

Taofeek K. Owonikoko¹, Andrew Elliott², Bhakti Dwivedi³, Andrey Ivanov³, Gabriel Sica⁴, Sonam Puri⁵, Abdul Rafah Naqash⁶, Kathleen Claire Kerrigan⁵, Shiven B. Patel⁵, Andreas Seeber⁷, Florian Kocher⁷, Dipesh Uprety⁸, Hirva Mamdani⁸, Amit Kulkarni⁹, Gilberto Lopes¹⁰, Balazs Halmos¹¹, Wallace L. Akerley¹², Stephen V Liu¹², Wolfgang Michael Korn², Hossein Borghaei¹³
¹UPMC Hillman Cancer Center, Pittsburgh, PA; ²Caris Life Sciences, Phoenix, AZ; ³Emory University, Atlanta, GA; ⁴Department of Pathology UPMC Hillman Cancer Center, Pittsburgh, PA; ⁵Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; ⁶Medical Oncology/ Stephenson Cancer Center, University of Oklahoma, Oklahoma City, OK; ⁷Department of Internal Medicine V (Hematology and Oncology), Medical University of Innsbruck, Innsbruck, Austria; ⁸Karmanos cancer institute, Detroit, MI; ⁹University of Minnesota, Minneapolis, MN; ¹⁰University of Miami Miller School of Medicine, Miami, FL; ¹¹Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY; ¹²Georgetown University, Washington, DC; ¹³Fox Chase Cancer Center, Philadelphia, PA

Background

- Effective treatment options for SCLC remain limited and new treatment approaches are needed to improve outcome. We sought to validate the initial observation in cell lines and limited tissue samples of SCLC of a differential expression of cancer/testis (CT) antigens and TACSD2 gene that encodes surface protein, Trop2 across various subtypes of SCLC. We also tested whether overall surfaceome profile as previously described in other tumor types will show hierarchical priority of expression between transcriptionally defined SCLC subtypes.

Methods

- We conducted a comprehensive surfaceome profiling of SCLC samples using data generated by RNA sequencing (whole transcriptome) at Caris Life Sciences (Phoenix, AZ).
- SCLC tumors were stratified into 5 subgroups (SCLC-A/N/Y/P and -mixed) based on the relative expression of the four transcription factors. Expression values were converted to z-scores (the expression value for each gene is normalized to the average expression of that specific gene such that the z-score reflects the number of standard deviations above or below the average). The highest positive z-score among the 4 transcription factors determined subgroup. If all transcription factor z-scores for a given sample were negative, the sample was assigned to 'Mixed' subgroup.
- Significance was tested by Chi-square, Fisher's exact test, or Mann-Whitney U test.

Results

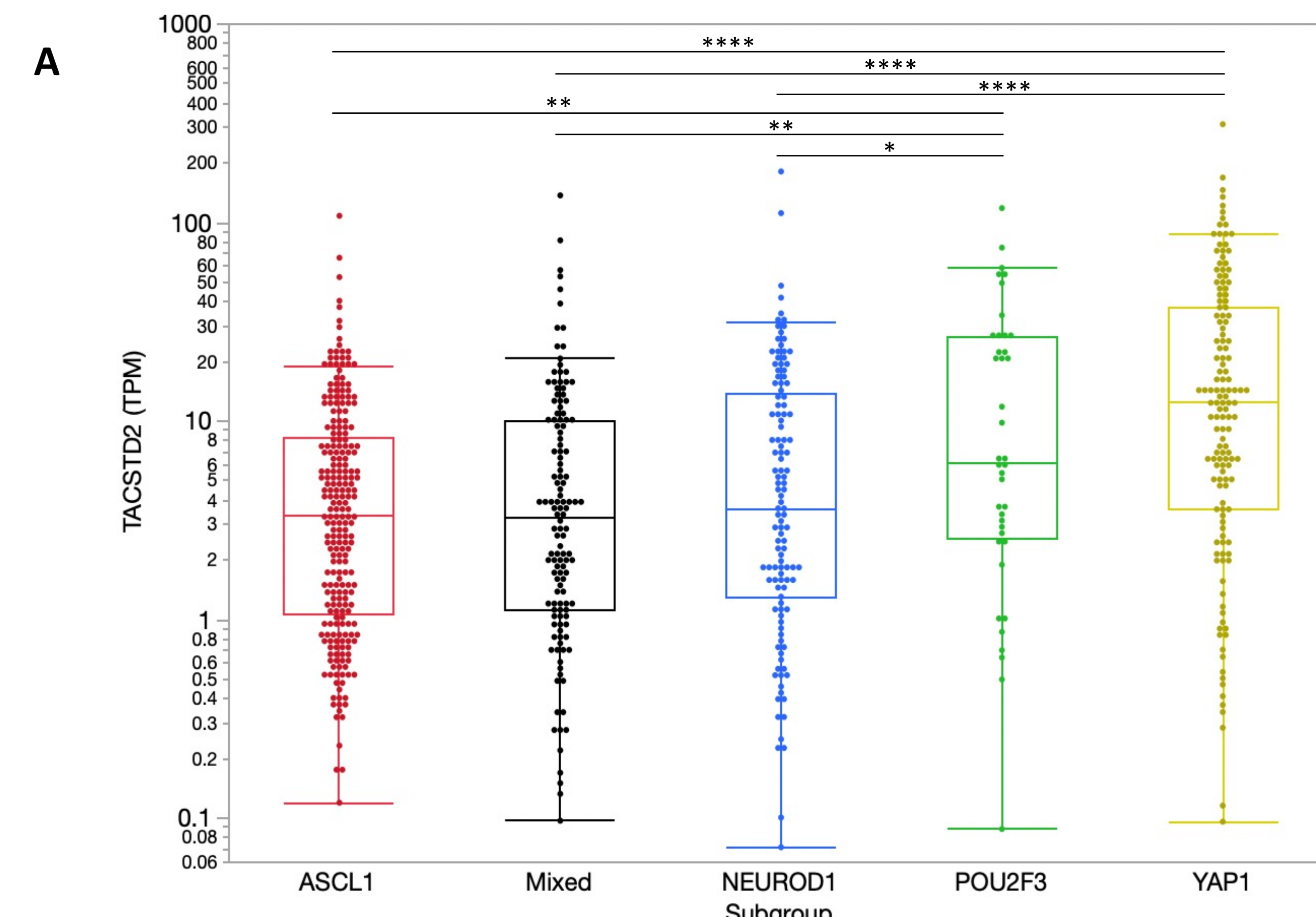
Table 1 – Cohort demographics by SCLC transcription factor-based subtype

Characteristic	All SCLC Subtypes	ASCL1	NEUROD1	POU2F3	YAP1	Mixed	P-value (test)
		SCLC-A	SCLC-N	SCLC-P	SCLC-Y		
Total, N cases (%)	674 (100%)	241 (35.8%)	120 (17.8%)	40 (5.9%)	143 (21.2%)	130 (19.3%)	-----
Median Age, years (SD)	66 (9.44)	65 (9.5)	68 (8.6)	67 (8.4)	66 (10.2)	65 (9.9)	P=0.4318 (Wilcoxon)
- Age Range, years	31-90+	41-87	44-89	56-89	31-85	18-85	
Male/Female, N cases	328/346	114/127	61/59	20/20	74/69	59/71	P=0.8200 (Chi-square)
- (% Male/% Female)	(48.7%/51.3%)	(47.3%/52.7%)	(50.8%/49.2%)	(50.0%/50.0%)	(51.8%/48.3%)	(45.4%/54.6%)	
Metastatic/Primary, N cases	461/213	174/67	88/32	30/10	71/72	98/232	P=4.99e-6 (Chi-square)
- (% Metastatic/% Primary)	(68.4%/31.6%)	(72.2%/27.8%)	(73.3%/26.7%)	(75.0%/25.0%)	(49.7%/50.3%)	(75.4%/24.6%)	

Figure 1 – TACSTD2 transcriptional expression across SCLC subtypes.

(A) TACSTD2 expression showed highest levels in YAP1 subtype and was overall significantly increased in SCLC-Y (~3-fold) and SCLC-P (~2-fold) subtypes compared to A, N and mixed subtypes.

(B) TACSTD2 expression positively correlated with YAP1 and POU2F3 expression



Gene	ASCL1		NEUROD1		POU2F3		YAP1	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
TACSTD2	-0.1186	2.03E-03	-0.018	6.40E-01	0.3174	3.04E-17	0.3677	5.32E-23

Figure 3 – Surfaceome and CT antigens gene expression profiling associated with SCLC subtypes. Top candidates from exploratory screen of 3699 surfaceome genes for each SCLC subtype; Top candidates from exploratory screen of 210 CT antigens for each SCLC subtype.

Surfaceome gene	SCLC Lineage-defining transcription factors							
	YAP1		ASCL1		NEUROD1		POU2F3	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
CYBRD1	0.8559	1.18E-194	-0.0765	4.72E-02	0.0591	1.25E-01	0.0853	2.69E-02

CT Antigen	SCLC Lineage-defining transcription factors							
	YAP1		ASCL1		NEUROD1		POU2F3	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
CTAGE5	0.5521	4.95E-55	0.1508	8.50E-05	0.0697	7.04E-02	0.1107	3.99E-03

Surfaceome gene	SCLC Lineage-defining transcription factors							
	YAP1		ASCL1		NEUROD1		POU2F3	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
SCN3A	0.0555	1.50E-01	0.7033	1.08E-101	-0.0731	5.78E-02	-0.1503	9.00E-05

Surfaceome gene	SCLC Lineage-defining transcription factors							
	YAP1		ASCL1		NEUROD1		POU2F3	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
SSTR2	0.108	4.99E-03	-0.1091	4.57E-03	0.742	8.18E-119	0.1187	2.03E-03

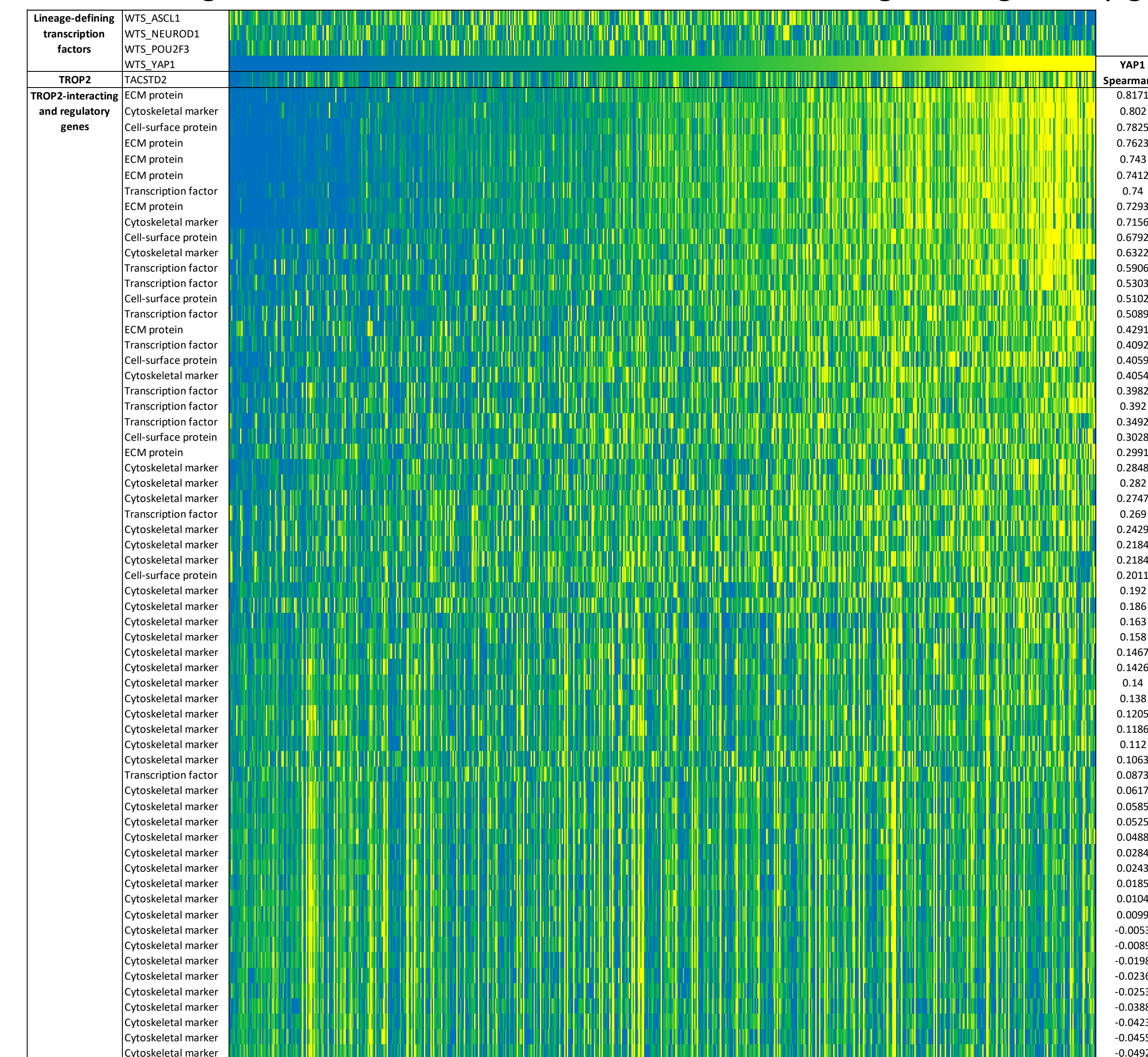
Surfaceome gene	SCLC Lineage-defining transcription factors							
	YAP1		ASCL1		NEUROD1		POU2F3	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
TMPRSS13	0.2397	2.89E-10	-0.0823	3.27E-02	-0.0165	6.68E-01	0.5699	2.64E-59

CT Antigen	SCLC Lineage-defining transcription factors							
	YAP1		ASCL1		NEUROD1		POU2F3	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
NOL4	0.019	6.22E-01	0.574	2.46E-60	0.0737	5.57E-02	-0.0605	1.17E-01

CT Antigen	SCLC Lineage-defining transcription factors							
	YAP1		ASCL1		NEUROD1		POU2F3	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
TMEFF1	0.0809	3.58E-02	0.2571	1.24E-11	0.3601	4.53E-22	0.022	5.68E-01

CT Antigen	SCLC Lineage-defining transcription factors							
	YAP1		ASCL1		NEUROD1		POU2F3	
	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value	Spearman ρ	P-value
LY6K	0.1126	3.42E-03	-0.1873	9.77E-07	0.3014	1.28E-15	0.4778	9.80E-40

Figure 2 – YAP1 expression is strongly correlated with TACSTD2-interacting and regulatory genes. SCLC-Y subtype showed the highest median expression as well as the strongest correlation with most TACSTD2-interacting and regulatory genes.



Conclusions

- SCLC-Y subtype showed the highest expression of TACSTD2 and its interacting and regulatory genes.
- This subtype could serve as an enrichment factor for antibody-drug-construct targeting TROP2.
- Several candidate CT antigens and surfaceome genes showing strong correlation with lineage-defining transcription factors offer additional therapeutic targets in SCLC

References

- Fonseca, A. L., da Silva, V. L., da Fonseca, M. M., Meira, I. T., da Silva, T. E., Kroll, J. E., Ribeiro-Dos-Santos, A. M., Freitas, C. R., Furtado, R., de Souza, J. E., Stransky, B., & de Souza, S. J. (2016). Bioinformatics Analysis of the Human Surfaceome Reveals New Targets for a Variety of Tumor Types. *International journal of genomics*, 2016, 8346198. <https://doi.org/10.1155/2016/8346198>
- Owonikoko, T. K., Dwivedi, B., Chen, Z., Zhang, C., Barwick, B., Ernani, V., Zhang, G., Gilbert-Ross, M., Carlisle, J., Khuri, F. R., Curran, W. J., Ivanov, A. A., Fu, H., Lonial, S., Ramalingam, S. S., Sun, S. Y., Waller, E. K., & Sica, G. L. (2021). YAP1 Expression in SCLC Defines a Distinct Subtype With T-cell-Inflamed Phenotype. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*, 16(3), 464–476. <https://doi.org/10.1016/j.jtho.2020.11.006>