

# HLA Class I Loss and Resistance to Immunotherapy in Pulmonary Metastasis of Hypopharyngeal Cancer

AKIRA MURAKAMI<sup>1</sup>, KAORI YUKINO<sup>1</sup>, YOSHIHIRO KOMOHARA<sup>2</sup>, YU SHIMODA<sup>1</sup>, HARUKI SAITO<sup>1</sup>, YUKIO FUJIWARA<sup>2</sup> and YORIHISA ORITA<sup>1</sup>

<sup>1</sup>Departments of Otolaryngology-Head and Neck Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan;

<sup>2</sup>Department of Cell Pathology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan


## Abstract

**Background/Aim:** Although immune checkpoint inhibitors (ICIs) can be remarkably effective in the treatment of unresectable recurrent or metastatic carcinoma of the head and neck, even in cases of a marked response, some lesions may remain partially refractory or new lesions may emerge. However, why ICIs sometimes produce such a patchy pattern of therapeutic effects remains unclear.

**Case Report:** A 62-year-old patient with advanced hypopharyngeal squamous cell carcinoma who developed pulmonary metastasis after surgery followed by postoperative chemoradiotherapy presented to our department. After initiating ICI therapy, the patient initially achieved complete remission; however, a new pulmonary lesion subsequently appeared and was surgically removed. The patient has since remained in durable remission with continued long-term ICI therapy. Immunohistochemical analysis comparing the new pulmonary lesion with the original hypopharyngeal tumor revealed that cancer cells in the primary lesion were positive for HLA class I and b2-microglobulin, whereas staining for these antigens was negative in cancer cells of the recurrent pulmonary lesion. Cancer cells in the primary lesion exhibited ectopic expression of HLA class II, but no expression was detected in cancer cells of recurrent lesions.

**Conclusion:** In the pulmonary lesion that did not respond to ICIs, a loss of HLA class I and b2-microglobulin expression was observed. These findings suggest that the reduced antigen-presenting capacity of cancer cells may contribute to immune escape.

**Keywords:** Immune checkpoint inhibitor, head and neck squamous cell carcinoma (HNSCC), pulmonary metastasis,  $\beta$ 2-microglobulin, immune escape.

 Yoshihiro Komohara, Department of Cell Pathology, Graduate School of Medicine, Kumamoto University, 1-1-1 Honjo, Kumamoto, 860-8556, Japan. Tel: +81 963735095, Fax: +81 963735096, e-mail: ycomo@kumamoto-u.ac.jp

Received January 7, 2026 | Revised February 8, 2026 | Accepted February 11, 2026



This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.  
©2026 The Author(s). Anticancer Research is published by the International Institute of Anticancer Research.

## Introduction

In recent years, immune checkpoint inhibitors (ICIs) have become more commonly used to treat unresectable recurrent or metastatic carcinoma of the head and neck. Although ICIs have contributed to improvements in the overall survival (OS) of patients with recurrent and/or metastatic head and neck carcinoma (RM-HNC) (1, 2), only approximately one-third of patients with RM-HNC respond to multimodal treatment using ICIs, and the response rate to ICIs alone is even lower (3). A major reason why ICIs contribute to improved OS in RM-HNC is the existence of so-called "super-responders"—patients who show an excellent response to ICIs and even achieve complete remission (CR). In such cases, surgeries that would have been unthinkable before the advent of ICIs are now being considered, such as resecting only the residual lesions that do not respond to ICIs or removing newly developed lesions that appear during ICI treatment (4). However, the reasons why some lesions respond to ICI therapy while others do not, even in super-responder cases, are still poorly understood. In the present case, we had the opportunity to observe the biological events in pulmonary metastasis that occurred following an initial CR to ICI therapy.

## Case Report

A 62-year-old Japanese man with no significant medical history initially presented with an enlarging mass in the right side of the neck. Right piriform sinus carcinoma was detected on magnifying electroendoscopy, and pathological evaluation of biopsy specimens was performed. A final diagnosis of hypopharyngeal non-keratinizing squamous cell carcinoma (right piriform sinus, cT4aN2bM0, stage IVA) was made. Although he was scheduled for surgery, he did not return, and we subsequently lost touch with him. Approximately 9 months later, he was brought to our emergency department as a result of severe dyspnea. Tracheotomy was immediately performed. The hypopharyngeal carcinoma had notably progressed (cT4aN2cM0, stage IVA, Figure 1A and B). After neoadjuvant

chemotherapy (docetaxel, cisplatin, 5-fluorouracil), total laryngopharyngectomy with interposition of the jejunum was performed. Extranodal invasion was noted based on the results of postoperative pathology; therefore, postoperative chemoradiotherapy using platinum-based regimens was performed. Six months later, follow-up computed tomography (CT) revealed left lung metastases (Figure 1C). The patient elected to start biweekly administration of nivolumab (200 mg/dose) for the platinum-refractory disease. Nivolumab showed remarkable efficacy, and CT at three months after starting nivolumab (total of seven doses) revealed that the lung metastases had nearly disappeared. The patient remained stable for an extended period, but approximately three years after starting nivolumab (total of 62 doses), a new lung metastasis appeared (Figure 1D). The lesion was surgically removed (partial pulmonary lobectomy), and the pathological diagnosis was non-keratinizing squamous cell carcinoma. Once the surgical site had stabilized, nivolumab was restarted. Since then, nivolumab therapy has been continued (total of 147 doses at the time of this report), and no recurrence or metastasis has been observed for over 3.5 years after lung surgery (total of 6.5 years after starting nivolumab). We have been discussing with the patient whether it is time to stop nivolumab, but a firm decision has yet to be made, thus the regimen continues.

*Histopathological examination.* An additional pathological examination of this patient was conducted to compare the primary hypopharyngeal and resected lung metastatic lesions. Additional immunohistochemical (IHC) analysis was performed to examine the resistant mechanism of the lung metastatic lesion compared with the primary lesion, as performed in previous studies (5, 6) (Figure 2). Immune cell infiltration and the expression of HLA antigens and PD-L1 were evaluated using anti-CD3 (Histofine, Tokyo, Japan), anti-CD8 (Histofine), anti-CD103 (Abcam, Cambridge, UK), anti-CD163 (Abcam), anti-HLA class I (A, B, and C antigens) (MBL, Tokyo, Japan), anti-HLA-DR (HLA class II antigen; Santa Cruz Biotechnology, Dallas, TX, USA), anti-beta-2 microglobulin (B2M; Santa Cruz Biotechnology),

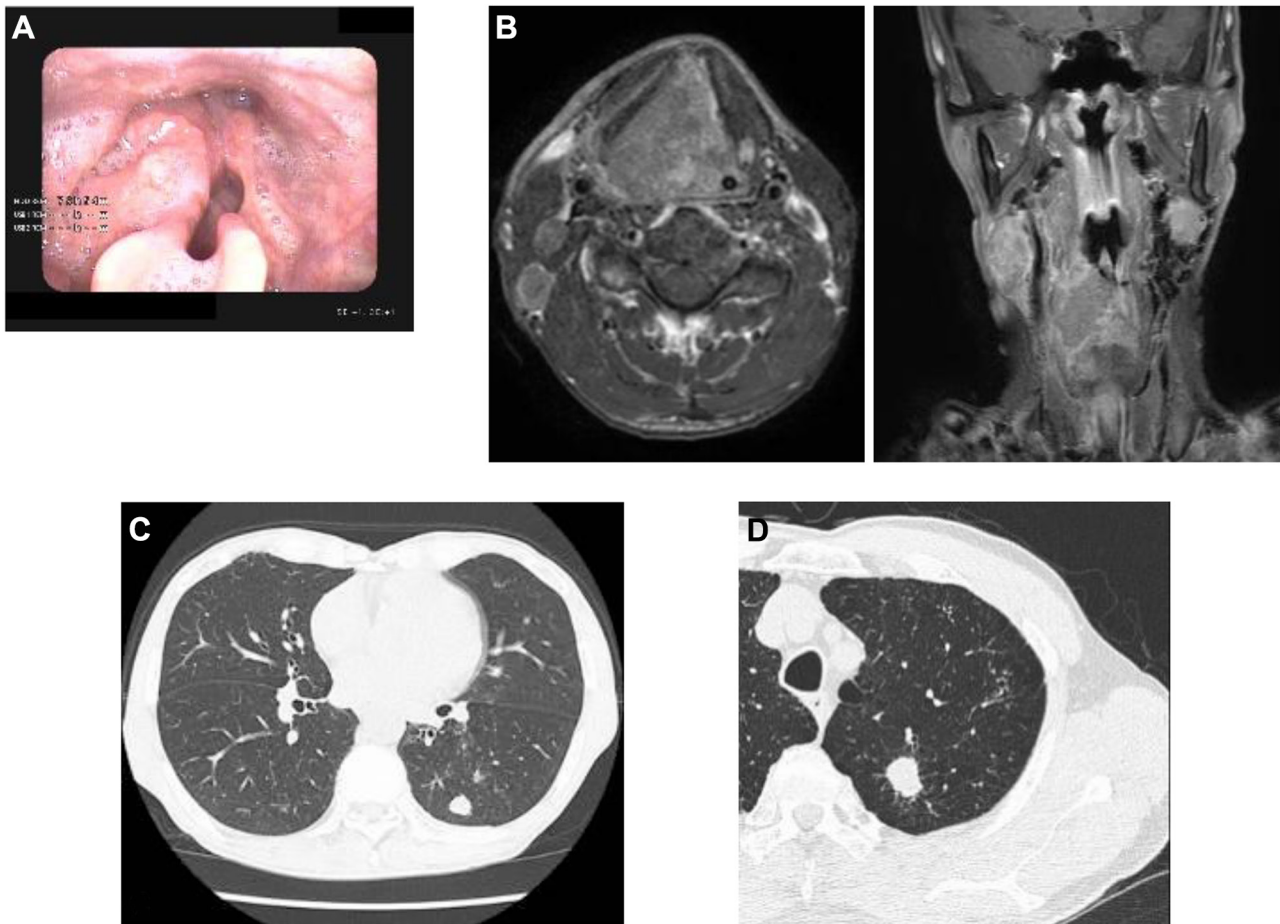


Figure 1. Clinical examination. (A) Laryngofiberscopic findings: Right vocal fold paralysis is noted, and airway stenosis is present because of compression by the hypopharyngeal carcinoma. (B) T1-weighted contrast-enhanced magnetic resonance imaging (B: axial) of hypopharyngeal carcinoma, likely originating from the right pyriform sinus, shows destruction of the thyroid cartilage. Multiple metastatic lymph nodes are noted in both sides of the neck. Lymph node metastases are seen in both sides of the neck. (C) Lung window computed tomography (CT) of a metastatic lesion in the left lung field. (D) Lung window CT of a new metastatic lesion appearing approximately three years after starting nivolumab therapy.

and anti-PD-L1 antibodies (Leica Biosystems, Nussloch, Germany) for the IHC examination. Infiltrating CD3- and CD103-positive lymphocytes were observed in the cancer nest and stroma; however, fewer infiltrating CD8- and CD163-positive cells were observed in the cancer nest and stroma of both the primary and recurrent lesions. Cancer cells positive for HLA class I and B2M expression were observed in the primary lesion; however, staining for these antigens was negative in the cancer cells of the recurrent lesions. Staining for HLA class I and B2M was positive in infiltrating immune cells in both the primary and recurrent

lesions. Cancer cells in the primary lesion exhibited ectopic expression of HLA-DR, but no HLA-DR expression was detected in cancer cells of recurrent lesions. Staining for PD-L1 was negative in both lesion types.

## Discussion

Several reports have indicated that lung metastases in various cancer types often show a favorable response to ICIs (7, 8). Because the lungs are continuously exposed to pollutants and pathogens, they harbor abundant antigen-

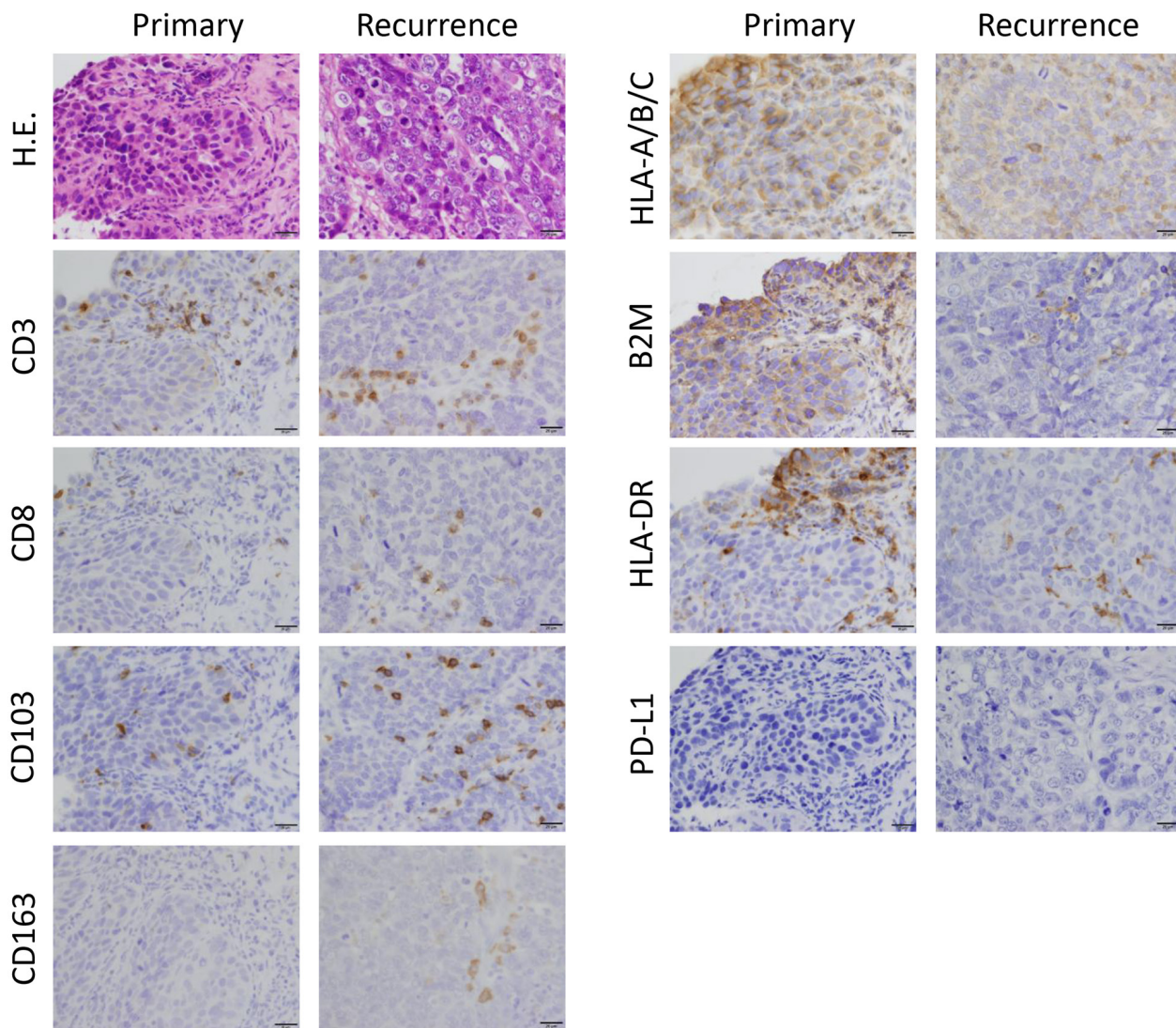


Figure 2. Pathological examination. Additional pathological analyses were performed using resected primary biopsy specimens and surgically resected pulmonary metastatic lesions. Hematoxylin and eosin staining and immunohistochemistry for CD3, CD8, CD103, CD163, PD-L1, HLA class I (A/B/C), B2M, and HLA-DR were conducted on serial sections. Representative images of the corresponding areas are shown. Scale bars=20  $\mu$ m.

presenting cells, including alveolar macrophages and dendritic cells (8). These specialized antigen-presenting cells can promote T-cell responses and may enhance the efficacy of ICI therapy. Nevertheless, a subset of patients ultimately develops resistance to ICIs after an initial response.

In the present case, we conducted a detailed pathological examination of a super-responder in whom lung lesions that had become resistant to ICI therapy

were surgically resected. IHC analyses of immune-related markers and cells were performed using pathological specimens from the primary hypopharyngeal tumor (before ICI therapy) and from the lung lesions (ICI-resistant, after ICI therapy). The density of lymphocyte infiltration in both the primary and lung metastatic lesions was not as high as that reported in some previous head and neck cancer cohorts (9). By contrast, the number

of CD163-positive tumor-associated macrophages (TAMs) was increased, and the expression of antigen presentation-related molecules such as HLA class I, HLA class II, and B2M was down-regulated in the lung lesions compared with the primary lesion.

CD163-positive TAMs are thought to exert pro-tumoral functions by secreting cytokines such as SPP1/osteopontin (9). Increased densities of CD163-positive TAMs have been associated with higher lactic acid levels in the tumor microenvironment and a worse clinical course (10, 11). Moreover, the loss of HLA antigens and B2M is known to correlate with a poor prognosis and resistance to ICI therapy in several cancers, including head and neck cancer (12–15). In the present case, the density of TAMs was not as high as that typically observed in primary lung cancers (9), suggesting that the loss of antigen-presenting capacity in cancer cells, rather than TAM infiltration alone, may have played a central role in immune escape in the ICI-resistant lung lesions.

In non-small-cell lung cancer, the development of resistance to ICIs has been linked to the downregulation of type I and type II interferon (IFN) pathways, along with reduced numbers of tumor-infiltrating CD8<sup>+</sup> T cells and reduced PD-L1 expression (16). Both type I and II IFNs are potent inducers of HLA gene expression, and this upregulation is critical for enabling tumor cells to be recognized and eliminated by CD8<sup>+</sup> T cells. Inactivating mutations in JAK1 and JAK2, key mediators of IFN signaling, have been identified in relapsing lesions of metastatic melanoma that initially show a CR to ICI therapy, but experience disease progression months to years afterward (17). These findings suggest that impaired IFN signaling may simultaneously reduce antigen presentation and undermine the cytotoxic T-cell-mediated antitumor response.

In the present case, we observed a comparable density of CD8<sup>+</sup> T cells in the lung metastases and primary lesion; however, irrespective of the absolute degree of infiltration, it is possible that the CD8<sup>+</sup> T cells in the lung metastases had become exhausted and functionally impaired after long-term ICI therapy (18). Within the tumor microenvironment, CD8<sup>+</sup> T cells comprise “progenitor

exhausted” cells, which retain greater polyfunctionality and persistence even in the absence of antigens, and “terminally exhausted” cells, which exhibit superior cytotoxicity but reduced long-term survival. The ultimate efficacy of checkpoint blockade therapy is thought to depend on the proportion of long-lived, terminally exhausted CD8<sup>+</sup> T cells within the overall exhausted T-cell pool (19). Our findings suggest that, in addition to CD8<sup>+</sup> T-cell exhaustion, the loss of HLA class I and B2M expression may collectively contribute to localized immune escape under ongoing ICI therapy.

## Conclusion

The findings of the present case suggest that, during ICI treatment, decreased antigen-presenting capacity contributes to a loss of ICI efficacy in a restricted area of the lung. However, why these changes occur only in a limited lung region remains unclear, and thus, further investigation is warranted.

## Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

## Authors' Contributions

Akira Murakami: Collection of clinical data, histological and immunohistochemical analyses, drafting of the manuscript; Kaori Yukino, Yukio Fujiwara: histological and immunohistochemical analyses support; Yu Shimoda: Responsible for diagnosis and treatment; Haruki Saito: Coordination of the treatment strategies; Yoshihiro Komohara: Writing – review and editing; Yori-hisa Orita: Supervise of the entire study.

## Acknowledgements

The Authors thank K.I. Stainer Inc. (Kumamoto, Japan) for their technical assistance.

## Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis, or presentation of this manuscript.

## References

- Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias Docampo LC, Haddad R, Rordorf T, Kiyota N, Tahara M, Monga M, Lynch M, Geese WJ, Kopit J, Shaw JW, Gillison ML: Nivolumab for recurrent squamous-cell carcinoma of the head and neck. *N Engl J Med* 375(19): 1856-1867, 2016. DOI: 10.1056/NEJMoa1602252
- Burtneß B, Harrington KJ, Greil R, Soulières D, Tahara M, de Castro G Jr, Psyrrri A, Basté N, Neupane P, Bratland Å, Fueeder T, Hughes BGM, Mesía R, Ngamphaiboon N, Rordorf T, Wan Ishak WZ, Hong RL, González Mendoza R, Roy A, Zhang Y, Gumuscu B, Cheng JD, Jin F, Rischin D, KEYNOTE-048 Investigators: Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. *Lancet* 394(10212): 1915-1928, 2019. DOI: 10.1016/S0140-6736(19)32591-7
- Magnes T, Wagner S, Kiem D, Weiss L, Rinnerthaler G, Greil R, Melchardt T: Prognostic and predictive factors in advanced head and neck squamous cell carcinoma. *Int J Mol Sci* 22(9): 4981, 2021. DOI: 10.3390/ijms22094981
- Tsushima N, Shinozaki T, Fujisawa T, Tomioka T, Okano W, Ikeda M, Tahara M, Higashino T, Hayashi R: Salvage reconstructive surgery during nivolumab therapy for a patient with hypopharyngeal cancer. *Clin Med Insights Case Rep* 13: 1179547620908854, 2020. DOI: 10.1177/1179547620908854
- Hosokawa A, Yamada R, Otsuki Y, Tamura H, Ichihara A, Fukushima T, Komohara Y: Remarkable and durable tumor response to pembrolizumab in locally advanced dMMR/MSI-H rectal cancer. *Anticancer Res* 45(11): 5069-5076, 2025. DOI: 10.21873/anticancer.17847
- Yukino K, Komohara Y, Zhao S, Yamada R, Fujiwara Y, Murakami A, Shimoda Y, Saito H, Orita Y: Anaplastic thyroid carcinoma with osteoclast-like giant cells: a case report and a study of a potential therapeutic approach. *Med Mol Morphol*, 2025. DOI: 10.1007/s00795-025-00443-1
- Lu LC, Hsu C, Shao YY, Chao Y, Yen CJ, Shih IL, Hung YP, Chang CJ, Shen YC, Guo JC, Liu TH, Hsu CH, Cheng AL: Differential organ-specific tumor response to immune checkpoint inhibitors in hepatocellular carcinoma. *Liver Cancer* 8(6): 480-490, 2019. DOI: 10.1159/000501275
- Khoja L, Kibiro M, Metser U, Gedye C, Hogg D, Butler MO, Atenafu EG, Joshua AM: Patterns of response to anti-PD-1 treatment: an exploratory comparison of four radiological response criteria and associations with overall survival in metastatic melanoma patients. *Br J Cancer* 115(10): 1186-1192, 2016. DOI: 10.1038/bjc.2016.308
- Matsubara E, Yano H, Pan C, Komohara Y, Fujiwara Y, Zhao S, Shinchi Y, Kurotaki D, Suzuki M: The significance of SPP1 in lung cancers and its impact as a marker for protumor tumor-associated macrophages. *Cancers (Basel)* 15(8): 2250, 2023. DOI: 10.3390/cancers15082250
- Almangush A, De Keukeleire S, Rottey S, Ferdinande L, Vermassen T, Leivo I, Mäkitie AA: Tumor-infiltrating lymphocytes in head and neck cancer: ready for prime time? *Cancers (Basel)* 14(6): 1558, 2022. DOI: 10.3390/cancers14061558
- Ohashi T, Aoki M, Tomita H, Akazawa T, Sato K, Kuze B, Mizuta K, Hara A, Nagaoka H, Inoue N, Ito Y: M2-like macrophage polarization in high lactic acid-producing head and neck cancer. *Cancer Sci* 108(6): 1128-1134, 2017. DOI: 10.1111/cas.13244
- Korytowski J, Sowiński H, Zakrzewski J: [Plasmocytoma of the nasal cavity and sphenoid sinus]. *Otolaryngol Pol* 25(3): 347-350, 1971.
- Ferris RL, Hunt JL, Ferrone S: Human leukocyte antigen (HLA) class I defects in head and neck cancer: molecular mechanisms and clinical significance. *Immunol Res* 33(2): 113-134, 2005. DOI: 10.1385/IR:33:2:113
- Paulson KG, Voillet V, McAfee MS, Hunter DS, Wagener FD, Perdicchio M, Valente WJ, Koelle SJ, Church CD, Vandeven N, Thomas H, Colunga AG, Iyer JG, Yee C, Kulikauskas R, Koelle DM, Pierce RH, Bielas JH, Greenberg PD, Bhatia S, Gottardo R, Nghiem P, Chapuis AG: Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA. *Nat Commun* 9(1): 3868, 2018. DOI: 10.1038/s41467-018-06300-3
- Jiang N, Yu Y, Wu D, Wang S, Fang Y, Miao H, Ma P, Huang H, Zhang M, Zhang Y, Tang Y, Li N: HLA and tumour immunology: immune escape, immunotherapy and immune-related adverse events. *J Cancer Res Clin Oncol* 149(2): 737-747, 2023. DOI: 10.1007/s00432-022-04493-1
- Hiltbrunner S, Cords L, Kasser S, Freiberger SN, Kreutzer S, Toussaint NC, Grob L, Opitz I, Messerli M, Zoche M, Soltermann A, Rechsteiner M, van den Broek M, Bodenmiller B, Curioni-Fontecedro A: Acquired resistance to anti-PD1 therapy in patients with NSCLC associates with immunosuppressive T cell phenotype. *Nat Commun* 14(1): 5154, 2023. DOI: 10.1038/s41467-023-40745-5
- Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, Torrejon DY, Abril-Rodriguez G, Sandoval S, Barthly L, Saco J, Homet Moreno B, Mezzadra R, Chmielowski B, Ruchalski K, Shintaku IP, Sanchez PJ, Puig-Saus C, Cherry G, Seja E, Kong X, Pang J, Berent-Maoz B,

- Comin-Anduix B, Graeber TG, Tumei PC, Schumacher TN, Lo RS, Ribas A: Mutations associated with acquired resistance to PD-1 blockade in melanoma. *N Engl J Med* 375(9): 819-829, 2016. DOI: 10.1056/NEJMoa1604958
- 18 Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, Jenkins RW, Lieb DJ, Chen JH, Frederick DT, Barzily-Rokni M, Freeman SS, Reuben A, Hoover PJ, Villani AC, Ivanova E, Portell A, Lizotte PH, Aref AR, Eliane JP, Hammond MR, Vitzthum H, Blackmon SM, Li B, Gopalakrishnan V, Reddy SM, Cooper ZA, Paweletz CP, Barbie DA, Stemmer-Rachamimov A, Flaherty KT, Wargo JA, Boland GM, Sullivan RJ, Getz G, Hacohen N: Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* 175(4): 998-1013.e20, 2018. DOI: 10.1016/j.cell.2018.10.038
- 19 Miller BC, Sen DR, Al Abosy R, Bi K, Virkud YV, LaFleur MW, Yates KB, Lako A, Felt K, Naik GS, Manos M, Gjini E, Kuchroo JR, Ishizuka JJ, Collier JL, Griffin GK, Maleri S, Comstock DE, Weiss SA, Brown FD, Panda A, Zimmer MD, Manguso RT, Hodi FS, Rodig SJ, Sharpe AH, Haining WN: Subsets of exhausted CD8(+) T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol* 20(3): 326-336, 2019. DOI: 10.1038/s41590-019-0312-6