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Original Research

Chromosome 3q arm gain linked to immunotherapy response in advanced cutaneous squamous cell carcinoma

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Abstract ***Aims:*** The activity that the immune checkpoint inhibitor (ICI) cemiplimab has recently demonstrated has led to a paradigm shift in the management of patients with advanced cutaneous squamous cell carcinoma (cSCC). To identify predictive biomarkers of response to ICIs in advanced cSCC, we studied 33 patients who received ICI therapy at the Dana-Farber/Harvard Cancer Center (DF/HCC) and analysed sequencing data for a subset of these patients.

Methods: We collected clinical data using electronic health records and genomic data using the institutional OncoPanel platform of the DF/HCC. We compared tumour genomics with data from previously sequenced cSCC cohorts.

Results: We observed high tumour mutational burden regardless of smoking status and response to ICI and longer median overall survival among those patients who achieved an ICI response. We compared the genetic data from our cohort with data from other cohorts that included fewer patients with distant metastatic disease. Although our cohort had a similar genetic landscape to those of comparator cohorts, mutations in *PIK3C2B* were more common in our study. In our cohort, copy number alterations (CNAs) in the 3q chromosomal arm appeared to predict response to ICI therapy.

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Conclusion: CNAs in the 21–27 bands of chromosome arm 3q, a region that includes *PIK3CA, ETV5* and *BCL6,* may represent predictors of response to ICI and may be candidates for drug targeting in combination or sequence with ICI agents.

1. Introduction

An estimated 95 of 100,000 individuals are diagnosed with cutaneous squamous cell carcinoma (cSCC) in the United States (US) each year [1]. Despite a relatively low rate of death across all patients (2.1% annually in the US), survival among those with advanced or metastatic incurable disease is poor, with an estimated 3-year survival of 55% [2]. Until recently, there has been little consensus on treatment recommendations for these patients other than extrapolating from management in other SCC subtypes.

The advent of immune checkpoint inhibitor (ICI) therapy has generated excitement for the treatment of locoregionally advanced and of recurrent or metastatic cSCC. Recently, Migden et al [3] reported an impressive 47% response rate to the programmed cell death-1 (PD-1) inhibitor cemiplimab in 59 patients with metastatic cSCC. With a median follow-up of 7.9 months, 1-year survival was estimated at 81%. The response exceeded 6 months in 57% of responders, suggesting that ICI therapy may be associated with particularly durable responses. In light of this promising data, the US Food and Drug Administration approved cemiplimab in September 2018. The perennial question, however, persists: how can we effectively select patients who we expect will derive benefit from ICI therapy in this disease?

Previous work has provided insight into the genomic landscape of aggressive or metastatic cSCC. The disease is characterised by a high mutational tumour burden attributable to ultraviolet (UV) exposure and DNA damage and is often associated with alterations in tumour suppressor genes [4,5]. In the present study, we seek to understand the clinical and molecular characteristics associated with ICI response in advanced cSCC.

2. Methods

2.1. Study cohort

Thirty-three patients with biopsy-proven cSCC treated with PD-1 blockade (either off-label or on protocol) at the Dana-Farber/Harvard Cancer Center from 2013 to 2018 were identified retrospectively following institutional review board approval. Sixteen patients (48%) had archival tumour material available for targeted massively parallel sequencing (MPS). We obtained tissue samples from the primary tumour (n = 9), a site of local recurrence (n = 1) or a site of distant metastasis (n = 6)—whichever was available (no matched primary and recurrent samples were available). We recorded patient demographics, clinicopathologic features and treatment outcomes. We recorded response data using Response Evaluation Criteria in Solid Tumors version 1.1 [6].

2.2. Targeted MPS

All sequenced patients separately consented to our institutional Cancer Research Study [7], and a clinical laboratory improvement amendments-certified laboratory performed molecular testing. For patient tumour samples to be eligible, we required the availability of greater than 20% tumour on haematoxylin and eosin slide review. As previously described, we used Qiagen kits to isolate and Qubit dsDNA detection (Invitrogen) to quantify isolated DNA. Libraries were prepared and hybridised to a custom biotinylated RNA bait set (Agilent SureSelect) targeting the full coding regions of 447 genes and selected intronic regions of 60 genes (OncoPanel version 3) [8,9]. Hybrid-capture libraries were sequenced on an Illumina HiSeq 2500 using 2 × 100 paired-end reads. The mean sequencing coverage was 318x unique, high-quality mapped reads per sample (range: 80x to 640x; 50x minimum required to pass quality control). We used an existing custom bioinformatics pipeline for data analysis [8–11]. We calculated total mutational burden (TMB) by determining the number of non-synonymous somatic mutations per megabase (Mb) across all genes on the panel.

2.3. Statistical analysis

We used Student's t-test for continuous variables and Fisher's exact test for categorical variables to compare clinical characteristics by response. We used Fisher's exact test to compare mutation frequencies between genomic cohorts. We used logistic regression analyses to calculate odds ratios for response with respect to clinical and molecular characteristics and used one-way analysis of variance to calculate differences in gene-level copy number alterations (CNAs) by response. We applied Kaplan–Meier statistics and log-rank testing to evaluate survival. Our multiple regression analysis used the Cox proportional hazards model. We conducted separate univariate analyses if sample numbers were limited. All statistical tests used a significance cut-off of less than 0.05 and were 2-sided. Data were analysed using Stata/IC (version 14.2).

3. Results

3.1. Clinical characteristics

The cohort predominantly comprised middle-aged men, with 55% of the cohort being current or former smokers (Table 1). Nine of 33 patients had a history of immune suppression, including five patients with history of haematologic malignancy, two with well-controlled human immunodeficiency virus and two with a history of organ transplant. The most common primary site of disease was the head and neck (61%). Over half of the patients had stage III or IV disease at diagnosis (57%). Surgery with adjuvant radiation (with or without concurrent chemotherapy) was the most common approach for initial therapy.

3.2. Response outcomes and survival

All 33 patients received single-agent PD-1 blockade either on-protocol or off-protocol. Time on therapy ranged from 0 months (one dose) to 22.1 months; 13 patients were still on therapy at the time of data reporting. Fifteen (45%) patients demonstrated a response (six with a complete response [CR] and nine with a partial response [PR]). Among the non-responders, six experienced stable disease (SD) with 67% (4/6) lasting 12 months or greater, and 12 of them had progressive disease (PD). The clinical benefit rate (CBR; defined as the aggregate of CR, PR and SD rates) was 64%. Patients with a smoking history of at least 10 pack-years were less likely to respond to ICI therapy ($p < 0.01$ in univariate analysis [Table 1] and $p = 0.02$ in multivariate analysis [Table 2]). Other clinicopathologic features were all similar among responders and non-responders. There were two renal transplant recipients, both being responders.

During a median follow-up of 17.8 months, 12 of 33 (36%) patients had died. Median overall survival (OS) was not reached in the full cohort or among responders, but median OS was 11 months for non-responders (hazard ratio, 0.18; confidence interval, 0.06–0.57; $p = 0.01$) (Fig. 1). The median OS was 5.3 months among patients with PD as best response. One-year OS was 58% for the entire cohort, 85% among those with clinical benefit to ICI therapy and 23% for patients with PD. On multivariate analysis, no single clinicopathologic feature correlated significantly with survival (Table 3). Histology (degree of differentiation) did not correlate with survival on univariate analysis. The two solid organ transplant patients were both alive at the time of data reporting. After 9 months on ICI therapy, one with a CR experienced transplant rejection. The other, with a PR, continued on ICI therapy and had not experienced organ rejection, now 4 months into therapy.

3.3. Molecular insights

Sixteen (48%) patients had sufficient archival tissue for targeted MPS: nine (56%) from the primary tumour and seven (44%) from a recurrent or metastatic focus. Eight responders and eight non-responders were sequenced. The median TMB was 33 mutations/Mb (range: 4–137) among sequenced patients, with the most common single nucleotide variants (SNVs) being *TP53* (81%), *NOTCH1* (63%) and *KMT2D* (63%) (Fig. 2). SNVs among the MD Anderson ($n = 39$) and Dana-Farber Cancer Institute ($n = 29$) advanced cSCC cohorts are shown for comparison [4,5]. Our cohort showed a trend towards a greater proportion of patients with SNVs in *PIK3C2B* (44% vs. 6%, $p = 0.06$) comparatively. These prior cohorts focused on those with lymph node metastases (10% of patients in comparator cohorts had distant metastatic disease versus 56% in our cohort). When separating our cohort by sequenced primary and recurrent/metastatic (R/M) sites of disease, we found that *NOTCH2* alterations were more common among R/M sites compared with primary sites (71% vs. 11%, $p = 0.02$). SNVs were similar between responders and non-responders. Alterations in *NF1* trended towards being more frequent among non-responders (63% vs. 13%, $p = 0.06$). The difference in median TMB by treatment response was not significant (median 41 vs. 27 mutations/Mb, $p = 0.67$), and TMB did not correlate with survival at this cohort size. Of the 11 samples for which UV and tobacco mutational signature analysis could be performed, all were positive for a UV signature and negative for a tobacco signature. Smokers had a lower TMB compared with non-smokers (median 14 vs. 54 mutations/Mb, $p = 0.01$).

CNAs occurred at a median rate of 65 per sample (range: 0–240) (Fig. 3). When accounting for primary and R/M disease sites among our sequenced cohort, CNAs in 10q23–24 were more often noted among R/M sites (50% vs. 0%, $p = 0.04$). There was a trend towards higher frequency of CNAs among responders (median of 97 vs. 47.5, $p = 0.12$). We found that, along bands 21–27 on the q arm of chromosome 3, 75% of responders had low-copy gains, whereas one (13%) non-responder had a single-copy deletion ($p < 0.01$). Furthermore, the two responders, with no CNAs in the 3q21–27 region, each had two SNVs in the region. CNAs in *CDKN2A* and *CDKN2B* (in band 9p21.3) were present in more than half of sequenced cases (10/16, 63%), but the difference in the number of CNAs in *CDKN2A/B* was not significant based on response ($p = 0.36$).

4. Discussion

We aimed to nominate clinical and molecular biomarkers that might predict benefit to ICI in advanced cSCC, given that fewer than half of patients demonstrate response. ICI therapy was associated with similar

Table 1
Demographics, clinical and survival characteristics of patients with advanced cSCC treated with an ICI (univariate analyses).

Characteristic	All (%)[a], $N = 33$	Responders (%), $N = 15$	Non-responders (%), $N = 18$	p-value*
Median age in years (range)	68 (52–96)	67 (52–89)	73.5 (54–89)	0.40
Sex				
Male	27 (82)	11 (73)	16 (89)	0.38
Female	6 (18)	4 (27)	2 (11)	
Smoking history				
Never or < 10 pack-year	15 (45)	11 (73)	4 (22)	<0.01
Former or current (≥10 pack-year)	18 (55)	4 (27)	14 (78)	
Immune suppression				
None	24 (73)	11 (73)	13 (72)	1.00[b]
Haematologic malignancy[c]	5 (15)	2 (13)	3 (17)	
Human immunodeficiency virus	2 (6)	0 (0)	2 (11)	
Solid organ transplant[d]	2 (6)	2 (13)	0 (0)	
Primary site of disease				
Head and neck	20 (61)	10 (67)	10 (56)	0.72[e]
Limb	7 (21)	4 (27)	3 (17)	
Torso	2 (6)	0 (0)	2 (11)	
Unknown	4 (12)	1 (7)	3 (17)	
Initial staging at diagnosis[f]				
Stage I	5 (15)	2 (13)	3 (17)	0.48
Stage II	9 (27)	3 (20)	6 (33)	
Stage III	4 (12)	3 (20)	1 (6)	
Stage IV	15 (45)	7 (47)	8 (44)	
Pathology				
Well differentiated	4 (12)	3 (20)	1 (6)	1.00[g]
Moderately differentiated	7 (21)	2 (13)	5 (28)	
Moderately to poorly differentiated	3 (9)	3 (20)	0 (0)	
Poorly differentiated	17 (52)	7 (47)	10 (56)	
Not known	4 (12)	3 (20)	1 (6)	
Initial treatment regimen				
None	1 (3)	1 (7)	0 (0)	–
Surgery	8 (24)	4 (27)	4 (22)	
Surgery + radiation	13 (39)	6 (40)	7 (39)	
Surgery + CRT	8 (24)	3 (20)	5 (28)	
Definitive CRT	2 (6)	0 (0)	2 (11)	
Chemotherapy	1 (3)	1 (7)	0 (0)	
Toxicity related to ICI				
Grade 1 or lower	26 (79)	10 (67)	16 (89)	0.20
Grade 2 or above	7 (21)	5 (33)	2 (11)	
Targeted sequencing data (N = 16)				
From the primary tumour	9 (56)	3 (38)	6 (75)	–
From a local recurrence	1 (6)	1 (13)	0 (0)	
From a metastatic focus	6 (38)	4 (50)	2 (25)	
Survival outcomes				
Number of deaths	12 (36)	2 (13)	10 (67)	
Median OS in months (95% CI)	NR (5.8-NR)	NR (NR–NR)	11.1 (2.7-NR)	0.01
1-year OS % (95% CI)	63 (42–78)	86 (54–96)	45 (20–67)	–
Median follow-up in months (range)	17.8 (2.6–87.1)	22.7 (13.8–35.7)	16.5 (2.6–87.1)	–

CRT, concurrent chemoradiation; cSCC, cutaneous squamous cell carcinoma; ICI, immune checkpoint inhibitor; NR, not reached; OS, overall survival.
(*) $p < 0.05$, Student's t-test for continuous variables, Fisher exact test for categorical variables, log-rank testing based on hazard ratios for survival data. All tests were univariate and two-sided.
[a] Except for age.
[b] Immunosuppressed vs. not.
[c] Two patients with history of chronic lymphocytic leukaemia, one with acute myeloid leukaemia, one with marginal zone lymphoma and one with prolymphocytic T-cell leukaemia.
[d] One kidney transplant patient received sirolimus and no systemic steroids, whereas the other kidney transplant patient received everolimus and a tapered regimen of 40 mg, 20 mg and 10 mg of prednisone scheduled around ICI treatments.
[e] Head and neck vs. any other (including unknown).
[f] American Joint Committee on Cancer (AJCC) 8th edition staging.
[g] Well/moderately vs. moderately to poorly/poorly.

Table 2
The effect of clinical, pathologic and genetic features on ICI response in patients with advanced cSCC (multivariate logistic regression modelling, unless otherwise noted).

Variable	Total (n = 33)		
	OR	95% CI	p-value
Older age	0.99	0.90–1.09	0.78
Female sex	3.87	0.31–48.17	0.29
Current/former smoker (≥10 pack-years)	0.10	0.01–0.69	0.02
Immunosuppression	0.83	0.10–7.05	0.87
Head/neck primary site (vs. other primary)	0.92	0.14–6.10	0.93
Stage III or IV disease at diagnosis	2.01	0.34–11.72	0.44
Poor or moderate-to-poor differentiation[a]	1.20	0.27–5.25	0.81
TMB[a]	0.99	0.96–1.02	0.65
NF1 mutation[a]	0.09	0.01–1.08	0.06
AR mutation[a]	0.14	0.01–1.76	0.13
PIK3CA copy number alteration[a,b]	–	–	0.02
3q arm copy number alteration[a,b]	–	–	<0.01

cSCC, cutaneous squamous cell carcinoma; CI, confidence interval; ICI, immune checkpoint inhibitor; OR, odds ratio (response vs. non-response); TMB, total mutational burden.
[a] Separate univariate analyses (data not available for all patients).
[b] One-way analysis of variance analyses (low-copy gain vs. no alteration vs. single-copy deletion).

response rates in our cohort compared with the phase II cemiplimab trial (45 vs. 47%), although the CBR was slightly lower in our study (64% vs. 73%). One-year survival in our cohort was also lower when compared with the cemiplimab phase II trial (63% vs. 81%). These differences likely result from variation in patient populations: the cemiplimab trial excluded patients with a life expectancy of <12 months, immunosuppressed patients, transplant recipients and patients with a lower performance status. Non-smokers in our cohort were more likely than smokers to respond to ICI therapy, contrary to data from patients with SCC of the head and neck and lung cancer [10,12]. We suspect this observation occurred because UV-induced DNA damage was the dominant driver of TMB in this cohort, as opposed to smoking status (smokers had a lower TMB relative to non-smokers). In support of this speculation, our prior work has shown that the median TMB among smokers in a sequenced SCC of the head and neck cohort was around 10.3 mutations/Mb which is similar to the median TMB of 14 in our cSCC smokers, whereas our cSCC non-smokers had a median TMB of 54. No other clinical characteristics were predictive of response, although the lack of significance may be related to our sample size. Although degree of histologic differentiation has been associated with the propensity for cSCC to metastasise [13,14], we did not find that histology correlated with survival among our ICI-treated patients (most of whom were already metastatic) as molecular findings may be more prognostic in this scenario. The fact that the two solid organ transplant patients, including one on daily steroids, were responders despite the risk of tranplant rejection was of interest. Whether long-term, low-dose steroids (in the setting of everolimus) help avoid rejection and whether their effects blunt ICI response remain open questions. Recent findings in a large non–small cell lung cancer population showed lower ICI response rates and survival among baseline corticosteroid users [15]. As expected, the difference in survival between responders and non-responders was significant. Eighty-six percent of responders were still alive at one year compared with 45% of non-responders. No recorded clinical features correlated significantly with survival, although future studies should incorporate more patients.

Compared with previously sequenced cSCC cohorts, our cohort had a higher proportion of patients with distant metastatic disease and a higher proportion of patients with *PIK3C2B* alterations. This is in line with previous data showing that *PI3K* pathway mutations are associated with more advanced disease in SCCs of the head and neck [16]. Of note, the *PIK3C2B* mutations in our cohort were observed at nearly equal frequency among primary and R/M disease sites sequenced, supporting that these alterations may occur early to promote metastatic potential. *PI3K* is associated with cellular switching from E-cadherin expression to N-cadherin expression, which in turn promotes epithelial-to-mesenchymal transition and, thus, metastasis [17].

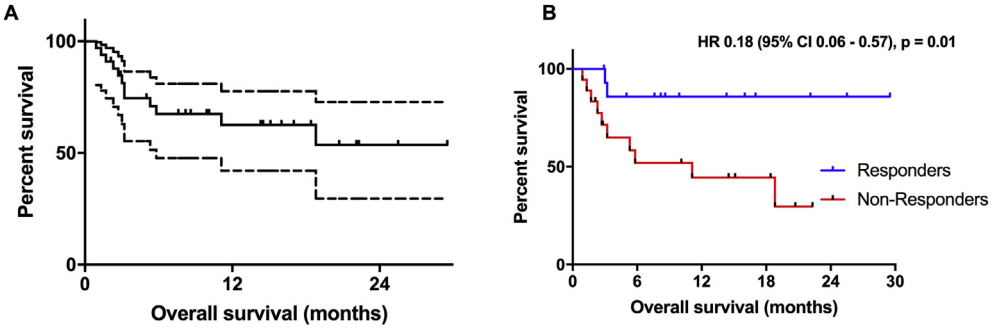

Fig. 1. Survival outcomes in cutaneous squamous cell carcinoma patients treated with an ICI. (A) Overall survival (in months) among 33 patients with cutaneous squamous cell carcinoma treated with an ICI. Dotted lines represent 95% CIs. (B) Overall survival among patients with cutaneous squamous cell carcinoma based on response to ICI. CI, confidence interval, HR, hazard ratio, ICI, immune checkpoint inhibitor.

Table 3
The effect of clinicopathologic features on survival outcomes in patients with cSCC treated with ICIs (Cox proportional hazards modelling, unless otherwise noted).

Variable	Total (n = 33)		
	HR	95% CI	p-value
Older age	0.99	0.92–1.07	0.84
Female sex	0.61	0.05–7.69	0.70
Current/former smoker (≥10 pack-years)	0.66	0.10–4.56	0.67
Immunosuppression	0.74	0.13–4.13	0.73
Head/neck primary site (vs. other primary)	0.42	0.09–1.98	0.28
Stage III or IV disease at diagnosis	0.90	0.54–1.49	0.69
Response to ICI	0.10	0.01–0.89	0.04
Poor or moderate-to-poor differentiation[a]	0.86	0.24–3.11	0.82
TMB[a]	1.01	0.97–1.04	0.64

CI, confidence interval; cSCC, cutaneous squamous cell carcinoma; HR, hazard ratio; ICI, immune checkpoint inhibitor; TMB, total mutational burden.
[a] Separate univariate analyses (data not available for all patients).

Although speculative, mutations in *PIK3C2B* or in other *PI3K* pathway genes may be useful as negative prognostic markers in cSCC. In addition, our sequenced tumours from R/M sites (as compared with primary sites) more often demonstrated alterations in *NOTCH2*—a highly conserved receptor and regulator of normal cellular signal transduction among epithelial cells [18], with inactivating mutations inhibiting its tumour suppressor function, to promote cellular growth.

The mutational profile of responders was overall similar to that of non-responders. It is worth noting that SNVs in *NF1* trended towards being more common among non-responders (p = 0.06). *NF1* mutations are linked to high absolute T-cell counts (because of *RAS*-mediated hyperplasia of the thymus and spleen) but with lower T-cell receptor and interleukin-2 receptor–mediated proliferation of thymocytes and mature T cells [19]. Although speculative, it may be that loss of *NF1* is associated with increased production of dysfunctional T cells, which might blunt response to PD-1 inhibition. Interestingly, *NF1* mutations are associated with PD-L1–positive tumours in melanoma [20]. Paradoxically, recent studies have shown that higher levels of PD-L1 expression are associated with greater risk of metastasis but with longer disease-free

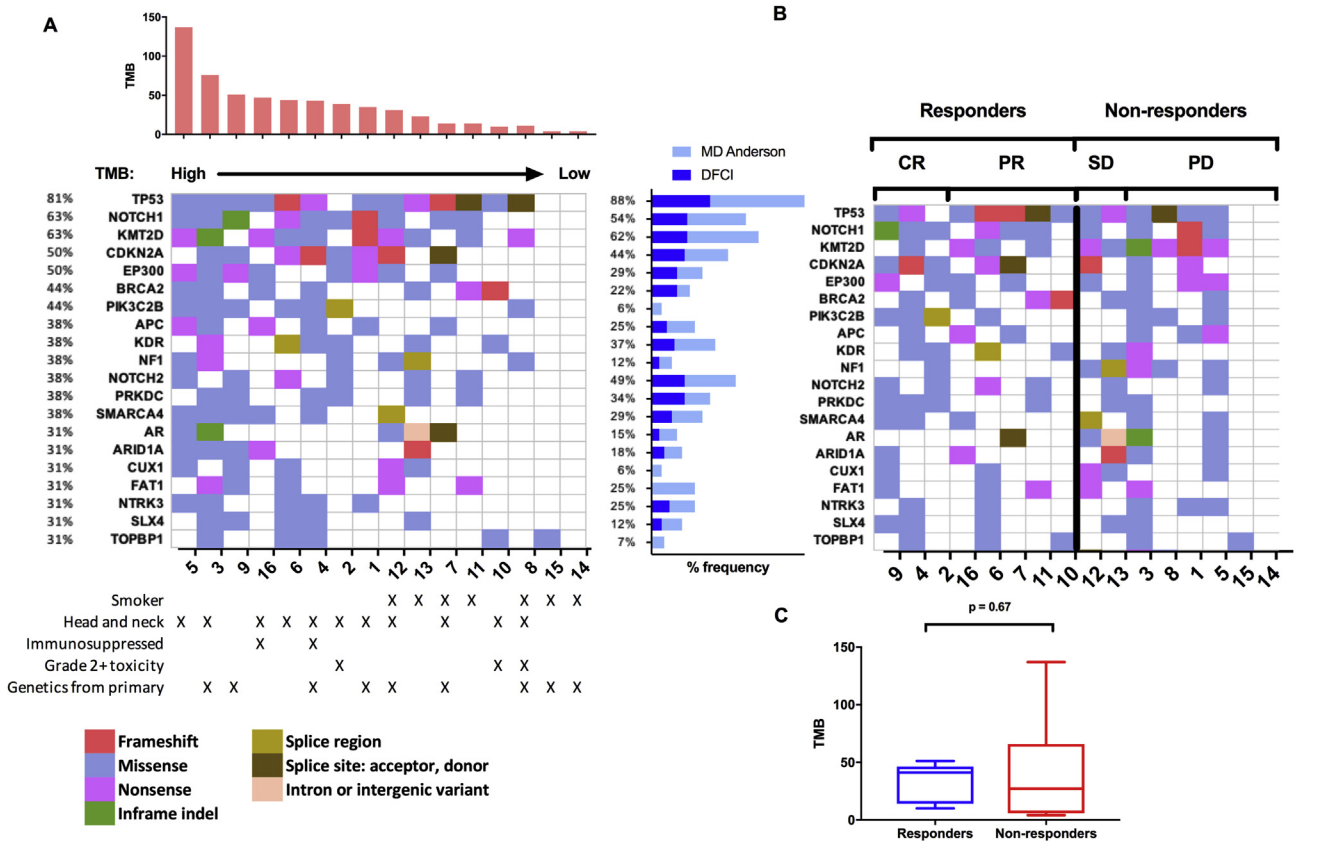

Fig. 2. Mutational landscape of cutaneous squamous cell carcinoma: overall and by response to ICI. (A) Mutational plot showing the most frequently mutated genes (top to bottom, >30% in the present cohort) with gene frequency listed at the left (%) among 16 patients with cutaneous squamous cell carcinoma who were treated with ICIs and had genetic sequencing performed. TMB is represented in the above plot, and clinical data is represented in the below plot. The vertical bar graph at right shows mutational frequency compared with sequencing results among 29 patients in the Dana-Farber Cancer Institute (DFCI) and 39 patients in the MD Anderson cohort. (B) Mutational plot with patients ordered by response to immune checkpoint inhibition. Genes are ordered by overall frequency rank among all patients in the cohort. (C) Difference in TMB for responders vs. non-responders. CI, confidence interval, CR, complete response; ICI, immune checkpoint inhibitor, PD, progressive disease; PR, partial response; SD, stable disease; TMB, total mutational burden.

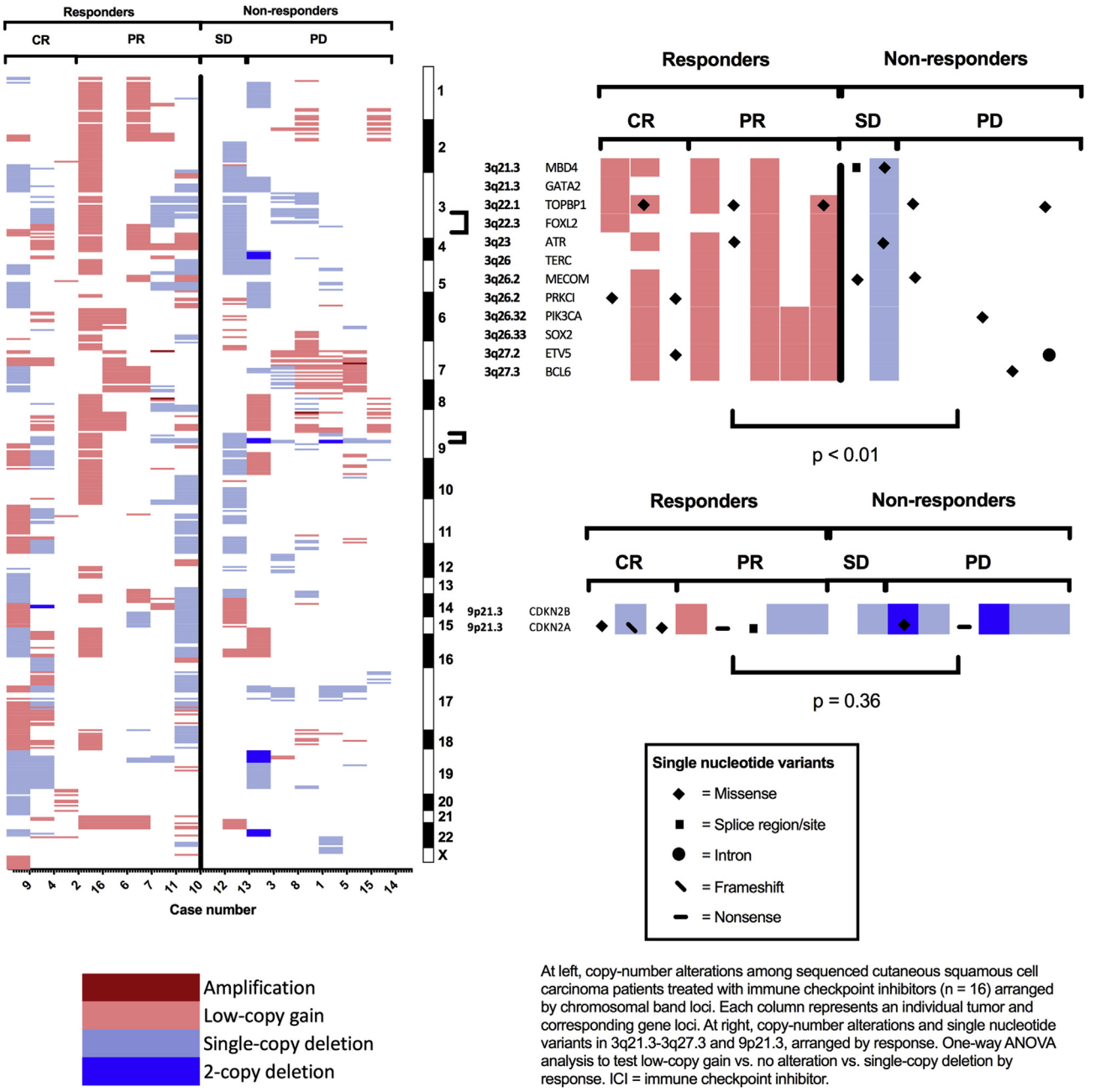

Fig. 3. Copy number alterations in cutaneous squamous cell carcinoma patients treated with ICIs. At left, copy number alterations among sequenced patients with cutaneous squamous cell carcinoma treated with ICIs (n = 16) arranged by chromosomal band loci. Each column represents an individual tumour and corresponding gene loci. At right, copy number alterations and single nucleotide variants in 3q21.3–3q27.3 and 9p21.3, arranged by response. One-way ANOVA analysis to test low-copy gain vs. no alteration vs. single-copy deletion by response. ANOVA, analysis of variance; CR, complete response; ICI, immune checkpoint inhibitor; PD, progressive disease; PR, partial response; SD, stable disease.

survival in immunotherapy-naive populations [21–25]. Future clinical trials in cSCC will surely seek to verify whether the presence of any amount of tumour and/or immune cell PD-L1 expression predicts response to ICI therapy or whether the degree of PD-L1 expression correlates with the degree of response.

R/M sites of disease among our sequenced cohort more often demonstrated CNAs in 10q23–24 compared with primary sites, a region of 10q with genes that function to control tumour growth—most notably *PTEN* which has important tumour suppressor function in cancer [26]. The landscape of CNAs was altogether similar between responders and non-responders. An exception was the 3q21–27 chromosomal region, in which six of eight responders had a low-copy gain and one of eight non-responders had a single-copy deletion. This region contains genes such as *ETV5, PIK3CA* and *BCL6* with importance in cancer. In neural stem cells,

ETV5 is upregulated downstream of the *NF1-RAS* pathway [27]. If it is the case in cSCC that *NF1* and *ETV5* act as part of the same pathway, then it is reasonable that a gain of function in *ETV5* is associated with ICI response, although loss of function in *NF1* is associated with lack of response. The fact that low-copy gains in *PIK3CA* are common among responders suggests that *PI3K* pathway signalling may be important for proper functioning of immune response to checkpoint inhibition. Indeed, the *PI3K* pathway has been shown to play a role in the differentiation of CD8 T cells [28]. Our finding suggests that patients in whom the *PI3K* pathway is particularly active may be, especially, appropriate candidates for ICI therapy. Furthermore, we speculate that *PI3K* inhibition may not be suitable in combination with ICI therapy in cSCC, but further studies are needed.

BCL6 is well known for influencing another component of the immune system, B cells [29]. In many lymphomas, *BCL6* is a transcriptional repressor that plays a role in B-cell proliferation. The fact that gains in *BCL6* are common in responders may indicate that functional B cells, in addition to T cells, are necessary for ICI therapy to stimulate effective immune-mediated anticancer activity. Although some B-cell populations (such as IgA+ and PD-L1+) may have immunosuppressive effects by binding PD-1 on cytotoxic T cells, others (such as IgG+) may contribute to an immune response by releasing antitumour antibodies [30]. Given that gains in *BCL6* were associated with response to immunotherapy in the present study, we hypothesise that *BCL6* is associated with the immune-stimulatory B-cell response.

The small size of our cohort was a limitation, but advanced cSCC is not particularly common. A limitation of our genetic analysis was the simultaneous consideration of genetic data collected using several editions of our sequencing platform in which genetic testing for some patients included more genes than others (from 275 in version 1 to 447 in version 3). However, all genes specifically mentioned here were present in all three versions of the sequencing panel. We did compare genomic differences among primary and R/M sites of disease sequenced among our cohort, but no matched patient samples were available. Although valuable to compare the molecular data between these subgroups, it also represents a limitation in terms of expected genomic heterogeneity among primary and metastatic tumour foci (although all biopsy samples were obtained before ICI exposure in our study). We also did not report tumour or immune cell PD-L1 expression among our cohort which has proven clinically meaningful in identifying ICI responders in other solid tumour types. Our observations about CNAs, although statistically significant, are preliminary observations and are speculative. In particular, gains were low-level, not true amplifications and were broad, not focal. However, because the CNAs were so different between responders and non-responders, the contrast may be meaningful.

Because response to ICI therapy predicts survival among cSCC patients, it is useful to identify which clinical and genetic characteristics can help predict response. We found that alterations in *NF1* and gains in the 3q21–27 region (a region that includes *ETV5, PIK3CA* and *BCL6*) are associated with response to ICI therapy. *PIK3CB* alterations may be associated with a worse prognosis (i.e. distant metastasis). Future research may help to validate these findings and provide further insight into their clinical and therapeutic potential.

Author contributions

A.J.K. and G.J.H. designed the study. A.J.K., E.J.H., J.H.L., R.I.H., N.G.C., G.R., N.R.L., C.D.S., M.T. and G.J.H. recorded and collected data. A.J.K. and G.J.H. analysed data. All authors were involved in writing the article and had final approval of the submitted and published versions.

Conflict of interest statement

A.J.K., E.J.H. and L.E.M. have no disclosures to report. J.H.L. receives consulting support from Bayer and Incyta and receives institutional research support from Bayer, Bristol-Myers Squibb and Novartis. R.I.H. receives consulting and research support from Merck & Co., Bristol-Myers Squibb, Pfizer, Genentech and AstraZeneca and consulting support from Loxo and Celgene. N.G.C. receives research funding from GlaxoSmithKline, Merck & Co. and Pfizer. G.R. consults for EMD Serono, Pfizer, Merck & Co., Regeneron, Sanofi and Castle and owns equity in Regeneron and Syros Pharmaceuticals. N.R.L. receives speaking fees from Bayer. C.D.S. receives research support from Genentech and Regeneron Pharmaceuticals and does consulting for Castle Biosciences and Regeneron Pharmaceuticals. M.T. consults for Merck & Co. G.J.H. receives institutional research support from BMS and EMD Serono and consulting honoraria from BMS, Regeneron and Sanofi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.03.004.

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