

Chapter 1

Promising Biomarkers for Early Diagnosis and Prognosis Prediction



Philip Sloan and Max Robinson

Introduction

There have been several recent reviews of biomarkers in relation to head and neck cancer [1–4] and although many markers show a degree of utility, none have so far translated into routine practice, apart from p16 testing for oro-pharyngeal squamous cell carcinoma [5] and PDL-1 prior to the administration of nivolumab or pembrolizumab in recurrent/metastatic squamous cell carcinoma [6]. Modern cellular pathology laboratories do routinely use a wide range of diagnostic biomarkers for immunohistochemical and molecular testing of biopsy material, however. Quality assurance is important, both for the laboratory processes and the interpretative diagnostic skills of the pathologist when using biomarkers [7]. For many types of cancer, accredited testing is routinely performed to guide therapy, but in head and neck cancer, such testing is only slowly finding applications. To achieve accreditation for such companion biomarkers, not only must the clinical utility be demonstrated by robust evidence but a health economic case also needs to be established. In essence the introduction of companion biomarkers into a pathology service depends on the drug therapies and practices being used by head and neck oncologists. Only if the biomarker can be used to select those who will benefit from a therapy or exclude those who will not benefit, can it find routine application. Increasingly, it is likely that tumour agnostic therapies based on molecular pathology will be used in clinical practice. An interesting example is the use of neurotrophic tropomyosin receptor kinase (NTRK) inhibitors which are licenced for use in a variety of tumours that carry the molecular signature. In head and neck cancer these include paediatric tumours and secretory carcinoma of the salivary glands [8]. The challenge for pathologists and oncologists is to identify which tumours to test and determine the

P. Sloan (✉) · M. Robinson

Department of Cellular Pathology, Newcastle upon Tyne Hospitals NHS Trust and Newcastle University, Newcastle upon Tyne, UK

e-mail: philip.sloan@newcastle.ac.uk; max.robinson@newcastle.ac.uk

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best testing method. Although genomic panel testing is attractive in that a wide spectrum of molecular signatures not identified by histology can be detected, turnaround time and cost are currently barriers, although these are likely to improve with time [9]. Immunohistochemistry is currently the most favoured option for NTRK detection because there are antibodies with high sensitivity and specificity ranging from 95–100% and 92–100%, respectively [10, 11]. Tumours with NTRK1/2 fusions demonstrate cytoplasmic expression and rarely perinuclear and nuclear membrane staining whereas those with NTRK3 fusions demonstrate cytoplasmic or nuclear expression [10–12]. Immunohistochemistry is relatively inexpensive and can offer a rapid turnaround time. However, as new drugs linked to molecular targets are developed, strategies for screening tumours to identify those that rarely contain a signature abnormality will have to be developed, so that patients can benefit [13].

Biomarkers that are used as companions to therapeutic drugs are often first identified as purely prognostic markers, and with accrual of further knowledge and drug development, they may become molecular targets or used to identify tumours that are likely to respond to a particular therapy. In this short review, a number of selected biomarkers that show promise for head and neck cancer therapies will be described.

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs, matrixins) are members of the metzincin protease superfamily of zinc-endopeptidases. It is five decades since the first MMP (MMP-1, collagenase) was identified from amphibian tissue. Currently a family with 28 members are classified as MMPs in vertebrates. The classification of human MMPs is based on their substrate specificities and the common structural domain architecture. Diverse biological functions are known within the subfamilies: collagenases, gelatinases, stromelysins, matrilysins, MMP membrane-type (MT)-MMPs and other MMPs are known. Numerous studies have found that MMP genes are frequently upregulated in cancer (reviewed in [14]). MMPs are thought to play significant roles in cancer progression, functional promotion of angiogenesis, invasion, metastasis and avoidance of immune surveillance. Indeed, much of the focus of research into MMPs in cancer has been related to tumour stroma and in particular angiogenesis, with a view to developing inhibitory drugs for MMPs and their natural inhibitors [15]. Drugs developed that targeted the MMP system and its inhibitors decades ago did not translate into practice. However, more specific targeting of small engineered molecules that can deliver payloads makes MMPs attractive targets [16, 17]. Recently, there has been interest in the membrane type MMPs and a systematic review of MT1-MMP (MMP-14) has demonstrated its potential as a

prognostic biomarker for cancer [16]. Over-expression of MMP-14 was significantly correlated with a poor overall survival in multiple cancers (HR: 2.22; 95% CI: 1.72–2.87). Also, high levels of MMP-14 were strongly associated with tumour progression and metastasis (HR: 1.83; 95% CI: 1.36–2.46) [16]. In ongoing studies, we are seeking to use MMP-14 as a target to guide entry of drugs to the cancer cells using novel circularised peptide molecules [17]. Various tumour types are known to have high MMP-14 expression including breast, ovarian, lung, and bladder cancer (reviewed in [16]) and MMP-14 has low expression in normal tissues. An immunohistochemical assay was developed on the Ventana platform using a Millipore MT1-MMP (MMP-14) primary antibody (MAB3328) at 1:6000 using Optiview chemistry as the detection system. When this was applied to tissue microarrays covering multiple cancer indications we found frequent overexpression in the malignant cells (membranous and cytoplasmic staining) and also in the stromal compartments. Expression levels were estimated by consensus review by two pathologists using an H-score which is the product of staining intensity (0–3) and percent positivity (0–100). H-scores (0–300) were derived separately for tumour membrane, cytoplasm and stroma in each case. Data modelling was used to identify a threshold for the identification of significant expression and the data could be used to define groups suitable for recruitment into a clinical trial. Interestingly, MMP14 was consistently overexpressed in squamous cell carcinoma, enabling head and cancer patients to enter into a phase1 trial (manuscript in preparation). Quality assurance is an important part of using an immunohistochemical test for entry into a clinical trial [7]. Cell lines showing a range of MMP-14 expression (0–3) were developed and used alongside tumour tissue as positive controls on each slide to ensure consistency of staining and stability (Figs. 1.1 and 1.2).

Fig. 1.1 Immunohistochemistry for MMP14 showing membranous staining intensity grade 3 in a cell line

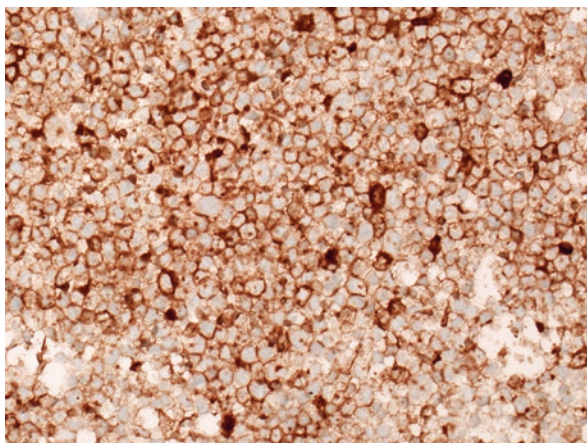
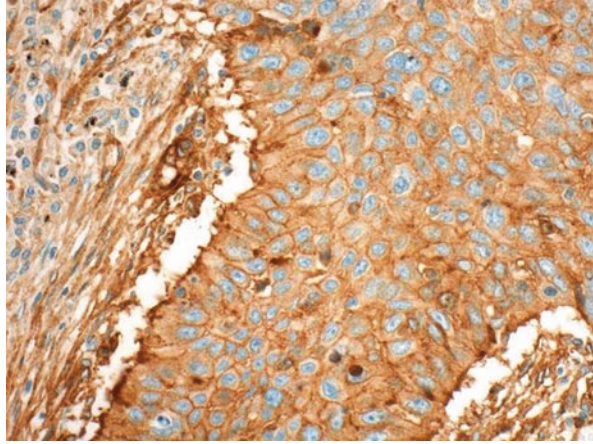


Fig. 1.2 Immunohistochemistry for MMP14 in a squamous carcinoma showing membranous and stromal overexpression



Autophagy Biomarkers

Autophagy is a self-degradative process that plays a role in removing misfolded or aggregated proteins and clearing damaged organelles. In cancer, autophagy is generally thought of as a promoting mechanism improving the survival of cancer cells by recycling nutrients, although its deregulation has been linked to non-apoptotic cell death. Strategies for both inhibiting and promoting apoptosis were therefore developed as potential cancer therapies. In this chapter, an interesting translational ongoing study in early stage cutaneous melanoma, including those arising in the head and neck, will be described (Fig. 1.3). The combination of an autophagy marker, Epidermal Autophagy and Beclin 1 Regulator 1 (AMBRA 1) and a cornified envelope differentiation marker, loricrin was used, where the biomarker expression was studied not in the tumour but in the overlying epidermis [18]. Most cases of cutaneous melanoma, including those in the head and neck, are diagnosed at an early stage. Detection and surgical excision results in high cure rates. Nevertheless, a small subset of patients with AJCC stage I disease progress and die from their disease. Initially three cohorts comprising a total of 455 AJCC stage I melanomas from the north east of England were studied. Immunohistochemistry for AMBRA1 and loricrin expression was validated and used to assess loss or downregulation of the markers in the epidermis overlying the melanoma, using adjacent non-tumour epidermis as an inbuilt control. The data indicate that the use of both markers in combination can stratify stage I patients at high and low risk of progression. In multivariate analysis of combined validation cohorts, the high-risk AMBRA1/loricrin (AMLo) expression pattern carried a HR of 3.89 (95% CI 1.8–8.41, $P < 0.001$) of melanoma recurrence [18]. The aim of our ongoing study is to validate AMLo in

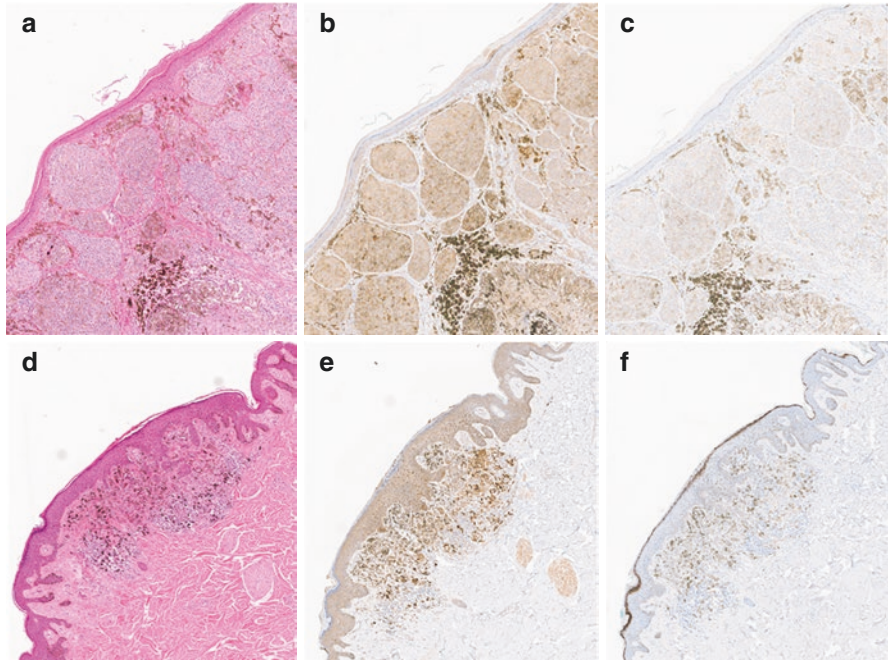


Fig. 1.3 Expression of AMBRA1 and Loricrin in cutaneous malignant melanoma. Top row: high risk melanoma, (a) haematoxylin and eosin, (b) loss of expression of AMBRA1 in the epidermis, (c) interrupted Loricrin staining in the upper epidermis. Bottom row: low risk melanoma, (d) Haematoxylin and eosin, (e) maintenance of AMBRA1 expression in the overlying epidermis, (f) intact band of Loricrin staining over the melanoma

several large international cohorts using digital pathology and consensus scoring in order to develop an accredited test. Preliminary data suggest that such a test may be able to replace sentinel node biopsy in early stage melanoma, avoiding an invasive and expensive investigation.

The study is of particular interest because the biomarkers used reflect changes not in the melanoma cells but in the tumour microenvironment. It may be that epidermal keratinocytes play an important role in melanoma switching between radial and vertical growth-phase and developing invasive growth as suggested by in-vitro studies [19]. The epidermis and stroma may not be simple bystanders and play crucial roles in early melanoma progression.

It is also possible that autophagy biomarkers could be used for head and neck squamous cell carcinoma and this is currently under investigation.

Intra-tumoural Immune Cells as Biomarkers

As in many human cancers, the presence of tumour infiltrating lymphocytes (TILs) is a prognostic biomarker in head and neck cancer [20, 21]. Currently, TILs are not quantified routinely in pathology services and do not form part of datasets for reporting head and neck cancer [21]. Increasingly, pathologists describe their presence or absence along with recognised histological prognostic biomarker features including peri-neural spread, lympho-vascular space invasion and pattern of invasive front in their reports. The presence of high levels of TILs is at least as powerfully prognostic as HPV status in oro-pharyngeal cancer [22]. The International Immuno-Oncology Biomarkers Working Group has published guidelines for pathologists to enable some standardisation of methods and facilitate consistency for TILs evaluation in cancer, including head and neck squamous carcinoma [23]. Two principal methods of quantification can be used. Classically, stromal TILs can be assessed over the whole tumour, recording the average percentage of TILs per stromal area at 200× magnification. An alternative approach is to assess maximum lymphocytic infiltration ('TIL hotspots') in a single field at 200× magnification where TILs are most dense. The values between average and hotspot counts can be dramatically different and there is a further limitation imposed by the biopsy size. Intra-tumoural heterogeneity is well described and small core biopsies may not be representative of the whole tumour volume. Further research is needed to clarify which is the most prognostic method, or if combined with immunotherapy, which is the most predictive method. Digital platforms are increasingly being used and when validated algorithms become available it may be possible to use artificial intelligence (AI) systems to provide both types of TIL count to the oncologist.

The success of CAR-T cell therapy for haematological malignancy has accelerated interest in using adoptive T cell therapies for solid tumours, though responses are more limited [24]. The wide use of immunotherapies has also driven interest in adoptive T cell therapy strategies [25]. Over decades, TIL therapy has demonstrated consistent success in treating metastatic malignant melanoma. Response rates greater than 50% and complete lasting response rates of over 20% were reported almost a decade ago [26]. Such findings have promoted interest in the development of similar adoptive T cell strategies in other cancers including head and neck squamous cell carcinoma [25].

Non-acral melanoma has a high mutational burden presumably due to years of exposure to ultra violet light. Mutations give rise to neo-antigens on the neoplastic cells that serve as potent stimulators of T cell-mediated anti-tumour responses within the host immune system. Squamous cell carcinoma of the head and neck also arises after years of exposure to mutagens in the form of tobacco and alcohol, and has been found to have a relatively high mutational burden [27, 28]. Both lung cancer and head and neck cancer show responses to PD-1 blockade adding further support to the concept that mutational burden is an important component of immunogenicity.

Table 1.1 Clinical trials using adoptive T-cell therapies for head and neck cancer

Therapy	Disease	Phase	Estimated dates ^a	Status ^a	Trial number
TIL	HPV associated cancer	II	2012–2016	Completed	NCT01585428
TIL	NPC	II	2015–2020	Unknown	NCT02421640
TIL	HNSCC	II	2017–2022	Active, recruiting	NCT03083873
TIL	HNSCC	II	2019–2024	Active, recruiting	NCT03645928
TIL	HNSCC	I	2020–2023	In set up	NCT03991741
Adoptive	HNSCC, solid tumours	I	2017–2025	Active, recruiting	EudraCT2017–002323-25
TCR	NPC (EBV+)	I	2001–2012	Completed	NCT00609219
TCR	EBV LMPs	I	2007–2011	Active	ACTRN12607000191493
TCR	HPV associated cancer	I/II	2014–2016	Completed	NCT02280811
TCR	NPC (EBV+)	III	2014–2023	Active, not recruiting	NCT02578641
TCR	HNSCC, all solid tumours	I	2015–2018	Active, not recruiting	NCT02366546
TCR	HPV associated cancer	I	2015–2033	Active, recruiting	NCT02379520
TCR	NPC (EBV+)	I	2015–2033	Active, not recruiting	NCT02065362
TCR	HNSCC	I	2016–2034	Active, not recruiting	NCT02989064
TCR	HPV associated cancer	I/II	2017–2026	Active, recruiting	NCT02858310
TCR	HPV associated cancer	I	2018–2021	Active, recruiting	NCT03578406
TCR	HNSCC	I	2018–2023	Active, recruiting	NCT03247309
CAR-T	HN cancer	I/II	2015–2020	Active, recruiting	NCT01818323

Data from [ClinicalTrials.gov](#), [anxtr.org.au](#) and [clinicaltrialsregister.eu](#)

^aAccessed June 11th 2020

TIL Autologous tumour infiltrating lymphocytes, *TCR* T cell receptor engineered T cells, *CAR-T* Chimeric antigen receptor T cells, *HNSCC* Head and neck squamous cell carcinoma, *NPC* Nasopharyngeal carcinoma

Currently, clinical trials are underway in head and neck cancer that employ conventional TIL therapy, T cell receptor engineered T cells and chimeric antigen receptor T cell therapy, many with promising responses (Table 1.1). One Phase III clinical trial is being conducted to assess if combined gemcitabine-carboplatin (GC) followed by adoptive T-cell therapy would improve clinical outcome for patients with advanced nasopharyngeal carcinoma. It follows a successful Phase II trial involving 38 patients at the National Cancer Centre, Singapore [29]. Thirty-eight

patients were enrolled, and 35 received GC and EBV-Cytotoxic T lymphocytes. A response rate of 71.4% with 3 complete responses and 22 partial responses was achieved. The 2-year and 3-year overall survival rates were 62.9% and 37.1%, respectively (median follow up of 29.9 months).

Much remains to be done in the field of T cell engineering for infusion therapies [30]. In the era of predictive, preventative, personalized, participatory (P4) medicine, advances in technology make identification of an individual profile of biomarkers a realistic possibility and in time precision profiles may supplant the use of single molecule predictive biomarkers [31]. During the early development of many cancers it is known that a series of mutations occur, that may be described as founder mutations [32, 33]. With time, a complex pattern of mutations occurs resulting in separate clones with differing mutation patterns. It is often one of these clones that leads to relapse or recurrence after oncological therapy. Key to the future of adoptive T cell therapy is the identification of founder mutations, present in all clones of the neoplasm, and the targeting of the engineered T cells to these neo-antigens. Head and neck cancer is a good candidate for adoptive T cell therapy and the presence of virus in both oropharyngeal (HPV) and nasopharyngeal carcinoma (EBV) offers additional non-host proteins that may be exploited for cell therapies.

References

1. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. *Nat Rev Cancer*. 2018;18:269–82. <https://doi.org/10.1038/nrc.2018.11>.
2. Budach V, Tinhofer I. Novel prognostic clinical factors and biomarkers for outcome prediction in head and neck cancer: a systematic review. *Lancet Oncol*. 2019;20:e313–26. [https://doi.org/10.1016/S1470-2045\(19\)30177-9](https://doi.org/10.1016/S1470-2045(19)30177-9).
3. Tonella L, Giannoccaro M, Alfieri S, Canevari S, De Cecco L. Gene expression signatures for head and neck cancer patient stratification: are results ready for clinical application? *Curr Treat Options in Oncol*. 2017;18:32. <https://doi.org/10.1007/s11864-017-0472-2>.
4. Galot R, Machiels JH. Current applications and challenges of circulating tumor DNA (ctDNA) in squamous cell carcinoma of the head and neck (SCCHN). *Cancer Treat Rev*. 2020;85:101992. <https://doi.org/10.1016/j.ctrv.2020.101992>.
5. el-Naggar AK, Chan JKC, Grandis JR, Takata T, Slootweg PJ, editors. WHO classification of tumours of the head and neck. 4th ed. Lyon: IARC Press; 2017.
6. Sloan P, Robinson CM. Cellular and molecular pathology in head and neck cancer. In: Critical issues in head and neck oncology: key concepts from the Sixth THNO meeting, Vermorken JB, Budach V, Leemans CR, Machiels J-P, Nicolai P, O’Sullivan B, editors. New York: Springer; 2018. ISBN: 3319988549, 9783319988542.
7. Sloan P, Robinson M. Quality assessment across disciplines in head and neck cancer treatment. Diagnostic pathology in HNSCC. *Front Oncol*. 2020;10:364. <https://doi.org/10.3389/fonc.2020.00364>.
8. Baranov E, Hornick JL. Soft tissue special issue: fibroblastic and myofibroblastic neoplasms of the head and neck. *Head Neck Pathol*. 2020;14:43–58. <https://doi.org/10.1007/s12105-019-01104-3>.
9. Solomon P, Hechtman JF. Detection of NTRK fusions: merits and limitations of current diagnostic platforms. *Cancer Res*. 2019;79:3163–8. <https://doi.org/10.1158/0008-5472.CAN-19-0372>.

10. Hechtman JF, Benayed R, Hyman DM, et al. Pan-Trk Immunohistochemistry Is an efficient and reliable screen for the detection of NTRK fusions. *Am J Surg Pathol.* 2017;41:1547–51. <https://doi.org/10.1097/PAS.0000000000000911>.
11. Rudzinski ER, Lockwood CM, Stohr BA, et al. Pan-Trk immunohistochemistry identifies NTRK rearrangements in pediatric mesenchymal tumors. *Am J Surg Pathol.* 2018;42:927–35. <https://doi.org/10.1097/PAS.0000000000001062>.
12. Hung YP, Fletcher CDM, Hornick JL. Evaluation of pan-TRK immunohistochemistry in infantile fibrosarcoma, lipofibromatosis-like neural tumour and histological mimics. *Histopathology.* 2018;73(4):634–44. <https://doi.org/10.1111/his.13666>.
13. Kurzrock R, Bowles DW, Kang H, et al. Targeted therapy for advanced salivary gland carcinoma based on molecular profiling: results from MyPathway, a phase IIa multiple basket study. *Ann Oncol.* 2020;31:412–21. <https://doi.org/10.1016/j.annonc.2019.11.018>.
14. Gobin E, Bagwell K, Wagner J, et al. A pan-cancer perspective of matrix metalloproteinases (MMP) gene expression profile and their diagnostic/prognostic potential. *BMC Cancer.* 2019;19:581.
15. Quintero-Fabián S, Arreola R, Becerril-Villanueva E, et al. Role of matrix metalloproteinases in angiogenesis and cancer. *Front Oncol.* 2019. <https://doi.org/10.3389/fonc.2019.01370>.
16. Zhang L, Jin S, Wei Y, et al. Prognostic significance of matrix metalloproteinase 14 in patients with cancer: a systematic review and meta-analysis. *Clin Lab.* 2020;66. <https://doi.org/10.7754/Clin.Lab.2019.190831>.
17. Gelb T, Bacon C, Sloan P, et al. MT1-MMP Immunohistochemistry (IHC) analysis of tumor microarrays (TMAs) using a novel scoring system guides patient selection for BT1718 expansion cohorts. AACR-NCI-EORTC international conference on molecular targets and cancer therapeutics, October 26–30, 2019 Abst. 561.
18. Ellis R, Tang D, Nasr B, et al. Epidermal autophagy and beclin 1 regulator 1 and loricrin: a paradigm shift in the prognostication and stratification of the American Joint Committee on Cancer stage I melanomas. *Br J Dermatol.* 2020;182:156–65.
19. Kharbili ME, Cario M, Béchetoille N, et al. Tspan8 drives melanoma dermal invasion by promoting ProMMP-9 activation and basement membrane proteolysis in a keratinocyte-dependent manner. *Cancers (Basel).* 2020;12:E1297. <https://doi.org/10.3390/cancers12051297>.
20. Nguyen N, Bellile E, Thomas D, et al. For the, head and neck SPORE program investigators. Tumor infiltrating lymphocytes and survival in patients with head and neck squamous cell carcinoma. *Head Neck.* 2016;38:1074–84. <https://doi.org/10.1002/hed.24406>.
21. Bullock M, Beitler JJ, Carlson DL, et al. Nodal excisions and neck dissection specimens for head & neck tumours, histopathology reporting guide, 1st edn. Sydney: International Collaboration on Cancer Reporting; 2018. ISBN: 978-1-925687-24-8.
22. de Ruiter EJ, Ooft ML, Devriese LA, Willems SM. The prognostic role of tumor infiltrating T-lymphocytes in squamous cell carcinoma of the head and neck: a systematic review and meta-analysis. *Oncol Targets Ther.* 2017;6(11):e1356148. <https://doi.org/10.1080/02162402X.2017.1356148>.
23. Hendry S, Salgado R, Gevaert T, et al. Assessing tumor-infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the international immuno-oncology biomarkers working group: Part 2: TILs in melanoma, gastrointestinal tract carcinomas, non-small cell lung carcinoma and mesothelioma, endometrial and ovarian carcinomas, squamous cell carcinoma of the head and neck, genitourinary carcinomas, and primary brain tumors. *Adv Anat Pathol.* 2017;24:311–35. <https://doi.org/10.1097/PAP.0000000000000161>.
24. Newick K, O'Brien S, Moon E, Albelda SM. CAR T cell therapy for solid tumors. *Annu Rev Med.* 2017;68:139–52. <https://doi.org/10.1146/annurev-med-062315-120245>.
25. Qureshi HA, Lee SM. Immunotherapy approaches beyond PD-1 inhibition: the future of cellular therapy for head and neck squamous cell carcinoma. *Curr Treat Options in Oncol.* 2019;20:31. <https://doi.org/10.1007/s11864-019-0630-9>.

26. Rosenberg SA, Yang JC, Sherry RM, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. *Clin Cancer Res.* 2011;17:4550–7.
27. Meucci S, Keilholz U, Tinhofer I, Ebner OA. Mutational load and mutational patterns in relation to age in head and neck cancer. *Oncotarget.* 2016;7:69188–99. <https://doi.org/10.18632/oncotarget.11312>.
28. Stransky N, Egloff AM, Tward AD, et al. The mutational landscape of head and neck squamous cell carcinoma. *Science.* 2011;333(6046):1157–60.
29. Chia WK, Teo M, Wang WW, et al. Adoptive T-cell transfer and chemotherapy in the first-line treatment of metastatic and/or locally recurrent nasopharyngeal carcinoma. *Mol Therapy.* 2014;22:132–9. <https://doi.org/10.1038/mt.2013.242>.
30. Ren L, Matsuda T, Deng B, et al. Similarity and difference in tumor-infiltrating lymphocytes in original tumor tissues and those of *in vitro* expanded populations in head and neck cancer. *Oncotarget.* 2017;9:3805–14.
31. Tian Q, Price ND, Hood L. Systems cancer medicine: towards realization of predictive, preventive, personalized and participatory (P4) medicine. *J Intern Med.* 2012;271(2):111–21. <https://doi.org/10.1111/j.1365-2796.2011.02498.x>.
32. Turajlic S, Sottoriva A, Graham T, Swanton C. Resolving genetic heterogeneity in cancer. *Nat Rev Genet.* 2019;20:404–16. <https://doi.org/10.1038/s41576-019-0114-6>.
33. McGranahan N, Swanton C. Neoantigen quality, not quantity. *Sci Transl Med.* 2019;11:eaax7918.

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