# Supplementary Material

## A Novel 2-Metagene Signature to Identify High-Risk HNSCC Patients amongst Those Who Are Clinically at Intermediate Risk and Are Treated with PORT

**Table S1.** Univariable Cox regression of loco-regional tumour control for the clinical parameters in the PORT and PORT-C cohorts before propensity score matching.

| Parameter | PORT cohort | | | PORT-C cohort | | |
|---|---|---|---|---|---|---|
| | Coefficient (ß) | Loco-regional control HR (95 % CI) | p-value | Coefficient (ß) | Loco-regional control HR (95 % CI) | p-value |
| **Clinical parameters** | | | | | | |
| Age (≥57 vs <57 years [b]) | -0.61 | 0.54 (0.32-0.94) | **0.028** | -0.97 | 0.38 (0.17-0.83) | **0.016** |
| Gender (female vs male[b]) | 0.04 | 1.04 (0.56-1.94) | 0.90 | 0.49 | 1.63 (0.72-3.69) | 0.28 |
| Tumour localization (oral cavity vs others[b]) | 0.71 | 2.03 (1.17-3.53) | **0.015** | 1.28 | 3.62 (1.73-7.57) | **<0.001** |
| T stage (3,4 vs 1,2[b]) | 0.88 | 2.41 (1.41-4.13) | **0.001** | 0.96 | 2.62 (1.25-5.46) | **0.011** |
| N stage (2,3 vs 0,1[b]) | 0.43 | 1.54 (0.90-2.62) | 0.11 | 0.26 | 1.30 (0.53-3.19) | 0.57 |
| Tumour grade (3 vs 1,2[b]) | -0.32 | 0.73 (0.42-1.25) | 0.25 | -0.61 | 0.55 (0.24-1.23) | 0.15 |
| R status (1 vs 0[b]) | 0.17 | 1.18 (0.50-2.78) | 0.70 | 0.04 | 1.05 (0.50-2.18) | 0.91 |
| ECE status (1 vs 0[b]) | 0.86 | 2.37 (1.15-5.03) | **0.025** | 0.43 | 1.54 (0.73-3.27) | 0.26 |
| Dose (Gy) | 0.07 | 1.08 (0.97-1.19) | 0.17 | 0.10 | 1.11 (0.93-1.32) | 0.23 |
| p16 overexpression (1 vs 0[b]) | -1.31 | 0.27 (0.01-0.75) | **0.012** | -1.49 | 0.27 (0.08-0.65) | **0.006** |

[b] Baseline class.

**Table S2**. Patient characteristics for the PORT and PORT-C cohorts before propensity score matching.

| Characteristics | PORT cohort (1999-2016) | | PORT-C cohort (2004-2011) | | p-value |
|---|---|---|---|---|---|
| | Median(range) | | Median(range) | | |
| Age (years) | 57.7 (27.7-88.8) | | 57 (24-75) | | **<0.001** |
| Dose (Gy) | 60.0 (60-66) | | 64.0 (56-68.4) | | **<0.001** |
| | **S** | **%** | **Number of pts** | **%** | |
| Gender<br>Male/female | 99/59 | 77.1/22.9 | 156/39 | 80.0/20.0 | 0.46 |
| Tumour localization<br>Oral cavity/<br>Oropharynx/Hypopharynx/Larynx | 135/88/13/22 | 52.3/34.1/5.1/8.5 | 55/115/25/0 | 28.2/59.0/12.8/0 | **<0.001** |
| Grading<br>1,2/3 | 133/124/1 | 51.6/48.1/0.3 | 78/117 | 40.0/60.0 | 0.098 |
| R status<br>0/1/missing | 230/21/7 | 89.2/8.1/2.7 | 85/109/1 | 43.6/55.9/0.5 | **<0.001** |
| ECE status<br>0/1/missing | 233/24/1 | 90.3/9.3/0.4 | 89/106/0 | 45.6/44.4 | **<0.001** |
| p16 overexpression<br>0/1/missing | 204/51/3 | 79.1/19.8/1.1 | 118/73/4 | 60.5/37.4/2.1 | **<0.001** |
| T stage<br>1,2/3,4 | 186/72 | 72.1/27.9 | 72/123 | 36.9/63.1 | **0.038** |
| N stage<br>0,1/2,3 | 167/91 | 64.7/35.3 | 47/148 | 24.1/75.9 | **<0.001** |
| Locoregional control | 55 | 21.3 | 29 | 14.9 | 0.082[a] |
| Distant metastases | 113 | 43.8 | 63 | 32.3 | 0.24[a] |
| Overall survival | 34 | 13.2 | 37 | 18.9 | 0.16[a] |

[a]Log-rank test

**Table S3**. Comparison of the clinical parameters between PORT and PORT-C patients in the original sample and in the propensity score matched sample. The standardized mean and the standardized mean difference between the cohorts are shown.

| Clinical parameter | Original sample | | | Matched sample (nearest method) | | |
|---|---|---|---|---|---|---|
| | Mean PORT-C (193) | Mean PORT (257) | Mean difference | Mean PORT-C (108) | Mean PORT (108) | Mean difference |
| Distance* | 0.617 | 0.288 | 1.212 | 0.440 | 0.433 | 0.0272 |
| Age (≥57 vs <57 years) | 0.513 | 0.524 | -0.021 | 0.509 | 0.528 | -0.037 |
| Tumour localization (oral cavity/others) | 0.288 | 0.528 | -0.529 | 0.287 | 0.287 | 0.000 |
| T stage (3,4 vs 1,2) | 0.377 | 0.276 | 0.209 | 0.361 | 0.315 | 0.096 |
| ECE status (1 vs 0) | 0.545 | 0.091 | 0.912 | 0.213 | 0.213 | 0.000 |
| p16 overexpression (1 vs 0) | 0.382 | 0.201 | 0.373 | 0.380 | 0.343 | 0.076 |

*Distance between propensity scores of PORT and PORT-C patients.

**Table S4.** Multivariable Cox regression of overall survival for the 2-metagene signature and their interaction term with treatment type.

| Parameter | Coefficient (ß) | HR (95 % CI) | p-value |
|---|---|---|---|
| **Overall survival** | | | |
| Gene classifier (high vs low risk [b]) | 0.60 | 1.81 (1.00-3.29) | **0.049** |
| Treatment status (PORT-C vs PORT [b]) | -0.16 | 0.85 (0.43-1.68) | 0.64 |
| Gene classifier * Treatment | -0.53 | 0.59 (0.23-1.49) | 0.26 |

[b] Baseline class.

**Table S5.** Multivariable Cox regression of freedom from distant metastases for the 2-metagene signature and their interaction term with treatment type.

| Parameter | Coefficient (ß) | HR (95 % CI) | p-value |
|---|---|---|---|
| **Freedom from distant metastases** | | | |
| Gene classifier (high vs low risk [b]) | 0.61 | 1.85 (0.75-4.57) | 0.18 |
| Treatment status (PORT-C vs PORT [b]) | 0.005 | 1.01 (0.41-2.48) | 0.99 |
| Gene classifier * Treatment | -0.65 | 0.52 (0.13-2.14) | 0.37 |

[b] Baseline class.
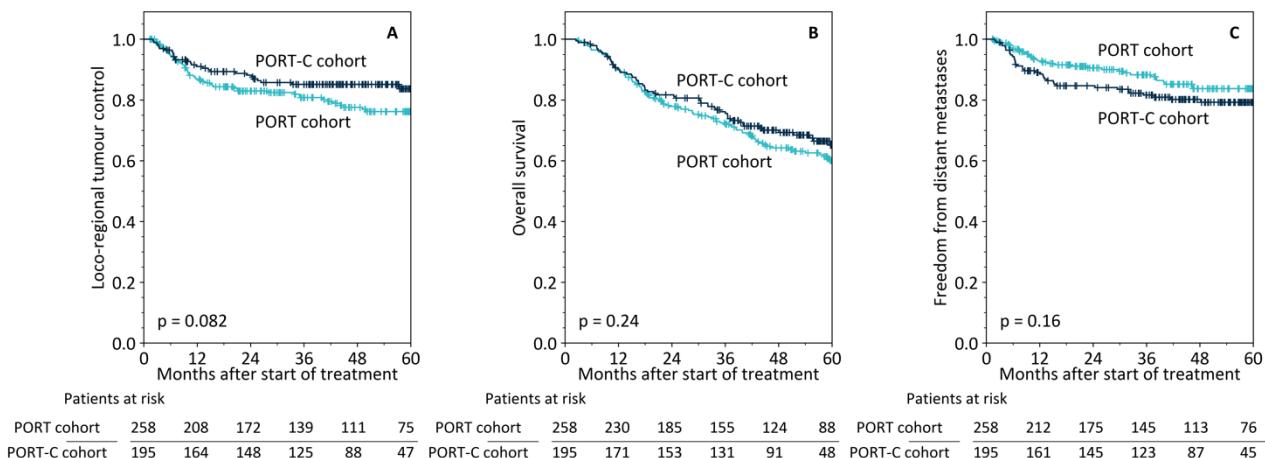
**Figure S1.** Loco-regional tumour control **(A)**, overall survival **(B),** and freedom from distant metastases **(C)** on the PORT and the PORT-C cohort before propensity score matching.
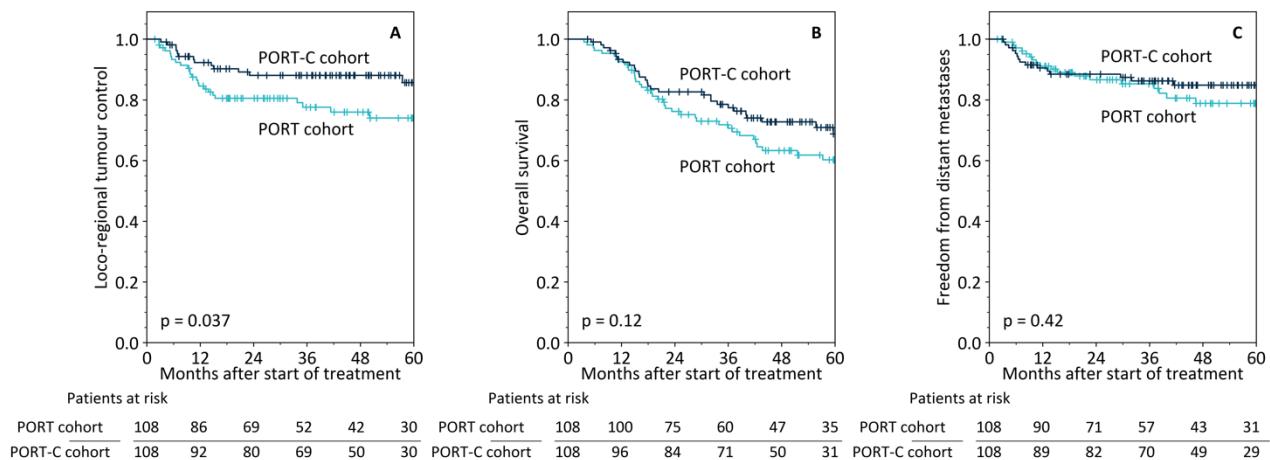


**Figure S2.** Loco-regional tumour control **(A)**, overall survival **(B),** and freedom from distant metastases **(C)** on the PORT and the PORT-C cohort after propensity score matching.
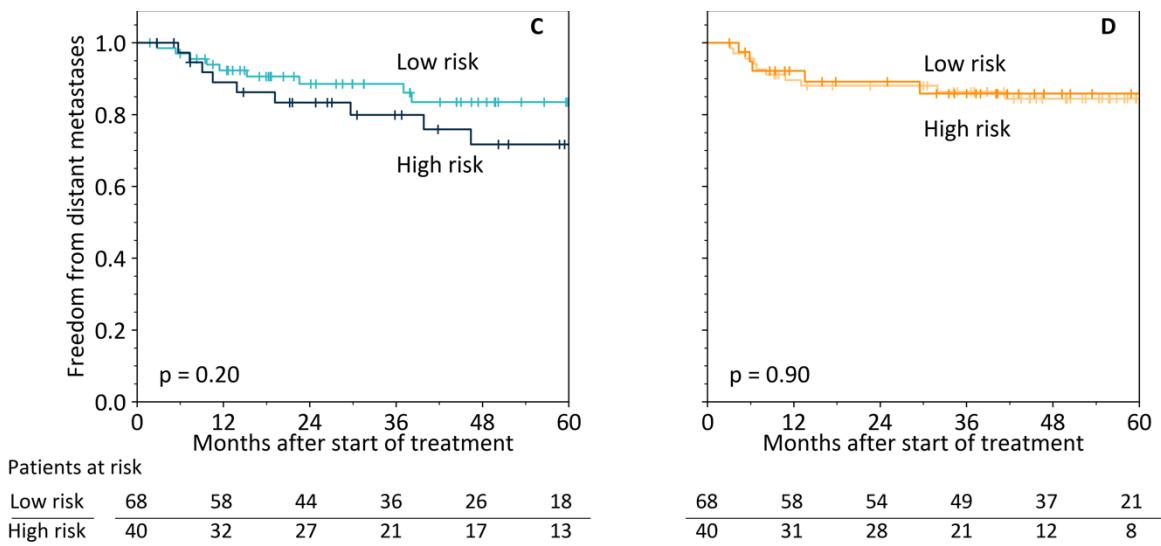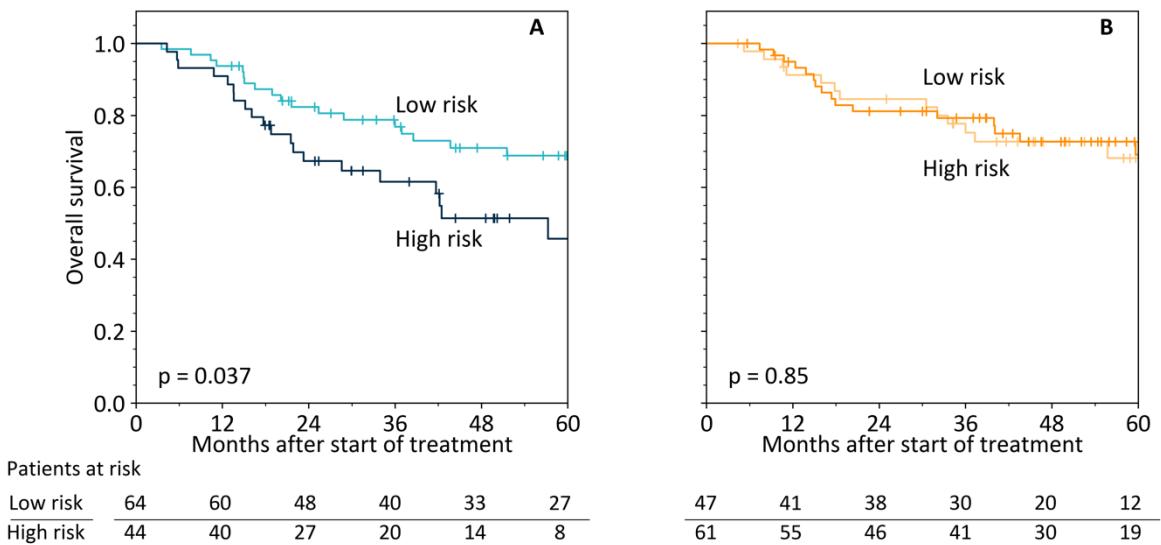
**Figure S3.** Patient stratification by the 2-metagene signature for overall survival (OS) in the PORT **(A)** and the PORT-C cohort **(B)** and for freedom from distant metastases (DM) in the PORT **(C)** and the PORT-C cohort **(D)**.