Genomic landscape of advanced basal cell carcinoma: Implications for precision treatment with targeted and immune therapies

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ABSTRACT
Metastatic basal cell cancer (BCC) is an ultra-rare malignancy with no approved therapies beyond Hedgehog inhibitors. We characterized the genomics, tumor mutational burden (TMB), and anti-PD-1 therapy responses in patients with locally advanced or metastatic BCC. Overall, 2,039 diverse cancer samples that had undergone comprehensive genomic profiling (CGP) were reviewed. Eight patients with locally advanced/metastatic BCC were identified (two had two CGP analyses; total, 10 biopsies). Two tumors demonstrated PD-L1 amplification. Seven patients had >1 actionable alteration. The TMB (mutations/mb) (median (range)) was 90 (3-103) for the BCCs versus 4 (1-860) for 1637 cancers other than BCC (P < 0.0001). Median progression-free survival (PFS) for all four patients treated with PD-1 blockade was 10.7 months (range, 3.8 to 17.6+ months); three patients had an objective response. In conclusion, advanced/metastatic BCC often has biological features (high TMB; PD-L1 amplification) predictive of immunotherapy benefit, and patients frequently respond to PD-1 blockade.

Introduction
Basal cell carcinoma is the most common malignancy with approximately 2.8 million cases diagnosed annually in the United States. Rarely, basal cell carcinomas can become locally destructive and/or metastasize.1 The development of metastatic basal cell carcinoma is highly dependent on the primary tumor size. Previous studies have shown that there is an approximately 2% incidence of metastasis for tumors larger than 3 cm in diameter. The incidence increases to 25% for tumors larger than 3 cm in diameter and is 50% for tumors larger than 10 cm in diameter. In addition, a greater than 90% metastasis rate has been observed for tumors that are greater than 25 cm.2,3

Most basal cell carcinomas harbor mutations in the Hedgehog pathway.4 Currently, the Hedgehog inhibitors vismodegib and sonidegib are approved by the Food and Drug Administration (FDA) for treating locally advanced/metastatic basal cell carcinoma.5,6 However, there are no approved therapies for patients who progress on Hedgehog inhibitors. Furthermore, response rates to Hedgehog inhibitors are only 30–45%, with a median duration of response of approximately six months.5,6

Antibodies blocking programmed death receptor (PD-1)/programmed death receptor – ligand 1 (PD-L1) show efficacy in both solid tumors and lymphoma.7,8 Biomarkers for response include PD-L1 positivity by immunohistochemistry, microsatellite instability, and high tumor mutations burden (TMB).7,8 Interestingly, approximately 90% of basal cell carcinomas are positive for PD-L1 by immunohistochemistry,9 and the intensity of PD-L1 staining on basal cell carcinoma cells increases with the number of prior treatment modalities.9 Furthermore, basal cell carcinoma is one of the most mutated types of human cancer.10 Similar to melanoma, the majority of mutations identified in basal cell carcinoma have an ultraviolet (UV) signature. Therefore, advanced basal cell carcinomas have features (PD-L1 positivity and high TMB) that would predict checkpoint inhibitor response.

There are a few case reports of successful treatment of refractory basal cell carcinoma with anti-PD-1 therapy.1,11,12 Herein, we present the genomic correlates of the largest case series to date of patients with advanced/metastatic basal cell cancer treated with anti-PD-1 therapy.

Results
Patient Characteristics: Nine biopsies from eight patients with locally advanced or metastatic basal cell carcinoma and a calculated TMB were identified (Supplemental Figure 1). Patient characteristics are listed in Table 1 and Supplemental Table 1. Five patients had locally advanced disease while three patients had metastatic disease.

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| Patient | Age (years) | Sex | Locally advanced or metastatic disease | TMB (mutations/mb) | Number of characterized alterations | Potentially actionable alterations with either an on or off-label FDA approved drug | Genomic alterations | Best response/PFS (months) to a PD-1 inhibitor (agent) | Best Response/PFS (months) to a Hedgehog inhibitor (agent) | Disease status at biopsy |
|---------|-------------|-----|----------------------------------------|-------------------|-------------------------------------|---------------------------------------------------------------------------------|------------------|-----------------------------------------------|---------------------------------------------------------------|--------------------------|
| 1       | 65          | M   | Locally advanced (large lesion of right auricle with recurrent disease after multiple resections and radiation) | 96                | 8                                   | 6 \(PTCH1\) Q576\* \(CB\) D861N \(CDKN2A\) p144ARF P105S \(EPHA3\) E930K \(KEAP1\) R169C \(TP53\) Q266R \(TP53\) R196 \(TP53\) S33ts\*10 \(PTCH1\) Q1366\* \(PTCH1\) W197\* \(CDKN2A\) P81L \(TP53\) P2785 \(CDKN1A\) R140Q \(CTNNAA1\) R383H \(LRP1B\) splice site 9121-1G\>-A \(NOTCH1\) W287\* \(SUT2\) K32S \(SMARCA4\) Q1166\* | PR/34.2+ (vismodegib) | PR/17.6+ (nivolumab) PD/2.0 (vismodegib/paclitaxel) Metastatic disease Also received sonidegib/buparlisib with progression at 1.9 months | Locally advanced disease |
| 2A      | 56          | M   | Metastatic                              | N/A               | 10                                  | 5 \(CD274 (PD-L1)\) amplification \(FLT1\) E487K \(JAK2\) amplification \(PDCD1LG2 (PD-L2)\) amplification \(PDGFR\) A E459K \(PIK3R2\) Q412 \(PTCH1\) Q1366\* \(PTCH1\) W197\* \(CDKN2A\) P81L \(TP53\) P2785 \(CDKN1A\) R140Q \(CTNNAA1\) R383H \(LRP1B\) splice site 9121-1G\>-A \(NOTCH1\) W287\* \(SUT2\) K32S \(SMARCA4\) Q1166\* | PR/3.8 (nivolumab) | PR/4.5 (vismodegib) | Metastatic disease |
| 2B      | 103         |     | Metastatic                              | 19                | 11                                  | 11 \(CD274 (PD-L1)\) amplification \(FLT1\) E487K \(JAK2\) amplification \(PDCD1LG2 (PD-L2)\) amplification \(PDGFR\) A E459K \(PIK3R2\) Q412 \(PTCH1\) Q1366\* \(PTCH1\) W197\* \(CDKN2A\) P81L \(TP53\) P2785 \(CDKN1A\) R140Q \(CTNNAA1\) R383H \(LRP1B\) splice site 9121-1G\>-A \(LRP1B\) W2334\* \(MLL2\) splice site 4132-1G\>-A \(NOTCH1\) W287\* \(SUT2\) K32S \(SMARCA4\) Q1166\* \(TERT\) promotor -139\_133CC\>-TT \* \(PTCH1\) E684 \(TP53\) R342 \(LRP1B\) 2553\* \(WT1\) S461F \(KDM5A\) P325S | PR/3.8 (nivolumab) | PR/4.5 (vismodegib) | Metastatic disease |
| 3A      | 53          | F   | Metastatic                              | 90                | 6                                   | 2 \(PTCH1\) E684 \(TP53\) R342 \(LRP1B\) 2553\* \(WT1\) S461F \(KDM5A\) P325S \(CREBBP\) H3976\* | PR/3.8 (nivolumab) | PR/4.5 (vismodegib) | Locally advanced disease |
| 3B      | 90          |     | Metastatic                              | 12                | 5                                   | 5 \(PTCH1\) E684 \(CD274 (PD-L1)\) amplification \(JAK2\) amplification – equivocal \(PDCD1LG2 (PD-L2)\) amplification \(TP53\) R342 \(CREBBP\) H3976\* | | | Metastatic disease |
|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|
| 4 | 66 | M | Locally advanced (involving left auricle and left lower extremity) | 52 | 6 | 3 |
|   |   |   |   |   |   |   |   |
| 5 | 62 | M | Locally advanced (unresectable 10 × 11 cm tumor located on back) | 53 | 12 | 6 |
|   |   |   |   |   |   |   |   |
| 6 | 69 | M | Locally advanced (lesion involving the right neck/submental area with recurrent disease following multiple surgeries and radiation) | 3 | 2 | 0 |
|   |   |   |   |   |   |   |   |
| 7 | 61 | M | Locally advanced (large nodular lesion involving the nose with patient refusing surgery and radiation) | 20 | 8 | 3 |
|   |   |   |   |   |   |   |   |
| 8 | 50 | F | Metastatic | 102 | 10 | 5 |

**Patient's age is at the time of locally advanced/metastatic disease.**

**Alterations in bold are considered potentially actionable by either an on- or off-label FDA approved drug.**

**Patients 2 and 3 each had multiple different biopsies sent for next generation sequencing. Patient 2 has been previously reported.**

**The variant GLI1 p.A670S is common in healthy people from European origin (1/333 individuals – 1000 Genomes database) and is considered neutral by several algorithms (SIFT, Provean, Polyphen-2). However, these algorithms only consider the similarities between amino acids (A and S are both polar uncharged amino acids). The addition of a serine residue within Gli1 sequence creates an additional phosphorylation site, and Gli1 is exclusively regulated by phosphorylation. The new phosphorylation site created by the A670S variant is not depending on PKA, and therefore may lead to the activation of the Hedgehog pathway. This variant might be pathogenic in the context of basal cell carcinoma.**

**Patient 5 received the combination of nivolumab and vismodegib.**

**All three patients who received immunotherapy as monotherapy received immunotherapy after treatment with a hedgehog inhibitor.**
Genomics: The median (range) number of genomic alterations (characterized alterations, excludes variants of unknown significance) found per patient biopsy was 9 (2-19). The most common alterations identified were in the TP53, PTCH1, and TERT genes (Fig. 1). All patients had ≥1 genomic alterations. Amplification of CD274 (PD-L1), PDCD1LG2 (PD-L2), and JAK2 (chromosome 9p24.1 amplification) was identified in two patients. The median (range) number of potentially actionable alterations with either an on- or off-label FDA approved drugs was 5 (0-11); seven patients had ≥1 such potentially actionable alterations (Supplemental Figures 2 and 3). Excluding PTCH1 and SMO alterations, seven patients had one or more actionable alterations with an off-label approved drug (median (range) = 3 (0-9)).

TMB: Nine biopsies from eight patients had TMB calculated. The median TMB (mutations/mb) (range) for nine basal cell carcinoma samples was 90 (3-103) while the median TMB (range) for 1637 samples with cancers other than basal cell carcinoma was 4 (1-860) (P < 0.0001) (Supplemental Table 1 and Figure 2). Of the eight patients evaluable for TMB, seven had a high TMB (≥20 mutations/megabase); six of these patients had a very high TMB (≥50 mutations/megabase).

Treatment with PD-1 blockade (Table 1): Four patients were treated with PD-1 blockade. Three of these patients had had tumor progression on ≥1 Hedgehog inhibitor while one patient received a combination of nivolumab and vismodegib.

Patient 2, with metastatic disease and a chromosome 9p24.1 amplification, has an ongoing near complete response (CR) on nivolumab (17.6 months duration). Patient 3, also with metastatic disease and a chromosome 9p24.1 amplification, had a partial response (PR) of 4.2 months duration on nivolumab. Patient 5, with locally advanced disease (10 × 11 cm unresectable tumor), has an ongoing CR of 8.1+ months duration on the combination of nivolumab and vismodegib. Patient 8, with metastatic disease, had progressive disease on pembrolizumab after 2.5 months. The median PFS for all four patients treated with PD-1 blockade was 10.7 months. For the 7 patients treated with a Hedgehog inhibitor, the median PFS was 11.1 months (P = 0.91) (Supplemental Figure 4).

Discussion

We demonstrate that the majority of patients with advanced and/or metastatic basal cell carcinoma have numerous genomic alterations that are potentially targetable by both on- and off-label FDA approved drugs. Importantly, seven of eight patients (84%) had a high TMB, which can be a marker of response to immunotherapy. Further, two of the patients had a high TMB (≥20 mutations/megabase) and achieved a near complete response and partial response with PD-1 blockade. Additionally, the combination of nivolumab and vismodegib resulted in an ongoing CR for Patient 2, and a partial response for Patient 3. For the patients treated with a Hedgehog inhibitor, the median PFS was 11.1 months. Our findings support the consideration of targeted therapies in the treatment of advanced and/or metastatic basal cell carcinoma.
amplification of PD-L1, which is the hallmark of Hodgkin lymphoma, known to be exquisitely responsive to checkpoint inhibitors in Hodgkin lymphoma.\textsuperscript{14} Finally, we present four patients with locally advanced/metastatic basal cell carcinoma treated with PD-1 blockade (seven of whom had previously failed ≥1 Hedgehog inhibitors, while one patient with an ongoing CR received concomitant vismodegib and nivolumab). The response rate in our cohort was 75% (3 of 4 patients), with a median PFS of 10.7 months (range, 3.8 to 17.6+ months).

TMB, measured by CGP, has been shown to correlate with response to PD-1/PD-L1 blockade in patients with melanoma\textsuperscript{15} and urothelial carcinoma.\textsuperscript{16} Herein, TMB reveals that advanced basal cell carcinoma has an extremely high mutational burden. Indeed, six patients had a TMB of ≥50 mutations/megabase (≥20 mutations/megabase is considered high). Prior studies have also demonstrated a high TMB and ultraviolet (UV) signature in basal cell carcinoma (Supplemental Table 3).\textsuperscript{10,17} This observation suggests that, like melanoma, basal cell carcinoma has a marker associated with a high likelihood of responding to checkpoint blockade.

Interestingly, two patients had amplification of chromosome 9p24.1, the region containing PD-L1, PD-L2, and JAK2. Chromosome 9p24.1 copy number alterations are a disease-defining feature in Hodgkin lymphoma, with 97% of patients having 9p24.1 copy number alterations.\textsuperscript{16} Heavily-pretreated Hodgkin lymphoma demonstrates response rates as high as 87% to PD-1 blockade.\textsuperscript{14}

Patients with advanced/metastatic basal cell carcinoma have a poor prognosis after progressing on Hedgehog inhibitor therapy, with limited therapeutic options. Our data presents a biologic rational and clinical data that support the use of checkpoint blockade with PD-1 inhibitors in these patients. Larger clinical trials are needed to confirm these findings.

Methods

Patient selection: We evaluated 2,039 cancer samples that underwent comprehensive genomic profiling (CGP). Only patients with a diagnosis of locally advanced/metastatic basal cell carcinoma confirmed by a dermatopathologist (PRC) are presented. This study was performed in accordance with UCSD Institutional Review Board guidelines for the PREDICT study (NCT02478931) and for any investigational treatments for which patients gave consent in accordance with the Declaration of Helsinki

Next Generation Sequencing and Assessment of Tumor Mutational Burden (TMB): Formalin-fixed paraffin embedded tumor samples were submitted for CGP to Foundation Medicine (N = 2039 patients) (clinical laboratory improvement amendments (CLIA)-certified lab): the FoundationOne (hybrid-capture-based CGP; 182, 236 or 315 genes, depending on the time period). (http://www.foundationone.com/).

For TMB (mutations per megabase (mb)), the number of somatic mutations detected on NGS (interrogating 1.2 mb of the genome) were quantified and that value extrapolated to the whole exome using a validated algorithm.\textsuperscript{16} Alterations likely or known to be bona fide oncogenic drivers and germline polymorphisms were excluded.

Definition of actionable alteration: An alteration was defined as potentially actionable if its protein product is a component of a molecularly defined pathway for which there is at least one available FDA-approved drug that may impact the function of the protein product of the alteration or the immediate downstream effectors of the protein product.

Statistical Analysis and Outcome Evaluation: Mann Whitney U test was used to assess continuous variables. Responses were assessed based on physician notation; physicians used RECIST criteria. Progression-free survival (PFS) was calculated by the method of Kaplan and Meier (P values by log-rank (Mantel-Cox) test). For patients who received multiple treatment regimens, the treatment with the longest PFS was chosen for analysis. Patients were censored at date of last follow up for PFS if they had not progressed. Statistical analyses were performed using Graph-Pad Prism version 7.0 (San Diego, CA, USA).

Abbreviations

| Abbreviation | Description |
|-------------|-------------|
| CR          | complete response |
| FDA         | Food and Drug Administration |
| f           | female |
| m           | male |
| mb          | megabase |
| N/A         | not available |
| PD          | progressive disease |
| PR          | partial response |
| TMB         | tumor mutational burden |

Conflict of interest

Dr. Frampton, Dr. Stephens, and Dr. Miller are employees and equity holders of Foundation Medicine. Dr. Kurzrock receives research funding from Genentech, Merck, Serono, Pfizer, Sequenom, Foundation Medicine, and Guardant, as well as consultant fees from X Biotech, and Actuate Therapeutics and has an ownership interest in Curematch Inc.

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