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PARP1 as a potential therapeutic target in Merkel cell carcinoma

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Abstract

Background—Patients with metastatic Merkel-cell carcinoma (MCC) are treated similarly to small-cell lung cancer (SCLC). PARP1 is overexpressed in SCLC and response to PARP inhibitors have been reported in SCLC patients. Our study explores PARP as a therapeutic target in MCC.

Methods—We evaluated PARP1 expression and Polyoma virus (MCPyV) in 19 MCC. Target exome-sequencing was performed in 14 samples. Sensitivity to olaparib was tested in 4 MCC cell lines.

Results—Most MCC (74%) express PARP1 at high levels. Mutations in DNA-damage repair (DDR) genes was identified in 9 samples (64%), occurred exclusively in head neck primaries, and correlated with *TP53/RB1* mutations. *TP53*|*RB1* mutations were more frequent in MCPyV-negative tumors. Sensitivity to olaparib was seen in the MCC line with highest PARP1 expression.

Conclusions—Based on PARP1 overexpression, DDR genes mutations, platinum sensitivity, and activity of olaparib in a MCC line, clinical trials with PARP inhibitors are warranted in MCC.

Keywords

Merkel cell carcinoma; Merkel cell polyomavirus; PARP; genomics; targeted therapy

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INTRODUCTION

Merkel cell carcinoma (MCC) is a rare and aggressive high-grade neuroendocrine tumor of the skin that affects predominantly the geriatric and immunocompromised populations. It has an incidence of 1,500 new cases per year in the United States and it is mostly seen in light-skinned males[1]. The most common primary sites for MCC are the head and neck or extremities[1]. Although the tumor's exact cell of origin and pathogenesis are unknown, Merkel cell polyomavirus (MCPyV) and ultraviolet (UV) radiation are associated with the development of MCC[2].

The MCPyV, a double-stranded DNA virus, is monoclonally integrated in 40 to 80% of MCCs[2]. MCPyV can be detected by polymerase chain reaction targeting the *LTA* coding gene or by immunohistochemistry (IHC) using a monoclonal antibody against the antigenic epitope.

While the majority of MCC patients present with local disease amenable to surgery and/or radiation therapy, tumor recurrence occurs in at least 35% of the cases[3]. Patients with stage IV disease have a dismal prognosis with a 5-year survival ranging from 0 to 18%[4]. Given its histological similarities with small cell lung cancer (SCLC), the combination of platinum and etoposide or single-agent topotecan are frequently used to treat patients with incurable disease. Platinum and etoposide in the first line setting renders responses in approximately 60% of the patients, usually short-lived, which is similar to that observed in patients with SCLC[5–7].

Recently, sequencing efforts in MCC have shed light into the genomic landscape of this disease[8–12]. Interestingly, while the mutational profile of MCPyV negative tumors mirrors SCLC, with the vast majority of cases harboring mutations in *TP53* and *RB1* and scarce actionable mutations in oncogenes, MCPyV positive tumors have a lower mutational burden, with virtually no mutations in *TP53* or *RB1*. Few mutations were identified in targetable oncogenes overall, however abnormalities in genes involved in DNA repair pathways have been described in approximately 30% of the cases[8,12].

Byers et al have reported a high expression of DNA repair proteins in SCLC at the mRNA and protein levels, particularly Poly (ADP-ribose) polymerase-1 (PARP1)[13]. High levels of PARP1 and other proteins involved in DNA Damage Repair (DDR) such as FANCD2 and pCHK2 are strongly associated with sensitivity of SCLC cell lines to PARP inhibitors[14]. Furthermore, responses to PARP inhibitors in metastatic SCLC patients have been reported, which led to a series of ongoing clinical studies testing PARP inhibitors as a single agent or in combination with chemotherapy in SCLC[13, 15–17].

Based on the similar histology, response to platinum, and mutational background between MCC, particularly MCPyV negative, and SCLC; the identification of mutations in genes involved in DNA-damage repair in MCC; and the promising anti-tumor activity of PARP inhibitors in SCLC, we aimed to explore PARP1 as a potential therapeutic target in MCC.

MATERIAL AND METHODS

Sample selection

The patient population consisted of 19 unequivocal MCC cases as determined by an experienced pathologist (DB) that were seen at MD Anderson Cancer Center in 2015 and had tissue available for immunohistochemistry studies. Fourteen patients had sufficient samples for target exome sequencing. Clinical data were retrospectively obtained from electronic medical records according to the IRB approved protocol RCR04-0030. Data acquisition was locked 12/1/2015. At the data of analysis, 18 patients were alive. Vital status was unknown for one patient.

Immunohistochemistry

Immunohistochemical (IHC) analysis for PARP1 (LSBio, clone A6.4.12) was performed in 4 micron paraffin embedded tissue sections using BOND MAX IHC staining protocol by Vision Biosystems platform. Polyoma virus status was evaluated by staining with anti-MCPyV antibody (Santa Cruz, clone CM2B4) in the tissue specimens. Intensity of the staining was graded as 0 (no staining), 1+ (weak), 2+ (moderate), or 3+ (strong). The percent of cells (0-100%) staining for each intensity was determined. H-score was calculated by multiplying the staining intensity (0-3+) by the percent of cells of each intensity (0-100%), and then by adding these together for a final score of 0-300. Only tumor tissue with strong nuclear staining in more than 10% of the neoplastic cells (H-score > 30) was considered positive. Tumor tissue with a H-score > 200 was considered strongly positive.

Genomic analysis

Targeted exome sequencing of 263 genes (T200 institutional panel) was done in DNA extracted from paraffin-embedded section of 14 patients using next-generation sequencing[18] and is available as Supplement Material. Paired peripheral blood mononuclear cells were not available for analysis of germline mutations. In order to select for mutation in genes of interest likely to impact protein function (oncogenic), we used the following approach: a) searched for the mutation in The Cancer Genome Atlas (TCGA)[19], b) utilized four established algorithms that predict the significance of the mutation, and only included the mutations in which at least three out of the four algorithms pointed as deleterious (CONsensus DELeteriousness score of missense mutations [Condel][20]), possibly or probably damaging (PoliPhen[21]), deleterious (Sorted Intolerant From Tolerant [SIFT][22]), or medium or high (Mutation Assessor[23]). If no information was found in the TCGA or provided by the mutation effect predictor algorithms, we included frame shift mutations, inframe codon loss or gain, and stop-gained mutations likely to impair protein function.

Cell Lines

Human MCC cell lines MKL-1, MS-1, MCC13, and MCC26 were obtained from Sigma-Aldrich (St. Louis, MO). All cells were grown in suggested medium supplemented with fetal bovine serum and penicillin/streptomycin. Cells were passaged for fewer than 6 months following receipt.

Western blot

Western blots of cell lysates were probed for PARP1 (cs9542, Cell Signaling Technology, Danvers MA), and actin (sc47778, Santa Cruz Biotechnology, Dallas TX).

Proliferation Assays

Cells were seeded in 96-well plates at 2,000 cells per well in triplicate for each cell line. After 24 hours, the cells in each well were treated for 24 hours with the PARP inhibitor olaparib (Selleck Chemicals, Houston, TX), cisplatin, or vehicle control. Four days later, proliferation was assayed by Cell Titer Glo (Promega, Fitchburg, WI). Median inhibitory concentration (IC50) values were estimated using the drexplorer software.

Statistical analysis

Descriptive statistics were used to report continuous and categorical variables. Fisher's exact test was applied to describe the association between PARP1 expression, MCPyV status, mutations in genes of interest, and patient's clinical characteristics. All *P* values were two-sided. P<0·05 was considered statistically significant.

RESULTS

Population Characteristics

Baseline characteristics of the 19 MCC patients are outlined in Table 1. The median age at diagnosis was 72 years old, and 68% (13/19) of the patients had a primary tumor in the head and neck region. Three patients were significantly immunosuppressed due to, leukemia (chronic lymphocytic or myelomonocytic), or therapy for rheumatoid arthritis. A previous history of solid tumor was positive in 14 patients, the most common being other skin cancers (basal cell or squamous cell carcinoma) diagnosed in eight, prostate cancer in four, and breast cancer in two patients. Overall, 15 patients (79%) had a history of liquid or solid malignancy.

Prevalence of IHC Markers of Interest

All 19 samples were available for MCPyV and PARP1 IHC staining, the majority (63%) representing the primary tumor. Approximately half of the tumors (47%) were associated with MCPyV. Interestingly, PARP1 expression was detected in the vast majority of cases (84%), as demonstrated in Figure 1. Out of the 16 samples that stained positive for PARP, 13 had a H-score of 300. PARP1 expression was detected in both, MCPyV positive (9/9) and negative (7/10) tumors, with no statistical correlation between PARP1 expression and MCPyV status or primary site of disease. The results of the IHC studies are outlined in Table 2.

Mutations in genes related with DNA damage repair

Out of the 19 patients, 14 had adequate tissue for targeted exome sequencing. Out of 263 genes analyzed (Supplemental Table 1), 12 (*ATM*, *ATR*, *ATRX*, *BRCA1*, *BRCA2*, *PALB2*, *RAD51*, *RAD51C*, *CHEK1*, *CHEK2*, *FANCA*, and *FANCD2*) are related to DNA-damage repair (DDR) and four (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) are involved in mismatch repair

(MMR)[24,25]. We also included mutations in the tumor suppressor gene *ARID1A*, a component of the SWI/SMF chromatin-remodeling complex, as loss-of-function mutation in this gene has been associated with sensitivity to PARP inhibitors pre-clinically[26]. Oncogenic mutations in the tumor suppressor genes *TP53* and *RB1* were annotated. They co-occurred in the majority of samples.

As demonstrated in Figure 2, nine of 14 patients (64%) had mutations in at least one gene involved in DDR and/or MMR. Predicted loss-of-function mutations in ARID1A were identified in 5 samples. The annotations of the mutations are specified in Supplemental Table 2.

Correlations between PARP staining, gene mutations, and clinico-histological characteristics

Using Fisher's exact test, no statistically significant correlation was found between PARP1 IHC staining (positive vs. negative) and MCPyV status (positive vs. negative), tumor primary site (head and neck vs. extremities), or mutations in any of the 17 genes of interest described above (present vs. absent).

Interestingly, mutations in genes involved in DDR, MMR, or *ARID1A*, were found exclusively in patients with a head and neck primary (Figure 2). *TP53* or *RB1* loss-of-function mutations were also restricted to tumors arising in the head and neck (P=0.03). Mutations in genes of interest were likely to co-occur with *TP53* or *RB1* mutations (P=0.03) (Supplemental Figure 1).

ARID1A mutations were found exclusively in the MCPyV negative samples (P=0.003). Mutations in *TP53* or *RB1* also correlated with MCPyV negative status (P=0.03) (Supplemental Figure 2).

PARP inhibitor activity in Merkel cell carcinoma cell lines

Having observed high PARP1 expression in patient samples, we assayed PARP1 expression in a panel of MCC cell lines that included two MCPyV positive (MKL-1 and MS-1) and two that were MCPyV negative (MCC13 and MCC26). Three SCLC cell lines with relatively low, moderate, and high PARP1 expression were included for comparison (Figure 3). In all four cell lines, Western blot analysis revealed PARP expression in MCC lines that was on a par with SCLC cell lines with moderate (H378) to high (DMS153) PARP1 expression (Figure 3A). To assay the potential sensitivity of MCC to PARP inhibition, we tested the efficacy of the FDA approved PARP inhibitor olaparib in our cell line panel. Of the four cell lines tested, the one with the highest level of PARP1 expression (MKL-1) was sensitive to olaparib (Figure 3B). Similar to observations in SCLC[14] sensitivity to olaparib and cisplatin were correlated (R=0.921 by pearson correlation).

DISCUSSION

New, more effective and well-tolerated therapy is greatly needed for patients with recurrent MCC, many of whom are elderly and chronically immunosuppressed. Recent trials have demonstrated benefit from immune checkpoint inhibition with pembrolizumab and

avelumab, anti-PD-1 and PD-L1 respectively, in a subset of patients with MCC[27,28]. Although these data are promising, it is notable that some patients with MCC require active immunosuppression for autoimmune disorders or organ transplant status, and thus, are not candidates for immune checkpoint inhibition.

In order to explore other novel therapeutic options for incurable MCC, we investigated the expression of PARP1 by IHC in MCC patients and found it to be strongly positive in 74% of patients. Our findings are similar to those in SCLC, in which PARP1 overexpression is identified in the majority of cases[13]. The mechanism of PARP1 overexpression in SCLC is thought to be secondary to *RB1* loss, which leads to increase expression of the transcription factor E2F1 and activation of its targets, including several components of DNA repair pathways[13].

In our series, mutations in the tumor suppressors genes *RB1* and/or *TP53* were identified in 6 out of 14 patients (43%), and were more frequent in the MCPyV negative tumors, consistent with the available literature[9,11]. Alternative mechanisms other than mutations leading to inactivation of *TP53* or *RB1* such as complexes gene rearrangements were not tested in our samples.

PARP1 is a key facilitator of single-stranded DNA breaks repair, therefore, the level of PARP1 protein generally increases in response to DNA damage[29]. In order to investigate the frequency of mutations in genes involved in DDR, we performed exome-sequencing of 263 genes including 16 known to be involved in DNA-damage repair pathways[30–32] in 14 available MCC samples and found that 9 (64%) patients had mutation in at least one of these genes. No correlation was seen between PARP1 expression and the presence of mutations in genes involved in DDR.

Other groups have also described mutations in DDR genes in MCC, however, at a lower frequency rate[10,12]. In our series, 53% of the patients were MCPyV negative, likely due to our center's geographic location subject to intense sun exposure. MCPyV negative tumors have a DNA-damage UV signature very distinct from MCPyV positive tumors[9], which may have accounted for the higher incidence of mutations in DDR genes identified in our series. Indeed, mutations in genes associated with DDR were exclusively identified in primary tumors of the head and neck, an area heavily exposed to the sun.

Interestingly, we identified frequent mutations in *ARID1A* (36%). *ARID1A*, a subunit of the SWI/SNF chromatin-remodeling complex, is involved in the regulation of the DNA damaging checkpoint via its interaction with ATR. Cell lines and xenograft models deficient in ARID1A are sensitive to PARP inhibitors, suggesting that specific loss-of-functions mutations in this gene might be a surrogate for benefit of this class of drugs[26].

Many PARP inhibitors are currently in late phase clinical development. Olaparib was the first PARP inhibitor to be FDA approved in refractory *BRCA1/2* mutant metastatic ovarian cancer[33]. This drug was also granted breakthrough therapy designation in *BRCA1/2* or *ATM* mutated castration-resistant prostate cancer[34]. Rucaparib and Niraparib, another two oral PARP inhibitor, are also approved for refractory *BRCA1/2* mutant (germline or somatic) ovarian cancer in the chemotherapy refractory and in the maintenance setting respectively.

Beyond *BRCA 1/2* mutations, various strategies are being explored to select patients who are likely to benefit from PARP inhibitors, such as molecular characterization of aberrations in DNA repair genes[34], gene-expression signatures[35], HDR score[36], loss of heterozygosity scoring system[37], prior sensitivity to platinum[30], PARP1 expression[38,39], SLFN11 expression, and epithelial-to-mesenchymal transition (EMT)[40].

Broadly speaking, PARP1 expression is elevated in SCLC but only a subset respond to single agent PARP inhibition[16]. On the basis of our experiments, MCC displays similar traits to SCLC, suggesting that better predictive markers and further testing including PARP inhibitor combinations may enhance the effectiveness of single agent PARP inhibitor. Given the recent approval of avelumab for patients with incurable MCC and the growing body of evidence demonstrating an increased therapeutic efficacy of PARP inhibitors combined with anti-PD-L1, this combination deserves clinical investigation in patients this orphan disease[41]. Other combinations such as PARP inhibitors and chemotherapy[42], PI3K inhibitors[43], or CHK1 inhibitors[44] has shown promising activity in SCLC and may be clinically active in MCC.

Taken together, in spite of the small sample size, the high prevalence of PARP1 overexpression in MCC, its sensitivity to platinum, the frequent mutations in genes involved in DNA-damage repair and ARID1A, particularly in the MCPyV negative cases, and the sensitivity of MCC cell line with high levels of PARP1 to olaparib suggest PARP inhibitors as single agent or in combination deserves clinical investigation in patients with MCC. Furthermore, based on the similarities between MCC and SCLC, basket trials for patient with rare high-grade neuroendocrine carcinomas irrespective of its organ of origin might be justified.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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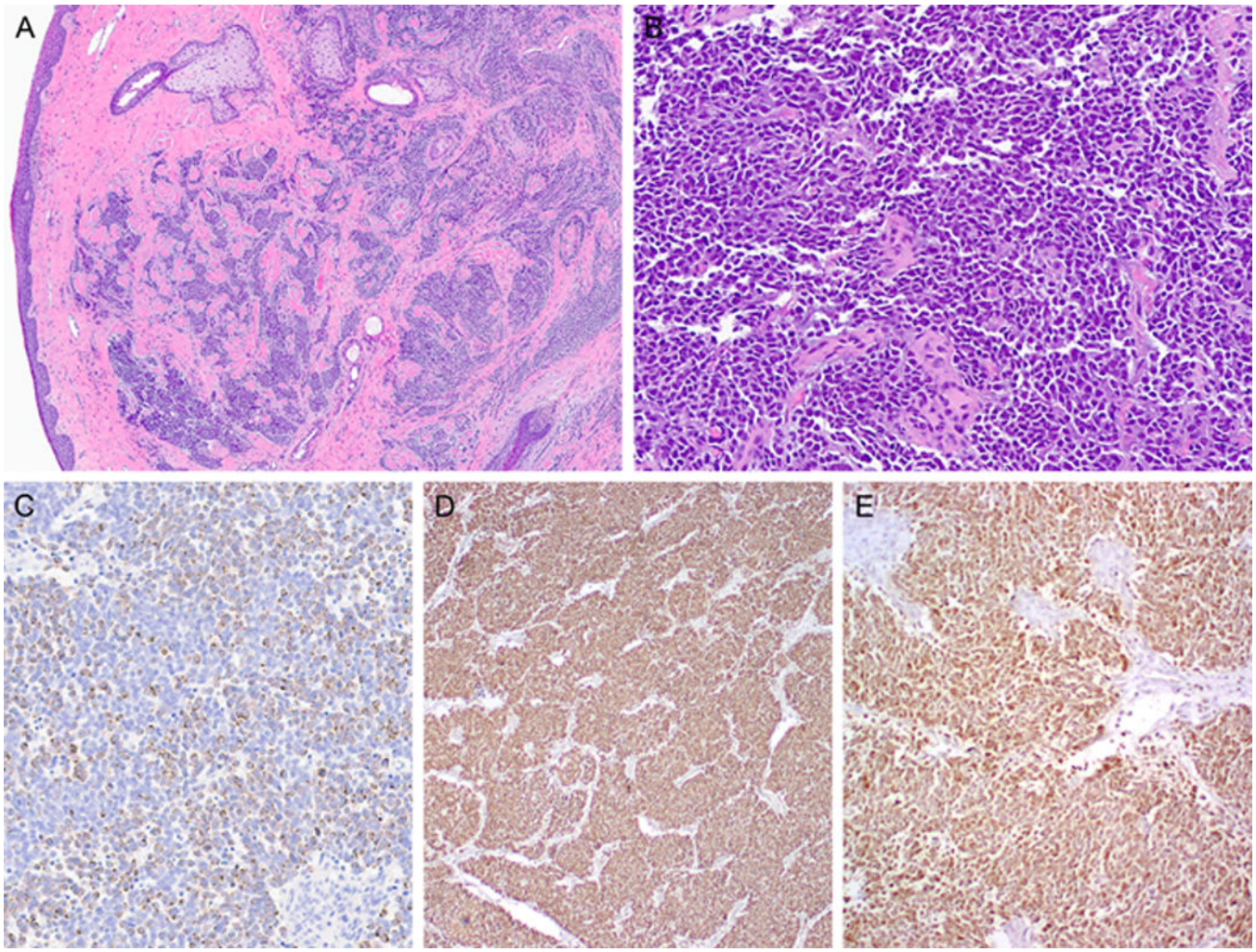

Figure 1.

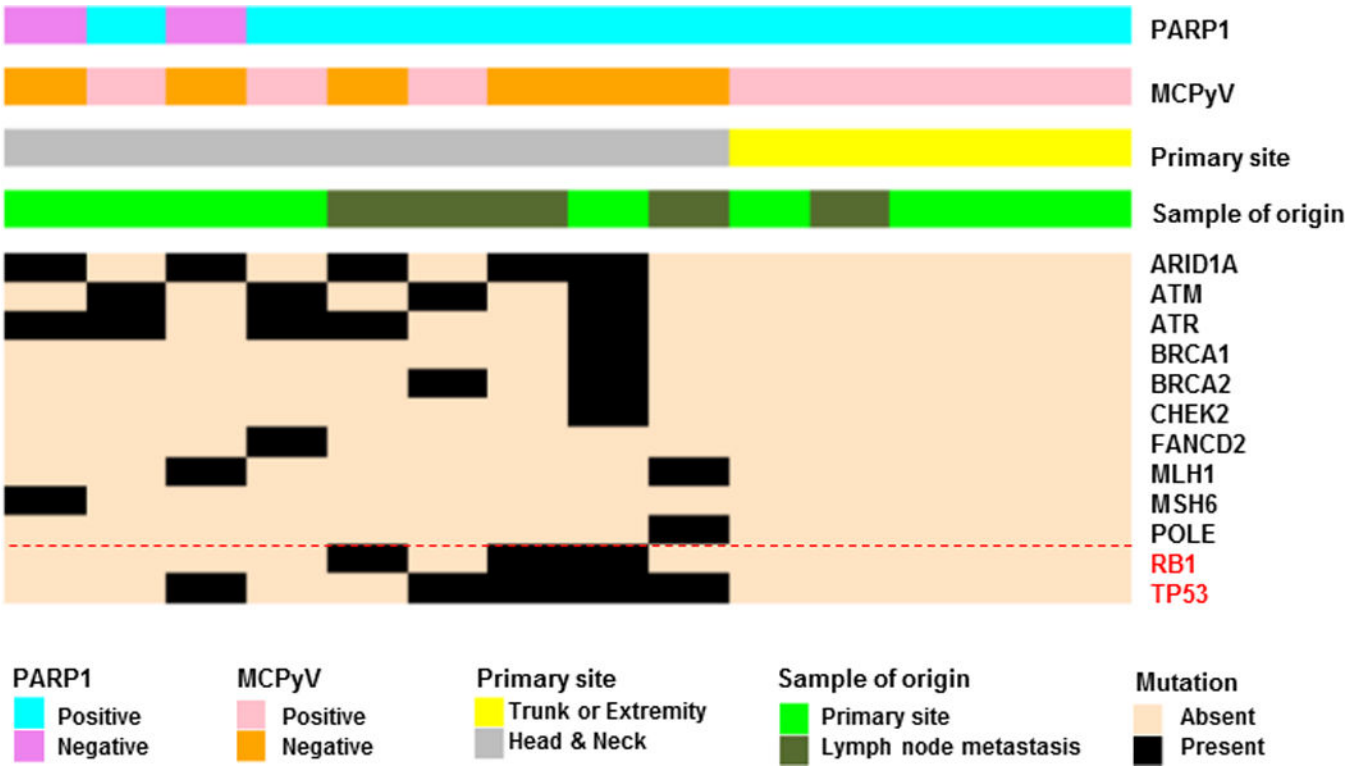

Figure 2.

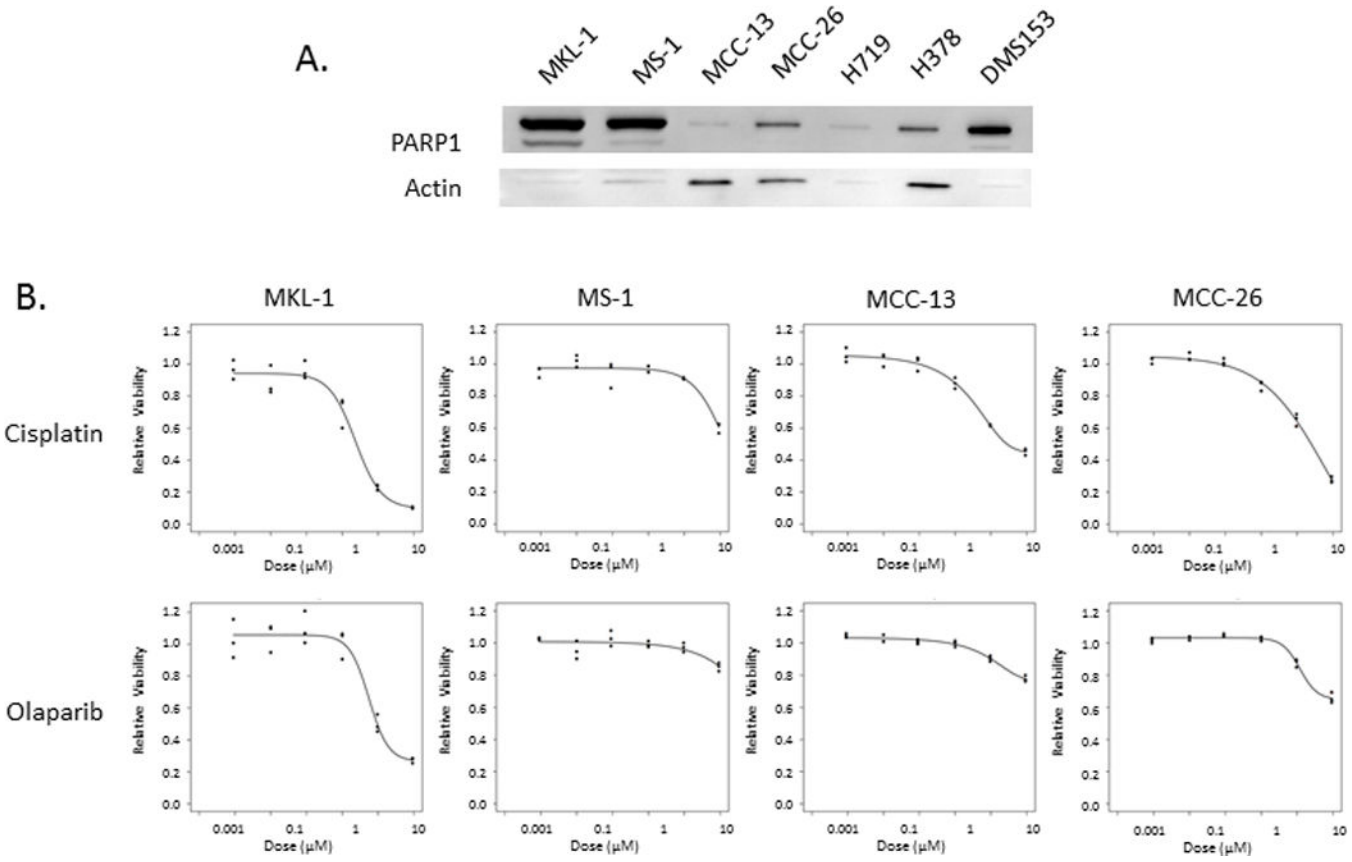

Figure 3.

Table 1

Baseline patient's characteristics

Characteristic	N or Median	% or Range
AGE	72	56-86
SEX		
Male	13	68
Female	6	32
DISEASE SITE		
Head and Neck	13	68
Trunk or Extremity	6	32
DISEASE STAGE AT DIAGNOSIS *		
I or II	6	32
III	12	63
IV	1	5
IMMUNOSSUPRESSION		
Yes	3	16
No	15	79
Unknown	1	5
HISTORY OF SOLID TUMOR MALIGNANCIES		
Yes	14	74
No	4	21
Unknown	1	5
TREATMENT MODALITY TO THE PRIMARY TUMOR		
Surgery	18	95
Radiation (concurrent or adjuvant +/− chemotherapy)	12	63
Unknown	1	5
RECURRENCE SITE		
No recurrence	11	57
Loco regional	2	11
Distant	4	21
Unknown	2	11

N=number,

*Staging according to the American Joint Committee on Cancer (AJCC) staging system 7th edition.

Table 2

Prevalence of IHC markers of interest

Variable	N	%
SAMPLE ORIGIN		
Primary site	12	63
Loco-regional metastasis (lymph node or parotid)	7	37
MCPyV IHC staining		
Positive	9	47
Negative	10	53
PARP1 IHC staining		
Negative (H-score ≤ 30)	3	16
Positive (H-score 30-200)	2	10
Strongly positive (H-score > 200)	14	74