ANTICANCER RESEARCH *41*: 1083-1087 (2021)
doi:10.21873/anticanres.14866

Clinical Applicability of the Proliferation Marker Thymidine Kinase 1 in Head and Neck Cancer Patients

AMICHAY MEIROVITZ[1*], MENACHEM GROSS[2*], VERA LEIBOVICI[3], KIM SHEVA[1], AHARON POPOVZER[1] and VIVIAN BARAK[1]

Departments of [1]Oncology, [2]Otolaryngology-Head and Neck Surgery, and [3]Dermatology, Hadassah – Hebrew University Medical Center, Jerusalem, Israel

Abstract. *Background/Aim: Prognostic factors serve as a vital tool in the treatment of patients with head and neck cancer (HNC). The aim of this study was to evaluate the clinical potential of Thymidine-kinase-1 (TK1) marker in the prognosis of HNC patients. Patients and Methods: We evaluated 366 blood samples from 278 HNC patients and 88 healthy controls, using an ELISA assay. Correlations of TK1 levels with disease stage, lymph node involvement and response to radiation therapy, were determined. Results: In HNC patients, TK1 levels were significantly higher compared to healthy controls. Significantly higher TK1 levels were demonstrated in node positive cases and in advanced disease stages compared to node negative and early disease stages. Levels were higher prior to radiation and decreased significantly thereafter, in patients responding to treatment. Increasing levels of TK1 post-radiation were indicative of recurrence or of non-response to treatment, while decreasing levels indicated a positive response. Conclusion: TK1 is a tumor marker in HNC patients with the ability to assess response to therapy. High or increasing levels correlated to a poor prognosis, whereas low levels correlated to an overall increased survival.*

Head and neck cancer (HNC) encompasses various epithelial malignancies arising in multiple sites including the larynx, pharynx, oral and nasal cavities and the paranasal sinuses. Approximately 90% of these cancers fall into the heterogeneous sub-group of head and neck squamous cell carcinomas (HNSCC), the main risk factors of which are tobacco and alcohol. About 2/3 of HNSCC patients present with advanced stage disease, commonly involving regional lymph nodes (1-4).

Treatment of HNSCC is often complicated due to tumor heterogeneity, site, stage and resectability, as well as patient factors such as swallowing, airway obstructions, organ preservation and comorbidities. While surgery and radiotherapy have long been the established methods of treatment, HNSCC patients suffer from high recurrent rates of disease, with significant risk of mortality. Reasons of mortality could be cardiac and respiratory diseases, as well as secondary primary tumors, which may affect the whole aero-digestive tract. Currently, no conclusive biomarker exists for the early detection and surveillance of HNSCC. Such markers are needed not only for earlier diagnosis of invisible tumors, but offer the potential of accelerating the developments of novel treatments (5, 6).

We have previously demonstrated serum tumor markers such as carcino-embryonic antigen (CEA), squamous cell carcinoma antigen (SCC), tissue polypeptide specific antigen (TPS) and Cyfra 21-1, as well as the immune marker sIL-2R, to be efficient in assessing response to therapies and providing insights into the prognosis of HNC patients in preliminary studies (7, 8).

While multiple serum markers have been found to show potential in revealing otherwise undetectable tumors, few have been proven clinically relevant in controlled clinical trials, including CEA, CA19-9 (9), CA125 (10), alpha-fetoprotein (AFP) and prostate specific antigen (PSA) (11).

Thymidine kinase 1 (TK1) is a key phosphotransferase enzyme in nucleotide synthesis that is involved in the salvage pathway of DNA synthesis and is activated in the G1/S phase of the cell cycle. Due to this imperative function, TK1 activity has been shown to correlate with the proliferative activity of tumor cells (12).

Serum TK1 activity has been investigated mainly in hematological malignancies. High TK1 levels have been shown to be a strong predictive factor of poor prognosis in T cell lymphoma as well as in ALL and AML patients (12, 13). Suzuki *et al.* (14) and also O'Neill *et al.* (15) showed such a prediction in ALL and AML patients.

*The Authors contributed equally to this study.

Correspondence to: Prof. Vivian Barak, Head, Immunology Laboratory for Tumor Diagnosis, Department of Oncology, Hadassah-Hebrew University Medical Center, Ein Kerem P.O.B. 12000, Jerusalem 9112001, Israel. Tel: +972 0522401916, e-mail: barakvivi@hadassah.org.il; barakvivi@gmail.com

Key Words: Head and neck cancer, TK1 marker, prognosis.

We have previously reported the good sensitivity of TK1 as a tumor marker in aggressive lymphoma, where serum TK1 levels were able to differentiate patients with a clinical suspicion of indolent to aggressive transformation (16).

Previous studies have shown that TK1 may be additionally used as a tumor marker in various types of solid tumors including lung cancer (17, 18), renal cancer (19), pancreatic cancer (20) and breast cancer (21, 22), amongst others (23-25).

HNC is considered an aggressive tumor type with an unfavorable prognosis and with a substantial number of cases of recurrent disease, despite improved new therapies emerging over the past few decades (26, 27). There is therefore an ongoing need to improve early detection methodologies and treatment approaches.

The aim of this study was to investigate the clinical potential of TK1 as a tumor marker in HNC patients.

Patients and Methods

This study included a cohort of 278 HNC patients and TK1 levels were determined in blood samples of these patients and compared to those of 88 healthy controls. Patients were evaluated at the first diagnosis, with follow-ups occurring before, during and/or after radiation therapy. Potential correlation of TK1 levels to: 1) disease stage (early *vs*. advanced); 2) node involvement (positive or negative); and 3) the effect of radiation therapy (pre- *vs*. post- radiation), was determined.

TK1 levels were assessed using ELISA kits (Diasorin, Italy).

The study was performed following the permission of the Hadassah Hospital IRB Committee.

Results

TK1 levels were evaluated in the HNC patient cohort and compared to those of the healthy control group, and its levels were found to be significantly higher in HNC patients (p=0.028) (Figure 1). To determine whether TK1 correlates to the clinical parameters of disease stage, patients were categorized as being either in the early disease group or the advanced disease group of HNC and the respective TK1 levels were evaluated. Significantly higher levels of TK1 were demonstrated in advanced disease (T3+T4) patients, as compared to early disease (T1+T2) patients (p=0.033) (Figure 2).

To determine whether any correlation existed between HNC patient nodal status and TK1 levels, TK1 levels were compared between the node-positive and -negative groups of patients. Node-positive HNC patients showed significantly higher TK1 levels as compared to node-negative patients (p=0.037) (Figure 3).

The effect of radiation therapy on TK1 levels was assessed by comparing its levels in the same patients prior and post treatment. Serum levels of TK1 were significantly higher prior to radiation therapy and decreased significantly (p=0.041) thereafter in all patients responding to the therapy (as per PET CT scans) (Figure 4).

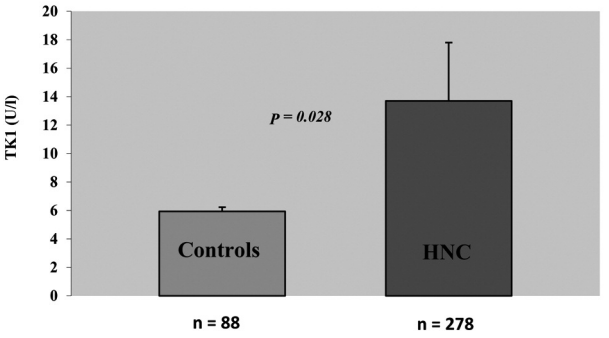

Figure 1. *TK1 levels in head and neck cancer patients and in healthy controls.*

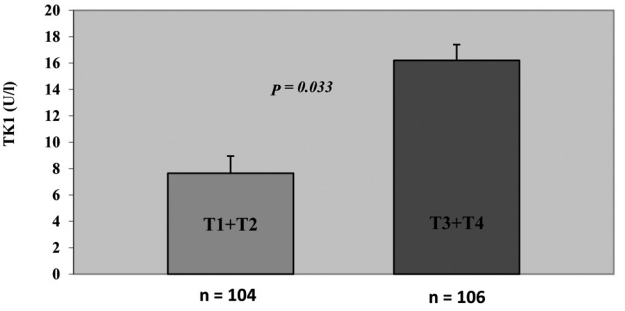

Figure 2. *TK1 levels in head and neck cancer patients according to stage.*

We compared TK1 levels in patients we considered as responsive to therapies *vs*. patients non-responsive. Indeed, higher levels of TK1 were found in non-responsive patients, as opposed to low levels in the responders (Figure 5).

We set out to better understand the changes occurring in TK1 levels of HNC patients and their potential correlation to earlier detection of HNC recurrence or earlier indication of a positive response to radiation therapy. Increasing levels of TK1 preceded disease recurrence or metastases that was observed only 6 – 12 months later by PET CT scans. In patients responding positively to radiation therapy, the trend of decreasing serum TK1 levels also preceded the findings revealed through PET CT scans.

Increasing levels of TK1 (day and month presented) indicated recurrence or no-response to therapies (Figure 6), while decreasing levels (day and month presented), indicated response to therapies (Figure 7). All TK1 levels (increasing, stabilized or decreasing) could be correlated to the therapeutic response seen in a specific patient.

Discussion

Previous studies have suggested that various serum tumor markers in HNC could serve as disease predictors for effective early therapeutic intervention, as well as to assess

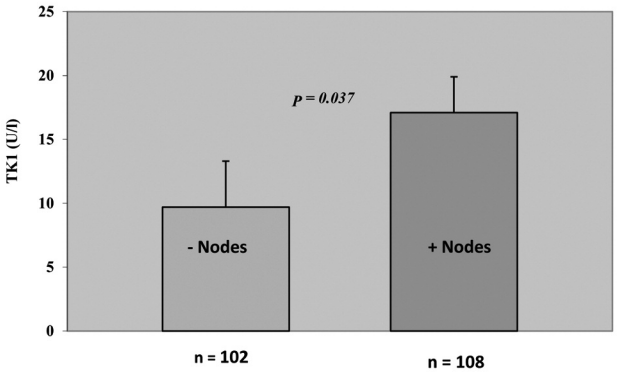

Figure 3. *TK1 levels in head and neck cancer patients according to nodes.*

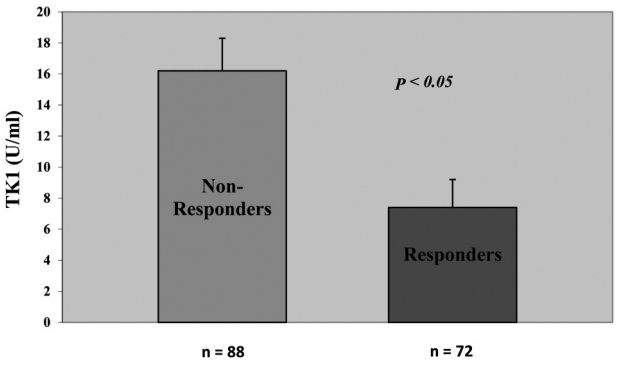

Figure 5. *TK1 levels in head and neck cancer patients in therapy responders.*

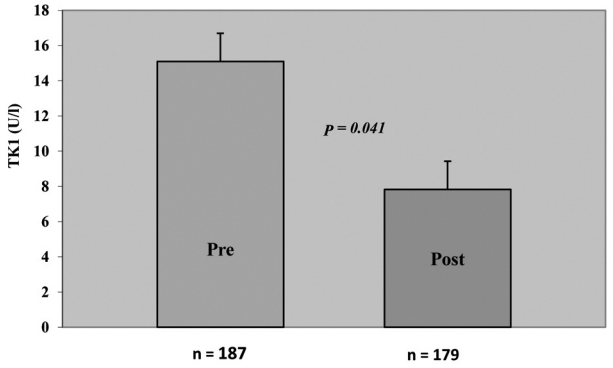

Figure 4. *TK1 levels in head and neck cancer patients according to radiation therapy.*

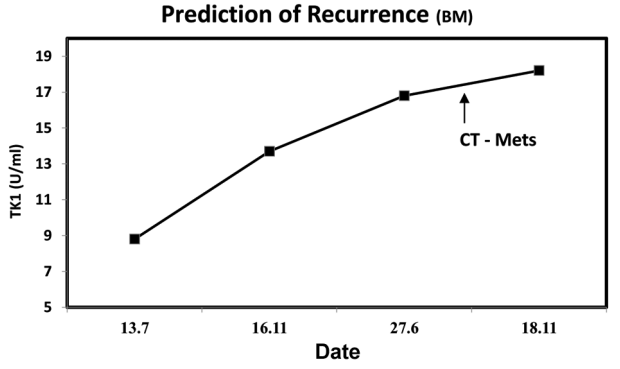

Figure 6. *Kinetics of TK1 levels of a head and neck patient.*

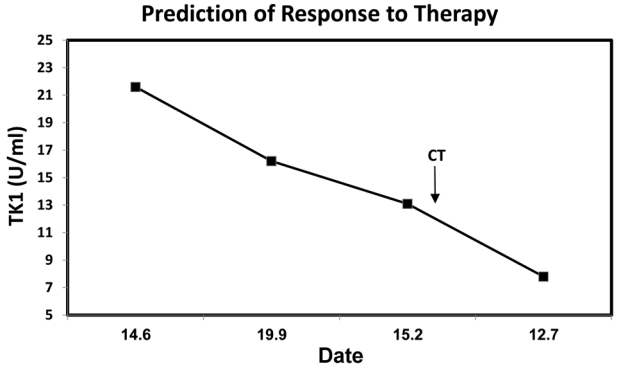

Figure 7. *Kinetics of TK1 levels of a head and neck patient – Decreasing levels.*

the response of individual patients to therapy (9). Selected molecules found in the sera of patients have been suggested as either single markers or as part of panels of markers for HNC patients depending on sensitivity. Multiple studies have suggested that advanced disease and lymph node involvement in HNC patients are correlated to higher levels of tumor markers (7, 27, 28).

We have also previously shown a panel of serum markers (CEA, SCC, TPS, Cyfra 21-1) that was successfully used to assess response to therapy in HNC patients (7). In addition, the immuno-marker sIL-2R has been shown to be a sensitive predictor of response in HNC patients (8).

Additional studies have addressed the ability of the TK1 marker to assess response to therapy in solid tumors. Holdenrieder *et al*. (17). have highlighted the clinical significance of TK1 in disease diagnosis, therapeutic monitoring and prognosis of non-operable lung cancer, where Jiang *et al*. (18) recommended the addition of the TK1 a panel of markers for improved sensitivity. The respective ROC curve analyses showed that the diagnostic value of TK1 combined with CEA, CYFRA21-1 and NSE in lung cancer was significantly higher than that of each biomarker alone (all $p<0.0001$). TK1 has also been recommended in the detection of early- and advanced-stage lung cancer, highlighting its importance in cancer detection (25, 26).

In pancreatic adenocarcinomas (20), TK1 was revealed to be significantly increased, accompanied by improved overall survival, especially in early tumor stages. Other studies have shown TK1 as a sensitive marker for breast cancer (21) and renal cell cancer detection (19).

While statistical analysis for progression-free survival was not possible in this study due to limited data for certain patients, it was found that TK1 predicted an approximately 80% survival in HNC patients. High levels of TK1 correlated to a shorter survival time, where in the majority of surviving patients TK1 levels were low.

In the present study, serum levels of TK1 were significantly elevated in HNC patients as compared with healthy individuals. These results are consistent with those of previously reported studies on various tumor markers levels (7, 29, 30). High TK1 levels, however, cannot serve as a definitive specific cancer type diagnostic tool, but rather as a marker of proliferation characterizing multiple malignancies and assisting in the clinical diagnosis of HNC patients. As expected, this study found TK1 to be elevated in advanced HNC, as well as in node-positive patients. The high sensitivity of the TK1 marker in HNC has the potential to significantly contribute to new therapeutic developments and early disease interventions (22, 27-29).

Despite recent advancements in HNC therapies (2, 29), the majority of patients are still diagnosed only in the more advanced stages of the disease. The potential of the serum TK1 marker as a sensitive and reliable approach to enable earlier diagnosis and subsequent therapeutic intervention of HNC patients is crucial. The detection of TK1 offers the added advantage of being used either as a single marker or as part of a panel of markers, to improve sensitivity in the accurate diagnosis and staging of HNC.

This is the first and only study analyzing serum TK1 in HNC patients and highlights how this sensitive marker could potentially be used as a treatment target, either using antibodies against TK1 or a drug-antibody conjugate.

Conclusion

In summary, this is the first study focusing on the clinical role of serum TK1 in HNC patients. TK1 was shown to correlate to disease stage as well as to nodal status. We revealed serum TK1 to be a useful and sensitive marker in assessing response to therapy and providing prognosis regarding HNC patient survival.

TK1 may be used as a single marker or as a part of a panel of markers for improved sensitivity and detection of HNC, providing clinicians with an improved ability to start effective therapeutic intervention as soon as possible.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

Meirovitz Amichay and Gross Menachem – patient's enrollment and therapy. Leibovici Vera patient's enrollment. Sheva Kim – paper design. Popovzer Aharon – data processing. Barak Vivian – PI of study and TK evaluations.

Acknowledgements

The Authors are thankful to Miss Shani Cohen for the technical assistance.

References

1 Siddiqui F and Gwede CK: Head and neck cancer in the elderly population. Semin Radiat Oncol *22(4)*: 321-333, 2012. PMID: 22985815. DOI: 10.1016/j.semradonc.2012.05.009

2 Rezende TM, de Souza Freire M and Franco OL: Head and neck cancer: Proteomic advances and biomarker achievements. Cancer *116*: 4914-4925, 2010. PMID: 20665484. DOI: 10.1002/cncr.25245

3 Argiris A, Karamouzis MV, Raben D and Ferris RL: Head and neck cancer. Lancet *371(9625):* 1695-709, 2008. PMID: 18486742. DOI: 10.1016/S0140-6736(08)60728-X

4 SEER Cancer statistics review 1975-2004. Available at: https://seer.cancer.gov/archive/csr/1975_2004 [Last accessed on January 7 2021]

5 Argiris A, Brockstein BE, Haraf DJ, Stenson KM, Mittal BB, Kies MS, Rosen FR, Jovanovic B and Vokes EE: Competing causes of death and second primary tumors in patients with locoregionally advanced Head and Neck Cancer treated with chemoradiotherapy. Clin Cancer Res *10(6)*: 1956-1962, 2004. PMID: 15041712. DOI: 10.1158/1078-0432.ccr-03-1077

6 Khuri FR, Lee JJ, Lippman SM, Kim ES, Cooper JS, Benner SE, Winn R, Pajak TF, Williams B, Shenouda G, Hodson I, Fu K, Shin DM, Vokes EE, Feng L, Goepfert H and Hong WK: Randomized phase III trial of low-dose isotretinoin for prevention of second primary tumors in stage I and II Head and Neck Cancer patients. J Natl Cancer Inst *98(7)*: 441-450, 2006. PMID: 16595780. DOI: 10.1093/jnci/djj091

7 Barak V, Meirovitz A, Rachmut K, Peretz T, Eliashar J and Gross M: The diagnostic and prognostic value of tumor markers (SCC, TPS, CYFRA 21-1, CEA) in head & neck cancer patients. Anticancer Res *35(10)*: 5519-5524, 2015. PMID: 26408719.

8 Gross M, Meirovitz A, Rachmut J, Kalichman I, Peretz T, Eliashar R and Barak V: The diagnostic and prognostic value of sIL-2R as an immune biomarker in head and neck cancers. Anticancer Res *36(8)*: 4347-4352, 2016. PMID: 27466555.

9 Fiala O, Finek J, Buchler T, Matejka M, Holubec L, Kulhankova J, Bortlicek Z, Liska V and Topolcan O: The association of serum carcinoembryonic antigen, carbohydrate antigen 19-9, thymidine kinase, and tissue polypeptide specific antigen with outcomes of patients with metastatic colorectal cancer treated with bevacizumab: a retrospective study. Target Oncol 10(4): 549-55, 2005. PMID: 25875421. DOI: 10.1007/s11523-015-0365-x

10 Dehaghani AS, Ghiam AF, Hosseini M, Mansouri S and Ghaderi A: Factors influencing serum concentration of CA125 and CA15-3 in Iranian healthy postmenopausal women. Pathol Oncol Res *13(4)*: 360-364, 2007. PMID: 18158573. DOI: 10.1007/BF02940317

11 Ulmert D, Cronin AM, Björk T, O'Brien MF, Scardino PT, Eastham JA, Becker C, Berglund G, Vickers AJ and Lilja H: Prostate-specific antigen at or before age 50 as a predictor of advanced prostate cancer diagnosed up to 25 years later: a case-control study. BMC Med *6*: 6, 2008. PMID: 18279502. DOI: 10.1186/1741-7015-6-6

12 Hallek M, Wanders L, Strohmeyer S and Emmerich B: Thymidine Kinase: a tumor marker with prognostic value for Non-Hodgkin's Lymphoma and a broad range of potential clinical applications. Ann Hematol *65(1)*: 1-5, 1992. PMID: 1643153. DOI: 10.1007/BF01715117

13 Hallek M, Langenmayer I, Nerl C, Knauf W, Dietzfelbinger H, Adorf D, Ostwald M, Busch R, Kuhn-Hallek I, Thiel E and Emmerich B: Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early non-smoldering chronic lymphocytic leukemia. Blood *93(5)*: 1732-1737, 1999. PMID: 10029603.

14 Suzuki K, Terui Y, Nakano K, Nara E, Nasu K, Ueda K, Nishimura N, Mishima Y, Sakajiri S, Yokoyama M, Takahashi S and Hatake K: High thymidine kinase activity is a strong predictive factor for poor prognosis in peripheral T-cell lymphoma treated with Cyclophosphamide, Adriamycin, Vincristine and Prednisone. Leuk Lymphoma *53(5)*: 849-854, 2012. PMID: 22035416. DOI: 10.3109/10428194.2011.635858

15 O'Neill KL, Zhang F, Li H, Fuja DG and Murray BK: Thymidine Kinase 1–a prognostic and diagnostic indicator in ALL and AML patients. Leukemia *21(3)*: 560-563, 2007. PMID: 17252017. DOI: 10.1038/sj.leu.2404536

16 Gatt M E, Goldschmidt N, Kalichman I, Friedman M, Arronson AC and Barak V: Thymidine Kinase levels correlate with prognosis in Aggressive Lymphoma and can discriminate patients with a clinical suspicion of indolent to aggressive transformation. Anticancer Res *35(5)*: 3019-3026, 2015. PMID: 25964590.

17 Holdenrieder S, Von Pawel J, Duell T, Feldmann K, Raith H, Schollen A, Nagel D and Stieber P: Clinical relevance of Thymidine Kinase for the diagnosis, therapy monitoring and prognosis of non-operable Lung Cancer. Anticancer Res 30(5): 1855–1862, 2010. PMID: 20592392.

18 Jiang ZF, Wang M and Xu JL: Thymidine kinase 1 combined with CEA, CYFRA21-1 and NSE improved its diagnostic value for lung cancer. Life Sci *194*: 1-6, 2018. PMID: 29247745. DOI: 10.1016/j.lfs.2017.12.020

19 Nisman B, Appelbaum L, Yutkin V, Nechushtan H, Hubert A, Uziely B, Pode D and Peretz T: Serum thymidine-kinase-1 activity following nephrectomy for renal cell carcinoma and radiofrequency ablation of metastases to lung and liver. Anticancer Res *36(4)*: 1791-1797, 2016. PMID: 27069161.

20 Felix K, Hinz U, Dobiasch S, Hackert T, Bergmann F, Neumüller M, Gronowitz S, Bergqvist M and Strobel O: Preoperative serum thymidine kinase activity as novel monitoring, prognostic, and predictive biomarker in pancreatic cancer. Pancreas *47(1)*: 72-79, 2018. DOI: 10.1097/MPA.0000000000000966

21 Svobodova S, Topolcan O, Holubec L, Treska V, Sutnar A, Rupert K, Kormunda S, Rousarova M and Finek J: Prognostic importance of thymidine kinase in Colorectal and Breast Cancer. Anticancer Res *27(4A)*: 1907-1909, 2007. PMID: 17649793.

22 Weagel EG, Burrup W, Kovtun R, Velazquez EJ, Felsted AM, Townsend MH, Ence ZE, Suh E, Piccolo SR, Weber KS, Robison RA and O'Neill KL: Membrane expression of Thymidine Kinase 1 and potential clinical relevance in lung, breast, and colorectal malignancies. Cancer Cell Int *18*: 135, 2018. DOI: 10.1186/s12935-018-0633-9

23 Zhou J, He E and Skog S: The proliferation marker Thymidine Kinase 1 in clinical use. Mol Clin Oncol *1(1)*: 18-28, 2013. PMID: 24649117. DOI: 10.3892/mco.2012.19

24 Chen Y, Ying M, Chen Y, Hu M, Lin Y, Chen D, Li X, Zhang M, Yun X, Zhou J, He E and Skog S: Serum Thymidine Kinase 1 correlates to clinical stages and clinical reactions and monitors the outcome of therapy of 1,247 cancer patients in routine clinical settings. Int J Clin Oncol *15(4)*: 359-368, 2010. PMID: 20354751. DOI: 10.1007/s10147-010-0067-4

25 Li Y, Wei R and Song S. Diagnostic and prognostic value of serum thymidine kinase 1 in cancer patients. Indian J Hematol Blood Transfus *34(1)*: 168-170, 2018. PMID: 29398824. DOI: 10.1007/s12288-017-0812-1

26 Alegre MM, Weyant MJ, Bennett DT, Yu JA, Ramsden MK, Elnaggar A, Robison RA and O'Neill KL: Serum detection of Thymidine Kinase 1 as a means of early detection of lung cancer. Anticancer Res *34(5)*: 2145-2151, 2014. PMID: 24778016.

27 Guo W and Song H: Development of gene therapeutics for Head and Neck Cancer in China. From bench to bedside. Hum Gene Ther *29(2)*: 180-187, 2018. PMID: 29334764. DOI: 10.1089/hum.2017.230

28 Topolcan O, Holubec L Jr, Finek J, Stieber P, Holdenrieder S, Lamerz R, Holubec Sen L, Svobodova S, Visokai V and Lipska L: Changes of Thymidine Kinase (TK) during adjuvant and palliative chemotherapy. Anticancer Res *25(3A)*: 1831-1833, 2005. PMID: 16033109.

29 Jagarlamudi KK and Shaw M: Thymidine Kinase 1 as a tumor biomarker: technical advances offer new potential to an old biomarker. Biomark Med *12(9)*: 1035-1048, 2018. PMID: 30039979. DOI: 10.2217/bmm-2018-0157

30 Prochazka V, Faber E, Raida L, Langova K, Indrak K and Papajik T: High baseline serum Thymidine Kinase 1 level predicts unfavorable outcome in patients with follicular lymphoma. Leuk Lymphoma *53(7)*: 1306-1310, 2012. PMID: 22263569. DOI: 10.3109/10428194.2011.654339

Received December 21, 2020
Revised January 6, 2021
Accepted January 7, 2021