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Impact of body mass index on the clinical outcomes of immune checkpoint inhibitor combination therapy for advanced renal cell carcinoma: insights from gene expression and tumor-infiltrating immune cell profile

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Abstract

Objective

To clarify the prognostic impact of body mass index (BMI) on outcomes with immune checkpoint inhibitor (ICI) combination therapy among patients with advanced renal cell carcinoma (RCC).

Methods

We retrospectively evaluated clinical data from 229 patients with advanced clear-cell RCC (ccRCC) treated with first-line ICI combination therapy. Patients were stratified by BMI as < 18.5 kg/m^2 (underweight), ≥ 18.5 to < 25 kg/m^2 (normal), and ≥ 25 kg/m^2 (overweight/obesity), and outcomes were compared among these subgroups. Bulk RNA-seq and flow cytometry were conducted on ccRCC samples to explore the mechanisms underlying the association between BMI and ICI efficacy.

Results

Underweight, normal, and overweight/obesity groups comprised 23 (10%), 137 (60%), and 69 (30%) patients, respectively. Among patients receiving ICIs plus VEGFR-tyrosine kinase inhibitors (TKIs) (n = 133), underweight patients had shorter progression-free survival and overall survival than normal patients and those with overweight/obesity ($p < 0.05$). BMI was not significantly associated with outcomes in those treated with dual ICI combinations (n = 96, $p > 0.05$). In transcriptome analyses of 94 ccRCC samples, angiogenesis-related pathways were downregulated in underweight patients compared with non-underweight patients, whereas tumor-infiltrating immune cell profiles assessed in 84 samples did not differ by BMI.

Conclusions

BMI differentially affected clinical outcomes in patients with ccRCC across ICI-combination regimens. Inactivation of angiogenesis-related pathways potentially reduced the efficacy of TKI-containing ICI combinations in underweight patients.

1. Introduction

The treatment landscape for advanced renal cell carcinoma (RCC) has changed dramatically with the introduction of immune checkpoint inhibitors (ICIs) (1–5). Current guidelines recommend either dual ICI regimens (i.e., immuno-oncology [IO]-IO) or ICI plus VEGFR-tyrosine kinase inhibitors (TKIs) combinations (i.e., IO-TKI) as the standard of care for first-line systemic therapy in advanced RCC (6, 7).

Extended follow-up from pivotal phase III clinical trials has demonstrated overall survival (OS) benefits with ICI combination therapy compared with sunitinib (8–11). Nonetheless, most patients eventually experience disease progression, even after achieving an objective response or durable response. Additionally, immediate disease progression can be observed in a subset of patients, particularly with IO-IO combinations (1). Therefore, effective biomarkers for outcome prediction and patient selection are urgently needed, although such factors have yet to be identified.

Obesity has been reported as a risk factor for RCC (12, 13). Conversely, elevated body mass index (BMI) has been reported to be associated with improved survival outcomes, a phenomenon known as the obesity paradox" (14, 15). In the current ICI era, several studies have documented the prognostic impact of BMI on outcomes in patients with RCC (16–20), although the findings remain controversial. In addition, it remains unclear whether the impact of BMI on outcomes differs across regimens, as the mechanism of antitumor effects differs between IO-TKI and IO-IO combinations (21, 22). Furthermore, the molecular mechanism underlying the association between BMI and outcomes in patients with RCC receiving ICIs has not been explored.

In this context, we retrospectively analyzed the impact of BMI on clinical outcomes in advanced clear-cell RCC (ccRCC) treated with ICI combination therapy, stratified by treatment regimen. Furthermore, we investigated the profiles of gene expression and tumor-infiltrating immune cells (TIICs) in ccRCC samples using RNA-seq and flow cytometry, respectively, to elucidate the molecular mechanism underlying the association between BMI and ICI outcomes.

2. Patients and Methods

2.1. Patient selection and RCC samples

Clinicopathological and laboratory data were obtained from electronic databases and patient medical records. Overall, 352 patients with advanced RCC who received ICI combination therapy as first-line therapy between September 2016 and February 2026 at five affiliated institutions (Tokyo Women's Medical University, Tokyo Women's Medical University Adachi Medical Center, Saiseikai Kawaguchi General Hospital, Saiseikai Kazo Hospital, and Jyoban Hospital) were evaluated. Of these, 123 patients were excluded because the pathological diagnosis was not established or was non-clear-cell RCC (n = 102), because baseline clinicopathological data, including BMI, were lacking or the post-treatment follow-up period was insufficient (< 3 months) (n = 8), or because systemic therapy was administered for locally advanced cases (i.e., cM0) (n = 13). Thus, the remaining 229 patients with advanced ccRCC were included in this retrospective analysis. BMI was evaluated within 1 month before the initiation of systemic therapy.

Ninety-four ccRCC samples obtained by radical or partial nephrectomy were collected from two institutions (Tokyo Women's Medical University and Tokyo Women's Medical University Adachi Medical Center) (Supplementary Table 1). All samples were freshly frozen, and histopathological diagnoses were

performed by a certified, experienced pathologist according to the 2016 WHO classification (23). Preoperative systemic therapy, such as ICIs or VEGFR-TKIs, was not administered to any patient. Using the 94 samples, genome-wide gene expression analysis was performed by RNA-seq. Furthermore, using 84 of the 94 samples, flow cytometry was performed to analyze the profile of TIICs (Supplementary Table 2).

The study protocol was approved by the Institutional Ethics Review Board of Tokyo Women's Medical University (IDs: 2020–0009 and 382), and tumor samples were obtained after all patients provided written informed consent. This study was conducted in accordance with the 1964 Declaration of Helsinki and its subsequent amendments.

2.2. Clinical outcomes

For efficacy, progression-free survival (PFS) and OS after initiation of ICI combination therapy were evaluated. Additionally, the magnitude of tumor response, including the objective response rate (ORR), was assessed. The best overall response and ORR for measurable target lesions were determined using RECIST version 1.1 (24). For tumor response assessment, post-treatment follow-up computed tomography scans of the chest, abdomen, and pelvis were performed at 4–12-week intervals, depending on the patient's condition. Magnetic resonance imaging, positron emission tomography/computed tomography, and brain scans were performed as necessary. Treatments were continued until radiographic or clinical disease progression was observed or until intolerable adverse events (AEs) occurred. ICI doses could not be modified. Nonetheless, administration intervals could be adjusted based on toxicity or patient condition. For TKIs, both the dose and the administration interval can be modified as needed. Safety was assessed by evaluating the incidence rates of AEs, treatment interruptions, treatment discontinuations, and glucocorticoid use. AEs were graded using CTCAE version 5.0 (25).

2.3. Gene expression analysis using RNA-seq

Total RNA samples with a RIN > 6, or those showing a prominent peak at appropriate nucleotide fragment sizes despite a RIN < 6, were subjected to RNA-seq. RNA-seq libraries were prepared and sequenced either at Macrogen (Tokyo, Japan) or at BGI (Shenzhen, China).

For samples processed at Macrogen, total RNA was extracted using QIAzol Lysis Reagent (QIAGEN), and RNA integrity was assessed using the TapeStation RNA ScreenTape system (Agilent Technologies, Santa Clara, CA, USA). Libraries were constructed from total RNA using the TruSeq Stranded mRNA Library Prep Kit (Illumina, Inc., San Diego, CA, USA). Libraries were quantified using the KAPA Library Quantification Kit (KAPA Biosystems) and qualified using the TapeStation D1000 ScreenTape system (Agilent Technologies). Indexed libraries were then sequenced on the Illumina NovaSeq platform (Illumina, Inc.), and 100-base-pair paired-end reads were obtained.

For samples processed at BGI, total RNA was extracted using TRIzol Reagent (Invitrogen), and RNA integrity was evaluated using the Fragment Analyzer system (Agilent Technologies, Santa Clara, CA,

USA). Libraries were prepared using the DNBSEQ Eukaryotic Strand-specific mRNA library protocol. The resulting DNA nanoballs, each carrying > 300 copies of the initial single-stranded circularized library molecule, were loaded onto a patterned nanoarray. Paired-end 100-base reads were generated on the G400 platform (BGI).

The sequencing data were subjected to quality-control filtering, trimming, and adapter removal using fastp. The filtered paired-end reads were aligned to the human reference genome (hg38) using STAR, and gene-level read counts were quantified using RNA-Seq by Expectation-Maximization (26). We previously confirmed that there were no substantial batch effects by sequencing provider (i.e., Macrogen or BGI), as demonstrated by principal component analysis (27).

Gene-level read counts were processed using the iDEP 2.0 web application (28). iDEP 2.0 was used for hierarchical clustering with a heatmap, identification of differentially expressed genes (DEGs), and gene ontology (GO) enrichment analysis. Low-expression genes were filtered out using a minimum threshold of 0.5 counts per million in at least the minimum group size, as implemented in iDEP. Data normalization and differential expression analysis were performed using the DESeq2 package implemented in iDEP. DEGs were extracted using an FDR cutoff of 0.1 and a minimum fold change of 2.

2.4. TIIC profiles analyzed using flow cytometry

Flow cytometry was performed according to a previously described protocol (27, 29). Specifically, ccRCC samples were obtained within 1 h of nephrectomy and immediately dissociated into single cells by mincing in RPMI 1640 medium. The resulting single-cell suspension was washed, passed through 40-μm cell strainers, suspended in CELL BANKER 1 (Nippon Zenyaku Kogyo, Co., Ltd., Fukushima, Japan), and stored at -80°C until flow cytometry. Cryopreserved cells were thawed and incubated with Fixable Viability Dye eFluor 506 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for 30 min at 4°C to stain dead cells, followed by staining with monoclonal antibodies (Supplementary Table 3) for 30 min at 4°C. For intracellular protein staining, including Foxp3, cells were fixed and permeabilized using a Foxp3 transcription factor staining buffer set (Thermo Fisher Scientific) and then stained with monoclonal antibodies. After washing, data were acquired using an LSRFortessa X-20 Cell Analyzer (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed using FlowJo software (BD Biosciences).

2.5. Statistical analysis

Continuous variables were analyzed using the Mann–Whitney U test, whereas categorical variables were analyzed using Fisher's exact test or the chi-square test, as appropriate. PFS was calculated from treatment initiation to disease progression or death, whichever occurred first. OS was calculated from treatment initiation to death from any cause. Patients who were lost to follow-up were censored at their last contact. Survival data through the end of February 2026 were obtained. Survival was estimated using the Kaplan–Meier method, and differences between groups were compared using the log-rank test. Univariate logistic regression analyses were used to identify factors associated with survival. Risks are expressed as odds ratios (ORs) with 95% confidence intervals (CIs). All analyses were performed

using the JMP software (version 19; SAS Institute Inc., Cary, NC, USA), and statistical significance was set at $p < 0.05$.

3. Results

3.1. Patient characteristics

Among 229 patients, 23 (10%), 137 (60%), and 69 (30%) were underweight, normal weight, and overweight/obese, respectively. Regarding ICI combination regimens, 133 (58%) received IO-TKI and 96 (42%) received IO-IO combination therapy.

Among 133 patients treated with IO-TKI, 16 (12%), 77 (58%), and 40 (30%) were underweight, normal weight, and overweight/obese, respectively. Baseline patient characteristics by BMI category are shown in Table 1. The proportions of younger patients (≤ 65 years) and female patients were higher in the underweight group ($p = 0.0001$ and $p = 0.0138$, respectively).

Table 1
Patient background based on BMI in the IO-TKI treatment group

Variable	Underweight (n = 16)	Normal (n = 77)	Overweight/obesity (n = 40)	p
Sex Male (ref. female)	7 (44%)	33 (83%)	56 (73%)	0.0138
Age, years >65 (ref. ≤65)	13 (81%)	55 (71%)	14 (35%)	0.0001
Height, m*	1.62 (1.51–1.69)	1.63 (1.58–1.71)	1.67 (1.62–1.74)	0.0482
Weight, kg*	45.5 (39.6–49.2)	60 (54.2–65.6)	78 (72.3–87.5)	< 0.0001
BMI, kg/m^2*	17.7 (16.5–18.3)	22.4 (20.6–23.7)	27.6 (26.0–30.0)	< 0.0001
Prior nephrectomy Presence (ref. absence)	13 (81%)	54 (70%)	35 (88%)	0.0976
Metastasis status Synchronous (ref. metachronous)	5 (31%)	32 (42%)	12 (30%)	0.416
IMDC risk	4 (25%)	14 (18%)	17 (43%)	0.0771
Favorable	9 (56%)	48 (62%)	19 (48%)	
Intermediate	3 (19%)	15 (19%)	4 (10%)	
Poor				
Regimen	8 (50%)	23 (30%)	16 (40%)	0.396
Lenvatinib plus pembrolizumab	5 (31%)	36 (47%)	14 (35%)	
Cabozantinib plus nivolumab	0	9 (12%)	6 (15%)	
Pembrolizumab plus axitinib	3 (19%)	9 (12%)	4 (10%)	
Avelumab plus axitinib				

*median (interquartile range)

BMI, body mass index; IO, immunotherapy; TKI, tyrosine kinase inhibitor; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium

Variable	Underweight (n = 16)	Normal (n = 77)	Overweight/obesity (n = 40)	p
Starting dose of TKIs Full (ref. reduced)	10 (63%)	52 (68%)	31 (78%)	0.423
Lung metastasis Presence (ref. absence)	11 (69%)	57 (74%)	31 (78%)	0.788
Bone metastasis Presence (ref. absence)	3 (19%)	17 (22%)	11 (28%)	0.725
Liver metastasis Presence (ref. absence)	2 (13%)	6 (8%)	5 (13%)	0.665
Lymph node metastasis Presence (ref. absence)	3 (19%)	18 (23%)	12 (30%)	0.614
*median (interquartile range)				
BMI, body mass index; IO, immunotherapy; TKI, tyrosine kinase inhibitor; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium				

Among 96 patients treated with IO-IO, seven (7%), 60 (63%), and 29 (30%) were underweight, normal weight, and overweight/obese, respectively. Patient background based on BMI is shown in Table 2. No significant differences were observed across BMI categories ($p > 0.05$).

Table 2
Patient background based on BMI in the IO-IO treatment group

Variable	Underweight (n = 7)	Normal (n = 60)	Overweight/obesity (n = 29)	p
Sex Male (ref. female)	3 (43%)	46 (77%)	23 (79%)	0.120
Age, years >65 (ref. ≤65)	5 (71%)	33 (55%)	14 (48%)	0.532
Height, m*	1.57 (1.54–1.72)	1.67 (1.59–1.72)	1.67 (1.61–1.74)	0.213
Weight, kg*	43 (40–48)	61.5 (55–65.1)	77 (69–88.5)	< 0.0001
BMI, kg/m^2*	17.3 (16.4–17.4)	22.1 (19.9–23.4)	28 (26.6–30.1)	< 0.0001
Prior nephrectomy Presence (ref. absence)	6 (86%)	42 (70%)	22 (76%)	0.617
Metastasis status Synchronous (ref. metachronous)	6 (86%)	12 (41%)	33 (55%)	0.0965
IMDC risk	3 (43%)	41 (68%)	24 (83%)	0.0894
Intermediate	4 (57%)	19 (32%)	5 (17%)	
Poor				
Lung metastasis Presence (ref. absence)	5 (71%)	39 (65%)	21 (72%)	0.764
Bone metastasis Presence (ref. absence)	2 (29%)	13 (22%)	5 (17%)	0.776
Liver metastasis Presence (ref. absence)	2 (29%)	6 (10%)	3 (10%)	0.336
Lymph node metastasis Presence (ref. absence)	4 (57%)	17 (28%)	9 (31%)	0.298

*median (interquartile range)

IO, immunotherapy; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium

3.2. Efficacy of ICI combination therapy based on BMI

During the median follow-up period of 23.5 (interquartile range: 11.4–38.8) months, 127 (55%) and 57 (25%) patients had disease progression and died, respectively.

In the IO-TKI treatment group, BMI was significantly associated with PFS and OS after treatment initiation. Specifically, underweight patients exhibited shorter PFS and OS than patients with normal weight and those with overweight/obesity (median PFS: 11.0 [95% CI: 5.39–20.6] vs. 30.4 [23.0 – not reached (N.R.)] vs. 24.9 [19.9–46.3] months, p = 0.0130; median OS: N.R. [95% CI: 14.3 – N.R.] vs. 79.6 [N.R. – N.R.] vs. N.R. [29.6 – N.R.] months, p = 0.0120) (Fig. 1a and 1b). Univariate analyses of patient characteristics showed that BMI was the only factor associated with PFS (p = 0.0378) and OS (p = 0.0352) (Table 3). Regarding tumor response, BMI was positively associated with the magnitude of tumor response, although the association was weak (R = 0.19, p = 0.0373) (Supplementary Fig. 1a). In addition, ORR increased numerically across BMI categories (56% vs. 69% vs. 83% in the underweight, normal, and overweight/obesity groups, respectively; not significant), but the difference was not statistically significant (p = 0.107) (Supplementary Fig. 1b).

Table 3
Univariate analyses for progression-free survival and overall survival in the IO-TKI treatment group

Variables	Progression-free survival HR (95% CI)	p	Overall survival HR (95% CI)	p
Sex Male (ref. female)	0.85 (0.49–1.49)	0.575	1.11 (0.44–2.83)	0.823
Age, years >65 (ref. ≤65)	1.03 (0.61–1.75)	0.908	0.85 (0.37–1.96)	0.709
BMI Underweight Normal Overweight/obesity	2.80 (1.36–5.76) Reference 1.13 (0.63–2.02)	0.0378 0.0050 - 0.673	4.37 (1.54–12.4) Reference 1.69 (0.64–4.44)	0.0352 0.0055 - 0.286
Prior nephrectomy Presence (ref. absence)	0.80 (0.44–1.45)	0.457	0.49 (0.20–1.21)	0.123
Metastasis status Synchronous (ref. metachronous)	1.32 (0.79–2.21)	0.286	2.01 (0.86–4.67)	0.106
IMDC risk Favorable Intermediate Poor	0.72 (0.39–1.32) Reference 1.05 (0.51–2.19)	0.500 0.284 - 0.891	0.86 (0.30–2.48) Reference 2.44 (0.90–6.64)	0.188 0.780 - 0.0807
Starting dose of TKIs Full (ref. reduced)	0.72 (0.42–1.22)	0.221	1.17 (0.46–3.00)	0.741
Lung metastasis Presence (ref. absence)	0.98 (0.54–1.76)	0.943	0.91 (0.37–2.25)	0.832
Bone metastasis Presence (ref. absence)	1.48 (0.82–2.66)	0.193	2.38 (1.00–5.69)	0.0508
Liver metastasis Presence (ref. absence)	1.31 (0.59–2.88)	0.505	2.01 (0.74–5.49)	0.174

HR, hazard ratio; CI, confidence interval; BMI, body mass index; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium

Variables	Progression-free survival HR (95% CI)	p	Overall survival HR (95% CI)	p
Lymph node metastasis Presence (ref. absence)	0.73 (0.38–1.41)	0.353	1.60 (0.65–3.95)	0.305

HR, hazard ratio; CI, confidence interval; BMI, body mass index; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium

In the IO-IO treatment group, PFS and OS were not significantly associated with BMI-defined categories (median PFS: 6.21 [95% CI: 1.97–21.5] vs. 12.5 [6.51–24.0] vs. 9.27 [4.31–44.6] months, p = 0.669, median OS: 40.3 [95% CI: 6.81–N.R.] vs. 77.6 [45.1–N.R.] vs. 76.7 [50.4–N.R.] months, p = 0.315) (Fig. 2a and 2b). Univariate analysis showed that BMI was not significantly associated with PFS (p = 0.698) or OS (p = 0.285) (Table 4). Regarding tumor response, BMI was not associated with the magnitude of tumor response (p = 0.554) or achievement of ORR (p = 0.285) (Supplementary Fig. 2a and 2b).

Table 4
Univariate analyses for progression-free survival and overall survival in the IO-IO treatment group

Variables	Progression-free survival HR (95% CI)	p	Overall survival HR (95% CI)	p
Sex Male (ref. female)	0.69 (0.39–1.19)	0.183	0.91 (0.42–1.97)	0.820
Age, years >65 (ref. ≤65)	1.27 (0.78–2.07)	0.329	1.68 (0.84–3.35)	0.143
BMI	1.48 (0.63–3.47)	0.698	1.18 (0.40–3.45)	0.285
Underweight	Reference	0.374	Reference	0.764
Normal	1.04 (0.60–1.80)	-	0.53 (0.22–1.30)	-
Overweight/obesity		0.892		0.164
Prior nephrectomy Presence (ref. absence)	0.95 (0.55–1.65)	0.864	1.14 (0.51–2.53)	0.754
Metastasis status Synchronous (ref. metachronous)	1.11 (0.69–1.80)	0.665	1.50 (0.75–3.03)	0.253
IMDC risk Poor (ref. intermediate)	0.93 (0.55–1.59)	0.801	0.99 (0.47–2.06)	0.969
Lung metastasis Presence (ref. absence)	0.80 (0.48–1.32)	0.380	0.96 (0.48–1.93)	0.911
Bone metastasis Presence (ref. absence)	1.34 (0.75–2.39)	0.317	1.67 (0.77–3.60)	0.195
Liver metastasis Presence (ref. absence)	2.66 (1.34–5.28)	0.0052	1.88 (0.77–4.57)	0.166
Lymph node metastasis Presence (ref. absence)	1.37 (0.82–2.29)	0.227	1.38 (0.68–2.79)	0.377

HR, hazard ratio; CI, confidence interval; BMI, body mass index; IMDC, International Metastatic Renal Cell Carcinoma Database Consortium

These data indicated that clinical outcomes were significantly associated with BMI in IO-TKI combination therapy but not in IO-IO combination therapy for advanced ccRCC.

3.3. Safety of ICI combination therapy based on BMI

We next analyzed the association between BMI and the safety profile of ICI combination therapy. In the IO-TKI combination therapy, all patients experienced at least one AE (Supplementary Table 4). When stratified by BMI, the rates of grade 3 or higher AEs, treatment interruption and discontinuation, and the need for glucocorticoids did not differ significantly ($p > 0.05$). Similar findings were observed for IO-IO combinations: the rates of grade 3 or higher AEs, treatment interruption and discontinuation, and glucocorticoid need were not significantly different by BMI ($p > 0.05$), whereas the rate of any-grade AE development was likely lower in underweight patients ($p = 0.0589$) (Supplementary Table 5).

These findings indicated that drug-induced toxicity was not affected by BMI in ICI combination therapy, regardless of the regimen.

3.4. Gene expression profile based on BMI

To explore the mechanism underlying the association between BMI and clinical outcome following ICI combination therapy, we conducted a genome-wide gene expression analysis using RNA-seq in 94 ccRCC samples.

Patients were classified into two subgroups based on BMI: <18.5 kg/m^2 (underweight) and ≥ 18.5 kg/m^2 (non-underweight). Compared with ccRCC in non-underweight patients, 373 genes were significantly upregulated, and 984 were significantly downregulated in ccRCC in underweight patients (Fig. 3a). Clustering analysis of DEGs showed that ccRCC in underweight patients had a distinct gene expression profile relative to ccRCC in non-underweight patients (Fig. 3b). GO analysis using DEGs further demonstrated that angiogenesis-related pathways were downregulated in ccRCC in underweight patients (Fig. 3c).

3.5. TIIC profile based on BMI

To further evaluate whether the TIIC profile differed by BMI, we performed flow cytometry in 84 ccRCC samples. The gating strategy for flow cytometry is shown in Supplementary Fig. 3. Unsupervised clustering analysis based on TIIC proportions revealed two major clusters; we defined the cluster with a high TIIC proportion as the immune-hot cluster and the cluster with a low TIIC proportion as the immune-cold cluster (Fig. 4a). The immune cluster assignment was not associated with BMI category ($p = 0.659$). When we compared individual TIIC proportions, no significant differences were observed between RCC in underweight patients and that in non-underweight patients ($p > 0.05$), except for NK cells ($p = 0.0090$) (Fig. 4b and Supplementary Table 6). These data indicated that BMI was not significantly associated with the TIIC profile.

Collectively, these gene expression and TIIC profiling data indicated that ccRCC in underweight patients showed inactivation of angiogenesis-related pathways but did not exhibit a different TIIC profile

compared with that in non-underweight patients, potentially leading to decreased efficacy of IO-TKI combination therapy.

4. Discussion

Real-world, multi-institutional clinical data showed that, in the first-line treatment of advanced ccRCC, the efficacy of ICI combination therapy stratified by BMI varied by regimen. Specifically, survival and tumor response were inferior in underweight patients who received IO-TKI combination therapy, whereas BMI was not significantly associated with outcomes of IO-IO. Genome-wide gene expression analysis further revealed that ccRCC in underweight patients exhibited downregulation of angiogenesis-related pathways. In contrast, the TIIC profile did not differ by BMI. To the best of our knowledge, this is the first study to demonstrate a regimen-dependent impact of BMI on outcomes of first-line ICI combination therapy for ccRCC, supported by transcriptomic and TIIC profiling.

The prognostic impact of BMI in ICI combination therapy for RCC has been highlighted. Sato et al. reported longer OS in overweight patients with RCC (BMI ≥ 22 and ≥ 18 kg/m^2 for men and women, respectively) who received first-line ICI combination therapy (16). Hara et al. reported that patients with BMI ≥ 25 kg/m^2 had longer OS with IO-TKI combination therapy than with IO-IO, whereas patients with BMI < 18.5 kg/m^2 exhibited longer OS with IO-IO combination therapy for RCC (17). Santoni et al. also reported that BMI > 25 kg/m^2 was an independent favorable factor for OS after ICI combination therapy, using a large cohort from an international, multi-institutional database (18). Given these findings, patients with elevated BMI may have a favorable prognosis after ICI combination therapy for RCC. However, in these previous studies, the differential impact of BMI according to the ICI combination regimen has not been assessed in patients with ccRCC (16–19). Here, we found that underweight patients with ccRCC had inferior outcomes, particularly PFS and OS, with IO-TKI but not with IO-IO combination therapy.

Molecular analyses of gene expression and TIIC profiling demonstrated that ccRCC in underweight patients exhibited inactivation of angiogenesis-related pathways. These molecular characteristics may reduce the efficacy of IO-TKI combinations, whereas no clear association between BMI and IO-IO efficacy was detected in this cohort. In patients with obesity, inflammation causes hypoxia, increasing local production of angiogenic factors and facilitating tumor progression (30, 31). Indeed, several clinical studies reported shorter survival with TKI monotherapy in underweight patients with RCC than in patients with normal weight or overweight/obesity (32–34), which appears consistent with the transcriptome data.

The TIIC profile did not differ between underweight and non-underweight patients with ccRCC, which also appeared reasonable given the absence of an association between BMI and outcomes with the IO-IO combination. Nonetheless, previous studies have indicated that obesity is associated with immunosuppression in the tumor immune microenvironment, such as impaired effector T-cell function or recruitment of immunosuppressive tumor-associated macrophages, through various pathways, including

metabolism, which contributes to the response to ICIs (35, 36). Thus, further studies are warranted to clarify the influence of obesity or underweight on the ccRCC tumor immune microenvironment.

In this cohort, the proportion of underweight patients was ≤ 10%, which was consistent with a previous study (32). However, the significant association between weight loss and outcomes should be emphasized, as the pathogenesis underlying this relationship, at least in part, may be identified by molecular analysis. Notably, we found that neither the starting TKI dose nor the drug-induced toxicity profile were associated with BMI, suggesting that reduced treatment intensity would not compromise efficacy in underweight patients. Therefore, careful monitoring is required for this high-risk subpopulation. We urgently need to identify effective treatment strategies and novel therapeutic targets.

This study has some limitations. First, its retrospective design and small sample size introduced selection bias, thereby potentially affecting outcomes. Second, the relatively short follow-up period may have influenced the interpretation of survival data, particularly OS. Third, undetected confounders, such as comorbidities or lifestyle history (e.g., hypertension, diabetes, smoking, or alcohol consumption), may have affected the clinical and molecular findings.

5. Conclusion

These real-world, multi-institutional clinical data showed that BMI had differential effects on the clinical outcomes of ICI combination therapy for advanced ccRCC. In underweight patients, ccRCC exhibited downregulation of angiogenesis-related pathways, potentially resulting in reduced efficacy of IO-TKI combination therapy. Careful monitoring and identification of effective treatment strategies would be required in patients with weight loss. In addition, further studies are warranted to clarify the mechanisms underlying the downregulation of angiogenesis-related pathways in underweight patients.

Declarations

Conflicts of interest

Kazuhiko Yoshida received honorariums from Bristol Myers Squibb, Ono Pharmaceutical Co., Ltd. Toshio Takagi received honorariums from Bristol Myers Squibb, Ono Pharmaceutical Co., Ltd., Merck KGaA, and Takeda Pharmaceutical Co., Ltd. Tsunenori Kondo received honoraria from Pfizer, Novartis, Bristol-Myers Squibb, and Ono Pharmaceutical Co., Ltd.

Competing Interests

Conflicts of interest Kazuhiko Yoshida received honorariums from Bristol Myers Squibb, Ono Pharmaceutical Co., Ltd. Toshio Takagi received honorariums from Bristol Myers Squibb, Ono Pharmaceutical Co., Ltd., Merck KGaA, and Takeda Pharmaceutical Co., Ltd. Tsunenori Kondo received honoraria from Pfizer, Novartis, Bristol-Myers Squibb, and Ono Pharmaceutical Co., Ltd.Funding This research was supported by the JSPS KAKENHI grants to Hiroki Ishihara, Hironori Fukuda, Tsunenori

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Author Contribution

Hiroki Ishihara, Hironori Fukuda, and Yu-Yu Liu performed the data analysis and wrote the manuscript. Hiroki Ishihra, Hironori Fukuda, Yuki Nemoto, Takayuki Nakayama, Koichi Nishimura, Shinsuke Mizoguchi, Hiroaki Shimmura, Yasunobu Hashimoto, Kazuhiko Yoshida, Tsunenori Kondo, and Toshio Takagi collected the data. Akihiro Kumuno, Yuki Nemoto, Takayuki Nakayama, Koichi Nishimura, Shinsuke Mizoguchi, Hiroaki Shimmura, Yasunobu Hashimoto, Kazuhiko Yoshida, Shigehisa Kitano, Kazunori Aoki, Tsunenori Kondo, and Toshio Takagi supervised the study and edited the manuscript. Hiroki Ishihara, Hironori Fukuda, Tsunenori Kondo, and Toshio Takagi received the funding.

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None.

Data Availability

Data AvailabilityRNA-seq data have been deposited to the NCBI Gene Expression Omnibus database under identifier GSE312695. All other data supporting the findings of this study are available from the corresponding author on reasonable request.Data availability statementThe data that support the findings of this study are available from the corresponding author (Hironori Fukuda) upon reasonable request.

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Figures

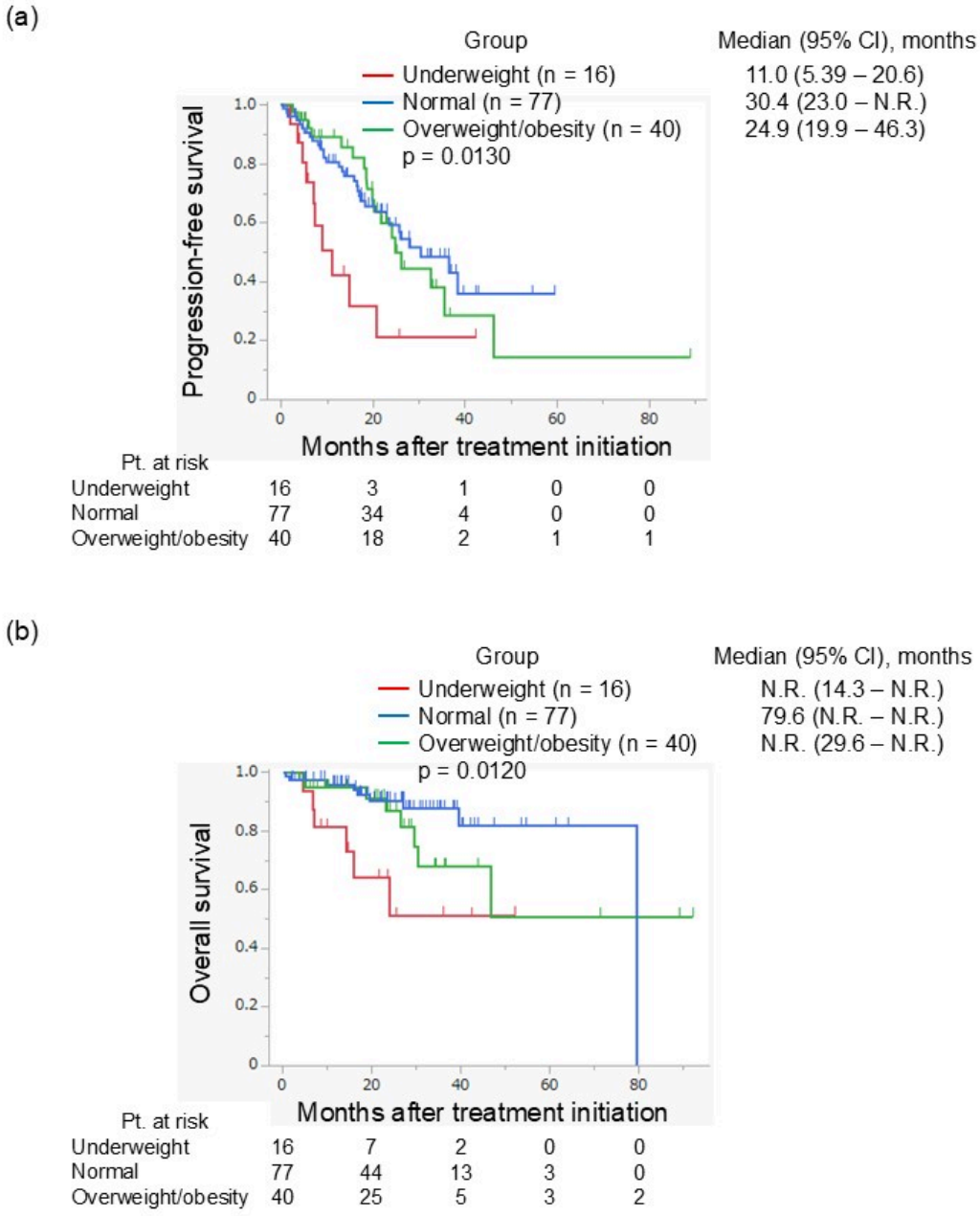


Figure 1

Figure 1

Comparison of survival after IO-TKI combination therapy based on BMI

(a) Progression-free survival. (b) overall survival.

IO, immunotherapy; TKI, tyrosine kinase inhibitor; BMI, body mass index; CI, confidence interval; N.R., not reached; Pt., patient

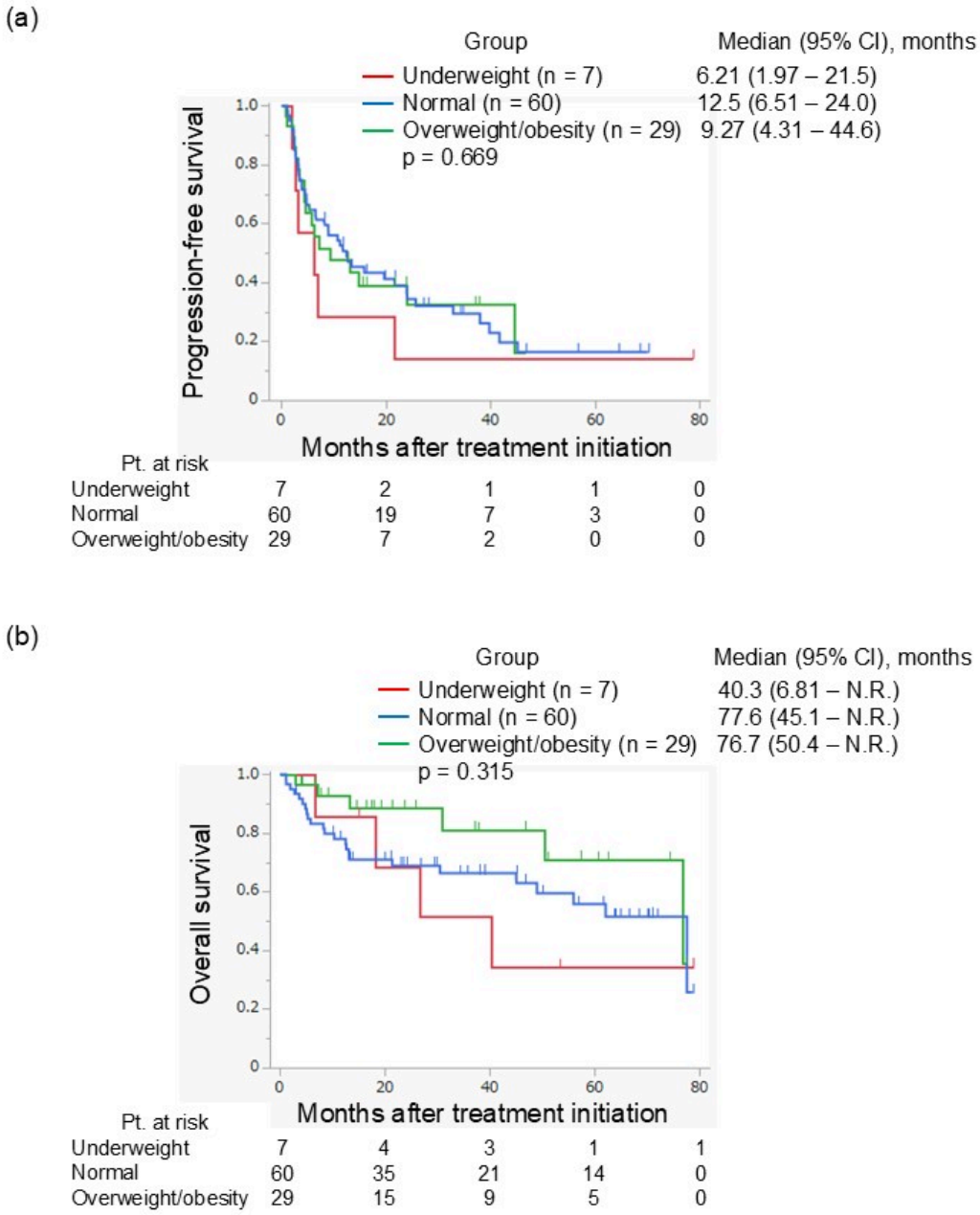


Figure 2

Figure 2

Comparison of survival after IO-IO combination therapy based on BMI

(a) Progression-free survival. (b) overall survival.

IO, immunotherapy; BMI, body mass index; CI, confidence interval; N.R., not reached; Pt., patient

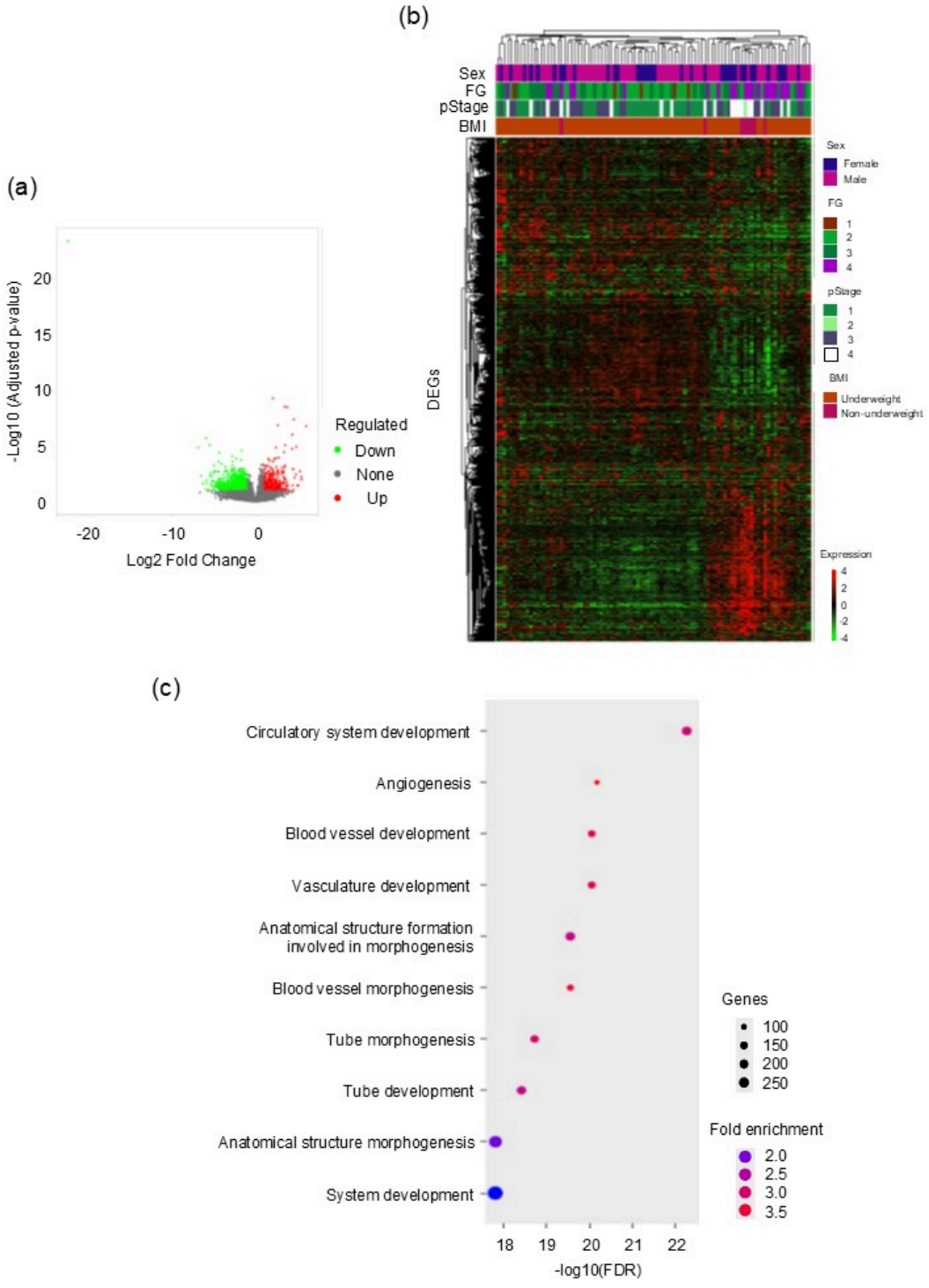


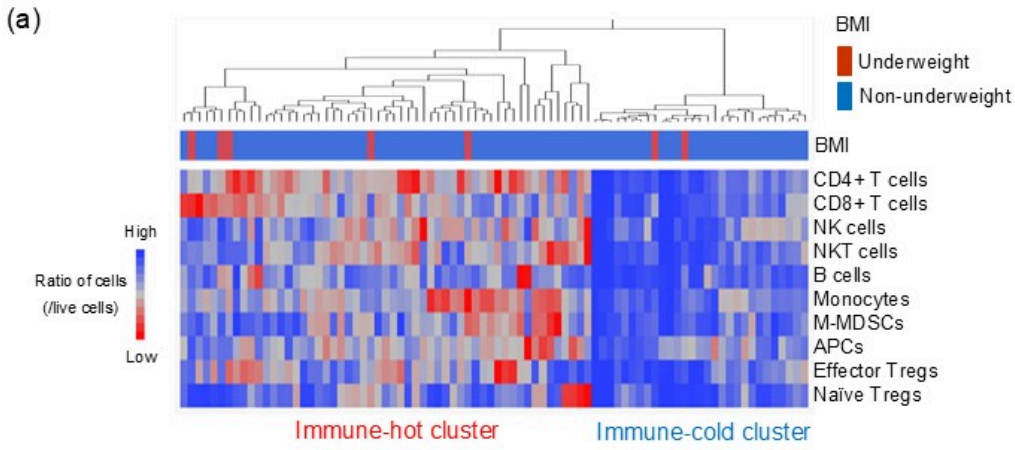
Figure 3

Figure 3

Comparison of gene expression profile between underweight and non-underweight patients with ccRCC

(a) Volcano plot shows DEGs between underweight and non-underweight patients. (b) Clustering analysis of DEGs between underweight and non-underweight patients. (c) Gene Ontology analysis.

FG, Fuhrman grade; pStage, pathological stage; BMI, body mass index; ccRCC, clear-cell renal cell carcinoma; DEGs, differentially expressed genes; FDR, false discovery rate



Cluster	Underweight	Non-underweight	p
Immune-hot	5 (9%)	50 (91%)	
Immune-cold	2 (7%)	27 (93%)	1.000

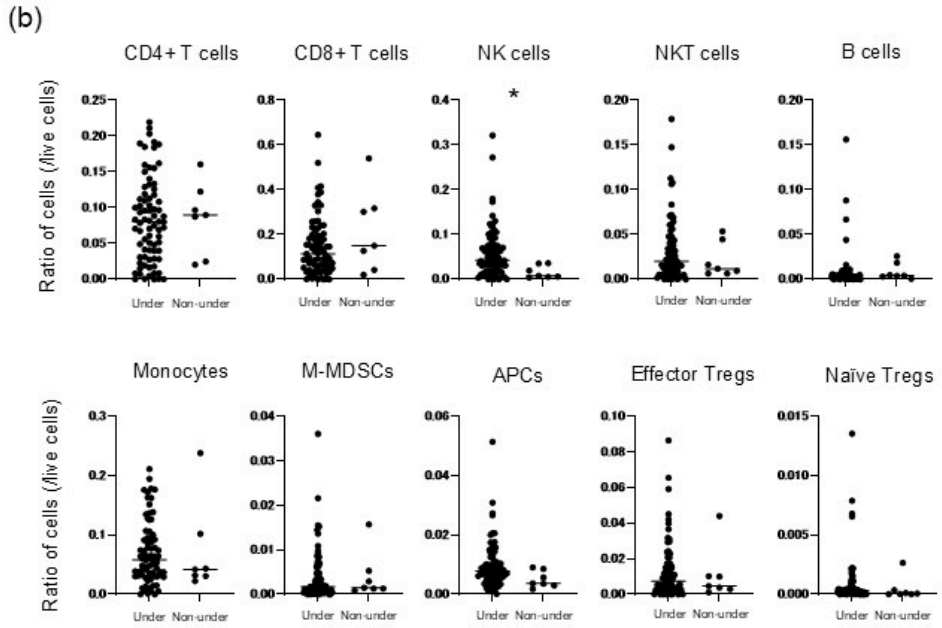


Figure 4

Figure 4

Comparison of TIICs between underweight and non-underweight patients with ccRCC

(a) Unsupervised hierarchical clustering analysis based on the ratio of TIICs.

TIICs, tumor-infiltrating immune cells; ccRCC, clear-cell renal cell carcinoma; BMI, body mass index; NK, natural killer; NKT, natural killer T; APCs, antigen-presenting cells; M-MDSCs, mononuclear myeloid-derived suppressor cells

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