CASE REPORT
ONCOLOGY

Diseases & Research

MRD-Guided PRaG 3.0 Therapy for HER-2 Positive Cervical Adenocarcinoma: A Case of Complete Remission

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Abstract: Advanced cervical adenocarcinoma with human epidermal growth factor receptor 2 (HER-2) expression poses significant treatment challenges. This case report explores the efficacy of PRaG 3.0 therapy, a novel combination of radiotherapy, immunotherapy agent [granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), and programmed death receptor 1 (PD-1) inhibitor], and disitamab vedotin (RC48), a targeted anti-HER2 antibody-drug conjugate. A patient with advanced, refractory cervical adenocarcinoma and HER-2 expression (immunohistochemistry 1 +), underwent PRaG 3.0 therapy, resulting in complete tumor remission after two cycles, sustained over 16 additional cycles without significant side effects. Post-treatment, ctDNA and lymphocyte monitoring confirmed continued remission. In conclusion, PRaG 3.0 therapy demonstrates promising potential for managing complex cases of cervical adenocarcinoma.

Keywords: Disitamab vedotin; PRaG 3.0 therapy; Minimal residual disease; HER-2 expression; Adenocarcinoma of cervix.

Introduction

Cervical adenocarcinoma with human epidermal growth factor receptor 2 (HER-2) expression presents unique challenges in treatment, with variable expression complicating targeted therapies like trastuzumab[1,2]. The heterogeneity of HER-2 across lesions adds to the complexity, especially in recurrent, chemotherapy-resistant cases[3,4]. However, new immunotherapies targeting programmed death receptor 1 (PD-1)/programmed cell death 1 ligand 1 (PD-L1) have shown promise[5–7]. PRaG therapy combines radiotherapy with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), and PD-1/PD-L1 inhibitors, enhancing antitumor immunity and showing efficacy in advanced malignancies[8–11]. Disitamab vedotin (RC48), China's first approved anti-HER2 antibody-drug conjugate, has shown potent antitumor effects, particularly when combined with PRaG therapy, suggesting a synergistic potential in treating HER-2 positive refractory solid tumors[12–14].

Global guidelines suggest a maintenance period for immunotherapy, with varying recommendations on duration post-CR[15,16]. Monitoring for minimal residual disease (MRD) with circulating tumor DNA (ctDNA) is critical in evaluating residual disease and adjusting treatment[17,18]. Lymphocyte counts further inform tumor progression and treatment efficacy[19].

This study highlights the effectiveness of PRaG 3.0 therapy and the role of MRD monitoring in managing advanced, refractory HER-2 positive cervical adenocarcinoma, contributing valuable insights to the treatment of chemotherapy-resistant metastatic condition.

Case presentation

Patient Background

A 51-year-old female with hypertension and diabetes underwent surgery for stage IIA cervical cancer at Fudan University Shanghai Cancer Center in September 2019. Pathology confirmed a

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5 × 4 cm adenosquamous carcinoma, without lymph node metastasis. Post-surgery, she received 46 Gy of radiotherapy and four cycles of chemotherapy (paclitaxel 270 mg and carboplatin 850 mg), resulting in grade III bone marrow and renal toxicity. By October 2021, her tumor markers increased, with CA125 at 323.5 U/mL, CEA at 41.62 ng/mL, SCC antigen at 2.0 ng/mL, and HER-2 expression [immunohistochemistry (IHC) 1 +], indicating potential disease activity.

Further evaluation through positron emission tomography/computed tomography (PET/CT) imaging indicated notable changes following cervical cancer surgery. Lymph nodes in different regions, including the right axilla, left supraclavicular area, paratracheal area adjacent to the aortic arch, and right external iliac vessel, exhibited varying degrees of enlargement with pronounced abnormal fluorodeoxyglucose (FDG) metabolism. Additionally, multiple nodules were observed in both lungs, highly suspicious of metastatic involvement. Subsequently, a biopsy procedure was performed on the right axillary lymph node, confirming the presence of cancer cell metastases. Immunohistochemical testing and clinico-pathological correlation validated the origin of the cancer cells from cervical tissue, with the metastatic component identified as adenocarcinoma, see Fig. 1.

Interventions and Treatment

The patient, who could not tolerate further chemotherapy, consented to participate in the "PRaG 3.0 Therapy" clinical trial (NCT05115500). The therapy commenced with the administration of RC48-antibody-drug conjugate (ADC, Disitamab Vedotin) on the first day, followed by segmented radiotherapy aimed at metastatic sites on the third and fourth days, GM-CSF (Molgramostim) to IL-2(I) treatment from the third to the twelfth day, and PD-1 immunotherapy (Sintilimab) on the fifth day, see Fig. 2. Subsequent imaging revealed the resolution of lymph node lesions in the left supraclavicular, paratracheal, and right external iliac areas. The two lymph node metastases in the right axilla, previously noted for abnormal FDG metabolism, were undetectable, and the lung nodules had regressed to near invisibility, see Fig. 3. Tumor markers significantly decreased, see Fig. 4, without causing major gastrointestinal or hematological side effects.

Post-Therapy Imaging and Clinical Observations

The therapy was continued for a full year, during which repeated follow-up exams uniformly indicated complete tumor remission. Imaging reviews revealed the total disappearance of the tumor (Fig. 5A-B). Although this was a positive outcome, further evaluations and ongoing maintenance immunotherapy were deemed necessary to confirm a full cure. In pursuit of this, MRD (minimal residual disease) detection, a cutting-edge and promising method, was employed. This included peripheral blood ctDNA-MRD testing, which scrutinized 165 genes linked to the potential for tumor relapse, progression, and response to the comprehensive treatment regimen.

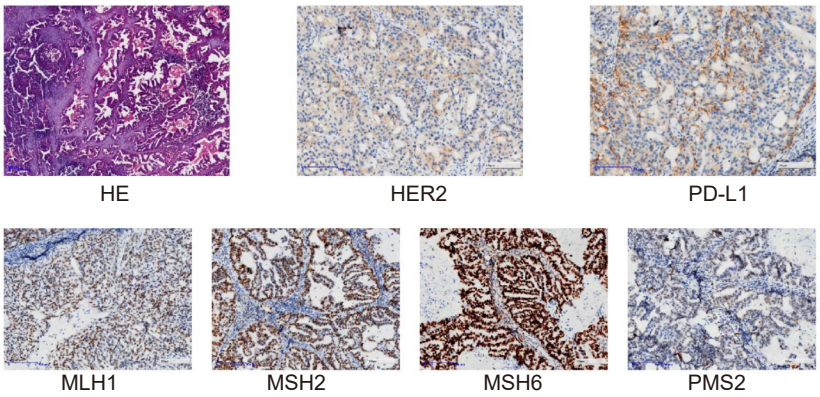
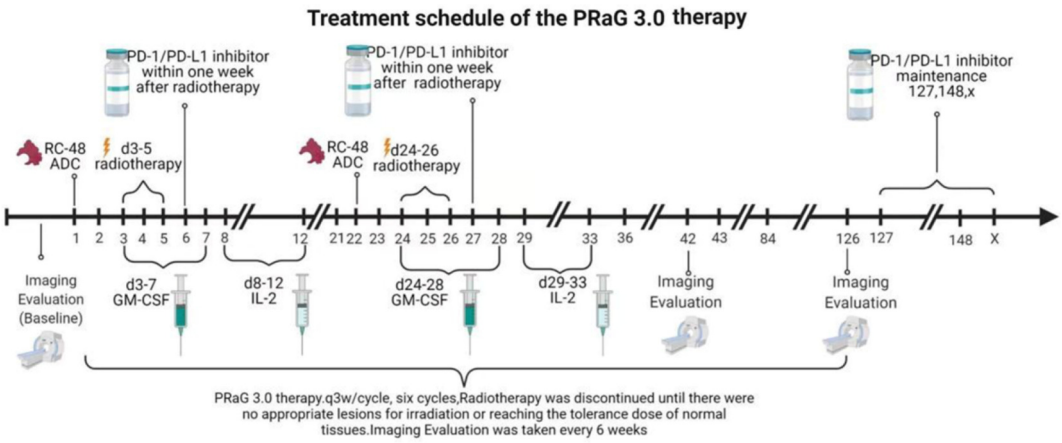


Fig. 1. Immunohistochemical (IHC) results: HER-2 (1 +), PD-L1 (+ 5%), PMS2 (weak +), MLH1 (+), MSH2 (+), MSH6 (+).



Cycle	RC-48 (Vedicetumab 120mg)	Radiotherapy	PD-1 inhibitor (Sintilimab200mg)	GM-CSF(200ug d3-7) +IL-2(2 million IU d8-12)
Cycle 1	2021.11.20	2021.11.22-11.23 (Para-arch lymph node metastasis)	2021.11.24	2021.11.22-11.31
Cycle 2	2021.12.11	2021.12.13-12.14 (Pelvic lymph node metastasis)	2021.12.15	2021.12.13-12.22
Cycle 3	2021.12.29	—	2021.12.30	2021.12.30-2022.01.08
Cycle 4	2022.01.20	—	2022.01.22	2022.01.22-2022.01.31
Cycle 5	2022.02.16	—	2022.02.18	2022.02.18-2022.02.27
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Cycle 18	2022.10.17	—	2022.10.24	2022.10.19-2022.10.28

Fig. 2. PRaG treatment process.

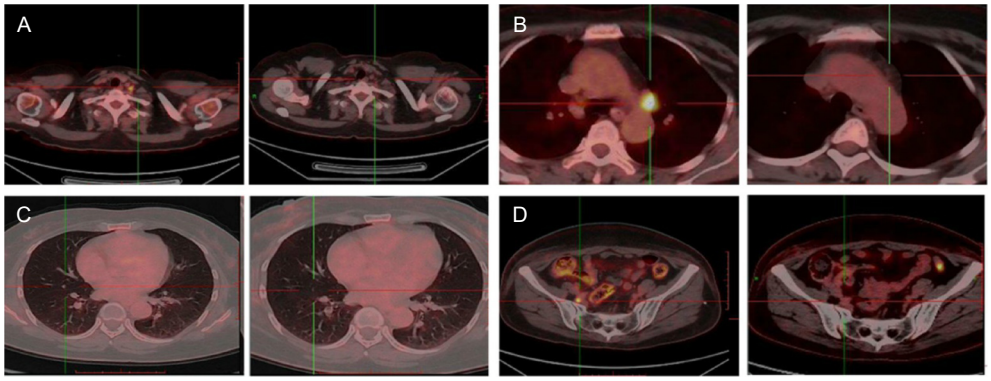

Fig. 3. Assessment of efficacy in irradiated and non-irradiated areas before and after PRaG 3.0 therapy for two cycles.
A. Left supraclavicular lymph nodes in the non-irradiated area before and after comparison.
B. Comparison of para-aortic arch lymph nodes before and after irradiation.
C. Before and after pulmonary nodules in the non-irradiated area.
D. Comparison of right external iliac lymph nodes before and after irradiation.

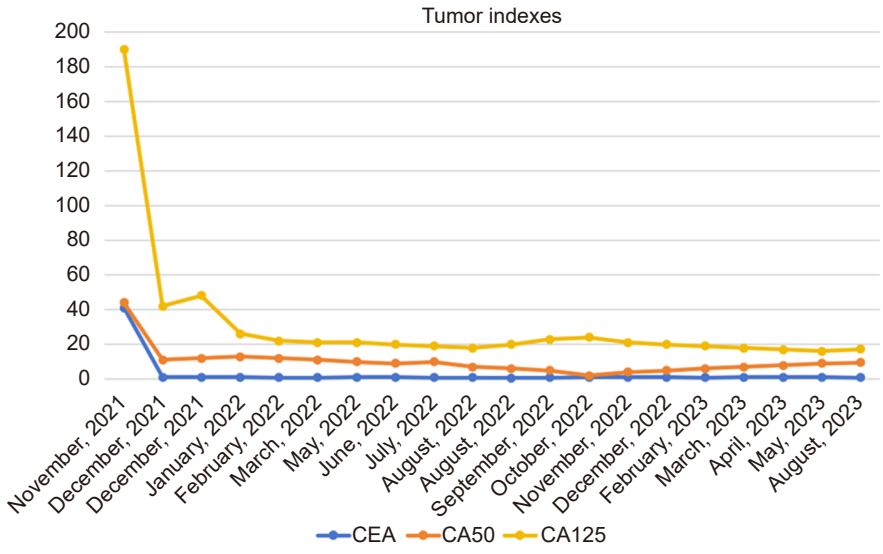

Fig. 4. Changes in tumor indexes before and after PRaG 3.0 therapy.

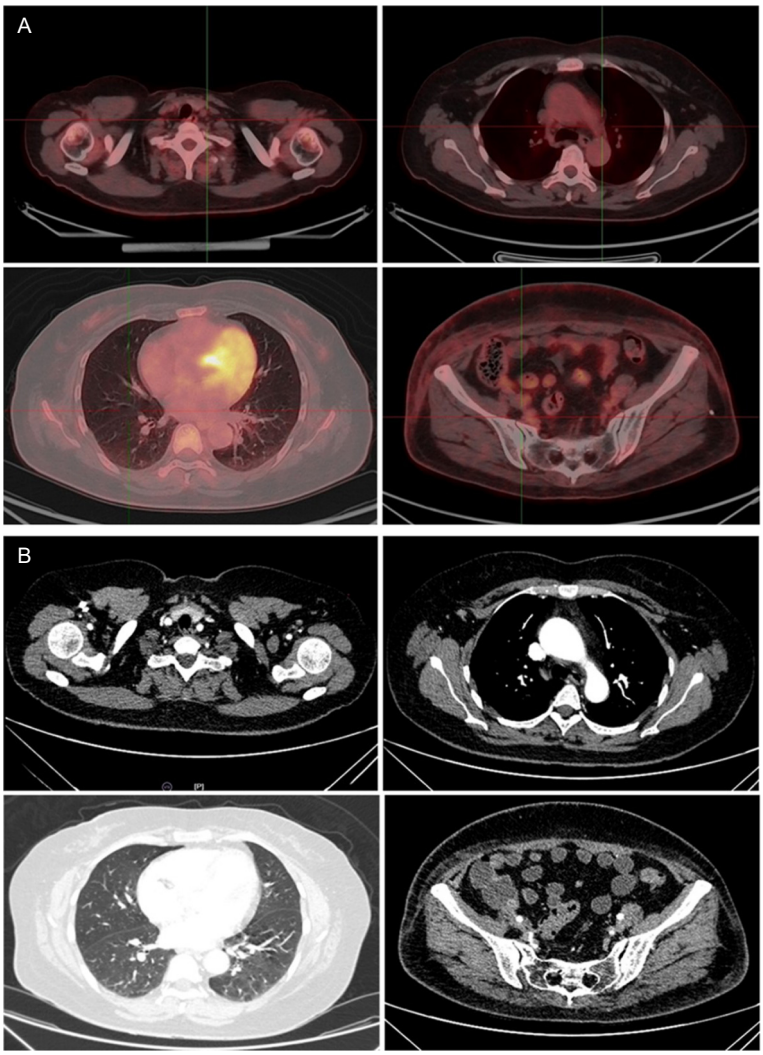

Fig. 5. Assessment of efficacy after PRaG 3.0 therapy discontinuance for ten months follow-up.
A. The PET-CT images of vanished tumor lesions correspond to the first ctDNA-MRD monitoring.
B. The CT images of vanished tumor lesions after PRaG 3.0 therapy discontinuance for ten months follow-up.

Sustained Remission and Follow-Up Strategy

The patient's monitoring continued for ten months after cessation of treatment. During this period, she underwent four MRD evaluations over six months, all of which returned negative results (Fig. 6). These findings corroborated the absence of minimal residual disease, underscoring the potential efficacy of the PRaG 3.0 therapy as evidenced by the sustained complete remission observed at the ten-month follow-up mark. Additionally, detailed lymphocyte subtyping revealed a normal immune profile, characterized by an increased count of CD4 + and CD8 + T cells, particularly noted during the initial two cycles of the PRaG 3.0 regimen (Fig. 7). This increase in lymphocyte levels was maintained even after the treatment concluded.

Discussion

Adenosquamous carcinoma of the cervix is a mixed cancer type, containing both adenocarcinoma and squamous carcinoma. The incidence of cervical adenosquamous carcinoma is extremely low. Moreover, the prognosis of cervical adenosquamous carcinoma is similar to that of squamous carcinoma. 2024 NCCN guidelines recommended chemotherapy combined with immunotherapy as the first-line treatment for advanced cervical cancer. There is currently a lack of suitable second-line treatment options. However, metastatic cervical adenocarcinoma generally has a worse prognosis than squamous carcinoma, because adenocarcinoma has higher incidence of infiltration and metastasis. PRaG 3.0 therapy combined with RC48-ADC, HFRT, PD-1/PD-L1 inhibitors sequential GM-CSF and IL-2 (PRaG3.0 regimen) further enhances the synergistic anti-tumor effect, and this precise paradigm of combination therapy has been proven to have good efficacy and safety by previous studies[20–22]. In this case, the patient, post-cervical cancer surgery, presented with multiple metastatic lymph nodes and low HER-2 expression. Despite initial standard treatments, the metastases persisted, prompting the transition to PRaG 3.0 therapy.

The abscopal effect in tumors refers to the phenomenon where localized irradiation ofa tumor causes regression of non-irradiated tumors at distant sites. However, radiotherapy alone induces

abscopal effects at relatively low rates. Recent studies have found that combining PD-1 inhibitors with radiotherapy significantly increases the incidence of abscopal effects. Radiation therapy activates the cyclic GMP-AMP synthase-Stimulator of Interferon Genes (cGAS-STING) signaling pathway, promoting type I interferon secretion and enhancing dendritic cell activation and antigen presentation. This process activates cytotoxic T lymphocytes. When combined with PD-1 inhibitors, these effects synergistically enhance anti-tumor immune responses, ultimately mediating more frequent abscopal effects. In this case, treatment consisted of two cycles of RC48-ADC, targeted radiotherapy, the immunotherapeutic drugs GM-CSF and IL-2, followed by PD-1 immunotherapy (Sintilimab), which resulted in a complete remission of the tumor and a return to normal tumor markers. The patient sustained 16 additional cycles of this regimen, with follow-up imaging confirming the resolution of both irradiated and non-irradiated metastases. Subsequent MRD monitoring via peripheral blood ctDNA consistently returned negative, indicating effective disease control. Immune profiling showed normal function with an increase in activated memory T cells, suggesting a durable immune response post-therapy.

HER-2 is pivotal in the pathology of various cancers, yet its variable expression presents therapeutic challenges[23,24]. Our study contributes to this understanding by demonstrating the effectiveness of RC48-ADC, an innovative antibody-drug conjugate targeting HER-2 positive tumors, which, when combined with radiotherapy, significantly enhances both local and systemic treatment efficacy[25,26]. Since its launch in China in 2021, RC48-ADC has shown greater receptor affinity and cellular uptake compared to trastuzumab, thereby offering a new therapeutic promise for HER-2 positive malignancies[27,28]. Particularly in this study, RC48-ADC has shown potential in treating HER-2 low-expressed tumors, an area where traditional therapies have had limited success. The agent's cleavable linker and ability to induce bystander cytotoxic effects make it a suitable candidate for IHC 1 + cases, as observed in our patient cohort[29]. This aligns with the FDA's recent approval of Tivdak for resistant cervical cancer, indicating a broader applicability of ADCs like RC48-ADC[30]. Our study's findings are supported by ongoing clinical trials that are evaluating RC48-ADC's efficacy in HER2-positive metastatic breast cancer and its comparative effectiveness against TPC in HER2-low metastatic breast cancer. Early results have been encouraging, particularly in advanced urothelial carcinoma, suggesting that RC48-ADC could be effective even in patients with

A	List of MRD genes					
ABL1	AKT1	ALK	APC	AR	ARID1A	ARID1B
ATM	ATR	ATRX	AXIN1	BAP1	BARD1	BRAF
BRCA1	BRCA2	BRIP1	BTK	CBL	CCDC6	CCND1
CCNE1	CD274	CDH1	CDK12	CDK4	CDK6	CDKN1B
CDKN2A	CDKN2B	CHEK1	CHEK2	CRKL	CSF1R	CTNNB1
DDR2	DNMT3A	EGFR	EP300	EPCAM	EPHA3	ERBB2
ERBB3	ERBB4	ESR1	EZH2	FANCA	FANCB	FANCD2
FANCI	FANCL	FAT1	FBXW7	FGFR1	FGFR2	FGFR3
FLT3	FOXA1	FOXL2	GATA3	GNA11	GNAQ	GNAS
H3-3A	HNF1A	HRAS	IDH1	IDH2	JAK2	JAK3
KDM6A	KDR	KEAP1	KIT	KMT2A	KMT2C	KMT2D
KRAS	LRIG3	MAP2K1	MAP2K2	MAP2K4	MAPK1	MAPK3
MDM2	MET	MLH1	MPL	MSH2	MSH6	MTOR
MYC	MYCL	MYD88	NBN	NF1	NF2	NFE2L2
NKX2-1	NOTCH1	NPM1	NRAS	NTRK1	NTRK2	NTRK3
PALB2	PBRM1	PDGFRA	PDGFRB	PIK3CA	PIK3R1	PMS2
POLE	PPP2R2A	PTCH1	PTEN	PTPN11	PTPRD	PTPRT
RAD51B	RAD51C	RAD51D	RAD54L	RAF1	RB1	RBM10
RECQL4	RET	RHOA	ROS1	SDHA	SDHB	SDHC
SDHD	SETD2	SLC34A2	SMAD4	SMARCA4	SMARCB1	SMO
SPOP	SRC	STK11	TCF7L2	TERT	TFG	TP53
TSC1	TSC2	VHL	BAT-25	BAT-26	MONO-27	NR-21
NR-22	NR-24	NR-27	BAT-40	D2S123	D5S346	D17S261
D17S520	D18S34	BAT-RII	D17S250			

B

Personal Information		Disease Information	
Diagnosis ID	ZXAM20061	Clinical Diagnosis	Cervical Cancer
Name	[Hidden for Privacy]	Pathological Diagnosis	NA
Gender	NA	TNM Staging	NA
Date of Birth	NA	Family History	NA
Ethnicity	NA	Sending Institution	NA

Note: The above disease information is based on the information provided by the patient at the time of sample submission. This test will not interpret or read these contents.

Sample Information

Sample Type	Date of Submission	Report Date	Number of Submissions
Blood	NA	2022/11/14	1

Monitoring Indicator	Test Result		
MRD	Tumor-Derived Molecular Variants (count)	Result Interpretation	
	0	MRD Negative	
ctDNA	Current ctDNA Equivalent (hGE/mL)	Trend	
	0	-	

C

Personal Information		Disease Information	
Diagnosis ID	ZXCM10027	Clinical Diagnosis	Cervical Cancer
Name	[Hidden for Privacy]	Pathological Diagnosis	NA
Gender	NA	TNM Staging	NA
Date of Birth	NA	Family History	NA
Ethnicity	NA	Sending Institution	NA

Note: The above disease information is based on the information provided by the patient at the time of sample submission. This test will not interpret or read these contents.

Sample Information

Sample Type	Date of Submission	Report Date	Number of Submissions
Blood	NA	2023/4/17	4

Monitoring Indicator	Test Result		
MRD	Tumor-Derived Molecular Variants (count)	Result Interpretation	
	0	MRD Negative	
ctDNA	Current ctDNA Equivalent (hGE/mL)	Trend	
	0	-	

Fig. 6. ctDNA-MRD monitoring of peripheral blood after PRaG 3.0 therapy.
A. The examination of 165 genes associated with tumor recurrence, progression, and comprehensive treatment.
B. The first ctDNA-MRD monitoring with negative MRD finding after PRaG 3.0 therapy.
C. The last ctDNA-MRD monitoring over a span of six months with negative MRD finding.

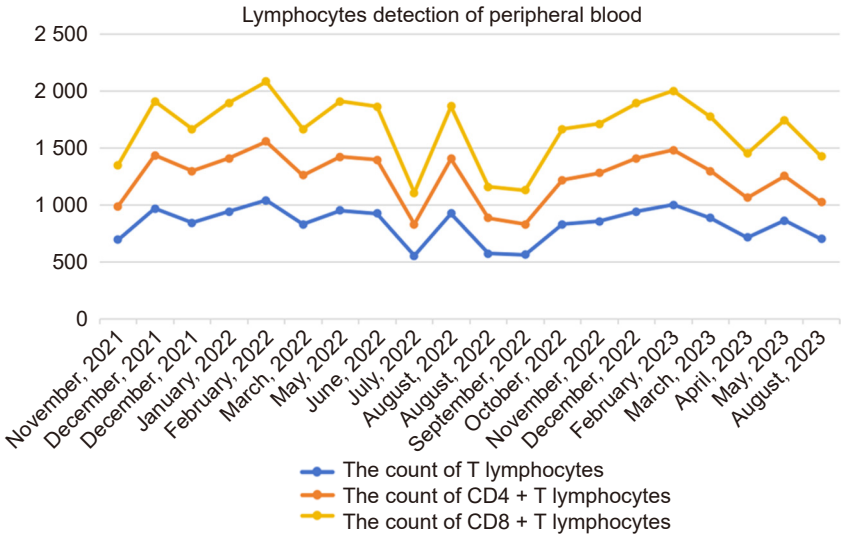

Fig. 7. Changes of T lymphocytes, $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes count in peripheral blood before and after PRaG 3.0 therapy.

impaired renal function, a significant consideration in cancer treatment[31].

In light of our study's results, the discussion on the role of PD-1/PD-L1 inhibitors in cancer immunotherapy can be expanded to illustrate the significant impact of these treatments on patient outcomes. The case reported demonstrated an exceptional response to PRaG 3.0 therapy, incorporating PD-1 inhibitor, which led to marked improvements in the patient's condition and quality of life. These inhibitors have revolutionized treatment paradigms for a range of malignancies, including melanoma and various carcinomas, and have been increasingly incorporated into clinical practice[32–34]. However, when used as monotherapy, PD-1/PD-L1 inhibitors often exhibit limited efficacy in the long term, with response rates typically between 15% to 25% for most tumor types[35–37]. This limitation highlights the urgent need for more innovative treatments. Our study contributes to this narrative by showing that combining these inhibitors with other therapies, like the novel ADC RC48, can significantly enhance treatment outcomes. Therefore, this case report not only supports the current use of PD-1 inhibitor in cancer therapy but also underscores the potential benefits of combining them with other treatment modalities. It calls for further research to optimize treatment regimens, improve long-term remission rates, and ultimately elevate the standard of care for cancer patients[38].

Incorporating the results from our study, the discussion on the augmented efficacy of combining radiotherapy with PD-1 inhibitor (Sintilimab), including the novel RC48-ADC, can be significantly expanded. Our findings demonstrate that radiotherapy, known for its precision and reduced side effects, serves not only as a treatment modality but also as an enhancer of the immune response[39]. This enhancement is particularly evident when radiotherapy is combined with PD-1 inhibitors, a synergy that is gaining recognition for its ability to initiate the 'abscopal effect', thus amplifying systemic antitumor immunity[34,40,41]. In this context, radiotherapy is not just a treatment but an immune response enhancer[42]. Clinical trials, including those by Kotecha *et al*.,[43] report improved outcomes when combining radiotherapy with PD-1 inhibitors, significantly impacting various metastatic cancers, with some trials showing a doubling in progression-free survival rates[44,45].

In addition, MRD monitoring via ctDNA is crucial for assessing treatment response and recurrence risk[18,46]. It guides real-time, non-invasive, personalized treatment decisions, evidenced by low recurrence rates in lung cancer patients with negative MRD[47]. Mutation tracking post-surgery can predict relapse, as demonstrated in non-small cell lung cancer patients[48,49], highlighting the potential for drug holidays in patients achieving complete remission to reduce treatment burden and preserve future treatment options.

PRaG therapy integrates PD-1/PD-L1 inhibitors, radiotherapy, and cytokines like GM-CSF and IL-2, to bolster systemic antitumor effects. This multimodal strategy enhances tumor immunogenicity, facilitates immune system activation, and disrupts cancer-induced immune suppression[9]. For example, GM-CSF plays a role in hematopoiesis and macrophage activation, while IL-2 promotes T-cell proliferation, collectively potentiate antitumor response[50,51]. The targeted effect of radiotherapy on the tumor microenvironment, combined with GM-CSF, can induce the abscopal effect, further evidenced by tumor regression beyond irradiated zones[52]. This case underscores PRaG therapy's potential, leveraging the interplay of various therapies to tailor treatment to the tumor's unique profile[20–22].

Conclusions

This case report highlights the efficacy of PRaG 3.0 therapy in treating advanced refractory cervical adenocarcinoma with low HER-2 expression, where MRD detection via ctDNA played a crucial role in confirming the therapeutic outcome. The case underscores the potential of RC48-ADC to induce a robust immune response and achieve prolonged remission in cases resistant to standard chemotherapy. While these results are promising, further studies are warranted to fully establish the clinical viability of this approach. This report contributes valuable insights into the therapeutic landscape for patients with chemotherapy-resistant solid tumors, supporting the use of the PRaG protocol.

Abbreviations

ADC, Antibody-drug conjugate; ctDNA, Circulating tumor DNA; FDG, Fluorodeoxyglucose; GM-CSF, Granulocyte-macrophage colony-stimulating factor; HER-2, Human epidermal growth factor receptor 2; IHC, Immunohistochemistry; IL-2, Interleukin-2; MRD, Minimal residual disease; PD-1, Programmed death receptor 1; PD-L1, Programmed cell death 1 ligand 1; PET/CT, Positron emission tomography/computed tomography; RC48, Disitamab vedotin.

Ethics approval and consent to participate

This is a case report that does not contain any identifiable patient information. The subject of this case report is enrolled in a clinical trial that has been approved by the Institutional Review Board of The Second Affiliated Hospital of Soochow University (No. JD-LK-2022-121-02) and registered in ClinicalTrails.gov. (https://www.clinicaltrials.gov, No. NCT05115500). Patient provided informed consent for participation in the clinical trial.

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Authors' contributions

JJZ, YHK and MLX contributed equally to this work and contributed to study conception and design, enrolled and took care of the patient, collected clinical data, performed the experiments and analyzed the data, wrote and revised the manuscript and figure; TTF and GQC enrolled and took care of the patient; ZHH, HZ, XXD, YFM, XRZ, CYZ, and RZC collected clinical data, performed the experiments and analyzed the data; GQC and ZHH performed imaging analysis; XXD and XRZ conducted pathological analysis; HZ, YFM and JJZ helped flow cy-tometry data analysis; PFX and LYZ contributed equally to this work and contributed to study conception and design, project administration, funding acquisition, revised the manuscript and figure; All authors reviewed and approved the final version of the manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent for publication

Written informed consent has been obtained from the patient to publish this paper.

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