The rapid adoption of next-generation sequencing (NGS) has enabled a low-cost detection of cancer associated mutations. While mutation-based tumor profiling is rapidly transforming the clinical management, only a minority of patients currently benefit by druggable mutations in a personalized treatment approach. It becomes clear that a multilevel molecular analysis that also incorporates transcriptome data will provide a deeper tumor profiling and will expand the proportion of patients who can have a benefit.

Microarray and RNA-seq gene expression data has been extensively used for the molecular classification of head and neck squamous cell carcinoma (HNSCC), providing insight into the molecular heterogeneity of this tumor. In the last few years, specific gene expression signatures associated with human papilloma virus status (HPV+)/HPV-, histological origin (basal/luminal) and epithelial-mesenchymal transition [1] have been generated for a better characterization of HNSCC.

Besides the great importance of gene expression analysis for the characterization of head and neck heterogeneity, transcriptome profiling has also a high prognostic or predictive value for patients and can be used to assist in treatment decisions for HNSCC. Head and neck cancer is characterized by heterogeneous clinical behavior and response to therapies. Despite the aggressive treatment with surgery, chemoradiotherapy and radiation, 40–50% of patients with advanced disease, recur [2]. Therefore, there is an urgent need to define optimal treatment approaches according to patient stratification. In this direction, gene expression signatures associated with chemoradiotherapy or radiotherapy resistance, metastasis, recurrence, immunotherapy response, cetuximab response and tumor aggressiveness have been developed. Moreover, expression signatures corresponding to specific activated oncogenic pathways that can be targeted therapeutically, such as EGFR and RAS pathways, have also been identified.

Despite the great contribution of such studies for understanding the role of pathways represented by these signatures, poor progress has been made in their translation for use in the clinics. The lack of robust validation in independent clinical trials and in multicenter settings is probably an important reason for that. Criticism on these studies also include the lack of appropriate sample size as well as the lack of overlapping genes among the different prognostic signatures.

In this article of EBioMedicine, Liu and colleagues report a 60 gene mRNA expression signature for predicting the risk of oropharyngeal squamous cell carcinoma (OPSCC) progression [3]. This signature was developed by RNA-seq profiling of 408 OPSCCs from different institutes and its prognostic power was validated by multiple independent cohorts including TCGA OPSCC data. The set of 60 genes was significantly predictive of 5-year overall, 5-year recurrence-free, and 5-year metastasis-free survival and remained also prognostic among the HPV-positive patients of the cohort.

The large sample size used, the high number of the selected genes for the signature and the fact that their statistical properties were conserved across different datasets indicate the robustness of the presented signature.

The development of this signature is very important as the incidence of OPSCC and particularly the p16+ HPV-related OPSCC has increased markedly by 40–60% in North America and Northwestern Europe during the last few decades [4]. The oropharynx is the predominant primary site for HPV-associated head and neck cancer and it is estimated that in North America approximately 60% of OPSCC is HPV positive while in Europe, the high-risk HPV prevalence in OPSCC is approximately 40% [5].

HPV+ OPSCC comprises a distinct disease entity that displays significantly better locoregional control and prognosis compared to HPV-OPSCC [6] and for this reason, de-intensification of cisplatin-based chemoradiotherapy which is the standard of care for locally advanced tumors, is evaluated in ongoing clinical trials aiming to reduce overtreatment. However, more recently, it became accepted that HPV+ OPSCC is not clinically uniform and that a subset of HPV+ tumors, is evaluated in ongoing clinical trials aiming to reduce overtreatment. However, more recently, it became accepted that HPV+ OPSCC is not clinically uniform and that a subset of HPV+ tumors displays high potential for distant recurrence and therefore has poor oncologic outcome [7]. Interestingly, distant metastasis in patients with aggressive HPV+ OPSCC occurs significantly later (more than two years) after completion of chemoradiotherapy than in patients with HPV− disease and is the leading cause of death for HPV− initiated OPSCC [8].

Therefore, the signature by Liu and colleagues provides a valuable tool for robust prognostic stratification of HPV+OPSCCs that may help to determine which patients might be at higher risk for distant recurrence. This is crucial for developing individualized treatment plans. For instance, high-risk patients could be excluded from de-escalation treatment protocols and follow a more intensive follow-up for a longer period than what is usually practiced in HNSCC. On the other hand, this gene signature could contribute to ongoing trials by the selection of low-risk HPV+ OPSCC patients for deintensification treatment.

Commentary
Oropharyngeal cancer: the dawn of the RNA era in precision medicine

Theodoros Rampias
Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece
Glucose transport and inflammatory response were identified by Liu and colleagues as the most enriched biological pathways represented by their gene expression signature. Numerous studies have demonstrated the critical role of these pathways in modulating cancer development and progression. Accelerated glycolysis is one of the metabolic characteristics of cancer cells. Recent work indicates that advanced glucose transport and metabolism in cancer cells are associated with chemoresistance and survival of tumor cells under hypoxia, leading to poor prognosis [9]. Furthermore, in head and neck cancer, inflammatory mediators have been demonstrated as strong prognostic factors as they affect both the tumor infiltration by immune cells and the cancer transcriptome [10].

It is certain that for the majority of the genes reported on the signature, more work is needed in order to establish their functional role on survival, since are not well-known biomarkers. In any case, the next several years are expected to bring an increased focus on incorporating transcriptome, proteomic and immunological data in tumor profiling analysis, revolutionizing the field of precision medicine.

Author's contribution
TR designed the outline of this manuscript and the major points to be presented; TR drafted, edited and finalised the manuscript.

Declaration of Competing Interest
The author declares no conflict of interest.

Acknowledgments
The author apologises to the many scientists whose work is not specifically referenced in this commentary due to space limitations.

TR is funded by the Hellenic Foundation for Research and Innovation (472-EpiNotch). The funder had no role in the writing of this commentary.

References
[1] Cancer Genome Atlas, N. Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 2015;517:576–82.
[2] Jones KR, Lodge-Rigal RD, Reddick RL, Tudor GE, Shockley WW. Prognostic factors in the recurrence of stage I and II squamous cell cancer of the oral cavity. Arch Otolaryngol Head Neck Surg 1992;118:483–5.
[3] Liu X, Liu P, Chemock R, Kuo K, Lewis J, Luo J, Gay H, W T, Wang X. A prognostic gene expression signature for oropharyngeal squamous cell carcinoma. EBioMedicine 2020. doi:10.1016/j.ebiom.2020.102805.
[4] Fan C, Issaeva N, Yarbrough WC. HPV-driven oropharyngeal cancer: current knowledge of molecular biology and mechanisms of carcinogenesis. Cancers Head Neck 2018;3:12.
[5] de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer 2017;141:664–70.
[6] Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tan PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24–35.
[7] O’Sullivan B, Huang SH, Siu LL, Waldron J, Zhao H, Perez-Ordonez B, Weinreb L, Kim J, Ringash J, Bayley A, Dawson LA, Hope A, Cho J, Irish J, Gilbert R, Guillein P, Hui A, Liu FF, Chen E, Xu W. Deintensification candidate subgroups in human papillomavirus-related oropharyngeal cancer according to minimal risk of distant metastasis. J Clin Oncol 2013;31:543–50.
[8] Trosman SJ, Koyfman SA, Ward MC, Al-Khudari S, Nwizu T, Grekovitch JF, Lamarre ED, Scharp J, Khan MJ, Lorenz RR, Adelstein DJ, Burke BB. Effect of human papillomavirus on patterns of distant metastatic failure in oropharyngeal squamous cell carcinoma treated with chemoradiotherapy. JAMA Otolaryngol Head Neck Surg 2015;141:657–62.
[9] Moldogazieva NT, Mokhosoev IM, Terentiev AA. Metabolic heterogeneity of cancer cells: an interplay between HIF-1, GLUTs, and AMPK. Cancers (Basel) 2020;12.
[10] Bonomi M, Patsias A, Posner M, Sikora A. The role of inflammation in head and neck cancer. Adv Exp Med Biol 2014;816:167–27.