Manuscript ID: biomedicines-1646385

Title: Serum Levels of Stromal Cell-derived Factor-1α and Vascular Endothelial Growth Factor Predict Clinical Outcomes in Head and Neck Squamous Cell Carcinoma Patients Receiving TPF Induction Chemotherapy

Reply to the reviewers' comments

Dear reviewers:

We thank the reviewers for their thorough review of our proposal, and their valuable and constructive comments. We have carefully addressed the reviewers' comments by including additional descriptions. Here, we have indicated the changes made to the manuscript to account for the comments from the reviewers.

Our point-by-point replies to each comment from the reviewers are as follows:

Reviewer #4 comments:

<< COMMENT 1 from Reviewer #3 >>

As there is a separate section of materials please list all materials listed in this manuscript in one section

- ANSWER to comment 1: Thanks for your comments. In our study, only "Serum SDF-1α and VEGF measurement" belong to "materials", and others such as "Induction chemotherapy with TPF" and "Chemoradiotherapy planning" were regarded as "methods". Therefore, we have listed all materials in only one section in my manuscript according to your suggestion for more clear presentation to readers. Please evaluate it.

<< COMMENT 2 from Reviewer #3 >>
Circulating levels of VEGF are induced by hypoxia and are angiogenic in vivo. Would the expression levels be influenced by ELISA measurements?

- ANSWER to comment 2:

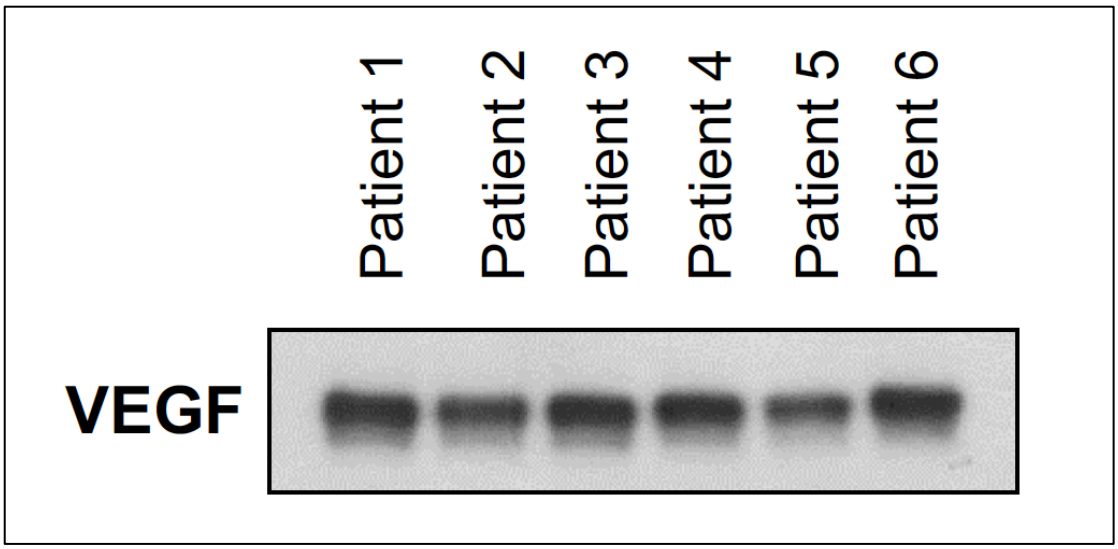
Thanks for your comments. VEGF are induced by hypoxia status and secreted into blood, so we could draw peripheral blood for VEGF test. In real-world clinical practice, the values of VEGF were frequently determined using commercially available sandwich ELISA kits, mostly Quantikine Human VEGF ELISA kits, which were used in our study. Such sandwich ELISA kits use specific capture and detection antibodies, and are used for quantitative measurement of human VEGF in cell culture supernatants, serum, plasma, CSF and urine. In our study, all assays of VEGF were performed in triplicate according to each manufacturer's instructions to minimize the influence. Please evaluate it.

<< COMMENT 3 from Reviewer #3 >>

Would it be possible to include western blot analysis for the VEGF for example?

- ANSWER to comment 3:

Thanks for your comments. It is executable for western blot analysis of VEGF. We also examined the expression of VEGF by wstern blot for some patients in our study, and the results showed below. However, the detection of serum VEGF by ELISA kit had better advantage than the western blot of VEGF. First, detecting VEGF by ELISA kit is very fast and cheap; in contrast, western blot approach took much time, cost, and complicated experimental procedures. Second, if we had many blood samples for examination (such as our study, 77 patients with blood samples before and after TPF treatment, performing in triplicate), ELISA kits may be a better choice for data available. Third, the expression of western blot is relatively difficult for quantitative comparison, but it is very easy to perform quantitative comparison using serum VEGF levels detected by ELISA kits. Therefore, it is executable to present western blot analysis of VEGF but we preferred ELISA kits for serum VEGF detection. Please evaluate it.



<< COMMENT 4 from Reviewer #3 >>

As the sample population was 77 patients, why there were no healthy volunteers to compare the expression levels of VEGF and SDF1

- ANSWER to comment 4:

Thanks for your comments. Growing evidences have confirmed the values of VEGF and SDF-1α are higher in patients with disease than healthy control, such as malignancy, pneumonia, osteoporosis, etc.[1-3] Therefore, we did not enroll healthy volunteers for comparison in our study. Please evaluate it.

References

1. Riedel F, Gotte K, Schwalb J, Wirtz H, Bergler W, Hormann K. Serum levels of vascular endothelial growth factor in patients with head and neck cancer. Eur Arch Otorhinolaryngol 2000;257:332-6.
2. Tsai PK, Hsieh MJ, Wang HL, Chou MC, Yang SF, Yeh CB. Elevated plasma stromal-cell-derived factor-1 protein levels correlate with severity in patients with community-acquired pneumonia. Dis Markers 2014;2014:829706.
3. Yang XW, Huang HX, Wang F, Zhou QL, Huang YQ, Qin RZ. Elevated plasma CXCL12/SDF-1 levels are linked with disease severity of postmenopausal osteoporosis. Innate Immun 2020;26:222-30.

<< COMMENT 5 from Reviewer #3 >>

In the last paragraph of the introduction please expand on the use of TPF for non-MD readers

- ANSWER to comment 5:

Thanks for your comments. We have added the description about the use of TPF for non-MD readers in the Introduction section in my revised manuscript. Please evaluate it.

Introduction/*Fourth paragraph*

In general, induction chemotherapy with TPF, the powerful regimen, incorporated both induction chemotherapy with PF and CRT in an attempt to improve locoregional control, eliminate distant metastases, and prolong survival. However, the role of SDF-1α and VEGF in HNSCC induction chemotherapy remains unclear. The aim of the present study was to explore the role of SDF-1α and VEGF in the prognosis of patients with HNSCC who underwent induction chemotherapy with TPF.

- CHANGE: Introduction/*Fourth paragraph*, **page 2, line 91**

<< COMMENT 6 from Reviewer #3 >>

In table 1 would it be possible to incorporate the grading of the patients and the T stage, and the lymph node

- ANSWER to comment 6:

Thanks for your comments. We have added the distribution of histological grade and performed the analysis of PFS and OS in the revised manuscript according to your suggestion. Please evaluate it.

Results/*Patient characteristics*

Between January 2010 and December 2016, 77 patients with HNSCC received TPF induction chemotherapy followed by CRT …… The distribution of histological grade included grade 1 in 19 patients (24.7%), grade 2 in 43 patients (55.8%) and grade 3 in 15 patients (19.5%). The characteristics of these 77 HNSCC patients

Table 1. Characteristics of 77 locally advanced head and neck squamous cell carcinoma patients who received TPF induction chemotherapy followed by CRT

Characteristics	
Age	53 years old (29-82)
Gender	
Male	73 (94.8%)
Female	4 (5.2%)
Location	
Oral cavity	22 (28.6%)
Oropharynx	28 (36.4%)
Hypopharynx	11 (14.3%)
Larynx	16 (20.7%)
HPV status	
Positive	**6 (7.8%)**
Negative	**71 (92.2%)**
T status	
2	19 (24.7%)
3	9 (11.7%)
4	49 (63.6%)
N status	
0	18 (23.4%)
1	12 (15.6%)
2	41 (53.2%)
3	6 (7.8%)
Stage	
II	5 (6.5%)
III	7 (9.1%)
IVA	34 (44.2%)
IVB	31 (40.2%)
Grade	
1	**19 (24.7%)**
2	**43 (55.8%)**
3	**15 (19.5%)**

TPF: Docetaxel, Cisplatin and Fluorouracil; CRT: chemoradiotherapy; HPV: Human papillomavirus

Table 2. Univariate and multivariate analysis of progression-free survival (PFS) in 77 patients with locally advanced head and neck squamous cell carcinoma patients who received induction chemotherapy of TPF followed by CRT

Characteristics	No. of patients	Univariate analysis		Multivariate analysis	
		PFS (months)	P-value	HR (95% CI)	P-value
Age			0.23		
< 60 years	65 (84.4%)	18.0			
≥ 60 years	12 (15.6%)	18.8			
Gender			0.41		
Male	73 (94.8%)	18.0			
Female	4 (5.2%)	22.6			
Location			0.06		
Oral cavity	22 (28.6%)	10.9			
Oropharynx + Hypopharynx + Larynx	55 (71.4%)	24.1			
HPV status			**0.014***		
Positive	**6 (7.8%)**	**116.6**			
Negative	**71 (92.2%)**	**16.1**			
T status			0.003*		
2	19 (24.7%)	71.3			
3 + 4	58 (75.3%)	13.9			
N status			0.61		
0 + 1	30 (39.0%)	26.8			
2 + 3	47 (61.0%)	13.3			
Tumor stage			0.17		
II	5 (6.5%)	98.1			
III + IV	72 (93.5%)	16.4			
Grade			**0.25**		
1 + 2	**62 (80.5%)**	**22.6**			
3	**15 (19.5%)**	**11.9**			
VEGF decrease after TPF treatment			0.001*		
Yes	42 (54.5%)	38.7		0.46 (0.27-0.52)	0.003*
No	35 (45.5%)	9.9			
Post-TPF VEGF ≥ 150 pg/mL			0.002*		
Yes	41 (53.2%)	13.3			
No	36 (46.8%)	41.0		0.50 (0.29-0.86)	0.011*
SDF-1α decrease after TPF treatment			<0.001*		
Yes	21 (27.3%)	116.6		0.38 (0.18-0.77)	0.007*
No	56 (72.7%)	11.2			
Post-TPF SDF-1α ≥ 1500 pg/mL			<0.001*		
Yes	59 (76.6%)	14.0			
No	18 (23.4%)	115.1		0.43 (0.19-0.95)	0.036*

TPF: Docetaxel, Cisplatin and Fluorouracil; CRT: chemoradiotherapy; HR: hazard ratio; CI: confidence interval; HPV: Human papillomavirus; VEGF: vascular endothelial growth factor; SDF-1α: stromal cell-derived factor-1α. *Statistically significant.

Table 3. Univariate and multivariate analysis of overall survival (OS) in 77 patients with locally advanced head and neck squamous cell carcinoma patients who received induction chemotherapy of TPF followed by CRT

Characteristics	No. of patients	Univariate analysis		Multivariate analysis	
		OS (months)	P-value	HR (95% CI)	P-value
Age			0.11		
< 60 years	65 (84.4%)	35.3		0.47 (0.24-0.90)	0.024*
≥ 60 years	12 (15.6%)	18.8			
Gender			0.40		
Male	73 (94.8%)	30.1			
Female	4 (5.2%)	43.8			
Location			0.032*		
Oral cavity	22 (28.6%)	16.3			
Oropharynx + Hypopharynx + Larynx	55 (71.4%)	35.7			
HPV status			**0.007***		
Positive	**6 (7.8%)**	**NR**			
Negative	**71 (92.2%)**	**28.6**			
T status			0.003*		
2	19 (24.7%)	71.3			
3 + 4	58 (75.3%)	25.6			
N status			0.26		
0 + 1	30 (39.0%)	44.7			
2 + 3	47 (61.0%)	21.8			
Tumor stage			0.05		
II	5 (6.5%)	NR			
III + IV	72 (93.5%)	28.6			
Grade			**0.99**		
1 + 2	**62 (80.5%)**	**30.1**			
3	**15 (19.5%)**	**32.9**			
VEGF decrease after TPF treatment			0.002*		
Yes	42 (54.5%)	55.4		0.43 (0.25-0.74)	0.002*
No	35 (45.5%)	18.4			
Post-TPF VEGF ≥ 150 pg/mL			<0.001*		
Yes	41 (53.2%)	20.3			
No	36 (46.8%)	58.3		0.38 (0.22-0.65)	0.001*
SDF-1α decrease after TPF treatment			<0.001*		
Yes	21 (27.3%)	116.6		0.40 (0.20-0.83)	0.013*
No	56 (72.7%)	23.3			
Post-TPF SDF-1α ≥ 1500 pg/mL			<0.001*		
Yes	59 (76.6%)	27.0			
No	18 (23.4%)	NR		0.42 (0.18-0.95)	0.037*

TPF: Docetaxel, Cisplatin and Fluorouracil; CRT: chemoradiotherapy; NR: not reached; HR: hazard ratio; CI: confidence interval; HPV: Human papillomavirus; VEGF: vascular endothelial growth factor; SDF-1α: stromal cell-derived factor-1α. *Statistically significant.

- CHANGE: Results/*Patient characteristics*, page 4, line 183

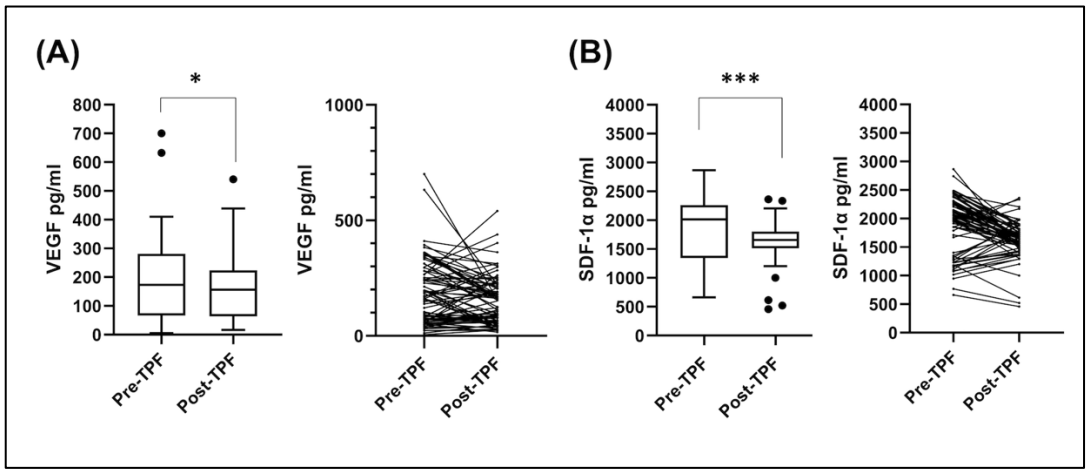
Table 1, Table 2 and Table 3

<< COMMENT 7 from Reviewer #3 >>

Figure 1 please enhance the axis labeling for readability and clarity

- ANSWER to comment 7:

Thanks for your comments. We have revised Figure 1 according to your suggestion. Please evaluate it.



- CHANGE: Figure 1

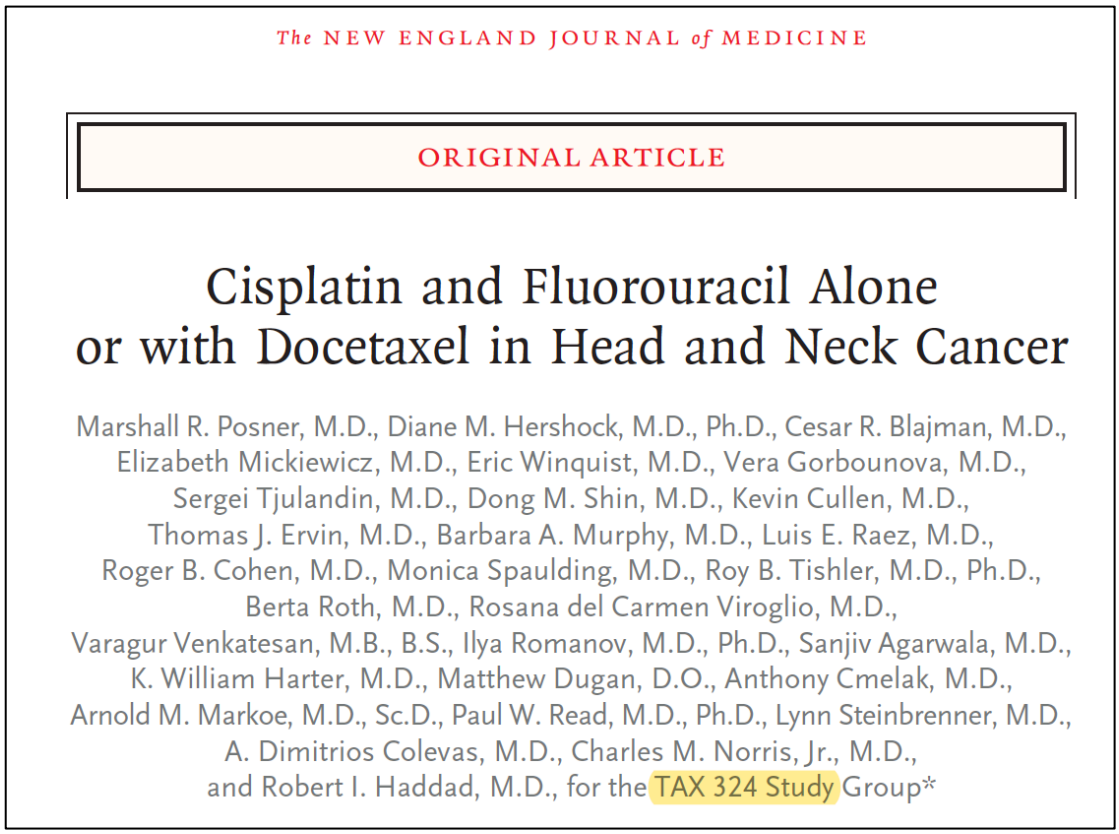
<< COMMENT 8 from Reviewer #3 >>

What was the patient enrollment approach adopted by the authors

- ANSWER to comment 8:

Thanks for your comments. The eligibility criteria of our study were followed by the TAX324 study, which was published in NEJM (2007) and focused on the TPF induction chemotherapy in head and neck cancer. Please evaluate it.

Posner MR, Hershock DM, Blajman CR, et al. Cisplatin and fluorouracil alone or with docetaxel in head and neck cancer. N Engl J Med 2007;357:1705-15



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cisplatin and Fluorouracil Alone or with Docetaxel in Head and Neck Cancer

Marshall R. Posner, M.D., Diane M. Hershock, M.D., Ph.D., Cesar R. Blajman, M.D., Elizabeth Mickiewicz, M.D., Eric Winquist, M.D., Vera Gorbounova, M.D., Sergei Tjulandin, M.D., Dong M. Shin, M.D., Kevin Cullen, M.D., Thomas J. Ervin, M.D., Barbara A. Murphy, M.D., Luis E. Raez, M.D., Roger B. Cohen, M.D., Monica Spaulding, M.D., Roy B. Tishler, M.D., Ph.D., Berta Roth, M.D., Rosana del Carmen Viroglio, M.D., Varagur Venkatesan, M.B., B.S., Ilya Romanov, M.D., Ph.D., Sanjiv Agarwala, M.D., K. William Harter, M.D., Matthew Dugan, D.O., Anthony Cmelak, M.D., Arnold M. Markoe, M.D., Sc.D., Paul W. Read, M.D., Ph.D., Lynn Steinbrenner, M.D., A. Dimitrios Colevas, M.D., Charles M. Norris, Jr., M.D., and Robert I. Haddad, M.D., for the TAX 324 Study Group*

Squamous-cell carcinoma of the head and neck accounts for 5% of newly diagnosed cancers in adults in the United States and 8% of cancers worldwide.[1] The disease is potentially curable at an early stage, but most patients present with locally advanced disease. After standard therapy (surgery and irradiation), only 30 to 50% of patients with locally advanced disease live for 3 years, and locoregional recurrences or distant metastases develop in 40 to 60% of them.[2-6] Various strategies to improve outcomes by coordinating chemotherapy with surgery and radiotherapy have been tried, but the optimal schedule for integrating chemotherapy into the management of this disease has yet to be defined.[7]

Although chemoradiotherapy (radiotherapy plus concurrent chemotherapy) has become the standard of care for patients with unresectable squamous-cell carcinoma of the head and neck[2] and for organ preservation,[3,8] induction chemotherapy with cisplatin and fluorouracil (PF) also has benefits in this disease.[9-11] A comprehensive meta-analysis showed that induction chemotherapy (i.e., chemotherapy as the initial treatment) with PF significantly improved the rate of survival at 5 years, as compared with standard radiotherapy plus surgery in patients with locally advanced disease.[11]

Docetaxel (Taxotere, Sanofi-Aventis) has substantial activity when administered alone in patients with recurrent or incurable disease.[12,13] In phase 1 and phase 2 studies of docetaxel plus cisplatin and fluorouracil (TPF) in the treatment of locally advanced squamous-cell carcinoma of the head and neck, including phase 2 studies of treatment with curative intent, clinical and pathological response rates have been high and survival has been prolonged.[14-18] Two phase 3 trials in which induction chemotherapy with TPF or PF was followed by radiotherapy (the European Organization for Research and Treatment of Cancer [EORTC] 24971/TAX 323 study by Vermorken et al.[19]) or chemoradiotherapy (TAX 324) in locally advanced disease have now been completed. The results of the study by Vermorken et al. are reported in this issue of the *Journal*. We report on the results of the TAX 324 study here.

METHODS

PATIENTS

Patients who had measurable, nonmetastatic, histologically proven stage III or IV squamous-cell carcinoma of the oral cavity, larynx, oropharynx, or hypopharynx were eligible if the tumor was deemed to be either unresectable (because of tumor fixation, involvement of the nasopharynx, or fixed lymph nodes) or of low surgical curability on the basis of advanced tumor stage (3 or 4) or regional-node stage (2 or 3, except T1N2), or if the patient was a candidate for organ preservation. Patients had to be at least 18 years of age with a World Health Organization (WHO) performance status of 0 or 1 and adequate bone marrow, liver, and renal function. Exclusion criteria were any previous chemotherapy or radiotherapy, a cancer diagnosis within the previous 5 years, another active cancer, any previous definitive surgery for squamous-cell carcinoma of the head and neck, severe weight loss (>20% of body weight) in the preceding 3 months, and chronic obstructive pulmonary disease requiring hospitalization within the previous 12 months.

Disease was staged according to the criteria of the American Joint Committee on Cancer.[20] All patients provided written informed consent, and each study center was required to have approval from its institutional review board before randomization.

STUDY DESIGN

In a randomized, open-label phase 3 trial, we compared three cycles of TPF induction chemotherapy with three cycles of PF induction chemotherapy; both regimens were followed by 7 weeks of chemoradiotherapy (Fig. 1 of the Supplementary Appendix, available with the full text of this article at www.nejm.org). Randomization was performed centrally with the use of a biased-coin minimization technique. At study entry, patients were stratified according to the site of the primary tumor, nodal status (N0 or N1 vs. N2 or N3), and institution. Patients with progressive disease after induction chemotherapy or chemoradiotherapy were treated according to the institution's choice of salvage therapy (Fig. 1 of the Supplementary Appendix). Neck dissections were performed after the completion of chemoradiotherapy. The primary end point was overall survival. Secondary end points included progression-free survival, response rates after induction chemotherapy, and toxic effects.

One of the academic investigators designed, wrote, and implemented the study protocol and managed the study in collaboration with employees of the sponsor, Sanofi-Aventis. Lead investiga-

<< COMMENT 9 from Reviewer #3 >>

Were there any differences in the serum levels of VEGF before and after the TPF administration?

- ANSWER to comment 9:

Thanks for your comments. In Figure 1, the mean VEGF values in the post-TPF treatment is lower than it in the pre-TPF treatment with significantly statistical difference ($P < 0.05$). Please evaluate it.

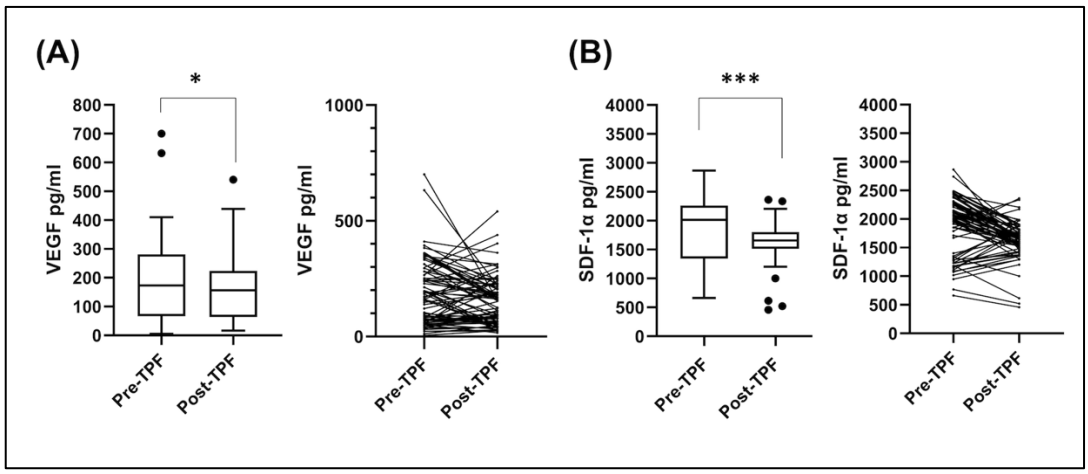


Figure 1 legend. Comparison of serum SDF-1α and VEGF concentrations between pre-TPF and post-TPF treatments in head and neck squamous cell carcinoma patients. (A) The distribution and kinetic change of serum VEGF in the pre-TPF and post-TPF status; (B) The distribution and kinetic change of serum SDF-1α in the pre-TPF and post-TPF status. • means extreme values; *** means P < 0.05** and *** means P < 0.001. SDF-1α: stromal cell-derived factor-1α; VEGF: vascular endothelial growth factor; TPF: docetaxel, cisplatin, and 5-fluorouracil.

<< COMMENT 10 from Reviewer #3 >>

Could the authors please comment on the survival and progressing response in the study samples

- ANSWER to comment 10:

Thanks for your comments. We have added the description to discuss about the survival in the Discussion section in my revised manuscript according to your suggestion. Please evaluate it.

Discussion/*Six paragraph*

In our study, we found that poor prognosis, including PFS and OS, were mentioned in patients with well-known poor prognostic factors, such as advanced T or N status, high tumor grade, negative HPV status, etc. In addition, patients without VEGF or SDF-1α decrease after TPF treatment, or higher post-TPF VEGF or SDF-1α values were also mentioned to have worse prognosis, whether in the univariate or multivariate analysis. Moreover, we found that patients with both VEGF and SDF-1α decrease after TPF treatment, and higher both post-TPF VEGF and SDF-1α values had the worst PFS and OS than the other groups, indicating the role of combination of serum VEGF and SDF-1α in the prediction of clinical outcome.

- CHANGE: Discussion/*Six paragraph***, page 13, line 398**

In addition, we have modified our manuscript using a professional language editing service. Please evaluate it.

editage

Editing Certificate

This document certifies that the manuscript listed below has been edited to ensure language and grammar accuracy and is error free in these aspects. The edit was performed by professional editors at Editage, a division of Cactus Communications. The author's core research ideas were not altered in any way during the editing process. The quality of the edit has been guaranteed, with the assumption that our suggested changes have been accepted and the text has not been further altered without the knowledge of our editors.

MANUSCRIPT TITLE

Serum Levels of Stromal Cell-derived Factor-1α and Vascular Endothelial Growth Factor Predict Clinical Outcomes in Head and Neck Squamous Cell Carcinoma Patients Receiving TPF Induction Chemotherapy

AUTHORS

Yen-Hao Chen, M.D., Ph.D., Chih-Yen Chien, M.D., Yu-Ming Wang, M.D., Ph.D., and Shau-Hsuan Li, M.D.

ISSUED ON

February 18, 2022

JOB CODE

YOPDO_16

THE EDITAGE Promise QUALITY GUARANTEE

Vikas Narang

Vikas Narang
Chief Operating Officer - Editage

editage

Editage, a brand of Cactus Communications, offers professional English language editing and publication support services to authors engaged in over 1300 areas of research. Through its community of experienced editors, which includes doctors, engineers, published scientists, and researchers with peer review experience, Editage has successfully helped authors get published in internationally reputed journals. Authors who work with Editage are guaranteed excellent language quality and timely delivery.

GLOBAL :
+1(833) 979-0061 | request@editage.com

TAIWAN :
226570306 | submitjobs@editage.com

CACTUS

impact.science

researcher.life

lifesciences.cactusglobal.com

Finally, we thank you again for your patience and valuable time

Sincerely

Shau-Hsuan Li, M.D.
Division of Hematology-Oncology, Department of Internal Medicine, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan.
No.123, Dapi Rd., Niaosong Dist., Kaohsiung City 833, Taiwan
Tel: 886-7-7317123 ext.8303, Fax: 886-7-7322402
E-mail: lee.a0928@msa.hinet.net