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ORIGINAL ARTICLE

Polycomb repressive complex 2 mutations predict survival benefit in advanced cancer patients treated with immune checkpoint inhibitors

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Background: Numerous biomarkers are being tested to enhance the ability of clinicians to predict responses and prognosis after treatment with immune checkpoint inhibitors (ICIs). Polycomb repressive complex 2 (PRC2) is a histone methyltransferase family that plays a major role in chromatin silencing. Preclinical evidence implicates PRC2 components such as enhancer of zeste homolog 2 (EZH2) in immune resistance. This study aimed to assess the clinical relevance of *PRC2* mutations in the clinical outcome of ICI-treated patients.

Materials and methods: Next-generation sequencing (NGS) data from tumor samples of patients treated with ICIs (anti-PD-1/PD-L1, anti-CTLA-4 or both) were interrogated for alterations in PRC2-related genes. The Kaplan—Meier method was used to assess the association between altered and unaltered *PRC2*-related genes with overall survival.

Results: Somatic NGS data from 1662 advanced-stage, ICI-treated patients with various primaries (lung, melanoma, bladder, kidney, head neck, esophagogastric, glioma, colorectal, breast, unknown primary) were examined. Seventy patients (4%) harbored truncating or missense mutations or fusions in *EZH2* (2.4%), *EZH1* (1.2%), *SUZ12* (0.9%) or *EED* (0.7%) genes. Patients carrying alterations in *PRC2* genes had significantly longer median overall survival (44 months) compared with those with unaltered tumors (18 months, log-rank P=0.0174). These findings were validated in two additional cohorts of patients (n=313) with various primaries (melanoma, lung, bladder, head neck, anal, sarcoma) who were treated with ICIs.

Conclusions: Inactivating mutations in the PRC2 chromatin silencing machinery, although rare, may predict favorable outcomes in ICI-treated patients with metastatic cancers. This warrants prospective confirmation, and suggests that epigenetic regulators could serve as surrogate markers to guide ICI treatment decisions.

Key words: PRC2, PD-1/PD-L1, CTLA-4, epigenetic regulation, overall survival, advanced cancer

INTRODUCTION

Immune checkpoint inhibitors (ICIs) have become part of the treatment armamentarium for patients with advanced cancer of different primaries.[1] Tissue-based biomarkers, such as programmed death ligand 1 (PD-L1) immunohistochemistry, are widely used to guide the selection of patients to receive anti-PD-1 or anti-PD-L1 antibodies.[2] However, PD-L1 has several limitations due to discordance in assay methodology and trial designs, as well as absence of prospective comparisons of how PD-L1-positive disease diagnosed using each assay relates to clinical outcomes.[3]

Besides PD-L1, numerous biomarkers are being tested to enhance the ability of clinicians to predict responses and prognosis of patients treated with ICIs. Other response biomarker candidates, including DNA mutations and neoantigen load, are only weak predictors of immune checkpoint blockade response.[4] Thus, identification of novel, more robust biomarkers that could help predict which patients could benefit from ICIs remains an unmet need. Of particular interest is the development of signatures from gene expression profiling of immune cells within the tumor microenvironment.[5] Additionally, gene alterations affecting chromatin remodeling and DNA methylation could play an important role in shaping response and resistance after treatment with ICIs.[6]

Polycomb repressive complex 2 (PRC2) is a histone methyltransferase family that plays a major role in chromatin silencing.[7] The broader family of Polycomb group genes is well conserved in animals, and its gene products assemble into large multimeric protein complexes

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functioning as negative regulators of gene transcription during development.[8] PRC2 is a methyltransferase with activity toward lysine 27 on histone H3. The SET-domain-containing component (EZH1 or EZH2) is closely associated with several other subunits. The core complex necessary for catalytic function consists of EZH1/2, the Zinc-finger protein SUZ12, and the WD40 protein EED.[9]

PRC2 can have both oncogenic and tumor-suppressive properties as a consequence of its molecular function in promoting a transcriptional repression state of numerous target genes.[10] The cell-cycle-coupled expression of PRC2 components, and the presence of PRC2 overexpression in the stem-cell compartment suggest that increased PRC2 expression in cancer cells may stem from the increased proliferative capacity and/or dedifferentiated phenotype of cancer cells.[9] Increased PRC2 activity may act as a driver in some cancers, evidenced by the occurrence of hyper-activating mutations as an early event through studies of clonality.[11] Meta-analyses of numerous studies have revealed an association between PRC2 overexpression and poor prognosis across 18 different types of cancer.[12] Recent *in vitro* and early clinical studies implicate key PRC2 components such as enhancer of zeste homolog 2 (EZH2) in immune resistance[13,14] through various mechanisms, including repression of T helper 1 (TH1)-type chemokines,[15] downregulation of interferon-gamma signaling,[14,16] downregulation of PD-L1 expression,[17] and major histocompatibility complex (MHC)-I and -II expression.[18,19] However, there is a paucity of evidence with respect to the clinical significance of these observations in patients treated with ICIs. This study aimed to assess the prognostic relevance of *PRC2* mutations in patients with advanced cancer of various primaries after treatment with anti-PD-1/PD-L1 or/and anticytotoxic T-lymphocyte-associated protein 4 (CTLA-4) ICIs.

MATERIALS AND METHODS

This study used a publicly available database, cBioportal for Cancer Genomics (www.cbioportal.org),[20] with targeted next-generation sequencing (NGS) data from a cohort of 1662 patients who were treated with at least one dose of ICI, representing a variety of cancer types.[21] The NGS assay used was MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets), and this identifies somatic exonic mutations in a pre-defined subset of 468 cancer-related genes using both tumor-derived and matched germline normal DNA.[22] NGS data from corresponding tumor samples of these patients were queried for mutations and fusions in *PRC2*-related genes. cBioPortal supports the annotation of variants from several different databases. These databases provide information about the recurrence of, or prior knowledge about, specific amino acid changes. For each variant, the number of occurrences of mutations at the same amino acid position present in the COSMIC database are reported. Furthermore, variants are annotated as 'hotspots' if the amino acid positions are found to be recurrent linear hotspots, as defined by the Cancer Hotspots method (cancerhotspots.org), or three-dimensional hotspots, as defined by 3D Hotspots (3dhotspots.org). Prior knowledge about variants, including clinical actionability information, is provided from three different sources: OncoKB (www.oncokb.org), CIViC (civicdb.org) and My Cancer Genome (mycancergenome.org). Copy number data sets within the portal are generated by the GISTIC or RAE algorithms. Both algorithms attempt to identify significantly altered regions of amplification or deletion across sets of patients. Both algorithms also generate putative gene/patient copy number specific calls, which are then input into the portal.

The BIOKARTA_PRC2_PATHWAY gene set from the Gene Set Enrichment Analysis molecular signatures database (http://www.gsea-msigdb.org/gsea/msigdb/index.jsp)[23] was used as a reference to include the most important *PRC2*-related genes in the analysis (Table 1).

The Kaplan—Meier method was used to assess the association between altered and unaltered *PRC2* pathway genes with overall survival (OS). OS was measured from the date of first ICI treatment to time of death or last follow-up.

Molecular data from two separate patient cohorts, consisting of patients with various primaries,[24,25] were analysed in a combined metadataset as a means to validate findings from the discovery dataset[21] with respect to the prognostic and predictive role of *PRC2* pathway genes. All results were reported at the 0.05 significance level.

RESULTS

In total, 1662 patients with advanced-stage cancers were examined. The majority of patients had metastatic disease (n=1446, 94%), while the rest had either locally advanced, unresectable disease (n=989, 59%) or locally recurrent disease (n=10, 0.6%), as described previously.[21] Primaries included non-small cell lung cancer (NSCLC, n=350), melanoma (n=321), bladder cancer (n=214), renal cell carcinoma (n=151), 138 head and neck cancer (n=138), esophagogastric cancer (n=126), glioma (n=117), colorectal cancer (n=110), cancer of unknown primary (n=90) and breast cancer (n=45) (Figure 1).

The majority of patients received a PD-1 or PD-L1 inhibitor: nivolumab, pembrolizumab, atezolizumab, avelumab or durvalumab (n=1256). One hundred and forty-six patients were treated with anti-CTLA-4 (ipilimumab or tremelimumab), and the rest were treated with combinations (n=260) (Figure 2).

Upon query of the cohort's genomic data for molecular alterations within the BIOKARTA_PRC2_PATHWAY gene set, tumors from 70 patients (4%) were found to harbor truncating or missense mutations in *EZH2* (2.4%), *EZH1* (1.2%), *SUZ12* (0.9%) or *EED* (0.7%) genes (Table 2 and Table S1, see online supplementary material). The presence of these alterations was most common in colorectal cancer (8.2%), followed by bladder cancer (6.2%), melanoma (5.6%), esophagogastric cancer (4.8%), glioma (4.3%), head and neck cancer (3.6%), cancer of unknown primary (3.4%), renal cell carcinoma (3.3%) and NSCLC (1.7%) (Figure 3). No

Table 1. Polycomb repressive complex 2 (PRC2) pathway genes

BIOCARTA_PRC2_PATHWAY	Gene family
BMI1	Transcription factor
CBX4	Transcription factor
COMMD3-BMI1	Read-through transcription
EED[a]	Transcription factor
EZH1[a]	Transcription factor
EZH2[a]	Transcription factor
HDAC1	Transcription factor
HDAC2	Transcription factor
PHC1	Transcription factor
RBBP4	Histone binding
RBBP7	Histone binding
RING1	Transcription factor
SUZ12[a]	Oncogene, translocated cancer gene
YY1	Transcription factor

[a] 'Core' *PRC2* genes.

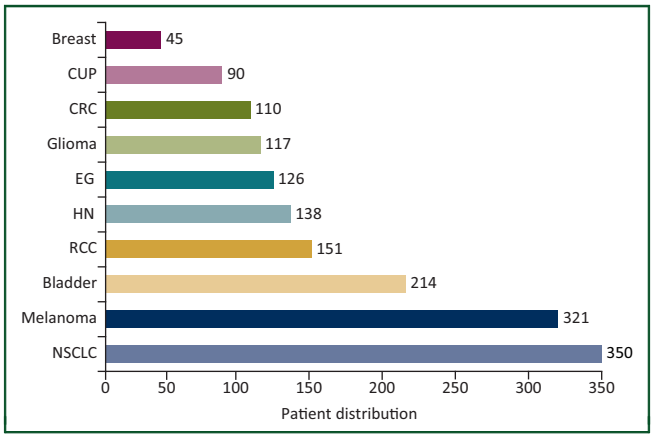


Figure 1. Patient distribution by cancer type (discovery cohort).
NSCLC, non-small cell lung cancer; CUP, cancer of unknown primary; RCC, renal cell carcinoma; EG, esophagogastric cancer; HN, head and neck cancer; CRC, colorectal cancer.

significant co-occurrence or mutual exclusivity of mutations or fusions was detected among *PRC2*-related genes.

The median follow-up was 19 months (range 0-80 months), with 830 (50%) patients alive and censored at last follow-up, as described previously.[21] Patients carrying somatic alterations in *PRC2*-related genes had a significantly longer median OS (44 months) compared with those without somatic alterations in *PRC2*-related genes (18 months, log-rank P=0.0174) (Figure 4).

To validate these findings, a query for alterations in these *PRC2*-related genes was performed in a validation meta-dataset derived from tumors of patients with melanoma (n=215), NSCLC (n=57), bladder cancer (n=27), head and neck squamous cell carcinoma (n=12), anal cancer (n=1) and sarcoma (n=1) treated with anti-PD-1 (n=74), anti-PD-L1 (n=20), anti-CTLA-4 (n=209), or a combination of anti-CTLA-4 and anti-PD-1/L1 therapies (n=10).[24,25] Queried genes were altered in 41 (13%) patients/samples (Figure S1, see online supplementary material). The presence of alterations in the same four *PRC2*-related genes (*EZH2* 8%, *EZH1* 2.2%, *SUZ12* 2.2%, *EED* 2.2%) was associated with longer median OS (48 months) compared with those without

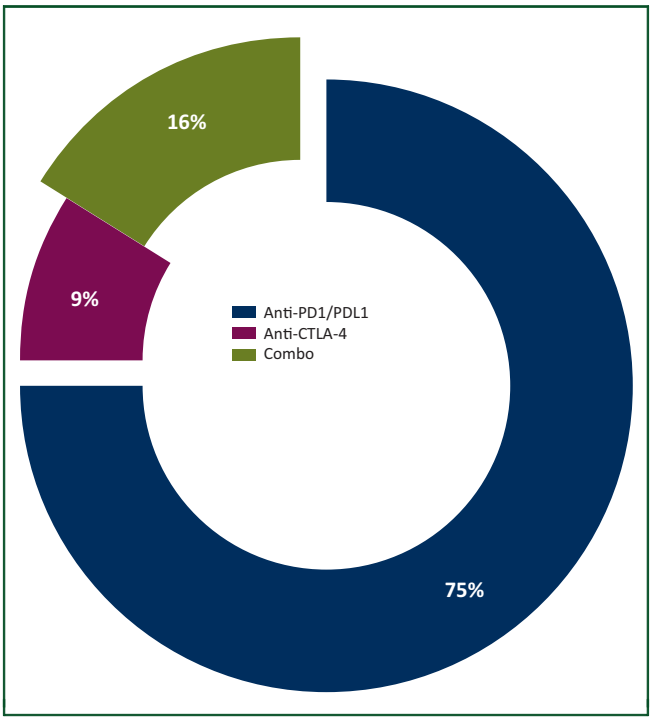


Figure 2. Patient distribution by treatment (discovery cohort).

Table 2. Mutations per Polycomb repressive complex 2 (PRC2) gene in tumor samples of patients treated with immune checkpoint inhibitors (discovery cohort)

PRC2 gene	Missense	Truncating	Inframe	Splice	Structural variant/fusion
EZH2 (2.4%)	29	6	2	3	0
EZH1 (1.2%)	4	0	0	0	1
SUZ12 (0.9%)	14	2	0	0	0
EED (0.7%)	9	2	1	0	1

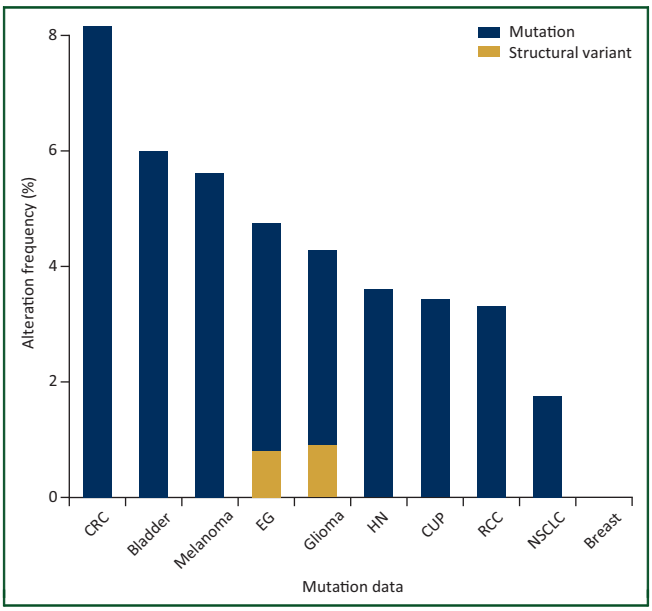
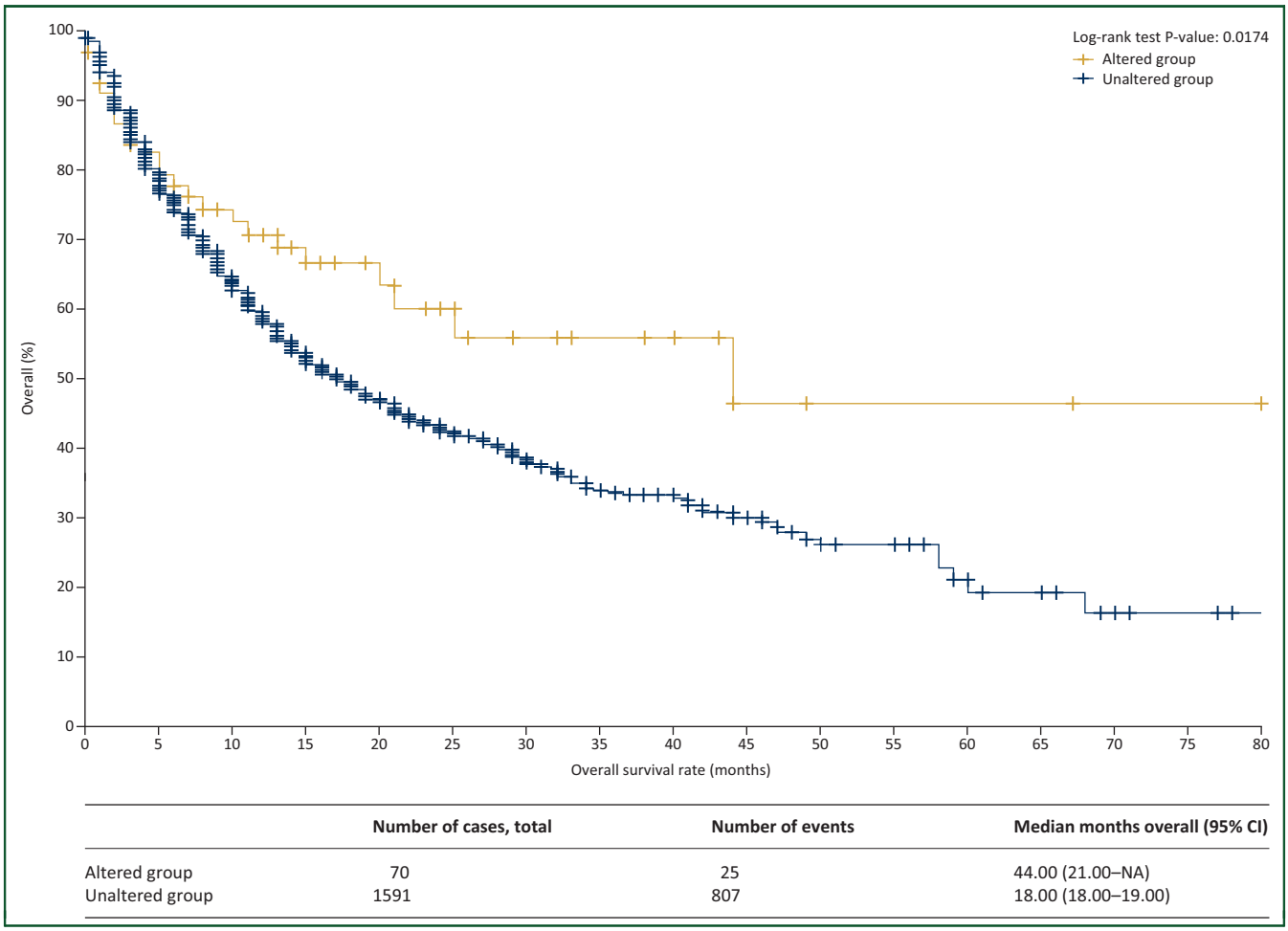


Figure 3. Frequency of alterations in Polycomb repressive complex 2 genes by cancer type (discovery cohort).
CRC, colorectal cancer; EG, esophagogastric cancer; HN, head and neck cancer; CUP, cancer of unknown primary; RCC, renal cell carcinoma; NSCLC, non-small cell lung cancer.



	Number of cases, total	Number of events	Median months overall (95% CI)
Altered group	70	25	44.00 (21.00–NA)
Unaltered group	1591	807	18.00 (18.00–19.00)

Figure 4. Kaplan-Meier survival plot of patients with and without alterations in *Polycomb repressive complex 2* genes (discovery cohort).
NA, not available; CI, confidence interval.

alterations (22 months, log-rank P=0.102) (Figure S2, see online supplementary material). There was also a trend towards longer progression-free survival of 22 versus 16 months, respectively (log-rank P=0.555) (Figure S3, see online supplementary material).

DISCUSSION

PRC2 is a histone methyltransferase family that plays a major role in chromatin silencing.[7] Increased PRC2 activity may act as a driver in some cancers, evidenced by the occurrence of hyperactivating mutations as an early event through studies of clonality.[11] Meta-analyses of numerous studies have revealed an association between PRC2 overexpression and poor prognosis across 18 different types of cancer.[12] The focus of this study was to examine the clinical value of molecular alterations in the PRC2 chromatin silencing machinery in patients with advanced cancer treated with ICIs. Mutations in key *PRC2*-related genes including *EZH1*, *EZH2*, *EED* and *SUZ12* were associated with favorable outcomes in both the discovery and validation cohorts used. It is plausible that attenuation of histone methylation induced by these transcriptional repressors may have a positive effect on the outcomes of these patients treated with ICIs.

An association between epigenetic features and response to PD-1 inhibitors was reported previously in stage IV NSCLC patients, whereby the microarray DNA methylation signature EPIMMUNE was positively correlated with improved progression-free survival and OS.[26]

While prospective confirmation of the results of these studies is warranted, it is conceivable that epigenetic regulators could serve as surrogate markers to guide ICI treatment decisions. Although the underlying mechanisms remain poorly understood, a previously described role of DNA methylation in silencing of PD-1, PD-L1, PD-L2 and CTLA-4 gene expression could explain the inhibition or attenuation of immunotherapy efficacy in such patients.[27,28]

Taken together, the results of this study and the positive effect of demethylation on transcriptional activity for some immune-related genes, including PD-L1 and genes of the interferon signaling cascade, pose important therapeutic implications for use of epigenetic modulation as a tool to sensitize patients to anti-PD-L1 ICIs.[29] For example, the demethylating drug azacytidine combined with an anti-

CTLA-4 ICI led to greater tumor regression compared with each drug alone by upregulating MHC-I components.[30] Increased lymphocyte infiltration and TH1-type chemokine and cytokine production were also observed after concurrent demethylation and anti-PD-L1 and CTLA-4 therapy in an ovarian cancer mouse model.[31]

This study was limited by heterogeneity of the discovery and validation patient cohorts with regard to different primaries, number of prior therapies, and timing of NGS testing relative to ICI initiation.[21] Additionally, as this was a hypothesis-generating analysis of previously reported genomic data[21,24,25] through the lens of epigenetic regulation, it underscores the effects of other genomic or/and epigenomic alterations on OS, focusing on mutations and fusions of *PRC2*-related genes. Additionally, the complex and very context-dependent effects of *PRC2* mutations on diverse sets of cancers, as well as on the immune system-PRC2 loss can either be tumor suppressive or tumor promoting, depending on cancer (sub) type and other co-occurring driver mutations- is also underestimated. Taken together with the rare occurrence of *PRC2* mutations in most cancers, the potential utility of *PRC2* mutations as a potential biomarker for the effects of immune checkpoint inhibition warrants confirmation in large prospective analyses.

Future studies that integrate other genomic or/and histopathologic biomarkers may allow for the development of an optimized predictive test to inform clinical decisions on the use of ICIs.[32] If confirmed and prospectively validated, knowledge of the mutation status of *PRC2*-related genes could help to better identify patients who could benefit from ICIs. Taking this a step further, modulating the expression of *PRC2* genes (e.g. *EZH2*) using small molecule inhibitors, such as CPI-1205, could improve antitumor responses elicited by anti-PD-1 or/and anti-CTLA-4 therapy.[33] Demethylating agents and histone deacetylases are currently being tested in combination with ICIs in numerous clinical trials and types of malignancies. Correlative studies on genomic and epigenomic surrogates of response and resistance, wherever available, will be of key importance to better target specific subpopulations.

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DISCLOSURE

The author has declared no conflicts of interest.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.iotech.2021.100035.

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