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Gamma Knife Stereotactic radiotherapy combined with tislelizumab as later-line therapy in hpMMR/MSS/MSI-L metastatic colorectal cancer: A Phase II Trial Analysis

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eLife Assessment

This **valuable** study found Gamma Knife SBRT combined with tislelizumab offers a safe and powerful later-line option for pMMR/MSS/MSI-L metastatic CRC patients who were unresponsive to the first and second-line chemotherapy. The authors implemented a well-structured experimental protocol and provide **convincing** evidence to substantiate the conclusions. This work would be of broad interest to oncologists working on colorectal cancer.

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Abstract

An immunosuppressive tumor microenvironment limits the efficacy of immunotherapy, thus patients with MSS and pMMR mCRC often face great challenges.In this phase II trial, patients received Gamma Knife SBRT combined with Tislelizumab. P Biomarker analysis was performed pre- and post-treatment. From November 2022 to July 2024, 13 of 20 patients achieved PR, 6 achieved SD. mPFS was 10.7 months (95% CI, 6.4-15.0). With no grade 4 events noted, common adverse events included nausea (65%), anemia (55%), and fatigue (45%). RNA sequencing indicated enhanced immune infiltration in PR patients. For patients with pMMR/MSS/MSI-L mCRC who had not responded to first and second-line therapies, the combo of Gamma Knife SBRT and tislelizumab showed high efficacy and reasonable safety. Significant post-radiotherapy improvements in the tumor's immunosuppressive

microenvironment, including lower fibrosis, normalizing of tumor vasculature, and activation of the PD-1/PD-L1 checkpoint pathway were revealed by biomarker analysis. These results imply that patients with pMMR/MSS/MSI-L mCRC who were unresponsive to the first and second-line chemotherapy, Gamma Knife SBRT with tislelizumab provides a safe and powerful later-line treatment alternative.

Statement of significance

This study offers a safe and powerful option for pMMR/MSS/MSI-L mCRC patients fail to first and second-line chemotherapy. And discover Gamma Knife SBRT contributed to potentially converting the suppressive “cold” tumor immune microenvironment into an activated “hot” microenvironment conducive to immunotherapy efficacy in pMMR CRC.

Introduction

Colorectal cancer continues to represent a significant threat to life. As reported in the 2020 Global Cancer Statistics, colorectal cancer accounts for 10% of all cancer cases, ranking third in incidence, while its mortality rate is 9.4%, second only to lung cancer (1,2). Especially, 20% of newly diagnosed colorectal cancer patients present show metastases, and 40% have recurrence and metastases after local treatment (3). The FOLFOX/FOLFIRI chemotherapy regimen, which comprises oxaliplatin, 5-fluorouracil, and irinotecan is the mainstay of clinical treatment for metastatic colorectal cancer (mCRC). For patients harboring wild-type RAS and BRAF, the addition of the epidermal growth factor receptor (EGFR) inhibitor cetuximab is recommended(4,5). For patients with RAS mutations, the anti-angiogenic agent bevacizumab is advised. Nevertheless, RAS-mutant patients exhibit poorer prognoses and shorter survival times compared to their wild-type counterparts (6,7). The efficacy of chemotherapy in combination with targeted therapy remains suboptimal (7).

The development of immune checkpoint inhibitors (ICIs) transforms cancer immunotherapy (8). Particularly CRCs with mismatch repair deficiency (dMMR) and high microsatellite instability (MSI-H) show a strong response to ICIs (9). But most CRC cases are either microsatellite-stable/low microsatellite instability (MSS/MSI-L) or mismatch repair-profile (pMMR), which reduces the efficacy of immunotherapy in a significant number of mCRC patients (9). Chemotherapeutic agents can cause immunogenic cell death in tumors, thus coordinating with ICIs improves anti-tumor efficacy (10). Additionally, anti-angiogenic therapies targeting VEGFR facilitate the normalization of tumor vasculature and promote immune cell infiltration, subsequently amplifying immune-mediated tumor eradication (11). Clinical studies, however, have revealed that t combining mFOLFOX6 or other chemotherapy regimens with anti-VEGF, anti-EGFR, and ICIs does not produce better clinical outcomes in mCRC (12,13). Consequently, identifying alternative strategies to augment the efficacy of immunotherapy remains a pivotal objective in the field of cancer immunotherapy in pMMR/MSS/MSI-L mCRC.

Stereotactic body radiation therapy (SBRT), effectively targets and eradicates tumor cells with high-dose radiation (14). Although traditional radiotherapy is sometimes linked with immunosuppressive effects (15), SBRT’s exact targeting can expose tumor neoantigens, mobilize and activate immune cells, increase their infiltration into the tumor, and improve the tumor immune microenvironment (16,17). The Gamma Knife is a principal modality in SBRT, employing gamma rays generated by cobalt-60 to deliver a single, high-dose focused irradiation to the target lesion. The Gamma Knife provides several benefits over conventional radiotherapy, including hiexact stereotactic targeting, increased delivery dose to the lesion, prevention of accelerated repopulation of tumor cells, and better local control rates of tumors(18). Our team

firstly observed a case with pMMR-type mCRC who exhibited local recurrence and distant metastasis following first-line and second-line chemotherapy combined with targeted therapy(19). After undergoing gamma knife SBRT followed by tislelizumab treatment, intrahepatic metastatic lesions were reduced and stabilized, the patient showed a partial response (PR) with notable reduction of recurrent lesions in the rectal wall and stabilization of intrahepatic metastases, so extending the progression-free survival (PFS) exceeded beyond 3 months (19). These findings suggest that Gamma Knife SBRT might improve ICBs sensitivity in mCRC.

The results of a phase II clinical trial assessing the combination of Gamma Knife SBRT combined with tislelizumab as a later-line therapy in patients with pMMR/MSS/MSI-L mCRC are presented in this report together with safety and efficacy. NanoString assay for transcriptome analysis was employed to elucidate changes in the tumor immune microenvironment during the combined treatment, offering insights into the therapeutic potential and mechanistic underpinnings of this integrated approach.

Results

Patients

In this clinical trial, twenty patients with pMMR/MSS/MSI-L tumors refractory to first or second-line treatment were enrolled. The cohort comprised 15 males and 5 females, with ages ranging from 47 to 77 years. Predominantly, the primary tumors were located in the left colon and rectum (17/20, 85%), with the liver being the most common site of metastasis, followed by the lung (3/20) (**Table 1**). Flowchart of therapeutic regimen and flow diagram of enrolled participants in the study were shown in **Figure 1A** and **Figure 1B**.

Molecular profiling revealed RAS mutations in 11 patients (55%), with 5 exhibiting KRAS mutations and 6 presenting NRAS mutations. PD-L1 expression was assessed in 18 patients, 12 (60%) patients had combined positive score (CPS) ≤ 1. Tumor mutation burden (TMB) data were available for 8 patients, with a median TMB of 4.62 mutations/Mb (IQR 3.08-8.97). Notably, only one patient exhibited a TMB > 10 mutations/Mb (**Table 1**).

Efficacy

In our cohort of 20 patients meeting inclusion criteria, 13 (65%) achieved a partial response (PR), and 6 (35%) maintained stable disease (SD), resulting in a robust disease control rate (DCR) of 95% (**Table 2**). Patients with liver metastases achieved 92.9% DCR, and patients with metastases in non-liver locations notably achieved a remarkable 100% DCR, only 1 patient with liver metastases experienced disease progression (PD) (**Figure 2A**), As of the data cutoff date, 7 patients remained on maintenance treatment, and 1 patient underwent surgery due to disease progression (**Figure 2A**). Remarkably, 3 patients refractory to first-line treatment responded to SBRT combined with tislelizumab, achieving rapid regression to NED status, with durations ranging from 6 to 18 months before progression. Encouragingly, 1 patient remains in a state of NED, under ongoing monitoring (**Figure 2A**).

Most patients exhibited favorable survival outcomes throughout the treatment (**Figure 2B**), and median progression-free survival (PFS) was 10.7 months (95% CI, 6.4, 15.0) (**Figure 2C**). Additionally, a comparative survival analysis included 23 patients who underwent first and second-line treatment and Gamma Knife SBRT without immunotherapy, revealing a median PFS of 6.7 months (95% CI, 5.6, 7.0), this data shown Gamma Knife SBRT combined with tislelizumab as later-line treatment prolong PFS in mCRC (Log-rank test = 5.638, P = 0.0176) (**Figure 2D**). These findings suggest Gamma Knife SBRT combined with tislelizumab can effectively inhibiting mCRC progression.

Characteristics	Patients (n=20)
Age, years, median (IQR), n (%)	60 (56-65)
<60	8 (40%)
≥60	12 (60%)
Sex, n(%)	
Male	15 (75%)
Female	5 (25%)
ECOG performance status, n (%)	
0	12 (60%)
1	8 (40%)
Primary tumor location, n (%)	
Left colon and rectum	17 (85%)
Right colon	3 (15%)
Number of metastatic organs[a], n (%)	
1	14 (70%)
≥2	6 (30%)
Metastatic organ, n (%)	
Liver	14 (70%)
Lung	7 (35%)
Lymph node	2 (10%)
Other	3 (15%)
Ras mutation type, n (%)	
KRAS	5 (25%)
NRAS	6 (30%)
Other	9 (45%)
PD-L1 expression, CPS, n (%)	
CPS≤1	12 (60%)
CPS > 1	6 (30%)
Unknown	2 (10%)
TMB (mut/Mb), median (IQR), n (%)	4.62 (3.08-8.97)
TMB<5	4 (20%)
TMB≥5, ≤10	3 (15%)
TMB>10	1 (5%)

Table 1

Baseline demographic and clinical characteristics.

Unknown	12 (60%)

Abbreviations: CPS, combined positive score; ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range; TMB, tumor mutation burden.
[a]Multiple answers allowed.

Table 1 (continued)

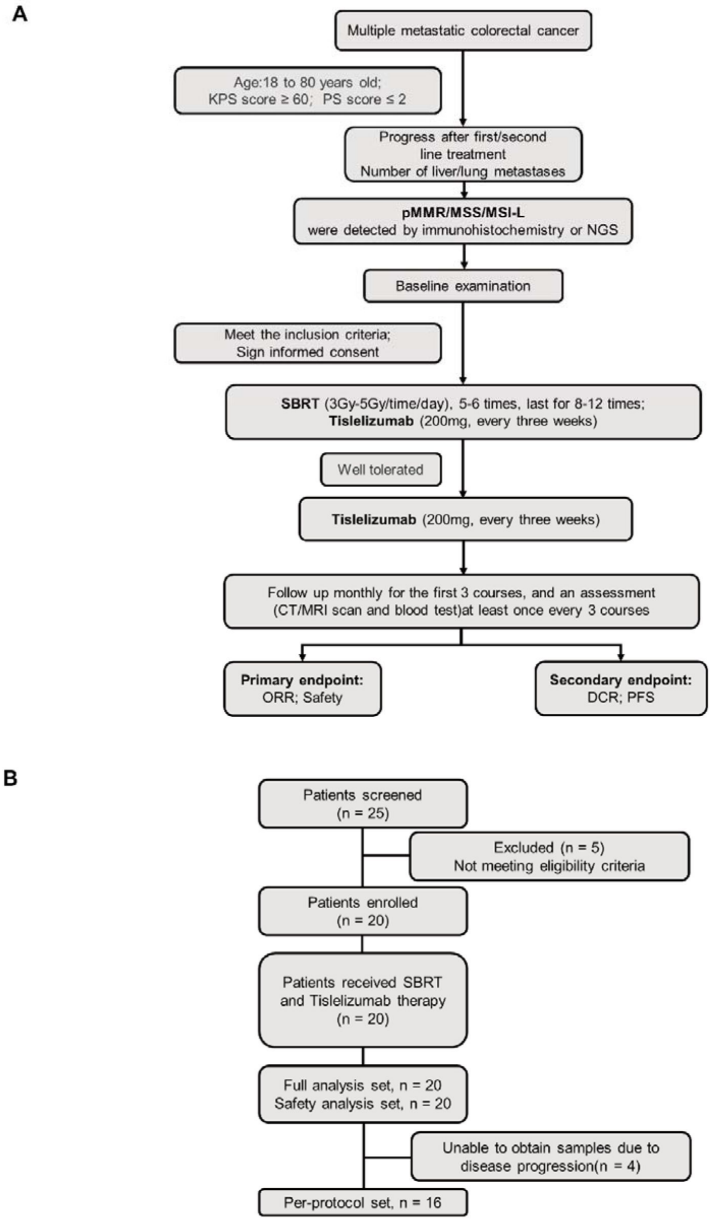


Figure 1.

Clinical trial flow chart.

A) Flowchart of therapeutic regimen. **B)** Flow diagram of participants in the study.

Table 2

Efficacy outcomes.

	All patients (N = 20)	Liver metastasis (N = 14)	Other metastasis (N = 6)
Best overall response			
Complete response (CR), n (%)	0 (0%)	0 (0%)	0 (0%)
Partial response (PR), n (%)	13 (65%)	8 (57%)	5 (83%)
Stable disease (SD), n (%)	6 (30%)	5 (36%)	1 (17%)
Progressive disease (PD), n (%)	1 (10%)	1 (7%)	0 (0%)
ORR, n (%, 95% CI)	13 (65%, 40.8-84.6%)	8 (57.1%, 28.9-82.3%)	5(83.3%, 35.9-99.6%)
DCR, n (%, 95% CI)	19 (95%, 75.1-99.9%)	13 (92.9%, 66.1-99.8%)	6(100%, 54.1-100%)

Abbreviations: ORR, objective response rate; DCR, disease control rate.

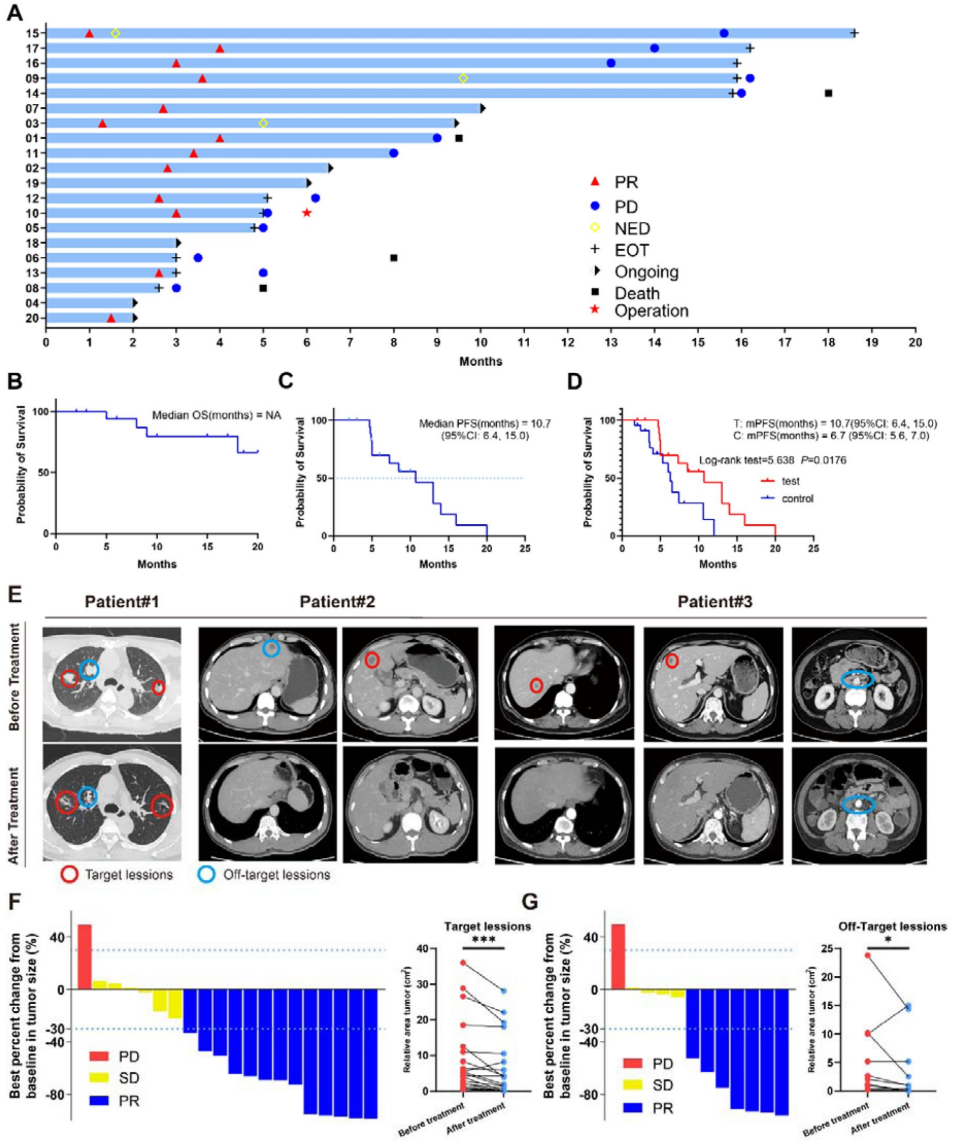


Figure 2.

Clinical trial results.

A) Swimmer plots of patients. **B)** Kaplan–Meier curves of OS for the per-protocol set (N = 20). **C)** Kaplan–Meier curves of PFS for the per-protocol set (N = 20). **D)** Kaplan–Meier curves of PFS for didn't receive immunotherapy set (control group) (N= 23) and per-protocol set (test group) (N=20). **E)** Radiological response from patient. **F)** Waterfall plot of best percent change from baseline in patient target lesion (N= 20). **G)** Waterfall plot of best percent change from baseline in patient off-target lesion (N= 12).

In our analysis of clinical characteristics and patient prognosis, we observed that the median progression-free survival (mPFS) of patients with wild-type RAS expression was significantly longer (14 months) compared to those with KRAS (7.3 months) and NRAS (5 months) mutations (**Supplementary Fig 1A**). These results are consistent with prior studies indicating that RAS mutations are associated with increased malignancy, diminished therapeutic response, and poorer overall prognosis in colorectal cancer. However, statistical analysis did not reveal a significant difference between the groups (Log-rank test = 4.278, P = 0.118), which may be attributed to the limited sample size and other factors inherent to the study design. Additionally, we assessed the PFS of patients with simple liver metastases versus those with metastases to other organs (**Supplementary Fig 1B**). Notably, the mPFS of patients with liver metastases (13 months) was longer than that of those with metastases at other sites (8.5 months). This difference could potentially be explained by the efficacy of localized gamma knife treatment in managing liver metastases. Despite this observation, statistical comparison between the two groups did not yield a significant difference (Log-rank test = 0.081, P = 0.776). Given these limitations, further investigation with larger sample sizes is essential to elucidate the factors contributing to these trends and to confirm the clinical significance of these findings.

In light of the abscopal effect of radiotherapy, we extended our observations beyond the lesions directly targeted by stereotactic radiotherapy to include non-irradiated lesions (**Supplementary Fig 1C**). Imaging examinations revealed significant tumor regression in both the irradiated target lesions (**Figure 2E**, **F**) and the non-irradiated lesions (**Figure 2E**, **G**) following Gamma Knife SBRT combined with tislelizumab. These findings suggest that SBRT not only impacts the irradiated lesions but also sensitize distant metastatic sites for ICBs through the abscopal effect, thereby enhancing the systemic antitumor response when combined with immunotherapy.

Safety

All 20 enrolled patients received the assigned treatment regimen, with safety assessments conducted every three treatment cycles. Treatment-related adverse events (TRAEs) and immune-related adverse events are summarized in **Table 3**. Predominantly, patients experienced mild to moderate adverse events, with the most common being nausea (65%), anemia (55%), electrolyte disturbances (55%), fatigue (45%), and anorexia (35%). Notably, only two patients experienced grade 3 events of increased blood bilirubin, while no grade 4 adverse events were reported throughout the study period.

Identification of differentially expressed genes between responder and non-response groups

To elucidate the impact of the tumor immune microenvironment on combination therapy outcomes, we employed NanoString assay for transcriptome analysis of tumor samples obtained from 16 enrolled patients before and after treatment, totaling 32 samples (**Figure 3A**). Patients were stratified into responder (PR) and non-responder (non-PR) groups based on treatment outcomes. Gene expression differential analysis between pre- and post-treatment samples within each group identified significant alterations, detailed in the **Supplementary Data** and illustrated in **Figure 3B**.

Our findings highlighted notable up-regulation of key genes involved in antigen presentation (CD40, TNFSF18, TNFSF4), immune checkpoint modulation (PDCD1LG2, CD274, IDO1, VTCN1), and T cell activation pathways (TNFRSF9, CD28, ICOS, CD40LG, CD2, GZMK, ENTPD1, ITGAE) in the responder group. Additionally, a diverse array of chemokine family genes (IL2, IL4, IL17A, CCR2, CCL22) showed enhanced expression in PR group (**Figure 3B**). Furthermore, immune cell abundance analysis based on 11 predefined immune cell types revealed significantly elevated levels in the PR group compared to non-PR. included T cells, B cells, mast cells, macrophages,

TEAEs, n (%)	Patient (N=20)				
	Grade 1	Grade 2	Grade 3	Grade 4	Any grade
Anemia	9 (45%)	2 (10%)	0	0	11 (55%)
Neutropenia	1 (5%)	0	0	0	1 (5%)
Nausea	10 (50%)	3 (15%)	0	0	13 (65%)
Poor appetite	3 (15%)	4 (20%)	0	0	7 (35%)
Electrolyte disturbance	7 (35%)	2 (10%)	0	0	11 (55%)
Hand-foot syndrome	0	0	0	0	0
Leukocytopenia	2 (10%)	0	0	0	2 (10%)
Aspartate transaminase increased	2 (10%)	2 (10%)	0	0	4 (20%)
Lipase increased	0	0	0	0	0
Proteinuria	0	0	0	0	0
Thrombocytopenia	2 (10%)	2 (10%)	0	0	4 (20%)
Vomiting	1 (5%)	3 (15%)	0	0	4 (20%)
Hypothyroidism	0	0	0	0	0
Triglycerides increased	0	0	0	0	0
Fatigue	6 (30%)	3 (15%)	0	0	9 (45%)
Blood bilirubin increased	0	0	2 (10%)	0	2 (10%)
Alanine transaminase increased	2 (10%)	1 (5%)	0	0	3 (15%)
Peripheral neurotoxicity	0	0	0	0	0
Hoarseness	0	0	0	0	0
Rash	4 (20%)	0	0	0	4 (20%)
Thyroiditis	0 (0%)	0	0	0	0
Diarrhea	1 (5%)	0	0	0	1 (5%)
Troponin increased	0	0	0	0	0
Fever	0	0	0	0	0
Alkaline phosphatase increased	0	0	0	0	0
Amylase increased	0	0	0	0	0
Hypertension	0	0	0	0	0

Table 3

Treatment-emergent adverse events (TEAEs) since the initiation of protocol-specified treatment

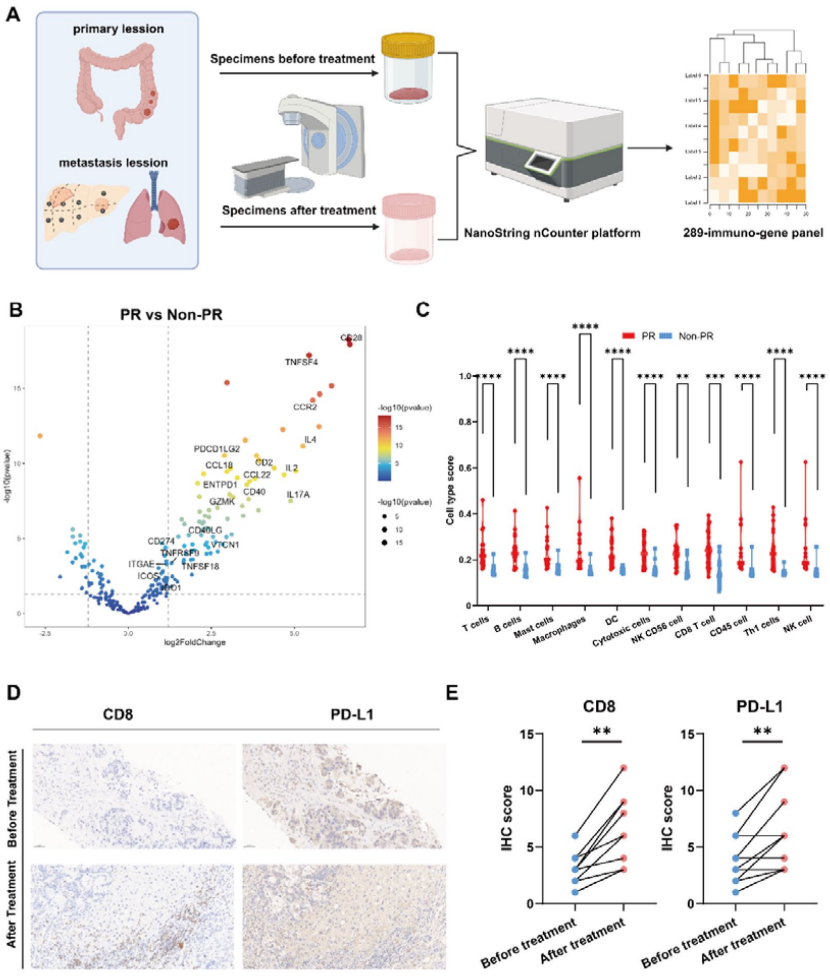


Figure 3.

Differential expressed genes analysis.

A) Specimens collection flowchart. **B)** Transcriptome analysis on differential expression genes between responders (PR) (n = 9) and non-responders (Non-PR) (n = 7), DESeq2 was provided to perform differential expression testing. **C)** The abundance of predefined 12 immune cells composition before and after treatment between responders (PR) (n = 9) and non-responders (Non-PR) (n = 7), Wilcoxon test was used to determine the statistical significance between subgroups. **D&E)** Representative CD8 & PD-L1 IHC staining of before and after treatment specimens of patient.

Dendritic Cell (DC), Cytotoxic cells, NK CD56 cell, CD8 T cell, CD45 cell, Th1 cells and NK cell (**Figure 3C**). This heightened immune activation in responders encompassed robust antigen presentation, T cell activation, and co-stimulation processes crucial for effective immune-mediated tumor control.

To investigate the impact of Gamma Knife treatment on patient outcomes, we conducted a differential expression analysis of pre- and post-treatment samples from both responder (PR) and non-responder (non-PR) groups (**Supplementary Fig 2A**). The results revealed limited gene expression alterations following treatment. However, intersecting the differentially expressed genes from both groups identified NOS2 as a gene that was consistently upregulated post-treatment in both cohorts (**Supplementary Fig 2B**). Although NOS2's increased expression may be associated with Gamma Knife treatment, its potential role in colorectal cancer treatment remains underexplored, warranting further investigation to elucidate the underlying mechanisms.

Additionally, to identify genes linked to treatment efficacy, we performed univariate and multivariate Cox regression analyses on the expression levels of 289 genes from sequencing data in relation to disease progression. Unfortunately, due to the limited sample size and sequencing depth, neither analysis yielded statistically significant results. The full Cox regression analysis of these genes is provided in the Supplementary Data.

Following the combination of stereotactic radiotherapy and immunotherapy, a striking reduction in liver metastasis target lesions was observed in two patients compared to baseline. To elucidate these findings, we conducted CD8 and PD-L1 immunohistochemical staining on liver metastasis biopsy specimens from some patients pre- and post-treatment (**Fig 3D-E**). The analysis revealed increased infiltration of T cells and improved immune microenvironment following treatment, aligning with our prior analytical findings.

Additional immune signatures analysis in predicting tumor response

We conducted gene expression analysis based on 12 predefined gene sets associated with immunotherapy and prognosis (**Figure 4A**). Notably, samples from the responder group exhibited higher expression of immune activation related genes compared to the non-responder group, include effector T cells (T-eff), T cell-Inflamed, IFN-γ(cytotoxic, Cytolytic activity score (CYT), chemokines, angiogenesis (AG), APC co-stimulation (APC co-sti), inflammation promoting (Inflam-pro), T cell co-stimulation (T cell co-sti), parainflammation (parainflam) and tumor-infiltrating lymphocytes (TIL) (**Figure 4A**).

Further compared the related-signature score, we found the responders had higher APC and T cell co-stimulation signature scores compared with the non-responder group (**Figure 4B**). Moreover, the responders had higher T cell-Inflamed, inflammation promoting and parainflammation signature scores compared with the non-responder group (**Figure 4C**). Additionally, increased expression of effector T cell, cytotoxicity, IFN-γ production, and cytolytic activity and TIL signature scores compared with the non-responder group (**Figure 4D**).

Further functional insights into differential gene expression between responder and non-responder groups were gained through gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. GO analysis highlighted enrichment in cytokine and chemokine receptor activities, alongside increased T cell and leukocyte proliferation and activation levels in responders (**Supplementary Fig 3A**). Correspondingly, KEGG analysis underscored enrichment in pathways involving antigen processing and presentation, T cell receptor signaling, chemokine interactions, and cytokine signaling (**Supplementary Fig 3B**).

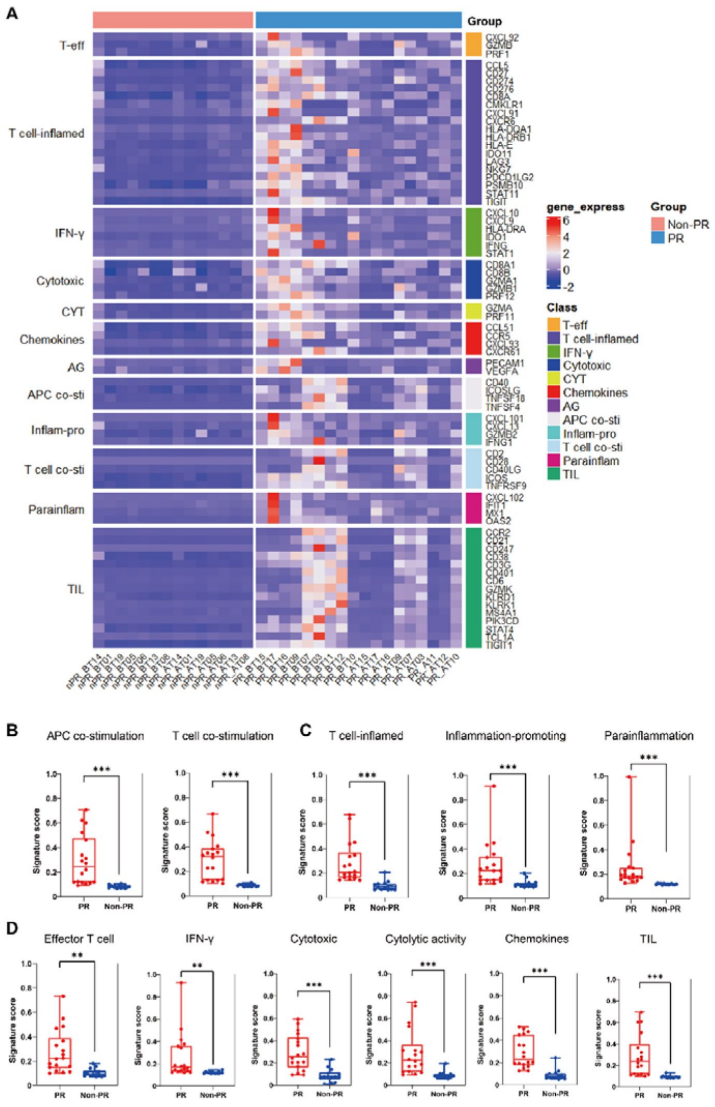


Figure 4.

Additional immune signatures analysis.

A) The expression of 12 gene sets previously reported to be associated with response to immunotherapy and prognosis between responders (PR) (n = 18) and non-responders (Non-PR) (n = 14). **BCD)** 11 gene sets of prognostic value were differentially expressed between responders (PR) (n = 18) and non-responders (Non-PR) (n = 14), box plots are indicated in terms of minima, maxima, centre, bounds of box and whiskers (interquartile range value), and percentile in the style of Tukey, Wilcoxon test was used to determine the statistical significance between subgroups.

Notably, these results indicated responders after combination of Gamma Knife SBRT and tislelizumab treatment will enhancing tumor antigen presentation and T cell mediated immune response in pMMR/MSS/MSI-L mCRC.

Analysis on differential expression genes before and after treatment in the responders

To unravel the mechanisms driving tumor regression in the responder cohort, we conducted comprehensive gene expression analysis before and after treatment, focusing on 7 gene groups known for their potential inhibitory effects on immunotherapy. Post-treatment analysis revealed significant reductions in exhausted T cells, Th2 cells, and Treg cells, indicative of a favorable shift away from a suppressive immune microenvironment (**Figure 5A**). Tumor resistance mechanisms, such as fibrosis and angiogenesis, play pivotal roles in limiting therapeutic efficacy(20,21). Initially, evaluation of immunotherapy-related gene groups in partial responders versus non-responders highlighted significantly higher angiogenesis scores in the former, albeit with no significantly difference (**Figure 5B**). Recognizing potential biases from pooling samples pre- and post-treatment, we conducted separate analyses within the responder group, expanding our gene set to include fibrosis-related genes. The findings underscored substantial inhibition of both angiogenesis and fibrosis within the tumor microenvironment following SBRT, targeted therapy, and immunotherapy (**Figure 5C**). We also analyzed tumor samples from select patients before and after treatment, employing immunohistochemical staining for CD31, a marker of vascular survival (**Figure 5D**). The results demonstrated a significant reduction in CD31 expression following Gamma Knife irradiation, further supporting the notion that Gamma Knife treatment, when combined with immunotherapy, can effectively inhibit tumor angiogenesis (**Figure 5E**).

Further stratified analysis of non-responder samples before and after treatment revealed no significant alterations in the expression levels of immunosuppression-related or angiogenesis/fibrosis-related gene sets (**Supplementary Fig 4**). These insights illuminate critical pathways through which combined therapies modulate the immune landscape and enhance treatment responses in pMMR/MSS/MSI-L mCRC.

Discussion

By successfully reaching its main endpoint, this phase II trial shows that for combined Gamma Knife SBRT with tislelizumab greatly increases progression-free survival (PFS) in pMMR/MSS/MSI-L mCRC, resistant to first and second-line therapies. For this patient population, the combo treatment has shown both safety and tolerability. By overcoming resistance to first treatment plans, our study presents a creative therapy approach for those unresponsive to conventional treatments that offers a suitable therapeutic option improving clinical outcomes.

Among the several cancers including nasopharyngeal carcinoma, esophageal cancer, liver cancer, and lung cancer, Tislelizumab, a new PD-1 inhibitor, has been shown especially therapeutic efficacy. Combining tislelizumab with chemotherapy has essentially extended PFS in patients across these cancers (22–25).While immunotherapy has proven beneficial for some patients, metastatic colorectal cancer (mCRC) presents unique challenges. Particularly in patients with MSS/pMMR tumors, which are marked by low immunogenicity and great resistance to immunotherapy, tumor cells in mCRC often evade immune detection and destruction (26). By directly targeting and destroying tumor cells, Gamma Knife SBRT presents a potential solution by releasing a significant volume of tumor neoantigens, and improving tumor immunogenicity, so optimizing maximizing the efficacy of subsequent immunotherapy(27). Furthermore demonstrated to extend survival in non-small cell lung cancer (NSCLC) with patients with brain

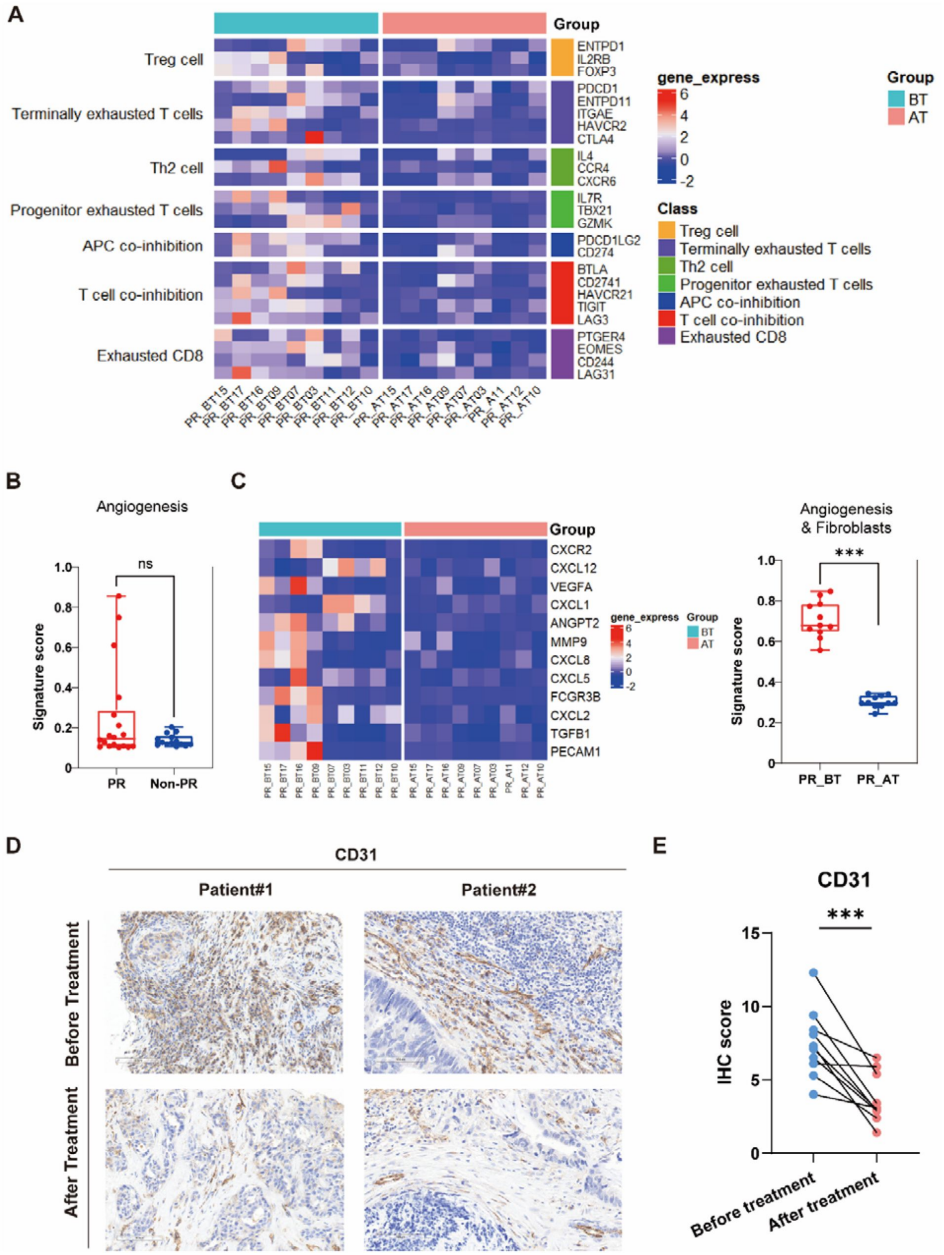


Figure 5.

Comparison of responders before and after treatment

A) The expression of 7 gene sets previously reported to be associated with response to immunosuppressive between before treatment (n = 9) and after treatment (n = 9) in the responders (PR). **B)** The expression of Angiogenesis sets between responders (PR) (n = 18) and non-responders (Non-PR) (n = 14). **C)** The expression of Angiogenesis & Fibroblasts sets between before treatment (n = 9) and after treatment (n = 9) in the responders. Box plots are indicated in terms of minima, maxima, centre, bounds of box and whiskers (interquartile range value), and percentile in the style of Tukey, Wilcoxon test was used to determine the statistical significance between subgroups. **D&E)** Representative CD31 IHC staining of before and after treatment specimens of patient.

metastases is the combination of Gamma Knife SBRT and immunotherapy (28). Still underreported, though, is the possibility of Gamma Knife SBRT coupled with ICIs to improve the response in pMMR/MSS/MSI-L CRC.

In our clinical observations, a notable therapeutic effect was achieved in a patient treated with combined SBRT and immunotherapy. We hypothesize that the addition of tislelizumab following SBRT could extend progression-free survival (PFS) compared to either modality alone. Tumor microenvironment post-radiotherapy showed significant changes revealed by sequencing analysis of tumor samples 'both before and after combined treatment. More precisely, the microenvironment transitioned from an immunosuppressive, angiogenesis- and fibrosis-promoting state to an immune-enhanced, angiogenesis- and fibrosis-attenuated state. Comparatively to non-responders, responders expressed genes linked to antigen presentation, tumor inflammation, and immune-mediated tumor killing more strongly. Further showing the activation of several signaling pathways associated with tumor cell death, including NF-κB, TNF, and JAK-STAT pathways was enrichment analysis. Furthermore, immunotherapy targets such as PD-L1, showed an elevation, which supports the possibility of efficient later immunotherapy. These findings substantiate our hypothesis that patients with MSS-type mCRC resistant to first-line treatment could benefit significantly from the combination of stereotactic radiotherapy and immunotherapy, with enhanced immunogenicity and a more favorable tumor microenvironment facilitating improved therapeutic outcomes.

This trial restrictions even if its outcomes show promise. First of all, our results could be biased as a single-arm study devoid of a control group. Second, the limited sample size and single-center design of the study lower its statistical power hence more robust conclusions depend on bigger studies. Furthermore, even though general survival (OS) was examined, the follow-up duration was insufficient to establish a reliable median OS. Subgroup analysis stratified by mutation type and metastatic site did not yield statistically significant prognostic results, likely due to limitations in both the available patient data and the sequencing depth. Given the importance of identifying reliable biomarkers for predicting the efficacy of Gamma Knife treatment, we attempted to address this gap by establishing a Cox proportional hazards regression model. This model aimed to correlate specific genetic and clinical features with treatment outcomes. Despite these efforts, no substantial or statistically significant findings emerged from the analysis, indicating that additional data and more comprehensive modeling may be required to uncover potential predictors of treatment response. To address these limitations, a multi-center, randomized controlled trial with a larger cohort and extended follow-up period is essential. This will provide a more comprehensive evaluation of the efficacy and safety of combining Gamma Knife SBRT and tislelizumab as a later-line therapy in pMMR/MSS/MSI-L mCRC patients.

Ultimately, for patients with pMMR/MSS/MSI-L mCRC who were unresponsive to first-line therapy regimens, the combination of Gamma Knife SBRT with tislelizumab demonstrated a high disease control rate (DCR) and manageable safety profile. Significant post-radiotherapy improvements in the tumor's suppressive immune microenvironment, reduced fibrosis, normalized tumor vasculature, and activation of the PD-1/PD-L1 checkpoint pathway revealed by biomarker analyses so improving the efficacy of immunotherapy.

Methods

Study design and participants

This single-arm, phase II trial was conducted at the First Affiliated Hospital of Jinan University to assess the antitumor efficacy and safety of a combined regimen consisting of SBRT and tislelizumab in patients with pMMR/MSS/MSI-L-type metastatic colorectal cancer (mCRC). The study is registered with *ClinicalTrials.gov* (identifier: ChicTR2200011777). Eligible patients, aged

18-75 years, had confirmed metastatic colorectal cancer. MSS and RAS mutation statuses were determined through gene sequencing, while clinical staging was based on imaging examinations and intraoperative findings. A total of 20 patients were enrolled in the study, with all providing written informed consent. Detailed inclusion and exclusion criteria are available in the Supplementary Materials.

Procedures

As illustrated in **Figure 1A**, eligible patients received SBRT (administered 5-6 times per week, 3-5 Gy per session) combined with tislelizumab (200 mg on day 1) was incorporated into the treatment regimen. Each three-week cycle comprised a maximum of 12 cycles of induction therapy. Patients achieving complete response (CR), partial response (PR), or stable disease (SD) transitioned to tislelizumab maintenance therapy (200 mg on day 1) until documented disease progression, death, unacceptable toxicity, or patient withdrawal of consent. Treatment response was evaluated using CT or MRI after each treatment cycle. Adverse events were systematically monitored and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0).

The study enrolled 20 eligible patients on November 24, 2022 (**Figure 1B**). All patients received at least one dose of the prescribed regimen. As of the data cutoff date (July 24, 2024), six patients continued to receive maintenance therapy. The median follow-up duration was 15 months (range: 3.4-20.0 months, IQR: 9.6-18.2 months). Due to disease-related complications, specimens could not be obtained from four patients, resulting in 16 patients being included in the per-protocol set (PPS).

Outcomes

The primary endpoints of the study were objective response rate (ORR) and safety, encompassing adverse events and serious adverse events, assessed according to RECIST version 1.1. Secondary endpoints included disease control rate (DCR) and progression-free survival (PFS). ORR was defined as the proportion of patients who achieved a best objective response of complete response (CR) or partial response (PR) per RECIST criteria (version 1.1). DCR was defined as the proportion of patients who achieved CR, PR, or stable disease (SD) according to RECIST criteria (version 1.1). PFS was defined as the time from enrollment to the first documented disease progression per RECIST version 1.1 or to death from any cause, whichever occurred first.

CD8, PD-L1 & CD31 expression level

Tumoral CD8 & CD31 expression was measured by immunohistochemistry (IHC) (22C3 pharmDx assays). The sections were scored for staining intensity according to the following scale: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellowish brown), and 3 (strong staining, brown), with 0 and 1 considered low expression, and 2 and 3 considered high expression.

The score is divided into 4 levels according to the percentage of positive cells: 0%≤positive cell percentage ≤ 25%, 1 point; 25%<positive cell percentage ≤ 50%, 2 points; 50%<positive cell percentage≤75%, 3 points; 75%<positive cell percentage≤100%, 4 points. IHC score = cell staining intensity score x positive cell percentage score. The PD-L1 combined positive score (CPS) was defined as the number of PD-L1 positive cells (tumor cells, lymphocytes, macrophages) as a proportion of the total number of tumor cells multiplied by 100. Positive PD-L1 expression was considered when the CPS was >1.

Nanostring panel RNA sequencing

Due to disease-related limitations, specimens could not be obtained from four patients, resulting in a cohort of 16 patients for combined analysis. Tumor tissue samples were collected both before treatment (BT) and after treatment (AT). Gene expression of each sample was measured using the

NanoString nCounter platform (NanoString Technologies, Seattle, WA). The quantitative transcriptome data were obtained based on the 289-immuno-gene panel, which includes 289 genes related to the tumor, tumor microenvironment, and immune responses in cancer. The samples that passed the quality control (QC), which included Imaging QC, Binding Density QC, Positive Control Linearity QC, and Positive Normalization QC can be processed in further analysis. Statistical analyses were conducted using R (version 3.6.1). The raw count data of 289 genes were normalized using the R package NanoStringNorm according to the geometric mean of five housekeeping genes. The log2 transformation was then performed on the normalized data. Differentially expressed genes were identified using the "DEseq2" package, employing criteria of log2|fold change| > 1 and false discovery rate < 0.05. Heatmaps depicting the expression patterns of these differentially expressed genes were generated using the "ComplexHeatmap" package.

Cox Proportional Hazards Model

Statistical analyses were conducted using R (version 3.6.1). The R package "survival" was used to generate univariate and multivariate COX analysis results, and the relevant data were saved in the **Supplementary Table**.

Immune cell profile analyses and Additional immune signatures analysis

The determination of immune cell types and gene sets associated with immunotherapy response was informed by established literature sources (29,30). We transformed each attribute (immune signature or gene set) value (GSVA score) xi into xi' by the equation xi' = (xi − xmin)/(xmax − xmin), where xmin and xmax represent the minimum and maximum of the ssGSEA scores for the gene set across all samples, respectively. The detailed gene signature list can be found in the Supplementary Table.

Gene set enrichment and pathway analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) / Gene Ontology (GO) enrichment analysis was performed using the Clusterprofiler R package. The list of gene IDs was used as the input file. The Benjamini-Hochberg method was employed to adjust the p-values. The cut-off threshold of p-values was set to 0.05. The enrichment results were visualized by the ggplot2 R package. The enrichment statistic was set to classic.

Statistical analyses

Progression-free survival (PFS) and OS was estimated utilizing the Kaplan-Meier method. Statistical analyses were conducted using R (version 3.6.1). Differences between subgroups in terms of efficacy response were assessed using the nonparametric Wilcoxon rank-sum test (Mann-Whitney U test), while comparisons between pre- and post-treatment samples were analyzed with the Wilcoxon signed-rank test. Confidence intervals (CIs) for response rates were calculated employing the Clopper-Pearson method, with all reported P values being two-sided. A P value < 0.05 was considered statistically significant.

Data availability

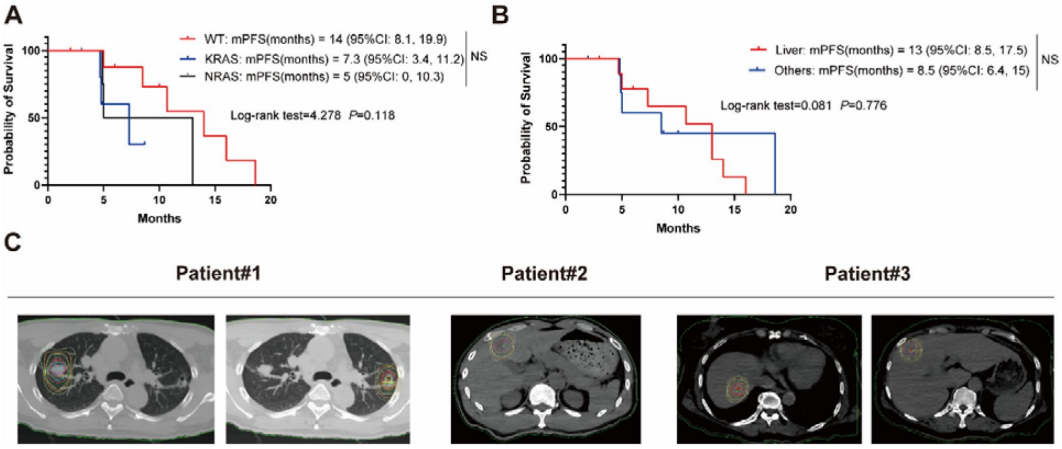
The data generated in this study are available within the article and its Supplementary Data. Additional data or resources related to this article are available upon reasonable request from the corresponding authors.

Supplementary Figures

Supplementary Figure 1.

Supplementary Clinical trial results.

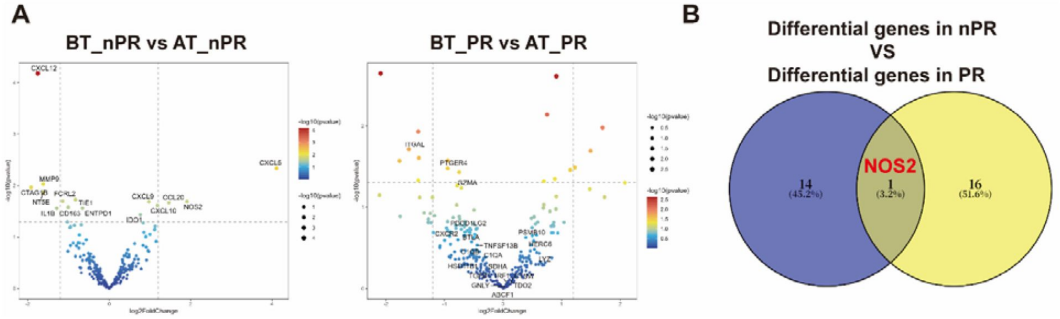
A) Kaplan–Meier curves of PFS for RAS wite-type set (n= 9), KRAS set (n= 5) and NRAS set (n=6). **B)** Kaplan–Meier curves of PFS for simple Liver metastasis (n= 10) and othe metastasis (n= 10). **C)** Schematic diagram of isodose lines of the planned treatment volume (PTV).

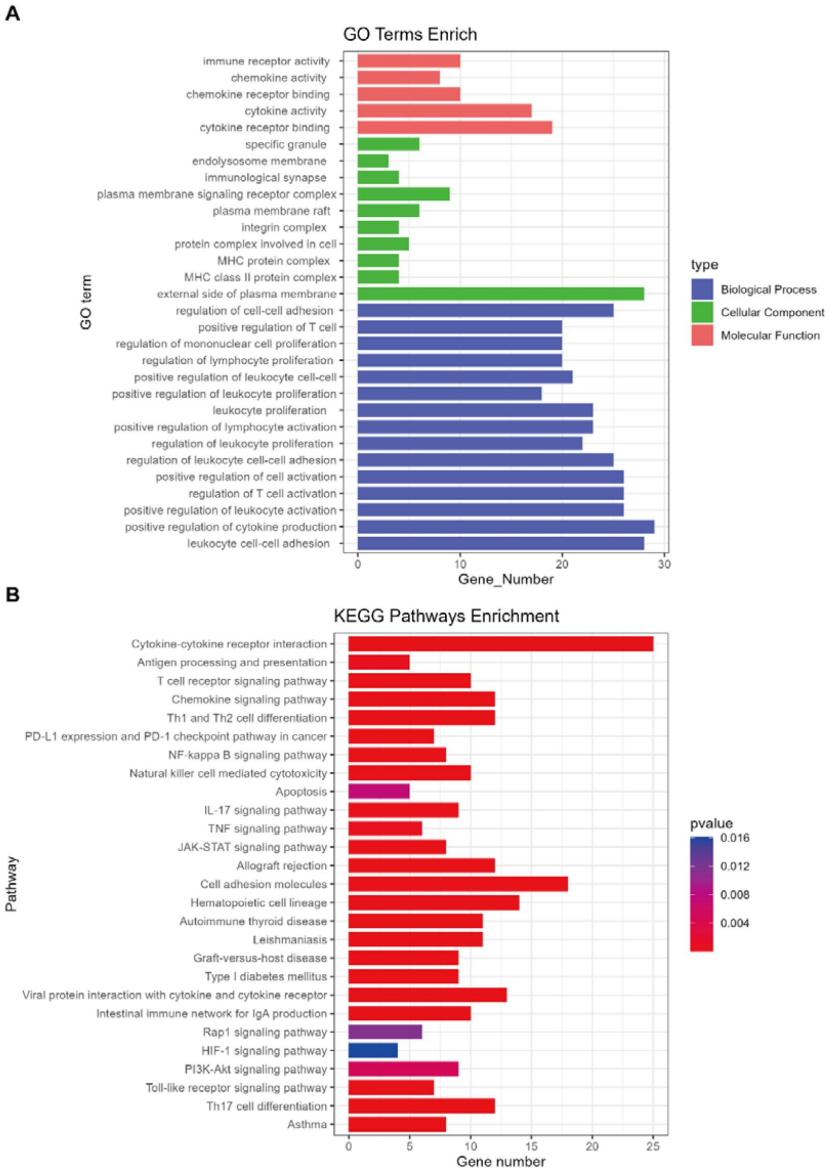


Supplementary Figure 2.

Supplementary Differential expressed genes analysis.

A) Transcriptome analysis on differential expression genes before and after treatment between non-responders (Non-PR) (n = 7 and responders (PR) (n = 9), DESeq2 was provided to perform differential expression testing. **B)** Venn diagram of two groups of differentially expressed genes.

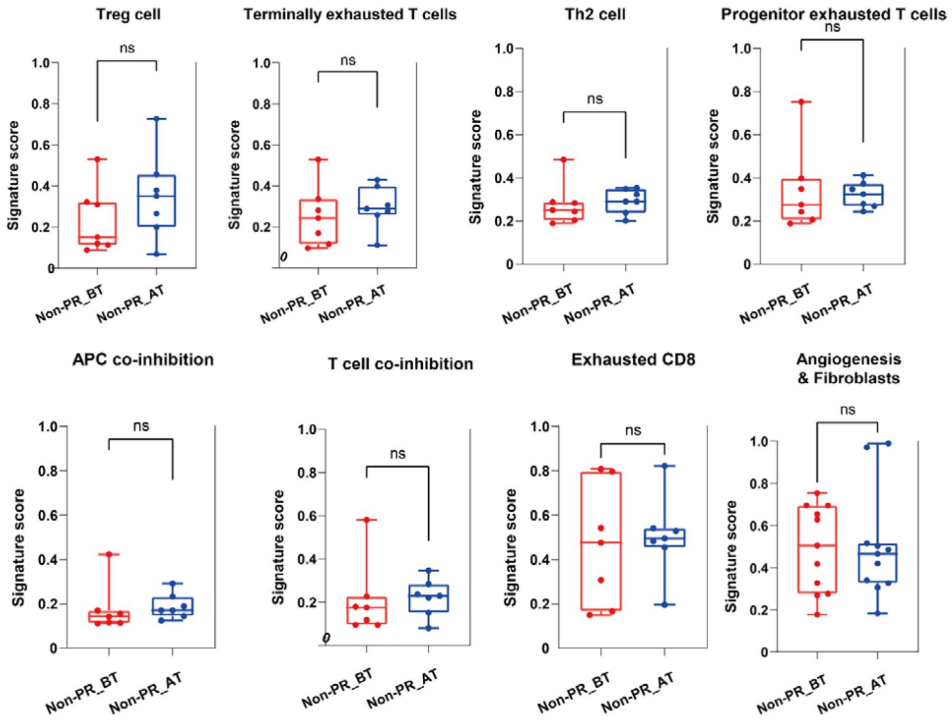




Supplementary Figure 3.

GO enrichment and KEGG pathways analysis of differential expression genes.

A) GO enrichment analysis were performed to identify the biological process, cellular component and molecular function of differential expression genes. **B)** H KEGG enrichment analysis of differential expression genes.



Supplementary Figure 4.

Comparison of non-responders before and after treatment.

Acknowledgements

This research was supported by the Clinical Frontier Technology Program of the First Affiliated Hospital of Jinan University (No. JNU1AF-CFTP-2022-a01223), the National Natural Science Foundation of China (82204436), Natural Science Foundation of Guangdong Province (2024A1515030010, 2022A1515011695), Science and Technology Projects in Guangzhou (2024A03J0825).

Additional information

Ethics approval and consent to participate

This trail was conducted in accordance with the Declaration of Helsinki after approval by the Institutional Review Board of The First Affiliated Hospital of Jinan University (KY-2022-236). All patients provided written informed consent. The *ClinicalTrials.gov* identifier was: ChiCTR2200066117.

Author Contributions

Y Zhang, H Guan and S Liu: acquisition of data, analysis of experimental data and drafted the manuscript. H Li and Z Bian: Investigation, visualization and methodology. J He, Z Zhao and S Qiu: data curation, software and formal analysis. T Mo, X Zhang and Z Chen: technical expertise inmanuscript editing. H Ding and X Zhao: assay optimization, acquisition, and analysis and interpretation of histology and pathology. L Wang, Y Pan and J Pan: funding acquisition, designed the study and writing-review and editing the draft.

Clinical trial number: *ChiCTR2200066117*.

Additional files

Supplementary Data

Supplementary material

Supplementary Table

Table

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Reviewer #1 (Public review):

Summary:

This study presents compelling evidence for a novel treatment approach in a challenging patient population with MSS/pMMR mCRC, where traditional immunotherapy has often fallen short. The combination of SBRT and tislelizumab not only yielded a high disease control rate but also indicated significant improvements in the tumor's immune landscape. The safety profile appears favorable, which is crucial for patients who have already undergone multiple lines of therapy.

Strengths:

The results underscore the potential of leveraging radiation therapy to enhance the effectiveness of immunotherapy, especially in tumor environments previously deemed hostile to immune interventions. Future research should focus on larger cohorts to validate these findings and explore the underlying mechanisms of immune modulation post-treatment.

Comments on revisions:

The author provided satisfactory responses to my queries, offering clarifications and additional explanations to address potential points of confusion. The supplementary experimental data further corroborate the author's conclusions. Although a more in-depth and detailed analysis did not yield significant results, this does not undermine the overall integrity of the article's structure or the reliability of its conclusions. Based on the content and the supporting evidence presented, I believe this article meets the necessary criteria for publication.

https://doi.org/10.7554/eLife.103559.2.sa2

Reviewer #2 (Public review):

Summary:

This Phase II clinical trial investigates the combination of Gamma Knife Stereotactic Body Radiation Therapy (SBRT) with Tislelizumab for the treatment of metastatic colorectal cancer (mCRC) in patients with proficient mismatch repair (pMMR). The study addresses a critical clinical challenge in the management of pMMR CRC, focusing on the selection of appropriate candidates. The results suggest that the combination of Gamma Knife SBRT and Tislelizumab provides a safe and potent treatment option for patients with pMMR/MSS/MSI-L mCRC who have become refractory to first- and second-line chemotherapy. The study design is rigorous, and the outcomes are promising.

Advantage:

The trial design was meticulously structured, and appropriate statistical methods were employed to rigorously analyze the results. Bioinformatics approaches were utilized to further elucidate alterations in the patient's tumor microenvironment and to explore the underlying factors contributing to the observed differences in treatment efficacy. The conclusions drawn from this trial offer valuable insights for managing advanced colorectal cancer in patients who have not responded to first- and second-line therapies.

Weakness:

(1) Clarity and Structure of the Abstract
- Results Section: The results section should contain important data, I suggest some important sequencing data should be shown to enhance understanding.
(2) As the author using the NanoString assay for transcriptome analysis, more detail should be shown such as the version of R, and the bioinformatics analysis methods.
(3) It is interesting for included patients that PD-L1 increase expression after Gamma Knife Stereotactic Body Radiation Therapy (SBRT) treatment, How to explain it?
(4) It would be helpful to include a brief discussion of the limitations of the study, such as sample size constraints and their impact on the generalizability of the results. This will give readers a more comprehensive understanding of the findings.
(5) Language Accuracy: There are a few instances where wording could be more professional or precise.

Revision comment:

The author had responded to all questions and improved the manuscript. The author's answers and revisions are very satisfactory to me. I believe it is an important study for the immunotherapy of colorectal cancer.

https://doi.org/10.7554/eLife.103559.2.sa1

Author response:

The following is the authors' response to the original reviews

> ***Public Reviews:***
>
> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *This study presents compelling evidence for a novel treatment approach in a challenging patient population with MSS/pMMR mCRC, where traditional immunotherapy has often fallen short. The combination of SBRT and tislelizumab not only yielded a high disease control rate but also indicated significant improvements in the tumor's immune landscape. The safety profile appears favorable, which is crucial for patients who have already undergone multiple lines of therapy.*
>
> *Strengths:*
>
> *The results underscore the potential of leveraging radiation therapy to enhance the effectiveness of immunotherapy, especially in tumor environments previously deemed hostile to immune interventions. Future research should focus on larger cohorts to validate these findings and explore the underlying mechanisms of immune modulation post-treatment.*
>
> *Weaknesses:*
>
> *I believe the author's work is commendable and should be considered with some minor modifications:*
>
> *(1) While the author categorized patients based on the type of RAS mutation and the location of colorectal cancer metastasis, the article does not adequately address how these classifications influence treatment outcomes. Such as whether KRAS or NRAS mutations, as well as the type of metastatic lesions, affect the sensitivity to gamma-ray treatment and lead to varying responses.*

Thank you very much for your question. Therefore, in the revised manuscript, we added an analysis of the impact of RAS mutation types and different metastatic sites on patient prognosis, but unfortunately, due to the limited number of samples, we were unable to obtain satisfactory results. We also placed the relevant results in the supplementary figure.

> *(2) In Figure 2, clarification is needed on how the author differentiated between on-target and off-target lesions. I observed that some images depicted both lesion types at the same level, which could lead to confusion.*

We sincerely apologize for any oversight in our previous submission. To clarify, during the process of radiotherapy planning, we pre-select target lesions at the CT image level, and subsequently define the planning treatment volume (PTV) by marking these pre-selected areas with the 50% isodose lines. In our efficacy evaluation, we distinguish between the target lesions inside the PTV and any lesions outside the target area. In response to your valuable feedback, we have now added the isodose lines for the target lesions to the supplementary figure for greater clarity.

> *(3) The author performed only a basic difference analysis. A more comprehensive analysis, including calculations of markers related to treatment efficacy, could offer additional insights for clinical practice.*

To identify potential markers associated with treatment efficacy, we attempted to establish a Cox proportional hazards model and conducted both univariate and multivariate Cox regression analyses. Unfortunately, due to the constraints of sample size and sequencing depth, the analyses did not yield statistically significant results, and we were unable to identify markers that could clearly predict treatment outcomes.

> *(4) The transcriptome sequencing analysis provides insights into how stereotactic radiotherapy sensitizes immunotherapy; however, it currently relies on a simple pre- and post-treatment group comparison. It would be beneficial to include additional subgroups to explore more nuanced findings.*

We acknowledge the limitations in the depth of our analysis. In addition to performing differential analysis between the responder group (PR) and the non-responder group (Non-PR), we also conducted differential gene expression analysis on samples before and after treatment. The results revealed a consistent increase in the expression of NOS2 in both groups following Gamma Knife combined with immunotherapy, suggesting that this gene may serve as a potential prognostic factor influencing treatment outcomes. However, given the limited number of studies exploring the role of NOS2 in this context, we recognize that further research is necessary to better understand its involvement and to substantiate its potential as a predictive marker.

> *(5) The author briefly discusses the effects of changes in tumor fibrosis and angiogenesis on treatment outcomes. Further experiments may be necessary to validate these findings and investigate the underlying mechanisms of immune regulation following treatment.*

We sincerely appreciate your thoughtful feedback on our results. In response, we conducted additional experiments, including immunohistochemical analysis of patient samples before and after combined treatment. The results demonstrated a reduction in the expression of CD31, a marker of tumor angiogenesis, following the combined treatment. This finding further supports our hypothesis that Gamma Knife treatment, in combination with immunotherapy, may effectively inhibit tumor angiogenesis, contributing to an improved therapeutic outcome.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *This Phase II clinical trial investigates the combination of Gamma Knife Stereotactic Body Radiation Therapy (SBRT) with Tislelizumab for the treatment of metastatic colorectal cancer (mCRC) in patients with proficient mismatch repair (pMMR). The study addresses a critical clinical challenge in the management of pMMR CRC, focusing on the selection of appropriate candidates. The results suggest that the combination of Gamma Knife SBRT and Tislelizumab provides a safe and potent treatment option for patients with pMMR/MSS/MSI-L mCRC who have become refractory to first- and second-line chemotherapy. The study design is rigorous, and the outcomes are promising.*
>
> *Advantage:*
>
> *The trial design was meticulously structured, and appropriate statistical methods were employed to rigorously analyze the results. Bioinformatics approaches were utilized to further elucidate alterations in the patient's tumor microenvironment and to explore the underlying factors contributing to the observed differences in treatment efficacy. The conclusions drawn from this trial offer valuable insights for managing advanced colorectal cancer in patients who have not responded to first- and second-line therapies.*

> *Weakness:*
>
> *(1) Clarity and Structure of the Abstract*
> *- Results Section: The results section should contain important data, I suggest some important sequencing data should be shown to enhance understanding.*

Thank you for your insightful question. In response, we have revised the content of the article and restructured the abstract to enhance its scientific clarity and make it more accessible to readers.

> *(2) As the author using the NanoString assay for transcriptome analysis, more detail should be shown such as the version of R, and the bioinformatics analysis methods.*

We have also addressed the missing details in our research methodology. The revised manuscript now includes a complete description of the research methods, along with the specific software and versions used.

> *(3) It is interesting for included patients that PD-L1 increase expression after Gamma Knife Stereotactic Body Radiation Therapy (SBRT) treatment, How to explain it?*

Thank you for your thought-provoking question. PD-L1 plays a crucial role in tumor cell immune evasion, and anti-PD-1/PD-L1 inhibitors have emerged as effective immune checkpoint inhibitors, widely used in cancer therapy. In our clinical trials, we observed an increase in PD-L1 expression in some patients following combined treatment. Existing literature suggests that activation of various carcinogenic and stress response pathways, along with post-transcriptional modifications of PD-L1 (such as phosphorylation, glycosylation, acetylation, ubiquitination, and palmitoylation), can influence its expression[1]. We hypothesize that the increase in PD-L1 expression may be attributed to the activation of specific signaling pathways induced by the radiation from Gamma Knife treatment, as well as the enhanced tumor stress in response to the treatment. However, the precise mechanisms underlying this observation require further experimental investigation. A deeper understanding of these processes could potentially optimize our clinical treatment strategies.

> *(4) It would be helpful to include a brief discussion of the limitations of the study, such as sample size constraints and their impact on the generalizability of the results. This will give readers a more comprehensive understanding of the findings.*

Thank you for highlighting the limitations of the article. In response, we have added a detailed discussion of the constraints arising from the limited number of experimental samples and insufficient sequencing depth. This addition aims to provide readers with a clearer understanding of the study's limitations and the context of our research findings.

> *(5) Language Accuracy: There are a few instances where wording could be more professional or precise.*

Regarding the language deficiency, we are very sorry that the wording of the professional content in the article is not careful and accurate enough due to the difference in the native language environment. We have checked our article again and revised the wording and grammar in the hope that you and other readers can grasp our research content more accurately.

> ***Recommendations for the authors:***
>
> ***Reviewer #1 (Recommendations for the authors):***
>
> *The research presented in this article is commendable; however, I would like to propose several revisions for consideration:*
>
> *Consideration of Concomitant Medications: It is imperative to ascertain whether enrolled patients utilized additional pharmacological agents alongside the trial regimen. Such concurrent drug use could potentially influence the final outcomes. A concise discussion of this aspect is warranted within the manuscript.*
>
> *Clinical Characterization of Response Groups: An examination of the clinical characteristics distinguishing the effective and non-responsive cohorts within the trial is essential. This inquiry merits further exploration, as it may elucidate factors influencing treatment efficacy.*
>
> *Tumor Microenvironment Analysis: The authors highlight the implications of tumor fibrosis and angiogenesis on therapeutic response. Identification of specific biomarkers associated with these phenotypes is crucial. I recommend undertaking straightforward testing and validation to substantiate these observations.*

Thank you very much for your valuable suggestions, many of which have been incorporated into the revised manuscript. Regarding the consideration of concurrent medication, we would like to clarify that all patients included in the study were advanced CRC patients who had progressed during first- or second-line treatments. As such, targeted therapy or chemotherapy was used concurrently in the trial. Previous studies have not indicated that different targeted therapies influence the efficacy of Gamma Knife treatment, though some chemotherapy agents may vary in their side effects. However, we believe these differences do not significantly impact the final outcomes. Given that existing chemotherapy regimens do not substantially affect patient prognosis, we considered the combined drug treatment regimen to be an irrelevant variable in our analysis.

Additionally, we have carefully examined the clinical characteristics of patients across different groups. We have also included an analysis of the impact of various mutation types and metastatic sites in the revised manuscript. Furthermore, we plan to perform CD31 staining on lesions from both the responder and non-responder groups before and after Gamma Knife treatment to assess the role of angiogenesis in treatment response.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *The abstract should be revised for greater clarity and include key results that substantiate the conclusions. The discussion section needs to more thoroughly address the limitations of the clinical trial, providing readers with a deeper understanding of the trial's findings and implications. Additionally, the methods section should be more rigorous and detailed, offering sufficient information to enhance the transparency and robustness of the experimental design.*

Thank you for your constructive suggestions regarding the shortcomings in our manuscript. In response, we have thoroughly reviewed the article and addressed the missing content, including revisions to the abstract, results, discussion, and methods sections. Additionally, we have refined the grammar and wording throughout the manuscript to enhance its professionalism and ensure it aligns with the standards expected for publication.

(1) YAMAGUCHI H, HSU J M, YANG W H, et al. Mechanisms regulating PD-L1 expression in cancers and associated opportunities for novel small-molecule therapeutics [J]. Nature

reviews Clinical oncology, 2022, 19(5): 287-305.

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