A loss-of-function polymorphism in ATG16L1 compromises therapeutic outcome in head and neck carcinoma patients

Julie Le Naour abc, Zsofia Szutoniszki de, Vincent Carbonnier de, Odile Casiraghi g, Virginie Marty h, Lorenzo Galluzzi i, Zoltan Szallas d,f, Guido Kroemer ablm, and Erika Vacchelli ab* 

*Equipe labellisée par la Ligue contre le cancer, Université de Paris, Sorbonne Université, INSERM U1138, Centre de Recherche des Cordeliers, Institut Universitaire de France, Paris, France; 6Metabolomics and Cell Biology Platforms, Gustave Roussy Cancer Campus, Villejuif, France; 6Université Paris Sud, Paris Saclay, Faculty of Medicine Kremlin Bicêtre, France; 6Computational Health Informatics Program (CHIP), Boston Children’s Hospital, Boston, MA, USA; 6Harvard Medical School, Boston, MA, USA; 6Danish Cancer Society Research Center, Copenhagen, Denmark; 6Department of Head and Neck Surgical and Medical Oncology, Gustave Roussy Cancer Campus, Paris-Saclay University, Villejuif, France; 6Experimental and Translational Pathology Platform (PETRA), AMMICA Inserm US23/UMS CNRS5655, Gustave Roussy Cancer Campus, Villejuif, France; 6Department of Radiation Oncology, Weill Cornell Medical College, New York, NY, USA; 6Sandra and Edward Meyer Cancer Center, New York, NY, USA; 6Caryl and Israel Englander Institute for Precision Medicine, New York, NY, USA; 6Institut du Cancer Paris CARPEMAP-HP, Hôpital Européen Georges Pompidou, Pôle de Biologie, Paris, France; 6Department of Cancer Medicine, Gustave Roussy Cancer Campus, Villejuif, France

ABSTRACT

The anticancer immune response is shaped by immunogenic cell stress and death pathways. Thus, cancer cells can release danger-associated molecular patterns that act on pattern recognition receptors expressed by dendritic cells and their precursors to elicit an antitumor immune response. Here, we investigated the impact of single nucleotide polymorphisms (SNPs) in genes affecting this cancer-immunity dialogue in the context of head and neck squamous cell carcinoma (HNSCC). We observed that homoyzosity for a loss-of-function SNP (rs2241880, leading to the substitution of a threonine residue in position 300 by an alanine) affecting autophagy related 16 like 1 (ATG16L1) is coupled to poor progression-free survival in platinum-treated HNSCC patients. This result was obtained on a cohort of patients enrolled at the Gustave Roussy Cancer Campus and was validated on an independent cohort of The Cancer Genome Atlas (TCGA). Homozygosity in rs2241880 is well known to predispose to Crohn’s disease, and epidemiological associations between Crohn’s disease and HNSCC have been reported at the levels of cancer incidence and prognosis. We speculate that rs2241880 might be partially responsible for this association.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a frequent cancer derived from the mucosal epithelium of the upper respiratory tract (nasal cavity and paranasal sinuses, larynx) and upper digestive tract (oral cavity and pharynx). HNSCC is determined by well-known risk factors (age, alcohol abuse, tobacco, and human papillomavirus (HPV)) that benefit from early detection, yet is difficult to treat due to the frequent incidence of comorbidities. Surgical removal of the tumor (if operable) is usually followed by adjuvant chemotherapy and/or radiotherapy, and prognosis is determined by localization, size, histological grade, HPV status, presence of local and distant metastases, as well as comorbidities. There is abundant evidence that infiltration of HNSCC by cytotoxic T lymphocytes (CTL) impacts prognosis in a favorable fashion, while immunosuppressive regulatory T cell (Treg) indicates poor prognosis. Moreover, HNSCC often responds to immunotherapy targeting the programmed death protein 1 (PD1)/programmed death-ligand 1 (PD-L1) interaction.

In the past, we observed that a set of single-nucleotide polymorphisms (SNPs) affecting a set of genes involved in the immunogenic cell death (ICD) process dictate the response to adjuvant chemotherapy of breast and colorectal cancer patients. This process involves pattern recognition receptors (such as formyl peptide receptor 1 (FPR1); purinergic receptor P2X, ligand-gated ion channel 7 (P2RX7) and toll like receptor 4 (TLR4)) and can be mutated in substantial fraction of the world population (allelic frequency of rs867228 in FPR1: ~20%; rs3751143 in P2RX7: ~19%; rs4986790 in TLR4: ~6%). Moreover, ICD involves autophagy, which can be compromised by a loss-of-function polymorphism (rs2241880, allelic frequency ~50%) in autophagy related 16 like 1 (ATG16L1). This SNP, which predisposes to inflammatory bowel disease (IBD) if present in homozygosity, compromises autophagic flux and related vesicular trafficking processes.

Driven by this consideration, we wondered whether such SNPs might impact the therapeutic response of HNSCC patients as well. Here, we show that a loss-of-function...
polymorphism in the gene coding for ATG16L1 is associated with poor prognosis of HNSCC patients undergoing platinum-based adjuvant chemotherapy.

Materials and methods

Patients and study design

Two-hundred forty-two head and neck squamous cell carcinoma (HNSCC) patients were enrolled in the study. In accordance with national and European legislation, the institutional review board approved the study, and written informed consent was obtained from all the included patients as to the use of their tissue samples for research purposes. A retrospective chart review followed by prospective survival data was conducted on all patients diagnosed between 2004 and 2009 at the Gustave Roussy Cancer Center. Paraffin-embedded tumor blocks (obtained at the time of surgery) were collected. The amount and quality of 193 paraffin blocks were judged adequate for histological evaluation. DNA from tumor specimens was extracted, yielding sufficient material to analyze specimens from 187 patients (180 for rs867228) related to missing information in the clinical database.

Tobacco and alcohol consumption were a common feature of all patients enrolled in this study. Tumor localization and human papilloma virus (HPV) status (for naso-and oropharyngeal cancer) were investigated to consider all relevant clinicopathological variables. Distant metastases were observed in only two patients, and this covariate has been discarded in the analysis. This cohort included mostly male patients (83.4% men versus 16.6% women), and tumors were usually located in the pharynx (60.4%) and larynx (25.1%). Most (80.2%) of the patients were diagnosed with stage IV and 19.8% with stage III disease. All patients received platinum/5-fluorouracil-based induction chemotherapy, followed by platinum-based maintenance chemoradiotherapy. Almost half of the patients received taxane-based therapy (8.3% docetaxel and 42.5% paclitaxel).

The Cancer Genome Atlas (TCGA) clinical data was downloaded from Huang et al.109

Genotyping. Genomic DNA was isolated from paraffin-embedded tumors by means of the DNeasy blood and tissue kit (Qiagen, Valencia, CA) or Maxwell 16 FFPE Tissue LEV DNA Purification kit (Promega, Madison, WI, USA). Gene-specific primers and genotype-specific probes (Life Technologies, Carisbad, CA, USA) were used to amplify the rs2241880 single nucleotide polymorphism (SNP) affecting autophagy related 16 Like 1 (ATG16L1), rs867228 in the formyl peptide receptor 1 (FPR1) gene, rs3751143 in the purinergic receptor P2X, ligand-gated ion channel, 7 (P2RX7) gene and rs4986790 in the toll like receptor 4 (TLR4) gene. Genotypes were determined by comparing the signals from fluorescent probes (FAM and VIC) and by calculating the natural logarithm of the ratio between FAM and VIC signals (log (FAM/VIC)).

Statistical analyses. Progression-free survival (PFS) and overall survival (OS) determined from the date of diagnosis were used as primary end-points. Cox proportional hazards regression modeling was employed to check the association between survival and SNPs coded by two different models (dominant or recessive). Dominant or recessive models were evaluated independently as long as the proportion of cases in the smallest group was greater than 5% (Gustave Roussy cohort, hereafter called IGR cohort) or if there were more than 10 platinum-treated patients in the subgroup (TCGA platinum-based patients). For the IGR cohort, the most suitable model was selected on the basis of the smallest p value as determined by the likelihood ratio test (LRT). Hazard ratios alongside their 95% confidence intervals are presented for the model including the SNP alone and after accounting for nodal status (pN, N0 versus N1 versus N2 versus N3), tumor stage (pT, T0-1 versus T2 versus T3 versus T4) and tumor location (oral cavity versus larynx versus pharynx). Association with clinicopathological parameters (age at diagnosis, tumor size, nodal status, tumor stage and tumor localization) was estimated using Firth’s penalized-likelihood logistic regression (dominant and recessive models) and reported as odds ratio alongside 95% confidence interval and p values. Survival rates were estimated using Kaplan-Meier method. For TCGA, as previously reported109 clinical data were accessed via the TCGAbiolinks R package. The groups were compared using the two-sided Mann-Whitney U test. PFS and OS were used as primary end-points and were determined either for the entire cohort of patients or the subgroups receiving platinum-based chemotherapy. According to the number of patients and events by genotype, PFS and OS for the whole HNSCC TCGA population were plotted over 200 months while PFS and OS for the platinum-based subgroups were plotted over 80 and 100 months, respectively.

Results and discussion

Driven by previous results from our laboratory, we profiled a cohort of 187 advanced HNSCC patients (Table 1) for SNPs affecting a set of ICD-relevant genes. DNA samples from these patients, treated at the Gustave Roussy Cancer Campus (also known as Institut Gustave Roussy (IGR)) with platinum-based induction chemotherapy followed by platinum-based maintenance chemoradiotherapy, were genotyped at several loci, including ATG16L1 (rs2241880),99 FPR1 (rs867228),66,110,67,110,112,113 P2RX7 (rs3751143)114,115,118–123 and TLR4 (rs4986790). All SNPs were found at frequencies that did not differ from those reported for the general Caucasian population.

The effect of each SNP on progression-free survival (PFS) and overall survival (OS) was determined (Table 2). Firstly, we compared patients that were homozygous for the wild type alleles of FPR1 (Table 3) or P2RX7 (Table 3) with
Table 1. Clinical and histopathological characteristics of HNSCC patients belonging to IGR cohort.

| Variable       | n (%) |
|----------------|-------|
| Sex            |       |
| Female         | 31    | 16.6 |
| Male           | 156   | 83.4 |
| Grade          |       |
| III            | 37    | 19.8 |
| IV             | 150   | 80.2 |
| T of TNM       |       |
| 0              | 1     | 0.5  |
| 1              | 9     | 4.8  |
| 2              | 24    | 12.9 |
| 3              | 77    | 41.4 |
| 4              | 75    | 40.3 |
| n.a.           | 1     | 0.5  |
| N of TNM       |       |
| 0              | 52    | 27.8 |
| 1              | 22    | 11.8 |
| 2              | 74    | 39.6 |
| 3              | 39    | 20.9 |
| M of TNM       | 187   | 100.0 |
| HPV            |       |
| no             | 173   | 92.5 |
| yes            | 14    | 7.5  |
| Localization   |       |
| Larynx         | 47    | 25.1 |
| Maxillofacial  | 5     | 2.7  |
| Oral cavity    | 22    | 11.8 |
| Pharynx        | 113   | 60.4 |

Abbreviations: HPV, human papilloma virus; M, metastasis; N, lymph node; n.a., not available; T, tumor size; TNM, Tumor-Node-Metastasis.

those bearing one or two copies of the loss-of-function alleles (GT/TT for FPR1 and AC/CC for P2RX7), and then plotted the Kaplan–Meier survival curves for PFS and OS. Notably, FPR1 (Table 4, Figures S1A and S1B) and P2RX7 (Table 4, Figures S2A and S2B) polymorphisms completely failed to influence progression-free or overall survival of HNSCC patients. Additionally, as previously described by Bergmann and colleagues,124 we noticed a weak but non-significant (p < .0658, HR 2.2, CI [1.03;4.7]) effect, of rs4986790 in TLR4 on PFS but not OS (Tables 3 and 4, Figures S3A and S3B). To corroborate these results in an independent cohort of patients, we interrogated The Cancer Genome Atlas (TCGA) database125 and found that neither FPR1 nor TLR4 nor P2RX7 polymorphisms impacted the investigated endpoints in HNSCC patients (Table 5, Figures S1C, S1D, S2C, S2D, S3C, and S3D). This held true for P2RX7 and TLR4 also upon the stratification of patients based on their allocation to platinum-based chemotherapy (Table 5, Figures S2E, S2F, S3E and S3F).

Interestingly, in the TCGA dataset, patients bearing FPR1<sup>GT</sup> and FPR1<sup>TT</sup> genotypes exhibited an improved OS and PFS compared to FPR1<sup>GG</sup> individuals (Table 5, Figures S1E and S1F). This effect was absent in larger and more homogeneous cohorts of HNSCC cancer (such as the IGR cohort where PFS p < .9802, HR 1.01, CI [0.6;1.68] and OS p < .3935, HR 0.82, CI [0.52;1.29]). Additionally, our group has recently described that FPR1-relevant SNP E346A correlates with early diagnosis in TCGA HNSCC patients,110 suggesting that in this cancer context only homozygosity might impact OS and PFS. Altogether, these data call for further analyses in other cohorts.

By analogy to previous results obtained for non-small cell lung carcinoma patients,114 we hypothesized that the intrinsic characteristics of HNSCC (which near-to-always is chemoresistant and associated with poor prognosis) and/or the type of therapy that is employed to treat this malignancy (which is mainly based oncisplatin, a DNA damaging agent that is weak ICD inducer) may explain why the aforementioned SNPs fail to influence the clinical progression of the HNSCC.126–130

One of the ICD-relevant pathways involves the autophagy-dependent lysosomal secretion of adenosine triphosphate (ATP), the ligand of P2RX7.131–136 The relevance of the autophagic pathway in the context of the HNSCC has already been described: rs1864183 in ATG10, rs3759601 in ATG2B and rs2241880 in ATG16L1 were found to be associated with an higher susceptibility to develop HNSCC (laryngeal, pharyngeal, and oral carcinoma, respectively) in a Spanish population.137 Given these premises, we decided to investigate the role of rs2241880 in our cohort of patients, knowing that rs2241880 affects ATG16L1, which encodes a central adaptor required for the formation of the autophagosome.135,136 Additionally, rs2241880, which consists in an A > G mutation, leading to the substitution of a threonine residue in position 300 to an alanine (the risk allele), sensitizes ATG16L1 to GGHomoe-3 mediated degradation, culminating in decreased autophagy.100,142 Since the most common genotype ATG16L1<sup>AG</sup> does not cause a full loss-of-function,100,142 a recessive genetic model was applied for this gene. Patients with the ATG16L1<sup>GG</sup> genotype exhibited significantly reduced PFS, independently of major clinicopathological variables both in IGR and in the TCGA (platinum-based) cohorts. We found an effect of the rs2241880 SNP on PFS (Tables 3 and 4, Figure 1a, p < .0062, HR 1.95, CI [1.22;3.12]), but not OS (Tables 3 and 4, Figure 1b, p < .9687, HR 0.99, CI [0.63;1.57]) of HNSCC IGR patients. Similarly, TCGA patients treated with platinum-based chemotherapy and carrying the ATG16L1<sup>GG</sup> genotype displayed reduced PFS (Table 5, Figure 2c, p < .042, HR 0.53, CI [0.28;0.98]) and OS (Table 5, Figure 2d, p < .005, HR 0.36, CI [0.18;0.74]). This effect was not observed for the entire cohort (Table 5, Figure 2a and 2b). Altogether, our results confirm that an impaired autophagic machinery culminates in an unsuccessful ICD, underscoring the likely relevance of this pathway in HNSCC patients receiving platinum-based chemoradiotherapy.

It should be noted that rs2241880 has been associated with inflammatory bowel disease, in particular Crohn’s disease.143–147 Several studies have been performed to evaluate the putative association between IBD and HNSCC.
susceptibility, development, or outcome. Particularly, in a large cohort of IBD patients (more than 7000), rs2241880 has been correlated with an increased risk of developing oral (especially tongue) carcinoma. Similarly, a Dutch study reported that IBD is associated with impaired survival of patients with oral cavity carcinoma and that advanced age at IBD diagnosis can be considered as a risk factor for the development of this malignancy. Additionally, IBD patients are more prone to develop mouth cancer, and the mechanisms of carcinogenesis may be linked to long-lasting inflammation, immunosuppressive treatments and to their HPV status. The role of ATG16L1 loss-of-function alleles has also been reported for other cancers than HNSCC. Indeed, rs2241880 has been described as a risk factor both for developing hepatocellular carcinoma in the context of cirrhosis, breast cancer, and gastric cancer. Moreover, the ATG16L1<sup>AG</sup> genotype was found to be associated with an earlier age at diagnosis of melanoma. All these observations underscore the implications of ATG16L1 T300A in several types of cancer and, more specifically, its prognostic value in HNSCC.

In summary, it appears that rs2241880 in ATG16L1 has a negative prognostic impact on a segment of patients with HNSCC, in particular those who undergo platinum-based chemotherapy. Although there is no formal evidence for this conjecture, it is tempting to speculate that the well-studied association between IBD and poor-prognosis HNSCC is in part determined by rs2241880, knowing that this SNP is among the major predisposing factors for the development of

| Variable | Distribution (%) | HR (95% CI) | p value | LRT (p value) | HR (95% CI) | p value | LRT (p value) |
|----------|-----------------|-------------|---------|--------------|-------------|---------|--------------|
| Gender   |                 |             |         |              |             |         |              |
| Female   | 31              |             |         |              |             |         |              |
| Male     | 16.6            |             |         |              |             |         |              |
| Age      |                 |             |         |              |             |         |              |
| 0-50     | 40              | 0.51        | 0.02    | 5.76         | 0.02        | 7.39    | 0.73 [0.44;1.22] | 0.24 |
| 51-65    | 21.4            | [0.38;1.14] | 0.14    | 2.17         | 0.033       | 0.68    | [0.41;1.11] | 0.8 |
| >65      | 31              | 0.77        | 0.47    | 6.42         | 0.0004      | 1.08    | [0.59;1.99] | 0.8 |
| HPV      |                 |             |         |              |             |         |              |
| negative | 173             | 0.35        | 0.05    | 3.74         | 0.005       | 0.47    | [0.16;1.36] | 0.11 |
| positive | 92.5            |             |         |              |             |         |              |
| T of TNM |                 |             |         |              |             |         |              |
| 0-1      | 10              | 0.72        | 0.61    | 11.74        | 0.008       | 1.56    | [0.2;2.54] | 0.3 |
| 2        | 24              | 0.64        | 0.46    | 0.56         | 0.196       | 0.49    | [0.2;1.24] | 0.16 |
| 3        | 77              | 0.21        | 0.98    | 1.13         | 0.494       | 1.3     | [0.46;2.78] | 0.77 |
| 4        | 75              | 0.49        | 0.48    | 1.48         | 0.494       | 1.48    | [0.46;2.78] | 0.48 |
| N of TNM |                 |             |         |              |             |         |              |
| 0        | 52              | 1.42        | 0.38    | 8.83         | 0.03        | 1.3     | [0.65;3.12] | 0.48 |
| 1        | 22              |             |         |              |             |         |              |
| 2        | 11.8            | 1.61        | 0.11    | 1.3          | 0.632       | 1.65    | [0.63;2.7] | 0.07 |
| 3        | 39              | 0.87        | 0.95    | 0.71         | 0.494       | 0.29    | [0.95;2.85] | 0.8 |
| 20.9     | 21.7            | [1.39;5.29] | 0.003   | 2.91         | [1.6;5.3]   | 0.004   |              | |
| Localisation | |         |         |              |             |         |              |
| Oral cavity | 22         |             |         |              |             |         |              |
| Maxillofacial | 5          | 1.63        | 0.4     | 13.55        | 0.004       | 1.74    | [0.54;4.95] | 0.55 |
| Larynx | 2.7            | [0.13;0.66] | 0.004   | 0.29         | [0.25;2.13] | 0.74    | [0.29;1.39] | 0.004 |
| Pharynx | 25.1           | [0.12;0.48] | 0.02    | 0.29         | [0.12;0.48] | 0.29    | [0.16;0.51] | 0.004 |

Abbreviations: CI, confidence interval; HPV, human papilloma virus; HR, hazard ratio; LRT, likelihood ratio test; M, metastasis; N, lymph node; OS, overall survival; PFS, progression-free survival; T, tumor size; TNM, Tumor-Node-Metastasis.
| Genotype | Genotype | Genotype | Genotype | Genotype |
|----------|----------|----------|----------|----------|
| ATG16L1 | FPR1 | P2RX7 | TLR4 |
| **Variable** | **n (%)** | **p value** | **n (%)** | **p value** | **n (%)** | **p value** | **n (%)** | **p value** |
| Gender | | | | | | | | |
| Female | 24 | 0.25 | 0.5669 | 25 | 6 | 3.26 | 0.0696 | 18 | 13 | 0.2 | 0.6672 | 26 | 5 | 6.87 | 0.0035 |
| Male | 17.5 | 0 < 0.001 | 95.5 | 0.45 | 0.07 | 97 | 59 | 0.84 | 0.651 | 151 | 5 | 0.17 | 0.009 |
| Age | | | | | | | | |
| 0–50 | 28 | 12 | 0.8709 | 30 | 9 | 2.36 | 0.3007 | 24 | 16 | 0.9 | 0.6451 | 40 | 0 | 2.91 | 0.2364 |
| 51–65 | 20.4 | 0.8604 | 25 | 15 | p < 0.001 | 20.9 | 22.2 | p < 0.001 | 22.6 | 0 | p < 0.001 |
| >65 | 16.8 | 0.2672 | 22.9 | 15.8 | 0.7617 | 14.8 | 0.4391 | 19.8 | 14.9 | 0.15 | 0.0298 |
| HPV | negative | 126 | 47 | 0.11 | 0.6407 | 113 | 53 | 1.89 | 0.1683 | 107 | 66 | 0.15 | 0.7278 | 163 | 10 | 0.21 | 0.3552 |
| positive | 8 | 6 | 0.1992 | 5.78 | 5.17 | 0.47 | 0.17 | 8 | 6 | 1.24 | 0.6980 | 14 | 0 | 0.54 | 0.64 |
| T of TNM | 0–1 | 6 | 4 | 0.73 | 0.6268 | 8 | 2 | 2.34 | 0.4715 | 6 | 4 | 1.72 | 0.6375 | 10 | 0 | 0.04 | 0.898 |
| 2 | 19 | 5 | 0.41 | 0.2521 | 13 | 10 | 0.38 | 0.22 | 13 | 11 | 1.23 | 0.7767 | 23 | 1 | 1.34 | 0.86 |
| 3 | 55 | 22 | 0.59 | 0.4259 | 53 | 22 | 0.70 | 0.63 | 45 | 32 | 1.032 | 0.9619 | 73 | 4 | 1.29 | 0.86 |
| 4 | 40.4 | 0.1612 | 22.95 | 44.2 | 36.7 | 0.1252 | 39.7 | 44.4 | 0.283 | 0.997 | 41.4 | 0 | 0.123 | 174.97 |
| 5 | 41.6 | 0.1261 | 8.41 | 38.3 | 41.7 | 0.0962 | 3.123 | 43.5 | 34.7 | 0.202 | 8.847 | 40.1 | 40 | 0.126 | 179.882 |
| n.a. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N of TNM | 0 | 15 | 2.87 | 0.3982 | 34 | 16 | 0.06 | 0.9961 | 35 | 17 | 1.44 | 0.6854 | 48 | 4 | 1.28 | 0.6123 |
| 1 | 77.5 | 0.76 | 0.63 | 14 | 17 | 0.93 | 0.88 | 14 | 8 | 1.19 | 0.7404 | 22 | 0 | 0.24 | 0.26 |
| 2 | 58 | 16 | 0.68 | 0.3351 | 47 | 24 | 0.93 | 0.85 | 42 | 32 | 1.55 | 0.2369 | 70 | 4 | 0.69 | 0.59 |
| 3 | 42.3 | 0.3041 | 13.35 | 39.2 | 40.1 | 0.4218 | 39.1 | 44.4 | 0.753 | 0.267 | 39.5 | 40 | 0.172 | 2.787 |
| 4 | 25 | 14 | 1.376 | 0.4752 | 25 | 13 | 0.90 | 0.82 | 24 | 15 | 1.283 | 0.5679 | 37 | 2 | 0.72 | 0.68 |
| 5 | 18.2 | 0.5713 | 3.139 | 20.8 | 21.7 | 0.3732 | 2.203 | 20.9 | 20.8 | 0.5433 | 20.9 | 0 | 0.123 | 13.434 |
| Localization | Oral cavity | 19 | 3 | 3.9 | 0.2336 | 12 | 9 | 0.99 | 0.7852 | 13 | 9 | 2.38 | 0.4564 | 20 | 2 | 3.37 | 0.3869 |
| Maxillofacial | 4 | 1 | 0.98 | 0.5858 | 3 | 1 | 1.77 | 0.58 | 4 | 1 | 0.474 | 0.4533 | 4 | 1 | 2.73 | 0.4 |
| Larynx | 2.9 | 2 | 0.15315 | 3.035 | 2.5 | 1.7 | 0.241 | 0.2954 | 3.5 | 1.14 | 0.0423 | 0.138 | 2.13 | 0.2652 | 10 |
| Pharynx | 57 | 27 | 1.56 | 0.5019 | 35 | 15 | 1.59 | 0.38 | 25 | 10 | 1.254 | 0.6583 | 45 | 2 | 0.45 | 0.49 |
| Abbreviations: ATG16L1, autophagy related 16-like 1; CI, confidence interval; FPR1, formyl peptide receptor 1; HPV, human papilloma virus; LRT, likelihood ratio test; M, metastasis; N, lymph node; n.a., not available; OR, odds ratio; P2RX7, purinergic receptor P2X, ligand-gated ion channel. 7; T, tumor size; TLR4, toll like receptor 4; TNM, Tumor-Node-Metastasis. |
**Table 4.** Correlation between SNPs mutational status and progression-free survival or overall survival in IGR patients affected by HNSCC.

| Gene     | ID        | Genetic model  | PFS HR | PFS p value | OS HR  | OS p value |
|----------|-----------|----------------|--------|-------------|--------|------------|
| ATG16L1  | rs2241880 | Recessive AA/AG vs GG | 1.95 [1.22, 3.12] | \( p < .0062 \) | 0.99 [0.63, 1.57] | \( p < .9687 \) |
| FPR1     | rs867228  | Dominant GG vs GT/TT | 1.01 [0.61, 1.68] | \( p < .9802 \) | 0.82 [0.52, 1.29] | \( p < .3935 \) |
| P2RX7    | rs23751143| Dominant AA vs AC/CC | 2.18 [0.83, 5.73]  | \( p < .1536 \) | 0.75 [0.49, 1.15] | \( p < .1779 \) |
| TLR4     | rs4986790 | Dominant AA vs AG   | 2.2 [1.03, 4.7]   | \( p < .0658 \) | 1.86 [0.87, 3.98] | \( p < .1363 \) |

Abbreviations: ATG16L1, autophagy Related 16 Like 1; FPR1, formyl peptide receptor 1; HR, hazard ratio; IGR, Gustave Roussy Cancer Campus; P2RX7, purinergic receptor P2X, ligand-gated ion channel; 7; PFS, progression-free survival; OS, overall survival; TLR4, toll like receptor 4. Significant \( p \) values are indicated in bold.

**Table 5.** Correlation between SNPs mutational status and progression-free survival (PFS) or overall survival (OS) in TCGA HNSCC patients. According to the number of patients and events by genotype, PFS and OS for the whole HNSCC TCGA population were plotted over 200 months while PFS and OS for the platinum-based subgroups were plotted over 80 and 100 months, respectively. Significant \( p \) values are indicated in bold.

| Gene     | PFS | OS |
|----------|-----|----|
| ATG16L1  |     |    |
| FPR1     |     |    |
| P2RX7    |     |    |
| TLR4     |     |    |

Abbreviations: ATG16L1, autophagy related 16-like 1; FPR1, formyl peptide receptor 1; HR, hazard ratio; P2RX7, purinergic receptor P2X, ligand-gated ion channel; 7; OS, overall survival; PFS, progression-free survival; TLR4, toll like receptor 4.

**Figure 1.** Kaplan-Meier estimates of progression-free survival (a) and overall survival (b) in HNSCC patients belonging to the IGR cohort and harboring AG (Thr300Ala) or AA (Thr300Thr) genotype of ATG16L1 rs2144880 SNP compared to patients carrying the variant alleles GG (Ala300Ala).
Crohn’s disease. Future studies should investigate this possible mechanistic link.

Acknowledgments

We acknowledge Dr. David P. Enot for his precious help with the statistical analyses of the IGR dataset. We thank Kariman Chaba for her contribution in the preparation of the IGR samples. We acknowledge Dr Stephan Temam for the IGR cohort samples and his supervision during the clinical analyses. The results shown here are partly based upon data generated by the TCGA Research Network; http://cancergenome.nih.gov. DK is supported by the Ligue contre le Cancer (équipe labellisée); Agence National de la Recherche (ANR – Projets blancs; AMM’ICA US23/CNRS UMS3655; Association pour la recherche sur le cancer (ARC); Association “Rubin Rose”; Cancéropolis Île-de-France; Fondation pour la Recherche Médicale (FRM); a donation by Elior; Equipex Onco-Pheno-Screen; European Joint Programme on Rare Diseases (EJPRD); Gustave Roussy Odyssée, the European Union Horizon 2020 Projects Oncobiome and Crimson; Fondation Carrefour; Institut National du Cancer (INCa); Inserm (HTE); Institut Universitaire de France; LabEx Immuno-Oncology (ANR-18-IDEX-0001); the Leducq Foundation; a Cancer Research ASPIRE Award from the Mark Foundation; the RHU Torino Lumière; Seerave Foundation; SIRIC Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); and SIRIC Cancer Research and Personalized Medicine (CARPEM). This study contributes to the IdEx Université de Paris ANR-18-IDEX-0001. The LG lab is supported by a Breakthrough Level 2 grant from the US DoD BRCP (#BC180476P1), by the 2019 Laura Ziskin Prize in Translational Research (#ZP-6177, PI: Formenti) from the Stand Up to Cancer (SU2C), by a mantle Cell Lymphoma Research Initiative (MCL-RI, PI: Chen-Kiang) grant from the Leukemia and Lymphoma Society (LLS), by a startup grant from the Dept. of Radiation Oncology at Weill Cornell Medicine (New York, US), by a Rapid Response Grant from the Functional Genomics Initiative (New York, US), by industrial collaborations with Lytix Biopharma (Oslo, Norway) and Phosplatin (New York, US), and by donations from Phosplatin (New York, US), the Luke Heller TECPR2 Foundation (Boston, US), Soto a.s. (Prague, Czech Republic), and by funding from the Sydney Brenner Institute for Biomedical Research at the University of the Witwatersrand, Johannesburg, South Africa.

Figure 2. Kaplan-Meier estimates of progression-free survival (PFS) and overall survival (OS) in HNSCC TCGA patients and harboring AG (Thr300Ala) or AA (Thr300Thr) genotype of ATG16L1 rs2144880 SNP compared to patients carrying the variant alleles GG (Ala300Ala). PFS (a and c) or OS (b and d) according to ATG16L1 genotype in all HNSCC (a and b) or platinum-based therapy treated (c and d) patients.
Czech Republic), Onexo (Paris, France), Ricerchiamo (Brescia, Italy), and Noxopharm (Chatswood, Australia).

**Disclosure statement**

GK has been holding research contracts with Daichi Sankyo, Eleor, Kaleido, Lytx Pharma, PharmaMar, Samsara, Sanofi, Sotio, Vascage and Vascucox/Tioma. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics, Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. The other authors declare no conflicts of interest. LG has been holding research contracts with Lytx Biopharma and Phosplatin, and has received consulting/advisory honoraria from Boehringer Ingelheim, AstraZeneca, OmniSEQ, Onexo, The Longevity Labs, Inzen, Sotio, and the Luke Heller TECPR2 Foundation.

**Funding**

The author(s) reported there is no funding associated with the work featured in this article.

**ORCID**

Julie Le Naour [http://orcid.org/0000-0002-3749-2171](http://orcid.org/0000-0002-3749-2171)
Zsofia Szutpinszki [http://orcid.org/0000-0002-8691-4086](http://orcid.org/0000-0002-8691-4086)
Guido Kroemer [http://orcid.org/0000-0002-9334-4405](http://orcid.org/0000-0002-9334-4405)
Erika Vacchelli [http://orcid.org/0000-0001-8010-0594](http://orcid.org/0000-0001-8010-0594)

**Author contributions**

E.V., J.L.N. performed the experiments. V.M. cut the paraffin blocks. Z.S., V.C. and S.Z., performed clinical analysis. L.G., S.T. helped in designing the clinical studies. O.G. evaluated the amount and quality of the tumor material and the HPV status. E.V., L.G. and G.K. conceived and directed the project. J.L.N., E.V. and G.K. wrote the manuscript.

**Data availability**

The IGR data that support the findings of this study are available on reasonable request from the corresponding authors. Patient-specific IGR data are not publicly available due to ethical restrictions. The TCGA data that support the findings of this study are openly available at [http://cancergenome.nih.gov](http://cancergenome.nih.gov).

**References**

1. Leemans CR, Snijders PJF, Brakenhoff RH. The molecular landscape of head and neck cancer. Nat Rev Cancer. 2018;18(5):269–282. doi:10.1038/nrc.2018.11.
2. Mody MD, Rocco JW, Yom SS, Haddad RI, Saba NF. Head and neck cancer. Lancet. 2021;398(10318):2289–2299. doi:10.1016/S0140-6736(21)01550-6.
3. Sundermann BV, Uhlimann L, Hoffmann J, Freier K, Thiele OC. The localization and risk factors of squamous cell carcinoma in the oral cavity: a retrospective study of 1501 cases. J Craniomaxillofac Surg. 2018;46:177–182. doi:10.1016/j.jcms.2017.01.019.
4. Ortu G, Mameli A, Denomits C, Rossi P, Batto D, Occhione A, Piras V, Kuoji L, Berretta M, Taibi R, et al. Oral human papilloma virus infection: an overview of clinical-laboratory diagnosis and treatment. Eur Rev Med Pharmacol Sci. 2019;23(18):8148–8157. doi:10.26355/eurrev_201909_19035.
5. Gregoire V, Grau C, Lapereyre M, Maingon P. Target volume selection and delineation (T and N) for primary radiation treatment of oral cavity, oropharyngeal, hypopharyngeal and laryngeal squamous cell carcinoma. Oral Oncol. 2018;87:131–137. doi:10.1016/j.oraloncology.2018.10.034.
6. Economidou P, de Bree R, Kotsantis I, Pyrrh D. Diagnostic tumor markers in head and neck squamous cell carcinoma (HNSCC) in the clinical setting. Front Oncol. 2019;9:827. doi:10.3389/fonc.2019.00827.
7. Kline ER, Muller S, Pan I, Tighiouart M, Chen ZG, Marcus AI. Localization-specific LKB1 loss in head and neck squamous cell carcinoma metastasis. Head Neck. 2011;33(10):1501–1512. doi:10.1002/hed.21638.
8. Szurt P, Cristina V, Herrera Gomez RG, Bourhis J, Simon C, Vermekken JB. Gisplatin eligibility issues and alternative regimens in locoregionally advanced head and neck cancer: recommendations for clinical practice. Front Oncol. 2019;9:464. doi:10.3389/fonc.2019.00464.
9. Dillon MT, Harrington KJ. Human Papillomavirus-Negative Pharyngeal Cancer. J Clin Oncol. 2015;33(29):3251–3261. doi:10.1200/JCO.2015.60.7804.
10. Ragin CC, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. J Dent Res. 2007;86(2):104–114. doi:10.1177/1540829X0708600202.
11. Sturgis EM, Wei Q, Spitz MR. Descriptive epidemiology and risk factors for head and neck cancer. Semin Oncol. 2004;31(6):726–733. doi:10.1053/j.seminoncol.2004.09.013.
12. Iasayev T, Li Y, Maswahdu D, Brandwein-Gensler M. Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review. Head Neck Pathol. 2012;6(1):S104–20. doi:10.1007/s12105-012-0368-1.
13. Stein AP, Saha S, Kraninger JL, Swick AD, Yu M, Lambert PF, Kimple RJ. Prevalence of human papillomavirus in oropharyngeal cancer: a systematic review. Cancer J. 2015;21(3):138–146. doi:10.1097/PPO.0000000000000115.
14. Bulbul MG, Genoves TJ, Hagan K, Rege S, Qureshi A, Varvares MA. Salvage surgery for recurrent squamous cell carcinoma of the head and neck: systemic review and meta-analysis. Head Neck. 2022;44(1):275–285. doi:10.1002/hed.26898.
15. Silverman DA, Lin C, Tamaki A, Puram SV, Carrau RL, Seib NM, Eskander A, Rocco JW, Old MO, Kang SY, et al. Respiratory and pulmonary complications in head and neck cancer patients: evidence-based review for the COVID-19 era. Head Neck. 2020;42(6):1218–1226. doi:10.1002/hed.26217.
16. Akhter M, Rahman QB, Rahman QB, Molla MR. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. J Oral Maxillofac Pathol. 2011;15(2):168–176. doi:10.1007/s11182-009-08448.
17. Dougherty MJ, Dougherty W, Kain JJ, Hughley BB, Shonka DC J, Fedder KL, Jameson MJ. Non-HPV-related head and neck squamous cell carcinoma in a young patient cohort. Ear Nose Throat J. 2021;100(10_suppl):S101S–S105S. doi:10.1177/0145561320935839.
18. Goldstein DP, Irish JC. Head and neck squamous cell carcinoma in the young patient. Curr Opin Otolaryngol Head Neck Surg. 2005;13(4):207–211. doi:10.1097/01.moo.0000170529.04759.4c.
19. van Monsjou HS, Wreesmann VB, van den Brekel MW, Balm AJ, van Monsjou HS, van den Brekel MWM. Head and neck squamous cell carcinoma in young patients. Oral Oncol. 2013;49(12):1097–1102. doi:10.1016/j.oraloncology.2013.09.001.
20. Zatterstrom UK, Wenerberg E, Ewers SB, Willen R, Attewell R. Prognostic factors in head and neck cancer: histologic grading, DNA ploidy, and nodal status. Head Neck. 1991;13(6):477–487. doi:10.1002/hed.2890130603.
21. Cardin GB, Bernard M, Bahig H, Nguyen–Tan PF, Ballivy O, Filion E, Soulieres D, Philouze P, Ayad T, Guertin L, et al. Single nucleotide polymorphism rs6942067 is a risk factor in young and in non-smoking patients with hpv negative head and neck squamous cell carcinoma. Cancers (Basel). 2019;12.
22. Guidi A, Codeca C, Ferrari D. Chemotherapy and immunotherapy for recurrent and metastatic head and neck cancer: a systematic review. Med Oncol. 2018;35(3):37. doi:10.1007/s12032-018-1096-5.
152. Reuken PA, Lutz P, Casper M, Al-Herwi E, Stengel S, Spengler U, Stallmach A, Lammert F, Nischalke HD, Bruns T, et al. The ATG16L1 gene variant rs2241880 (p.T300A) is associated with susceptibility to HCC in patients with cirrhosis. Liver Int. 2019;39(12):2360–2367. doi:10.1111/liv.14239.

153. El-Amir MI, Wahman MM, Khaled HA, El-Feky MA. Role of ATG16L1 (rs2241880) and Interleukin 10 (rs1800872) Polymorphisms in Breast Cancer Among Egyptian Patients. The Egyptian Journal of Immunology. 2020;27:65–76.

154. Burada F, Ciurea ME, Nicoli R, Streata I, Vilcea ID, Rogoveanu I, Ioana M. ATG16L1 T300A polymorphism is correlated with gastric cancer susceptibility. Pathol Oncol Res. 2016;22(2):317–322. doi:10.1007/s12253-015-0006-9.

155. White KA, Luo L, Thompson TA, Torres S, Hu CA, Thomas NE, Lilyquist J, Anton-Culver H, Gruber SB, From L, et al. Variants in autophagy-related genes and clinical characteristics in melanoma: a population-based study. Cancer Med. 2016;5(11):3336–3345. doi:10.1002/cam4.929.