCLC (n=3)	4 (3-5)	1 (33.3)	1 (33.3)	0 (0)	2 (66.7)	1 (33.3)
NSCLC (n=31)	4 (2-6)	14 (45.2)	6 (19.4)	5 (16.1)	25 (80.6)	11 (35.5)
LUAD (n=26)	4 (3-6)	13 (50)	6 (23.1)	4 (15.4)	23 (88.5)	10 (38.5)
LUSC (n=5)	3 (2-6)	1 (20)	0 (0)	1 (20)	2 (40)	1 (20)
Driver negative NSCLC (n=12)	4 (2-4)	9 (75)	0 (0)	0 (0)	9 (75)	0 (0)
Driver positive NSCLC (n=19)	5 (3-6)	5 (26.3)	6 (31.6)	5 (26.3)	16 (84.2)	11 (58)
EGFR mutant (n=4)	4 (3-5)	0 (0)	2 (50)	0 (0)	2 (50)	2 (50)
ALK fusion (n=4)	5.5 (4-6)	1 (25)	1 (25)	2 (50)	4 (100)	3 (75)
ROS fusion (n=4)	4 (3-4)	3 (75)	0 (0)	0 (0)	3 (75)	0 (0)
MET ex 14 mutation (n=5)	6 (4-6)	1 (20)	1 (20)	3 (60)	5 (100)	4 (80)
RET fusion (n=2)	5 (5-5)	0 (0)	2 (100)	0 (0)	2 (100)	2 (100)
Comparison of IRS by histology and oncogene drive						
LUAD vs LUSC			p=0.116			
Driver positive vs driver-negative NSCLC			p=0.009			

EGFR vs driver-negative NSCLC	p=0.64
ALK vs driver-negative NSCLC	p=0.008
ROS vs driver-negative NSCLC	p=1
MET exon 14 vs driver-negative NSCLC	p=0.003
RET vs driver-negative NSCLC	p=0.014