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Original Research Article

Prognostic stratification of HPV associated oropharyngeal cancer based on CD103+ immune cell abundance in patients treated on TROG 12.01 and De-ESCALaTE randomised trials

Running title: CD103 and prognosis in HPV oropharyngeal cancer

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Summary

Background

High CD103+ intratumoral immune cell (ITIC) abundance is associated with better prognosis in unselected patients with human papilloma virus associated oropharyngeal squamous cell carcinoma (HPV-associated OPSCC) treated with cisplatin and radiotherapy (CIS/RT). Substituting cetuximab (CETUX) for CIS with RT in HPV-associated OPSCC resulted in inferior efficacy. Our aim was to determine if quantification of ITIC CD103 could be used to identify a population of HPV-associated OPSCC with superior prognosis.

Patients and methods

We pooled data from the TROG 12.01 and De-ESCALaTE randomised trials that compared CETUX/70GyRT with CIS/70GyRT in low risk HPV-associated OPSCC: AJCC 7th Stage III (excluding T1-2N1) or stage IV (excluding N2b-c if smoking history >10 pack years and/or distant metastases), including all patients with available tumor samples. The primary endpoint was failure-free survival (FFS) in patients receiving CETUX/RT comparing CD103+ ITIC high (>30%) versus low (<30%). High/low CD103 were compared using Cox regression adjusting for age, stage and trial.

Results

Tumor samples were available in 159/182 patients on TROG 12.01 and 145/334 on De-ESCALaTE. CD103+ ITIC abundance was high in 27% of patients. The median follow-up was 3.2 years. The 3-year FFS in patients treated with CETUX/RT were 93% (95% CI: 79-98%) in high CD103 and 74% (95% CI: 63-81%) in low CD103, adjusted HR 0.22 (95% CI: 0.12-0.41);
p<0.001. The 3-year overall survival in patients treated with CETUX/RT was 100% in high CD103 and 86% (95% CI: 76-92%) in low CD103, p<0.001. In patients treated with CIS/RT there was no significant difference in FFS.

Conclusion

CD103+ ITIC expression separates CETUX/RT treated low risk HPV-associated OPSCC into excellent and poor prognosis subgroups. The high CD103 population is a rational target for de-intensification trials.

Keywords

Head and neck cancer

Oropharyngeal cancer

Human papillomavirus

CD103

Cetuximab

Cisplatin
Highlights

- Intratumoral immune cell CD103 expression separates CETUX/RT treated low risk HPV-associated OPSCC into excellent and poor prognosis subgroups.
- There was an increase in distant and locoregional failures in the low CD103 population treated with CETUX/RT.
- In a low risk HPV-OPSCC population weekly CIS/RT achieves excellent outcomes in both high and low CD103 groups.
- In the high CD103 population CIS/RT and CETUX/RT achieve similar excellent outcomes.
- CD103 expression also stratifies CETUX/RT treated patients with abundant CD8 TILs into distinct prognostic groups.
Introduction

Since the emergence of human papilloma virus (HPV)-associated oropharyngeal squamous cell carcinoma (OPSCC) as a distinct entity with a superior prognosis to HPV negative OPSCC, there has been considerable interest in de-intensification strategies for HPV-associated OPSCC[1]. The common goal of these trials has been to decrease acute and long term morbidity without a decrement in survival.

The first wave of randomised trials in HPV-associated OPSCC compared radiotherapy and cisplatin to radiotherapy and cetuximab[2-4]. It was believed based on the available data that cetuximab and radiotherapy, which was a regimen approved by regulatory authorities, would have similar efficacy and would result in less toxicity than cisplatin containing regimens[5]. However, several trials have now shown that cetuximab and radiotherapy results in inferior efficacy compared to cisplatin and radiotherapy[2-4, 6]. These results have raised concern about the existing criteria for defining low risk HPV-associated OPSCC candidates for de-escalation trials[7], and highlighted the need for improved criteria to identify accurately a genuinely low risk population[8].

We and others have previously reported that the presence of CD8$^+$ tumor infiltrating lymphocytes (TILs) is prognostic in HPV-associated OPSCC[9-13]. Tissue resident memory (T$_{RM}$) cells are a subset of T cells that occupy tissues without recirculating, and play roles in cancer immunosurveillance and local tissue immunity to infections[14]. T$_{RM}$ cells are characterised by expression of CD103 and CD69, and are usually CD8 positive but can also be CD4 positive. We have previously demonstrated that HPV-associated OPSCC tumors with high abundance of CD103$^+$ intratumoral immune cells (ITIC) have features consistent with
Furthermore, we found that in unselected HPV-associated OPSCC patients treated predominantly with platinum based chemoradiation, patients with high CD103 tumors had a superior prognosis to patients with low CD103 tumors in two independent cohorts[15]. In both cohorts the high CD103 patients represented an excellent prognosis population with 100% 3 year overall survival, and was superior to CD8+ TILs for identifying a low risk population.

We hypothesised that in low risk HPV-associated OPSCC patients treated with cetuximab and radiotherapy, patients with high CD103+ ITICs would have a superior prognosis compared to patients with low CD103+ ITICs. In order to test this hypothesis we combined data from two randomized trials that compared cisplatin/radiotherapy to cetuximab/radiotherapy, TROG 12.01 and De-ESCALaTE[3, 4].

Patients and methods

Patients
Two independent clinical trial cohorts of HPV-associated OPSCC were utilized for the current study and were combined for analysis. In both trials low risk HPV-associated OPSCC patients (as defined by Ang et al based on the American Joint Committee on Cancer (AJCC) 7 staging and smoking history criteria[7]) received 70 Gy radiotherapy conventionally fractionated with either cisplatin or cetuximab. Key differences between the trials was more restricted eligibility in TROG 12.01 with exclusion of T4 and/or N3 patients, and the use of high dose cisplatin (100mg/m² x 3) in De-ESCALaTE and weekly cisplatin (40mg/m² x 7) in TROG 12.01[3, 4]. The analysis population is defined as all eligible patients from TROG 12.01 and De-ESCALaTE trials who were randomised, commenced protocol treatment and had a tumor sample available for biomarker assessment.

Formalin-fixed paraffin embedded (FFPE) tumor blocks or unstained tissue sections were collected. All tissue samples were from primary resections or biopsies of primary tumors taken before the commencement of treatment. HPV status had been previously established
for both trials primarily by p16 immunohistochemistry, with HPV RNA in situ hybridization (ISH) for high-risk HPV E6/E7 mRNA using the RNAscope HPV assay also done for TROG 12.01[16] and HPV DNA ISH in De-ESCALaTE[3]. The study had Institutional Ethics Committee approval (PMCC HREC-12/144).

Objectives

The primary objective was to assess whether CD103 ITIC abundance can select a subgroup of patients suitable for de-intensification by comparing failure-free survival (FFS) by CD103+ ITIC abundance (≥30% versus <30%) in patients receiving cetuximab and radiotherapy.

Secondary objectives included comparing: FFS by CD103+ ITIC abundance in patients receiving cisplatin and radiotherapy, FFS by CD8+ TIL abundance (≥30% in either versus <30% intratumoral and stromal) in patients receiving cetuximab and radiotherapy, FFS by CD103+ ITIC abundance within each AJCC 8th stage group, overall survival (OS) by CD103+ ITIC abundance in patients receiving cetuximab and radiotherapy and the pattern of first failure according to CD103 group.

Immunohistochemistry

Immunohistochemistry (IHC) for CD103 and CD8 was carried out on FFPE sections as previously described[15]. Briefly, 4μm whole tumor sections were baked at 60°C for 1 hour followed by dewaxing through several xylene and alcohol baths. Antigen retrieval was performed in an EDTA buffer, pH 9.0 (Agilent), in a pressure cooker after which slides were placed onto a Dako autostainer (Agilent) for the following automated incubations: 3% H2O2 for 10 minutes, CD103 (clone EPR4166(2); Abcam) at a 1:1500 dilution or CD8 (clone 4B11; Leica Biosystems) at a 1:1000 dilution for 60 minutes; secondary detection with Dako EnVision+ rabbit for CD103 or EnVision+ mouse for CD8 for 60 minutes; 3,3′-diaminobenzidine (DAB) for 10 minutes for color detection. Slides were rinsed with TBST buffer between all incubations. To complete IHC slides were counterstained with hematoxylin, mounted, and coverslipped.

The abundance and location of CD103+ ITICs or CD8+ TILs was determined by semi-quantitatively scoring the proportion of positively stained cells located specifically within epithelial intratumoral nests or associated stroma, as previously described[15].
proportion of positively stained CD103+ or CD8+ cells was scored as a proportion of the total number of cells in tumor nests or stroma as 0%, 1%, 5%, 10%, 20%, 30%-100% (to the nearest 10% above 10%). The optimal cut points of 30% for CD103 ITIC and 30% for intratumoral and/or stromal CD8 TILs had been determined in our previous study[15]. All slides were independently scored by two observers blinded to the outcome data. Discrepant cases were reviewed together and consensus results used for analysis.

Statistical analysis

FFS was defined as the time from randomisation to failure (locoregional or distant) or death due to any cause. Patients alive without evidence of failure were censored at their last follow-up. Second primary cancers were not considered an event for FFS. OS was defined as the time from randomisation to the date of death due to any cause. Patients who were lost to follow-up were treated as censored at their last known follow-up date. Patients who were alive at the defined study close-out date were treated as censored at that date. Pattern of first failure was defined as the cumulative incidence of first failure, considering each failure type separately. Failures were classified as locoregional (recurrence at primary tumor site and/or regional nodes), distant, both (locoregional and distant) or death. Cumulative incidence of first failure was measured from the date of randomisation to the date of first failure or death (without preceding failure).

Patient demographics, baseline characteristics and treatment details were described using descriptive statistics such as minimum, maximum, median, interquartile range (IQR), mean and standard deviation (SD) for quantitative variables. Qualitative variables were described in tabular form as counts and percentages. Descriptive statistics were provided per trial and overall. Median follow-up was calculated using the reverse Kaplan-Meier method. Cox proportional hazard model was used to assess the impact of CD103 and CD8 on FFS and OS in
patients treated with cetuximab and in patients treated with cisplatin, adding age and stage as covariates, nested within trial. The analysis of CD103 and CD8 on FFS and OS in all patients was also assessed using Cox proportional hazard model, adding age, stage and treatment, nested within trial. Kaplan-Meier plots were provided. Estimates were provided with 95% confidence interval based on the log(-log(survival)). The proportional hazard assumptions were tested and verified graphically by plotting the Schoenfeld residuals.

Cumulative incidence of first failure according to CD103 group (high or low) were estimated considering each failure and death as competing events. There was no imputation of missing values and no adjustment for multiplicity. All statistical analyses were performed in R version 3.6.1.

Results

Patient characteristics

Slides were available for 159/182 TROG 12.01 eligible patients and 145/334 De-ESCALaTE eligible patients. Combined this resulted in a total of 304 patients for this analysis, 147 treated with cetuximab and 157 treated with cisplatin. Baseline characteristics in patients with biomarker data available and in the total population are shown in Table 1. De-ESCALaTE had a higher proportion of females and patients with unilateral treatment, and AJCC 8 stage 3 patients were only eligible for De-ESCALaTE. CD103 results were available from 299 patients and CD8 results from 292 patients. Tumors from 28% and 27% of patients had CD103+ ITIC $\geq$30%, and 40% and 41% of patients had CD8+ intratumoral and stromal TILs $\geq$30% in TROG 12.01 and De-ESCALaTE trials respectively. For each trial the characteristics of the patients with
biomarker samples available were very similar to the total population. Median follow up (both trials combined) was 3.2 years.

**Impact of CD103 intratumoral immune cell abundance on failure-free and overall survival**

Three year FFS in the combined population of 299 patients with CD103 data available was 89% with cisplatin and 79% with cetuximab adjusted HR 2.3 (95% CI: 1.6-2.3); p<0.001. (Supplementary Figure 1). For the primary aim of comparing FFS by CD103+ ITIC abundance in cetuximab treated patients, 3 year FFS in patients with high and low CD103 was 93% v 74% adjusted HR 0.22 (95% CI: 0.12-0.41); p<0.001 (Figure 1a). OS was also superior in cetuximab treated patients with high CD103, 3 year OS 100% versus 86% in low CD103, p<0.001 (Figure 1b). The superior FFS in the high CD103 population was independent of AJCC 8 stage: for stage 1 HR =0.27 (95%CI: 0.20-0.35); p<0.001 and for stage 2 adjusted HR=0.23 (95%CI: 0.10-0.55); p=0.001 Figures 2a and 2b). Patterns of first failure in patients treated with cetuximab are shown in Figures 2c and 2d, demonstrating that the increase in failures in the low CD103 population are approximately evenly split between distant and locoregional failures. Survival results were very good in cisplatin treated patients regardless of CD103 abundance with no significant differences between high and low CD103 patients, 3 year FFS was 90% and 89% adjusted HR = 0.89 (95% CI: 0.59-1.34), p=0.57 and 3 year OS was 100% and 95%; (adjusted HR = 0.49 (95% CI: 0.20-1.17), p=0.11) (Figures 3a and 3b). For patients with high ITIC CD103 there was no significant difference between patients treated with cetuximab or cisplatin, with 3 year FFS of 93% versus 90%; adjusted HR = 1.28 (95%CI: 0.82-2.02), p=0.28, and 3 year OS of 100% with both regimens, p=0.84 (Supplementary Figures 2a and 2b). While for patients with low ITIC CD103 comparing cetuximab and cisplatin, 3 year FFS was 74% versus 89%;
adjusted HR = 0.34 (95%CI: 0.24-0.49), p<0.001 and 3 year OS was 86% versus 94%; adjusted HR = 0.31 (95%CI: 0.19-0.51), p<0.001 (Supplementary Figures 3a and 3b)

The treatment outcomes of patients who were available for the current combined analyses were similar to those of the total population in each trial (Supplementary Figures 4a, 4b, 4c and 4d). In an exploratory analysis we also looked at the results separately for the 2 individual trials, showing results in each trial consistent with the combined analysis with better prognosis in high ITIC CD103 patients compared to low ITIC CD103 patients treated with cetuximab 3 year FFS in TROG 12.01 of 90% and 76%; adjusted HR = 0.33 (95%CI: 0.08-1.46), p=0.14, and 3 year FFS in De-ESCALaTE of 95% and 71%; adjusted HR = 0.13(95%CI: 0.02-0.96), p=0.046 (Supplementary Figures 5a and 5b), and no significant difference in high ITIC CD103 patients compared to low ITIC CD103 patients treated with cisplatin, 3 year FFS in TROG 12.01 of 90% and 93%; adjusted HR = 1.40 (95%CI: 0.25-7.94), p=0.70, and 3 year FFS in De-ESCALaTE of 83% and 85%; adjusted HR = 0.71(95%CI: 0.15-3.33), p=0.66 (Supplementary Figures 5c and 5d)

The addition of CD103 to the models including age, AJCC stage and trial improved the model fit measured by the C-statistic in cetuximab treated patients. For FFS the C-statistic improved from 0.55 to 0.63, and for OS it improved from 0.67 to 0.77. (Supplementary Table 1)

Impact of CD8+ tumor infiltrating lymphocytes on failure-free and overall survival

Three year FFS in cetuximab patients with high and low CD8+ TILs was 85% v 75%; adjusted HR 0.46 (95% CI: 0.24-0.87), p=0.017 (Figure 4a). In order to evaluate the additional prognostic value of CD103 we analysed cetuximab treated patients with high CD8+ TILs, and compared patients with high versus low CD103 tumors. Three year FFS was 93% in patients
with high CD8+ TILs/high CD103+ ITICs versus 76% in patients with high CD8+ TILs/low CD103+ ITICs; adjusted HR 0.23 (95% CI: 0.14-0.38), p< 0.001 (Figure 4b). Furthermore, to evaluate any additional prognostic value of CD8+ TILs we analysed cetuximab treated patients with high ITIC CD103, and compared high versus low CD8+ TILs tumors. Three year FFS was 92% in patients with high CD8+ TILs/high CD103+ ITICs versus 90% in patients with low CD8+ TILs/high CD103+ ITICs; adjusted HR = 0.80 (95% CI: 0.24-2.67), p=0.72 (Supplementary Figure 6)

Discussion

The key finding from our study was that in patients with HPV-associated OPSCC treated with the deintensified cetuximab/radiotherapy regimen, high abundance of CD103+ ITICs was predictive of superior FFS and OS, consistent with our hypothesis. Furthermore, in this low risk HPV-associated OPSCC population, the high CD103 group has an excellent prognosis irrespective of whether cisplatin or cetuximab are combined with radiotherapy. On the other hand our results suggest that the inferior survival now reported with cetuximab in several trials may be due to increased locoregional and distant failures in patients with low CD103 tumors. These results do not alter the current standard of care, cisplatin and radiotherapy. The randomized trials did not show a significant improvement in overall tolerability of cetuximab compared to cisplatin[2-4], so even in the high CD103 population cisplatin would still be preferred. In patients with a contraindication to cisplatin, cetuximab would be a reasonable option in the high CD103 population, but other alternatives such as carboplatin based regimens may be preferable in low CD103 patients[17, 18].

These results in cetuximab and radiotherapy treated patients build on our previous report of the excellent and superior prognosis of unselected patients with high CD103 tumors when
treated with platinum based chemoradiation[15]. However, in the current study we did not find any significant differences in outcome between high and low CD103 patients treated with cisplatin. Key differences between the patients treated with cisplatin in this study compared to our previous publication, are the restriction of eligibility to modified low risk patients by the Ang criteria[7] compared to the inclusion of all HPV-associated OPSCC patients in the previous study i.e., low and intermediate risk HPV-associated OPSCC and the inclusion of patients in the previous study who would not have met other TROG 12.01 or De-ESCALaTE trial eligibility criteria. This is reflected in the 3 year FFS for cisplatin treated patients with low CD103+ ITICs in the current study of 89% versus 82% in our previous study. Due to the low number of events in the cisplatin treated patients in the current study, the study is underpowered to detect any difference in outcome based on CD103 group. Our results in the cetuximab treated patients are consistent with our previous findings using the same CD103 criteria in HPV-associated OPSCC in a higher risk population treated predominantly with platinum based chemoradiation[15]. We have demonstrated that we can detect a difference based on CD103 abundance when analysing HPV-associated OPSCC cohorts with a significant number of events e.g, unselected patients treated predominantly with platinum based chemoradiation or low risk patients treated with cetuximab.

TILs, and CD8+ TILs specifically, have been found to be prognostic factors in many cancers[9, 19, 20]. This is also the case in our current study, but the effect size is less than what we observed with CD103. Moreover, CD103+ ITIC abundance could stratify cetuximab/radiotherapy patients with abundant CD8+ TILs into distinct prognostic groups. These findings are consistent with our previous findings that CD103+ ITIC abundance could
stratify platinum/radiotherapy patients with abundant CD8\(^+\) TILs into distinct prognostic groups\([15]\). The use of high CD103 improves identification of a lower risk excellent prognosis population compared to use of CD8\(^+\) T cells alone.

The inferior outcomes with cetuximab and radiotherapy in randomised trials were unexpected and concerning\([8]\). The results of the cisplatin versus cetuximab randomized trials reinforced the excellent efficacy of the standard of care cisplatin and radiotherapy, and highlighted that caution is required in the design of future de-escalation trials\([8]\). An important consideration is that the risk stratification systems in place e.g., Ang criteria\([7]\), were developed in patients treated with cisplatin and radiotherapy, and do not appear to be adequate to identify low risk patients suitable for de-intensification trials. The fact that a defined low risk population does well with standard intensive treatment, does not imply that they are genuinely low risk suitable for less intensive treatment. Our results suggest that CD103 may be a suitable biomarker for selection of a better defined low risk population for de-intensification trials. As patients with high CD103 tumors when treated with cetuximab and radiotherapy, a regimen with overall suboptimal efficacy in HPV-associated OPSCC, do very well with 92\% 3-year FFS and 100\% 3-year OS it supports the notion that the high CD103 population represents a genuine low risk population. In view of these results and our previous findings of superior outcomes in unselected patients with high ITIC CD103 tumors treated with cisplatin\([15]\), we speculate that high CD103 identifies a low risk population irrespective of the systemic therapy given concurrently with radiotherapy, i.e. whether it is the gold standard cisplatin or a now established inferior drug, cetuximab. The addition of ITIC CD103 abundance to a multivariable prognostic model resulted in a significant improvement in the C-statistic, consistent with our previous findings in the retrospective cohorts. These
results confirm the prognostic value of ITIC CD103, but the major potential clinical utility is the identification of an excellent prognosis subgroup. Our previously reported findings that high CD103 HPV-associated OPSCC is associated with high expression of immune checkpoint markers including PD1, TIM3 and LAG3, and also with gene signatures associated with responses to pembrolizumab and atezolizumab raise the possibility that these cancers may be responsive to strategies incorporating immunotherapy[15].

Limitations of this study include the fact that it was not prospectively planned as part of the original trial designs, although analyses were conducted on a formal protocol with pre-specified cut-offs and used prospectively collected clinical trial data. Other limitations include different eligibility criteria between the two trials with AJCC 8 stage 3 patients only eligible for De-ESCALaTE, different cisplatin schedules used in the two trials albeit with excellent outcomes in the control arms of both trials, and low total numbers especially for analysis of the cisplatin treated patients. There was a lower percentage of De-ESCALaTE patients with available samples for biomarker analysis (45% versus 87% of TROG 12.01 patients). However, the baseline characteristics of patients with biomarker data available were similar to the total population and the outcomes were similar as well. Therefore, it seems unlikely that there is selection bias in patients with available tissue. Due to the number of events reported in each trial an a priori decision was made to pool the data from both trials to have adequate power instead of using one trial as training cohort and the other trial as validation cohort. However, an exploratory analysis was performed showing similar findings in each individual trial to what was observed in the combined analysis.
In conclusion, we have established in patients treated on two randomised trials that CD103+ ITIC abundance separates cetuximab/radiotherapy treated patients into excellent and poor prognosis subgroups, and that the increase in locoregional and distant failures seen with cetuximab occurs predominantly in the low CD103+ ITIC population. The high CD103+ ITIC population is a rational target for future de-intensification trials.

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<td><strong>AJCC 8 N category, n (%)</strong></td>
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<td>N0</td>
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<td>N3</td>
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<td><strong>AJCC 7 stage, n (%)</strong></td>
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<tr>
<td>III</td>
<td>64 (19%)</td>
<td>14 (8%)</td>
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<td>IV</td>
<td>270 (81%)</td>
<td>168 (92%)</td>
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<tr>
<td><strong>AJCC 8 stage, n (%)</strong></td>
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<tr>
<td>I</td>
<td>188 (56%)</td>
<td>121 (66%)</td>
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<tr>
<td>II</td>
<td>88 (26%)</td>
<td>61 (34%)</td>
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<tr>
<td>III</td>
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<tr>
<td>Neck irradiation, n (%)</td>
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<tr>
<td></td>
<td>Bilateral</td>
<td>Unilateral</td>
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<tr>
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<td></td>
<td>266 (80%)</td>
<td>151 (83%)</td>
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<td>68 (20%)</td>
<td>31 (17%)</td>
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**Figures**

**Figure 1a** - Failure-free survival in cetuximab treated patients; high versus low CD103+ ITIC abundance

**Figure 1b** - Overall survival in cetuximab treated patients; high versus low CD103+ ITICs

**Figure 2a** - Failure-free survival in cetuximab treated AJCC 8 stage 1 patients; high versus low CD103+ ITICs

**Figure 2b** - Failure-free survival in cetuximab treated AJCC 8 stage 2 patients; high versus low CD103+ ITICs

**Figure 2c** - Pattern of failure; cetuximab treated patients with low CD103 tumors

**Figure 2d** - Pattern of failure; cetuximab treated patients high CD103 tumors

**Figure 3a** - Failure-free survival in cisplatin treated patients high versus low ITIC CD103

**Figure 3b** - Overall survival in cisplatin treated patients high versus low ITIC CD103

**Figure 4a** - Failure-free survival in cetuximab treated patients; high versus low CD8+ TIL abundance

**Figure 4b** - Failure-free survival in cetuximab treated patients with high CD8+ TILs; high versus low CD103+ ITICs

**Supplementary File**

**Supplementary Table 1** – C –statistics for multivariable model with and without CD103

**Supplementary Figure 1** - Failure-free survival in patients with biomarker data available; cisplatin versus cetuximab
**Supplementary Figures 2a** – Failure-free survival in patients with high ITIC CD103 abundance; cetuximab versus cisplatin

**Supplementary Figures 2b** - Overall survival in patients with high ITIC CD103 abundance; cetuximab versus cisplatin

**Supplementary Figures 3a** – Failure-free survival in patients with low ITIC CD103 abundance; cetuximab versus cisplatin

**Supplementary Figures 3b** - Overall survival in patients with low ITIC CD103 abundance; cetuximab versus cisplatin

**Supplementary Figure 4a** - Failure-free survival in cetuximab treated patients on TROG 12.01; patients with biomarker versus all patients

**Supplementary Figure 4b** - Failure-free survival in cetuximab treated patients on De-ESCALaTE; patients with biomarker versus all patients

**Supplementary Figure 4c** - Failure-free survival in cisplatin treated patients on TROG 12.01; patients with biomarker versus all patients

**Supplementary Figure 4d** - Failure-free survival in cisplatin treated patients on De-ESCALaTE; patients with biomarker versus all patients

**Supplementary Figure 5a** - Failure-free survival in cetuximab treated patients on TROG 12.01; high versus low CD103\(^+\) ITIC abundance

**Supplementary Figure 5b** - Failure-free survival in cetuximab treated patients on De-ESCALaTE; high versus low CD103\(^+\) ITIC abundance
**Supplementary Figure 5c** - Failure-free survival in cisplatin treated patients on TROG 12.01; high versus low CD103+ ITIC abundance

**Supplementary Figure 5d** - Failure-free survival in cisplatin treated patients on De-ESCALaTE; high versus low CD103+ ITIC abundance

**Supplementary Figures 6** - Failure-free survival in cetuximab treated patients with high ITIC CD103 abundance; high versus low CD8+ TILs
**Figure 1a** - Failure-free survival in cetuximab treated patients; high versus low CD103+ ITIC abundance

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Figure 2d - Pattern of first failure; cetuximab treated patients high CD103 tumors
Figure 3a - Failure-free survival in cisplatin treated patients high versus low ITIC CD103

Figure 3b - Overall survival in cisplatin treated patients high versus low ITIC CD103
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**Figure 4b** - Failure-free survival in cetuximab treated patients with high CD8⁺ TILs; high versus low CD103⁺ ITICs
Supplementary Table 1 – C –statistics for multivariable model with and without CD103

<table>
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<tr>
<th>Subset</th>
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<td>Cetuximab</td>
<td>FFS</td>
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<td>0.74</td>
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