Transcriptomic signatures related to the obesity paradox in patients with clear cell renal cell carcinoma: a cohort study

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Summary

Background Obesity is associated with an increased risk of developing clear cell renal cell carcinoma (RCC) but, paradoxically, obesity is also associated with improved oncological outcomes in this cancer. Because the biological mechanisms underlying this paradoxical association are poorly understood, we aimed to identify transcriptomic differences in primary tumour and peritumoral adipose tissue between obese patients and those at a normal weight.

Methods In this cohort study, we assessed data from five independent clinical cohorts of patients with clear cell RCC aged 18 years and older. Overweight patients were excluded from each cohort for our analysis. We assessed patients from the COMPARZ phase 3 clinical trial, a cohort from the Cancer Genome Atlas (TCGA), and a Memorial Sloan Kettering (MSK) observational immunotherapy cohort for their inclusion into our study. We assessed overall survival in obese patients (those with a body-mass index [BMI] ≥30 kg/m²) and in patients with a normal weight (BMI 18·5–24·9 kg/m², as per WHO's BMI categories), defined as the time from treatment initiation (in the COMPARZ and MSK immunotherapy cohorts) or surgery (in the TCGA cohort) to the date of any-cause death or of censoring on the day of the last follow-up. We also evaluated and validated transcriptomic differences in the primary tumours of obese patients compared with those of a normal weight. We compared gene-expression differences in peritumoral adipose tissue and tumour tissue in an additional, prospectively collected cohort of patients with non-metastatic clear cell RCC (the MSK peritumoral adipose tissue cohort). We analysed differences in gene expression between obese patients and those at a normal weight in the COMPARZ, TCGA, and peritumoral adipose tissue cohorts. We also assessed the tumour immune microenvironment in a prospective cohort of patients who had nephrectomy for localised RCC at MSK.

Findings Of the 453 patients in the COMPARZ trial, 375 (83%) patients had available microarray data, pretreatment BMI measurements, and overall survival data for analyses, and we excluded 119 (26%) overweight patients, leaving a final cohort of 256 (68%) patients from this study for our analyses. From 332 patients in the TCGA cohort, we evaluated clinical and demographic data from 152 (46%) patients with advanced (ie, stages III and IV) clear cell RCC treated by nephrectomy; after exclusion of 59 (39%) overweight patients, our final cohort consisted of 93 (61%) patients. After exclusion of 74 (36%) overweight patients from the initial MSK immunotherapy study population of 203 participants, our final cohort for overall survival analysis comprised 129 (64%) participants. We found that overall survival was longer in obese patients than in those with normal weight in the TCGA cohort, after adjustment for stage or grade (adjusted HR 0·41, 95% CI 0·22–0·75), and in the COMPARZ clinical trial after adjustment for International Metastatic RCC Database (IMDC) risk score (0·68, 0·48–0·96). In the MSK immunotherapy cohort, the inverse association of BMI with mortality (HR 0·54, 95% CI 0·31–0·95) was not significant after adjustment for IMDC risk score (adjusted HR 0·72, 95% CI 0·40–1·30). Tumours of obese patients showed higher angiogenic scores on gene-set enrichment analysis-derived hallmark gene set angiogenesis signatures than did those of patients at a normal weight, but the degree of immune cell infiltration did not differ by BMI. We found increased peritumoral adipose tissue inflammation in obese patients relative to those at a normal weight, especially in peritumoral fat near the tumour.

Interpretation We found aspects of the tumour microenvironment that vary by BMI in the tumour and peritumoral adipose tissue, which might contribute to the apparent survival advantage in obese patients with clear cell RCC compared with patients at a normal weight. The complex interplay between the clear cell RCC tumour and peritumoral adipose tissue microenvironment might have clinical relevance and warrants further investigation.

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Research in context

Evidence before this study

An inverse association between body-mass index (BMI) and mortality has been observed in patients with renal cell carcinoma (RCC) across several cohorts. A meta-analysis of 1543 patients with RCC who had a nephrectomy showed a higher overall survival in overweight or obese versus normal weight patients (pooled hazard ratio [HR] 0·57, 95% CI 0·43–0·76). We searched PubMed for studies that assessed primary tumour and peritumoral adipose tissue gene expression differences and were published in English from database inception to June 22, 2019, to understand the mechanisms underlying the obesity paradox in clear cell RCC. We used the search terms "obesity kidney cancer", "obesity renal cell carcinoma gene expression", and "obesity kidney cancer genomic". Previous studies found an inverse association between BMI and cancer-specific mortality in 2119 patients with clear cell RCC treated with nephrectomy (0·59, 0·42–0·83) and in 1975 patients with metastatic clear cell RCC treated with targeted therapy (HR 0·84, 95% CI 0·73–0·95), in which obese patients survived for longer than patients at a normal weight (as defined by WHO). In a smaller subset of patients in the localised (n=126) and metastatic clear cell RCC (n=61) cohorts, expression of the metabolic oncogene fatty acid synthase was significantly lower in the primary tumours of obese patients than in those at a normal weight. Another study evaluated the association of a previously published and validated 34-gene signature (ClearCode34-identified molecular subtype) with comorbidities in 282 patients with clear cell RCC, and the authors noted that obese patients were more likely to harbour ClearCode34 molecular subtype A tumours (*vs* ClearCode34 molecular subtype B tumours, which denote a more aggressive phenotype; 48% *vs* 34%; p=0·02). A report of 313 patients with metastatic RCC treated with second-line (or later) nivolumab showed shorter survival in patients with normal weight than in obese patients (HR 1·59, 95% CI 1·10–2·30). A previous study found that altered PD-1-mediated T-cell dysfunction might be partially driven by leptin, which has increased expression in obese patients. Finally, emerging evidence points to the importance of understanding microenvironmental differences in primary RCC (eg, angiogenesis and inflammation) to personalise treatment selection. We found no studies evaluating differences in primary tumour or peritumoral fat gene expression pathways between obese and non-obese patients in relation to the obesity paradox in clear cell RCC.

Added value of this study

The findings from our study lend biological support to the obesity paradox in clear cell RCC. Specifically, gene-expression differences in angiogenic and inflammatory pathways within the tumour microenvironment (ie, the tumour and peritumoral adipose tissue) could help to explain the survival advantage in obese patients versus those at a normal weight in this disease setting.

Implications of all the available evidence

The obesity paradox observed in patients with clear cell RCC might be driven by differences in the tumour microenvironment. The cross-talk between the tumour and peritumoral adipose tissue is incompletely understood, but investigation into this area might provide new, clinically relevant insights for patients with clear cell RCC. Our study highlights the need to evaluate the clinical relevance of host factors, such as body size, and their potential contribution to tumour biology, prognosis, and treatment selection. Future studies are required to evaluate the cross-talk between peritumoral adipose tissue and RCC, the impact of visceral fat inflammation on outcomes in patients receiving immunotherapy, and therapeutic interventions to address adverse characteristics of body size (eg, increased visceral fat).

Introduction

For **WHO's BMI categories** see https://www.who.int/dietphysicalactivity/childhood_what/en/

Obesity, defined as a body-mass index (BMI) of at least 30 kg/m² (according to WHO's BMI categories), is an established risk factor for developing clear cell renal cell carcinoma (RCC);[1] however, obesity has a counterintuitive association with prognosis.[2] Obese patients with localised clear cell RCC who are treated with nephrectomy survive for longer than those with normal weight in WHO's categorisation (BMI 18·5–24·9 kg/m²)—a phenomenon known as the obesity paradox.[1,3] We also observed the same pattern in patients with metastatic clear cell RCC who had been treated with targeted therapy.[4] McQuade and colleagues[5] reported the obesity paradox in patients with metastatic melanoma who had been treated with immunotherapy, and others have also found this phenomenon in cohorts of patients with mixed solid tumours who had been treated with immunotherapy.[6,7] De Giorgi and colleagues[8] found that, in patients with metastatic RCC who had been treated with second-line (or later) nivolumab, those who were a normal weight showed worse overall survival compared with those with a higher BMI (≥25·0 kg/m²). Why obese patients with clear cell RCC have better outcomes than patients who are of a normal weight, regardless of treatment type, is not known. Initial mechanistic insights suggest this finding could be due to BMI-related differences in the tumour transcriptome. We previously showed that the adverse metabolic oncogene, fatty acid synthase (*FASN*), was downregulated in the tumours of obese patients compared with those at a normal weight.[3] Wang and colleagues.[6] speculated that the adipocyte-derived hormone leptin in the tumour microenvironment of obese patients with colorectal cancer can alter T-cell function, thereby improving the patient's response to systemic immunotherapy.

We aimed to compare the angiogenic and immunological transcriptomic patterns of tumour and peritumoral adipose tissue in patients with clear cell RCC who were obese versus in patients who were at a normal weight, to explore and further understand the putative mechanisms underlying the obesity paradox.

Methods

Study design and participants

In this cohort study, we assessed data from five independent clinical cohorts. All cohorts included patients with clear cell RCC aged 18 years and older. Overweight patients (25·0–29·9 kg/m²) were excluded from each cohort for our analysis.

We analyzed survival, RNA/DNA sequencing data, and immunohistochemistry data from the COMPARZ phase 3 clinical trial[9] (in which patients were enrolled between August, 2008, and Sept 30, 2011). This international trial included patients with metastatic clear cell RCC randomly assigned to either first-line pazopanib or sunitib. The aim of the trial was to test the non-inferiority of pazopanib to sunitinib. In this study, formalin-fixed paraffin-embedded tumour blocks were collected at baseline from 453 patients who had sufficient tissue available for RNA microarray and detection of gene mutations. The cohort used in this retrospective study was approved by the institutional review boards of participating institutions. This study is registered with ClinicalTrials.gov, NCT00720941.

We also assessed survival and transcriptomic data from the Cancer Genome Atlas (TCGA) cohort,[10] in which primary tumours of patients with clear cell RCC were transcriptomically profiled between 1998 and 2010 (exact dates not available) and the results of which were published in 2013. This study collected 446 nephrectomy specimens from patients with histologically confirmed clear cell renal cell carcinoma treated across multiple institutions in the USA. This cohort study collected clinical and demographic data (including BMI) and tumour samples from 319 patients (of whom we excluded 165 patients with American Joint Committee on Cancer [AJCC] stage I/II and included the remaining 154) at the time of patients undergoing nephrectomy. We retrospectively collected these data and tumour specimens from several institutions in the USA, after appropriate institutional review board approval. For most TCGA participants, data about subsequent recurrences of clear cell RCC and the systemic therapy received (if cancer recurred) were unavailable.

In the Memorial Sloan Kettering (MSK) immunotherapy observational cohort we retrospectively investigated the association between BMI before treatment and overall survival in 203 patients with metastatic clear cell RCC who were treated with immunotherapy (anti-PD-1 monotherapy, anti-PD-L1 monotherapy, anti-PD-1 plus anti-CTLA-4 combination, or anti-PD-1 plus anti-PD-L1 combination) between July 26, 2011, and Dec 10, 2018. RNA and DNA samples were not available for analysis in this cohort. Approval for this study was given by MSK cancer centre institutional review board.

The MSK peritumoral adipose tissue cohort study was approved by the institutional review board of the MSK Cancer Center on Nov 7, 2016. This cohort, which had never been included in a publication before, included patients from oncology inpatient centres in the USA with localised RCC treated with surgery alone. The aim of this study was to understand the relationship between obesity and cancer. We prospectively obtained primary tumour and peritumoral fat specimens from 62 patients with non-metastatic clear cell RCC at the time of nephrectomy (appendix p 19). We harvested peritumoral fat immediately adjacent to the tumour (perinephric near [PNN]) and on the opposite unaffected pole (perinephric away [PNA]) to the tumour. We also extracted demographic and clinical data, including BMI at the time of nephrectomy, from patients' electronic medical records of the participating oncology inpatient cantres.

See Online for appendix

Finally, we included a small cohort of patients (seven patients at a normal weight and 16 obese patients) who had nephrectomy for localised RCC between June 11, 2015, and April 16, 2018. Fresh tissue collected from patients in this cohort was used for flow cytometry to assess the tumour immune microenvironment. This cohort was a separate prospective cohort at MSK under the direction of AAH. Other authors (AS, LV, DK, and RM) contributed to the study of this cohort and data analysis. Approval for this study was given by MSK institutional review board.

Procedures

In the COMPARZ clinical trial, RNA was extracted from tumour blocks by AltheaDx (San Diego, CA, USA) in 2013, using a Qiagen RNAeasy FFPE kit (Hilden, Germany) with a modified deparaffinisation step. Gene-expression profiles were derived via Affymetrix GeneChip HTA 2.0 (Affymetrix; Santa Clara, CA, USA). RNA microarray data was normalised to the log2 value. Probes that were found without a corresponding gene symbol were excluded from further analyses. For genes that matched with several probes, the probe with maximum median absolute deviation was chosen to represent the expression of the gene. The log2 normalised expression values were used in subsequent analyses. For differentially expressed genes (DEG) and individual gene analyses, the quantile normalisation values were used.

The authors of the COMPARZ trial extracted DNA from the primary tumours and matched normal tissue with the Qiagen DNeasy kit (Hilden, Germany) according to the standard protocol, and further analysed this DNA. Germline mutations were ruled out by analysis of adjacent non-tumoral tissue or normal germline samples for every sample. Samples from 377 patients with adequate DNA yields were extracted and sequenced using the 410-oncogene panel MSK mutation profiling of actionable targets assay. This assay is a hybridisation capture-based next-generation sequencing assay for targeted deep sequencing (approximately 500x) of all exons and selected introns of 410 oncogenes, tumour suppressor genes, and members of pathways deemed actionable by targeted therapies using Illumina HiSeq 2500 (Illumina; San Diego, CA, USA).[11] A

minimum of 40 ng of DNA was required for this sequencing. Our analysis focused on the three most clinically relevant mutations in clear cell RCC: *PBRM1*, *BAP1*, and *TP53*.[12] We did not analyse *VHL* mutations. *VHL* mutation calling is difficult and large published mutational analyses have shown that *VHL* loss is an early and essential event in the pathogenesis of clear cell RCC.[13,14] Therefore, an analysis of *VHL* mutation frequency by BMI groups would not be appropriate in this cohort of patients with clear cell RCC. The total mutational count was calculated as the total number of somatic mutations.

Publicly available RNAseq data for the TCGA cohort, including from the tumour and the normal adjacent kidney, were downloaded from the National Institutes of Health Genomic Data Commons. The methods for RNA extraction and processing for the TCGA cohort have previously been published.[10]

For the **National Institutes of Health Genomic Data Commons** see https://gdc.cancer.gov

RNA and DNA sequencing data were not available for the MSK immunotherapy cohort, so this cohort was only assessed for overall survival.

For the MSK peritumoral adipose tissue cohort, tissue samples were stored in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) at 4°C until RNA was extracted. We isolated total RNA from tumour and perinephric fat tissues using the RNeasy Mini Kit (Qiagen) and sequenced. We sequenced tumour and fat specimens with at least 500 ng of RNA and an RNA integrity number of more than 6·0 using RNA poly-A capture. Quality control of RNAseq using a principal component analysis did not indicate any clusters or similarity (data not shown). RNAseq raw read sequences were aligned against human genome assembly hg19 by STAR 2-pass alignment.[15] RNAseq gene-level count values were computed by using the GenomicAlignments R package[16] over aligned reads with University of California Santa Cruz KnownGene[17] in hg19 as the base-gene model. The union counting mode was used, and only mapped paired reads were considered. Fragments per kilobase-million values were then computed from gene-level counts by using the fragments per kilobase-million function from the DESeq2 R package.[18]

For **human reference sequence GRCh37 on the Genome Browser Gateway** see https://genome.ucsc.edu/cgi-bin/hgGateway?db=hg19

We used DEG results from the COMPARZ and TCGA cohort studies for gene-set enrichment analyses[19] and building gene-set enrichment plots against the Molecular Signatures Database Hallmark gene sets through the clusterProfiler R package.[20] The limma R package (version 3.29.0) was used for microarray data DEG analysis.[21] The limma package returns empirical Bayes moderated t statistic p values and adjusted p values.To correct for multiple comparisons testing, we used the Benjamini–Hochberg method to control the false-discovery rate. The DEG analysis, for the COMPARZ and TCGA cohorts, was done with the DESeq2 R package. In brief, DESeq2 provides methods to test for differential expression between conditions by use of negative binomial generalised linear models; the estimates of dispersion and logarithmic fold changes incorporate data-driven prior distributions. Given the raw count data and gene model used, DESeq2 normalised the raw count data by sample-specific size factor and took covariates, if any, into account while testing for significant differences in gene expression between conditions with multiple test correction through the false-discovery rate approach. The hallmark gene sets included angiogenesis and hypoxia gene signatures. We used two additional previously published and validated angiogenesis signatures[22,23] to measure an overall angiogenesis score (appendix p 14). These validated signatures have minimal overlap in the number of genes per signature, probably because each signature represents a different aspect of angiogenesis biology. One signature that we used was derived by Masiero and colleagues[23] and the other signature was used by McDermott and colleagues[22] to evaluate predictors of response to anti-VEGF treatment, immunotherapy, and combination therapy in the IMmotion150 phase 2 clinical trial.

For the **Molecular Signatures Hallmark Gene Sets** see http://software.broadinstitute.org/gsea/msigdb

For the COMPARZ, TGCA, and MSK peritumoral adipose tissue cohorts, we used a single-sample gene-set enrichment analysis (ssGSEA)[24] for immune deconvolution analyses, to estimate the abundance of immune cell types. In addition, we used T-cell infiltration score, immune infiltration score, and fraction of immune cells (ImmuneScore) to estimate the abundance of immune cells. ssGSEA takes the sample gene expression values as the input and computes an overexpression measure for the given gene list of immune cell types relative to all other genes in the transcriptome. Marker genes of immune cell types for ssGSEA were obtained from previous studies by Bindea and colleagues[25] and Senbabaoglu and colleagues.[26] The extent of infiltration by different immune cell types was quantified using the ssGSEA implementation by the gsva R package.[27] The estimate R package[28] was used to infer the fraction of stromal and immune cells (ImmuneScore) in tumour samples on the basis of a given gene-expression profile in fragments per kilobase-million function or normalised log2-transformed values. ssGSEA scores for each individual immune cell type were used to calculate total T-cell infiltration score and immune infiltration score, as previously describe by Senbabaoglu and colleagues.[26]

We used the DEG results for ingenuity pathway analysis (IPA; Qiagen), with the Ingenuity Knowledge Base as the reference set.[29] IPA was used to evaluate differences in canonical pathways that are predicted to change based on gene expression.[29] IPA analysis was used rather than gene-set enrichment analysis because of the smaller cohort sample size in the peritumoral fat cohort. Filters used in identifying DEG genes for IPA were a mean expression of more than 10 read counts, a 20% difference or more in gene expression between obese patients and patients with normal weight; and a p value for the difference in gene expression in obese patients versus patients with normal wight of less than 0·05. After filtering, 2517 DEG genes for PNN tissue,

675 DEG genes for PNA tissue, and 629 DEG genes for tumour tissue remained.

We derived an immune cytolytic score (for the COMPARZ, TCGA, and MSK peritumoral adipose tissue cohorts) based on the geometric mean expression of two key cytolytic effectors, granzyme A (*GZMA*) and perforin (*PRF1*).[30] We used previously published signatures of immune cell function to assess differences in T-cell function (Teff score,[22] including *CD8A*, *EOMES*, *PRF1*, *IFNG*, and *CD274*), in immune checkpoint expression (*CD274*, *CTLA4*, *HAVCR2*, *LAG3*, *PDCD1*, *PDCD1LG2*, *TIGIT*), and in myeloid expression (myeloid score,[22] including *IL6*, *CXCL1*, *CXCL2*, *CXCL3*, *CXCL8*, *PTGS2*), and to detect upregulation of macrophage M1 and M2 signals (which were described as M1_Up and M2_Up signatures by Chung and colleagues).[31]

PD-L1 expression quantification in FFPE samples from the COMPARZ trial was done using immunohistochemistry, as per previously validated protocols and as previously described.[32] Briefly, patients were categorised as positive for PD-L1 or B7H1 when any tumour cell positivity was detected (when the H score was >0).

Clear cell RCC tissue specimens from patients in the MSK nephrectomy cohort were prepared by mechanical disruption using a razor blade followed by treatment with 280 units per mL collagenase type 3 (Worthington Biochemical, Lakewood, NJ USA) and 4 µg/mL DNase I (Sigma, St Louis, MO, USA) at 37°C for 1 h with periodic vortexing. Digested tissues were passed through 70-µm filters. Resulting cells were resuspended in 44% Percoll–66% Percoll gradient (Sigma) and centrifuged for 30 min at 1900 × g with no brake. Mononuclear cells were collected and immediately stained for flow cytometry analysis following Fc blocking (BioLegend, San Diego, CA, USA) and live/dead staining (TONBO Biosciences, San Diego, CA, USA). Major immune cell populations were then assessed by flow cytometry (appendix p 17).

Outcomes

We calculated pretreatment BMI (in the COMPARZ and MSK immunotherapy cohorts) or presurgical BMI (in TCGA and MSK peritumoral adipose tissue cohorts), and we categorised participants as normal weight (BMI 18·5–24·9 kg/m²), overweight (25·0–29·9 kg/m²), or obese (≥30·0 kg/m²), in accordance with WHO criteria.[33] The main outcome of our analysis was overall survival, defined as the time from treatment initiation (in the COMPARZ and MSK immunotherapy cohorts) or surgery (in the TCGA cohort) to the date of any-cause death or of censoring on the day of the last follow-up visit. Progression-free survival (in the COMPARZ cohort) was defined as the time between treatment initiation and the earliest date of either disease progression or any-cause death.

Our secondary outcomes were to assess transcriptomic and genomic differences between obese patients and patients with normal weight in the COMPARZ cohort and then validate them in the TCGA cohort. In addition, we assessed transcriptomic differences in angiogenesis and immune pathways within the COMPARZ and TCGA cohorts. Finally, we characterised possible fat–tumour interactions in the MSK peritumoral fat tissue cohort and assessed the tumour immune microenvironment in the MSK nephrectomy cohort.

Statistical analysis

Transcriptomic and genomic differences between obese patients and patients with normal weight were tested using Fisher's exact and χ^2 tests (using SAS version 9.4) and the Wilcoxon rank-sum test (with the ggpubr R package version 0.1, to report p values in the plot for continuous variables, such as immune feature ssGSEA scores and total mutation count) after *Z* score normalisation between sample groups, or a Mann–Whitney test (used to compare immune scores between groups) in the COMPARZ, TCGA, and MSK peritumoral adipose tissue cohorts. Survival curves were created using the Kaplan–Meier method and analysed by log-rank test (with SAS 9.4). The TCGA cohort survival curves were adjusted for stage and grade because the patients in this cohort had a mixture of AJCC stage III and IV disease, whereas all the patients in the COMPARZ and MSK immunotherapy cohorts were stage IV and did not require stage or grade adjustment. However, we did adjust for International Metastatic RCC Database Consortium (IMDC) risk score for our MSK immunotherapy cohort. The hazard ratio (HR) estimates and 95% CIs were determined with Cox proportional hazards regression modelling (using SAS 9.4). Statistical significance was set at p values of less than 0·05.

	COMPARZ cohort (n=375)
Age, years	62 (55–69)
Sex	
Female	93 (25%)
Male	282 (75%)
Body-mass index	27·5 (24·0–31·6)
Normal	128 (34%)
Overweight	119 (32%)
Obese	128 (34%)
Memorial Sloan Kettering Cancer Center risk group	
Favourable	94 (26%)
Intermediate	207 (55%)
Poor	66 (18%)
Unknown	8 (2%)
Treatment	
Sunitinib	192 (51%)
Pazopanib	183 (49%)

Data are median (IQR) or n (%).

Table 1: **Demographic and clinical characteristics of the COMPARZ clinical trial cohort**

	TGCA cohort (n=152)
Age, years	62 (56–71)
Sex	
Female	53 (35%)
Male	99 (65%)
Body-mass index	28·0 (25·0–31·9)
Normal	38 (25%)
Overweight	59 (39%)
Obese	55 (36%)
American Joint Committee on Cancer stage	
III	88 (58%)
IV	64 (42%)

Data are median (IQR) or n (%). Advanced disease included American Joint Committee on Cancer stages III and IV. TCGA=The Cancer Genome Atlas.

Table 2: **Demographic and clinical characteristics of the TCGA cohort with advanced clear cell renal cell carcinoma**

	Memorial Sloan Kettering immunotherapy cohort (n=203)
Age, years	62 (42–90)
Sex	
Female	52 (26%)
Male	151 (74%)
Body-mass index	26·7 (18·8–55·0)
Normal	63 (31%)
Overweight	74 (36%)
Obese	66 (33%)
International Metastatic Renal Cell Carcinoma Database Consortium risk group	
Favourable	35 (18%)
Intermediate	128 (63%)
Poor	33 (16%)
Unknown	7 (3%)
Immunotherapy treatment line	
First-line	48 (24%)
Second-line or later	155 (76%)

Data are median (range) or n (%).

Table 3: **Demographic and clinical characteristics of the Memorial Sloan Kettering immunotherapy cohort**

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of the 453 patients in the COMPARZ cohort, 375 (83%) patients had available microarray data, pretreatment BMI measurements, and progression-free and overall survival data for analyses (table 1). After excluding 119 (26%) overweight patients, our final cohort from the COMPARZ trial comprised 256 (68%) patients. From 332 patients in the TCGA cohort, we evaluated clinical and demographic data from 152 (46%) patients with advanced (ie, AJCC stages III and IV) clear cell RCC that was treated by nephrectomy (table 2). After exclusion of 59 (39%) overweight patients, our final cohort from the TCGA cohort consisted of 93 (61%) patients. We prospectively obtained primary tumour and peritumoral fat specimens from 62 patients with non-metastatic clear cell RCC at the time of nephrectomy in the MSK peritumoral adipose tissue cohort. In this cohort, we sampled PNN peritumoral fat from 59 (95%) patients, PNA peritumoral fat from 25 (40%) patients, and tumour tissue from 55 (89%) patients. After exclusion of overweight patients (18 [31%] patients with PNN samples, eight [32%] with PNA samples, and 18 [33%] with tumour samples), our final cohort included 41 (69%) patients with PNN samples, 17 (68%) with PNA samples, and 37 (67%) with tumour samples (appendix p 19). After exclusion of 74 (36%) overweight patients from the initial MSK immunotherapy study population of 203 participants, our final cohort for overall survival analysis comprised 129 (64%) participants (table 3).

During the study periods, 19 (35%) of 55 obese patients versus 28 (76%) of 38 patients of normal weight in the TCGA cohort died; 57 (45%) of 128 obese patients and 76 (59%) of 128 patients of normal weight in the COMPARZ cohort died; and 25 (38%) of 66 obese patients and 36 (57%) of 63 patients of normal weight in the MSK immunotherapy cohort died. We found a longer overall survival in obese patients (ie, those with a BMI ≥30 kg/m²) than in those at a normal weight (ie, those with a BMI <25 kg/m²) in the TCGA cohort, after adjustment for stage or grade (median overall survival not reached [53·5–not reached] in obese patients *vs* 25·3 months [95% CI 14·2–39·5] in normal weight patients; adjusted HR 0·41, 95% CI 0·22–0·75; figure 1A), and in the COMPARZ clinical trial after adjustment for IMDC risk score (median overall survival 35·7 months [27·7–not reached] *vs* 19·1 months [15·3–27·8]; 0·68, 0·48–0·96; figure 1B). In the MSK immunotherapy cohort, the significant inverse association of weight with overall survival (median overall survival 49·9 months [31·8–not reached] in obese patients *vs* 15·6 months [95% CI 11·7–30·2] in normal weight patients; HR 0·54, 95% CI 0·31–0·95) was not significant after adjustment for IMDC risk score (adjusted HR 0·72, 95% CI 0·40–1·30; figure 1C).

In exporatory analyses, our findings for the inverse association between mortality and BMI in the COMPARZ, TCGA, and MSK immunotherapy cohorts were unchanged when the overweight category was included in the analysis (appendix pp 3–4) or when BMI was included as a continuous variable (appendix p 21). Notably, the association between BMI and overall survival in the MSK immunotherapy cohort did not differ

significantly between men and women in a model that included IMDC risk score (p=0·34; appendix p 21). In the COMPARZ clinical trial cohort, the longer overall survival (figure 1B) and progression-free survival (appendix p 1) in obese patients than in patients with normal weight was observed in all patients treated with first-line tyrosine kinase inhibitors (pazopanib or sunitinib). The inverse association between overall survival and BMI was stronger in the sunitinib-treated group than the pazopanib-treated group (appendix p 2).

After confirming the presence of the obesity paradox in our cohorts, we next assessed transcriptomic differences in tumours between obese and normal-weight BMI groups in the COMPARZ cohort. Tumours from obese patients, compared with those from patients with normal weight, in the COMPARZ cohort showed significant upregulation in hypoxia, TGF-β, epithelial–mesenchymal transition, and angiogenesis signalling pathways (appendix pp 5, 6). Increased expression of of TGF-β, Hedgehog, notch, and epithelial–mesenchymal transition pathways suggests activation of wound-healing pathways in obese patients. Obese patients also showed an enrichment of metabolic pathways (eg, adipogenesis, glycolysis, and fatty acid metabolism) compared with patients with normal weight (appendix p 5).

Notably, tumours of obese patients seemed to have distinct angiogenic differences compared with those of patients at a normal weight. We found higher angiogenesis scores in obese patients on gene-set enrichment analysis-derived hallmark gene set angiogenesis signatures (appendix p 14), which we confirmed using two independently published and validated angiogenesis signatures (figure 2, appendix p 14).

Because obesity is associated with a state of chronic systemic inflammation,[34] we hypothesised that obese patients harbour increased local inflammation in the primary tumour. Notably, in the COMPARZ cohort, we found that the tumours of obese patients had down-regulated interferon-γ pathway and no significant changes in other inflammatory pathways (interferon-α response and inflammatory response; appendix p 5). Therefore, tumours arising in an obesogenic environment do not seem to harbour increased local inflammation.

To better characterise differences in the tumour immune microenvironment by BMI, we performed immune deconvolution[35] using published immune cell signatures.[25] We found that total immune infiltration score (ImmuneScore) and macrophage, neutrophil, overall myeloid immune cell, or T-cell infiltration scores

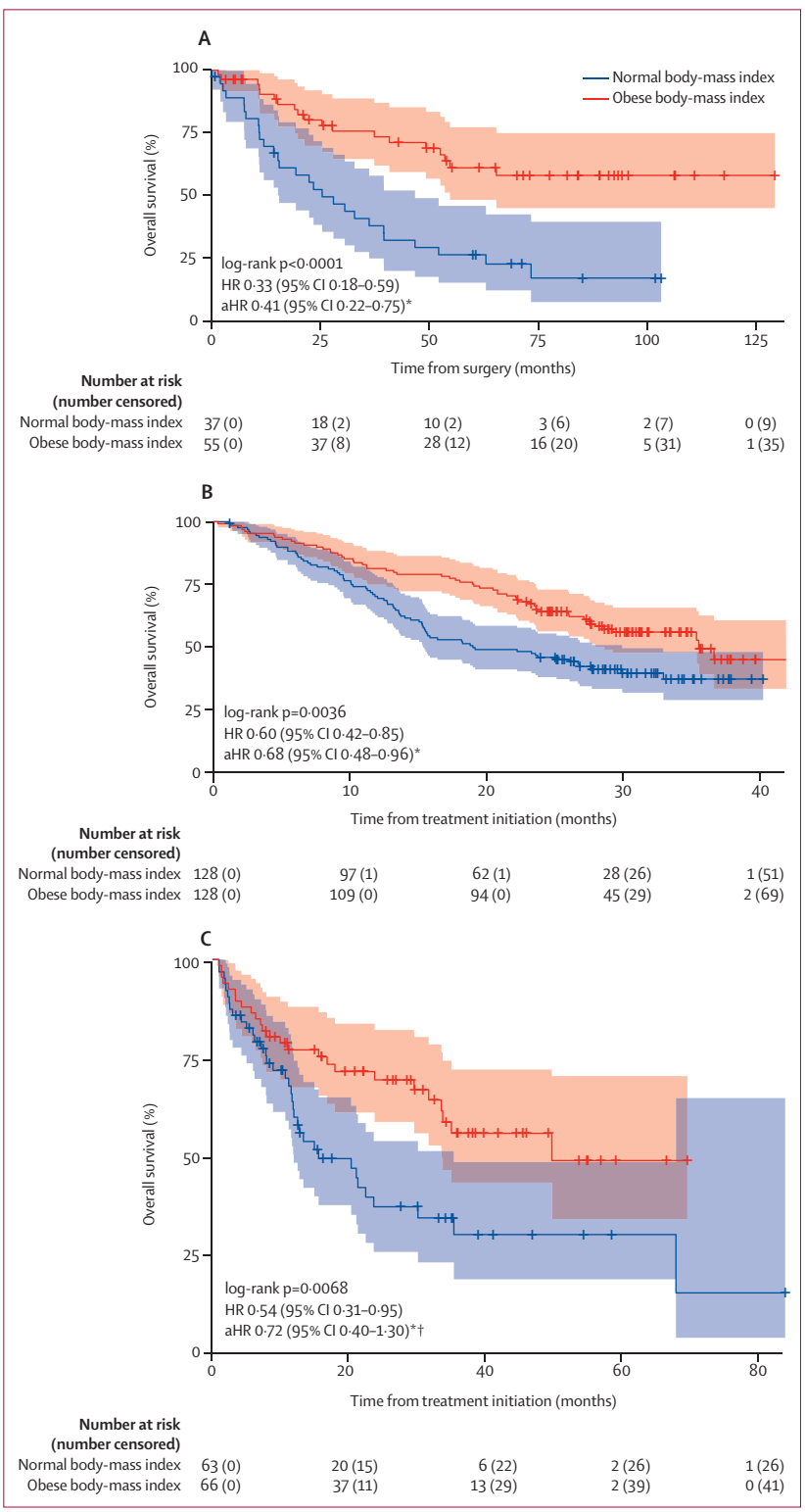

Figure 1: **Overall survival in the TCGA (A), COMPARZ (B), and MSK immunotherapy (C) cohorts**

Shaded areas are 95% CI. Shown are unadjusted Kaplan-Meier curves comparing patients with clear cell renal cell carcinoma who were of a normal weight versus those who were obese. aHR=adjusted hazard ratio. HR=hazard ratio. TCGA=The Cancer Genome Atlas. MSK=Memorial Sloan Kettering. *TCGA cohort was adjusted for stage and grade. The COMPARZ and MSK immunotherapy cohorts were adjusted for International Metastatic RCC Database (IMDC) alone. †The association of body mass index with overall survival in the MSK immunotherapy cohort was not significant after adjustment for IMDC, and showed no significant association with overall survival after further adjustment for age and sex (aHR 0·60, 95%CI 0·34–1·08).

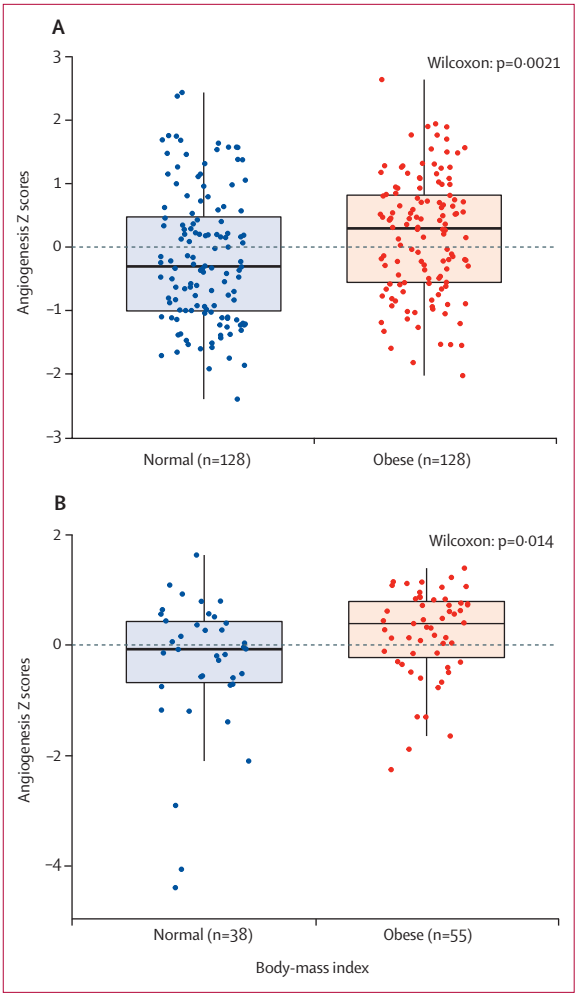

***Figure 2*: Angiogenesis *Z* scores in the primary tumours of obese patients versus those at a normal weight in the COMPARZ (A) and TCGA (B) cohorts**
Data are box plots of the differences in single-sample gene-set enrichment analysis angiogenesis scores. The angiogenesis RNA signature was derived from the study by Masiero et al.[23] TCGA=The Cancer Genome Atlas.

did not significantly differ between obese patients and those at a normal weight in the COMPARZ cohort. We found that obese patients had higher proportions of infiltrating plasmacytoid dendritic cells than patients of a normal weight in the COMPARZ cohort (appendix pp 7, 20), but this finding was not replicated in the TCGA cohort (appendix pp 15, 20). In a separate cohort of nephrectomy patients with advanced RCC (seven patients at a normal weight and 16 obese patients) analysed using flow cytometry, we found no differences in the proportion of effector immune cells expressing CD45, natural killer, or macrophage populations by BMI status (appendix pp 17–18).

Although we found no differences in the overall immune infiltration in the tumours of obese patients compared with those at a normal weight, we found some differences in RNAseq-derived immune checkpoint signature and PD-L1 immunohistochemistry in our analyses of the COMPARZ cohort. Specifically, obese patients showed a lower expression of several immune checkpoint molecules (appendix p 8) and decreased PD-L1 tumoural expression by immunohistochemistry (appendix p 9) than those of a normal weight. In the TCGA cohort, we found no differences in ImmuneCheckpoint expression in obese patients compared with patients at a normal weight (p=0·32) and PD-L1 protein staining by immunohistochemistry between obese and normal weight patients (appendix p 15).

Finally, because tumour mutational burden has been extensively studied as a biomarker for potential response to immunotherapy,[36] we examined whether mutations differed between obese patients and those at a normal weight in the COMPARZ cohort. We found no differences in total mutational burden or frequency of *PBRM1*, *BAP1*, or *TP53* mutations in the tumours of obese patients compared with those of a normal weight (appendix p 16).

Given the notable absence of differences in tumoral immune infiltration in obese patients compared with those of a normal weight, we hypothesised that the obese adipose tissue surrounding the kidney tumour (ie, the peritumoral fat) might harbour a distinct immunological milieu, possibly contributing to their paradoxical response to immunotherapy. As such, we did RNAseq and immune deconvolution on tumour and peritumoral fat specimens from the MSK peritumoral fat tissue cohort to characterise possible fat–tumour interactions. Our ingenuity pathway analysis results suggested that the tumour and the peritumoral fat (near and away from the tumour) in obese patients have higher expression of canonical inflammatory signatures (such as Th1 and Th2 pathways, CD28 signalling in T helper cells, and dendritic cell maturation) than these regions in patients at a normal weight (appendix pp 10, 11, 13, 20). We then used immune deconvolution to better characterise the immune microenvironment of the PNN and PNA regions around the tumour (appendix p 12). Obese patients had higher immune infiltration scores in the peritumoral fat than did patients with normal weight, regardless of its proximity to the tumour (appendix p 11). Compared with fat away from the tumour (ie, PNA fat tissue), peritumoral fat near the tumour (ie, PNN fat tissue) showed more significant immune infiltration (appendix p 12) in obese patients than in those at a normal weight.

Since hypoxia is a hallmark of dysregulated adipose tissue in obesity, we assessed the degree of hypoxia in peritumoral fat specimens and its potential association with immune infiltration in the MSK peritumoral fat tissue cohort. Within peritumoral fat, we found higher hypoxia gene-expression scores near the tumour and hypoxia was correlated with higher total immune infiltration scores and type 1 macrophage infiltration scores in obese patients versus those at normal weight (appendix p 12). We found no difference in hypoxia, immune infiltration, or macrophage scores within the

tumours of obese patients compared with those at a normal weight (appendix p 12, 13).

Discussion

In this study, we investigated the potential mechanisms underlying the inverse association between BMI and survival that is observed in clinical cohorts of patients with localised and metastatic clear cell RCC, regardless of treatment regimen. Our observations suggest that, compared with the tumours of patients at a normal weight, clear cell RCC tumours in obese patients have higher angiogenesis scores. Contrary to our initial hypothesis, obese patients did not harbour increased inflammation within their primary tumours. However, tumours of obese patients in the COMPARZ cohort had lower immune checkpoint expression than tumours from patients at a normal weight, but we observed no difference in gene mutational profile or tumour mutational burden by BMI. Finally, our analyses of peritumoral fat revealed higher hypoxia and inflammation close to the primary tumour in obese patients. Taken together, our initial mechanistic findings suggest that differences in the tumour microenvironment might underlie the apparent survival advantage of obese patients with clear cell RCC compared with patients at a normal weight that has been observed in clinical cohorts.

In the COMPARZ trial cohort, we found improved survival in obese patients versus patients with normal weight treated with tyrosine kinase inhibitor therapy, after adjustment for IMDC risk score, with the strongest association being noted among patients treated with sunitinib. These findings are consistent with a 2019 report, in which patients with higher angiogenesis scores are more likely to benefit from sunitinib than pazopanib.[37] Notably, the inverse significant association of BMI with immunotherapy in the MSK immunotherapy cohort was attenuated after adjustment for IMDC risk score. The IMDC risk score model encompasses a composite of some host factors that indirectly reflect systemic inflammatory effects of the cancer (eg, anaemia, neutrophilia, and thrombocytosis), which might overlap with mechanisms occurring concurrently with the association between immunotherapy outcomes and BMI.

Using three published and validated angiogenesis signatures, our finding that tumours of obese patients harbour higher angiogenesis scores than those of patients at a normal weight is in line with previous studies showing that obese patients are more likely to have ClearCode34 molecular subtype A (which is associated with improved prognosis relative to ClearCode34 molecular subtype B), indicating enrichment in genes involved in angiogenesis, β-oxidation pathways, organic acid metabolism, fatty acid metabolism, and pyruvate metabolism.[38] Furthermore, patients with high angiogenesis scores show improved survival outcomes with sunitinib compared with other VEGF-directed therapy, immunotherapy, or a combination of the two.[22,37] Visceral adipose tissue in obese patients is characterised by adipocyte hypertrophy, which leads to regions of hypoxia and subsequent increases in angiogenesis and immune cell infiltration.[39] We noted increased hypoxia scores in the adipose tissue closest to the primary tumour (PNN) and increased expression of canonical inflammatory pathways. Adipocytes produce angiogenic factors that can alter the underlying biology of tumours, both locally (at the adipocyte–tumour interface) and systemically.[40] Additionally, leptin secreted by adipocytes induces activation, proliferation, and migration of endothelial cells by upregulating VEGF and VEGF receptor-2.[41] Therefore, we hypothesise that obesity might create an environment that facilitates clear cell RCC growth via angiogenesis while simultaneously making these tumours more susceptible to tyrosine kinase inhibitors; and that increased angiogenesis could enhance local drug delivery. Similar findings have been reported in obese patients with metastatic colorectal cancer who have been treated with anti-VEGF drugs (eg, bevacizumab and ramucirumab).[42] Therefore, differences in angiogenesis expression could partly explain the superior survival outcomes in obese patients receiving tyrosine kinase inhibitor therapy.

Obesity-associated inflammation in other solid malignancies can cause tumour progression and therapeutic failure.[43] Chronic inflammation in visceral adipose tissue leads to immune cell infiltration, extracellular matrix remodelling and, eventually, fibrosis. We did not find increased overall immune cell infiltration in clear cell RCC tumours from obese patients compared with those at a normal weight. We noted a higher expression of plasmacytoid dendritic cells in obese patients in the COMPARZ cohort, but this finding was not validated in the TCGA cohort. Using a mouse model with intrarenal RCC, James and colleagues[44] showed that diet-induced obesity led to increased expression of tumour-suppressive dendritic cells and accelerated tumour growth, prompting a poor response to dendritic cell-directed immunotherapy. Further exploration into the impact of obesity on dendritic cell function is required, given these counter-intuitive findings.

In the COMPARZ cohort, we found lower expression of immune checkpoint molecules in the tumours of obese patients. These findings are counter to findings from Wang and colleagues,[6] who found that diet-induced obesity in tumour-bearing mice cause increased CD8-positive T-cell infiltration and T-cell dysfunction, as measured by increased immune checkpoint molecule expression (of Lag3, Tim3, and PD-1).[6] However, these results differed in human specimens, in which colorectal cancer tumour specimens from obese patients showed lower CD8-positive T-cell infiltration and melanoma specimens showed higher CD8-positive T-cell infiltration and immune checkpoint expression than equivalent specimens from those of normal weight.[6] Therefore, the underlying mechanism by which obesity alters the tumour microenvironment might differ depending on

the origin of each cancer. Like colorectal cancer, clear cell RCC arises near to visceral adipose tissue (ie, peritumoral fat), and how fat–tumour interactions affect a patient's response to immunotherapy are not known.

Notably, the peritumoral fat of obese patients showed increased immune infiltration and hypoxia, especially near the tumour. The potential importance of the interaction between peritumoral adipose tissue inflammation and clear cell RCC tumours is unclear. In ovarian, breast, and prostate cancer, substantial cross-talk between the primary tumour and adipocytes has been noted. Studies such as ours require further exploration in clear cell RCC. In our hypothesis-generating study, although the primary tumours of obese patients did not show more immune infiltration than those of patients at a normal weight, which would explain their response to immune checkpoint blockade, the surrounding peritumoral fat did. We speculate that the peritumoral adipose tissue might act as an immune reservoir of activated cells that are made available for mobilisation upon administration of various systemic therapies (appendix p 13). Therefore, peritumoral fat might act locally to affect the biology and, ultimately, survival of patients with clear cell RCC.

Our study has a few limitations. Our study is restricted to patients with clear cell RCC because less is known about the association of obesity with non-clear cell RCC. Because ours was exploratory analysis of the microenvironmental difference between obese patients and those at a normal weight, we did not fully adjust for all clinical covariates associated with clinical response. We used bulk immune deconvolution to estimate immune cell composition within primary tumours, and our results were not validated with multiplex immunohistochemistry. However, we validated our results across several cohorts, using different gene-expression platforms (microarray and RNAseq) and flow cytometry. We used published and validated gene signatures for our analysis, and we did not seek to generate novel signatures in our study because each angiogenic signature might represent a different aspect of angiogenesis biology. We analysed results from a single area of primary tumours, and acknowledge the potential for substantial heterogeneity to exist in clear cell RCC.[46] We recognise that we are correlating primary tumour specimens with the treatment of metastatic disease, which might not be representative of the microenvironment of metastatic lesions.[46] Our MSK immunotherapy cohort consisted primarily of patients receiving second-line immunotherapy, and therefore our results cannot be generalised to patients receiving first-line treatment. Finally, although BMI is a useful marker for visceral adiposity, it is not indicative of other elements of body composition (eg, muscle mass).[47]

For **trial data access** see www.clinicalstudydatarequest.com

In summary, our findings lend biological support to the obesity paradox, which is observed among patients with clear cell RCC regardless of treatment. We detected differences in the tumour microenvironment in obese patients relative to patients of a normal weight. Specifically, the tumours of obese patients with clear cell RCC harboured increased angiogenic pathways and they did not differ significantly in overall inflammation relative to the tumours of patients at a normal weight. Notably, the peritumoral fat of obese patients showed increased inflammation and hypoxia conditions relative to patients at a normal weight. Although our study is hypothesis-generating and our results cannot be translated directly to clinical practice, differences in transcriptomic pathways associated with obesity and other body composition features should be further investigated so that they can be leveraged to improve outcomes for patients with clear cell RCC. Future studies should focus on the usefulness of body size (such as through BMI) or body composition measures as factors that are prognostic, in combination with clinicopathological and tumour-specific features (such as mutational status), and they should explore mechanisms of fat–tumour cross-talk that can be used to help improve patient outcomes.

Contributors

AS, HF, AJD, and AAH conceived and designed the study and drafted the manuscript. FK and SP did the transcriptomic analyses, produced the figures, and interpreted the data in collaboration with AS. AS and CHL extracted RNA from the tumours and perinephric fat in the prospective cohort. All authors contributed to data acquisition and interpretation and critically reviewed and approved the final manuscript.

Declaration of interests

AJD reports membership of the scientific advisory boards of SynDevRx and Quentis Therapeutics. TC reports commercial research grants from Illumina, Bristol-Myers Squibb, Eisai, AstraZeneca, and An2H Discovery; ownership interest (including stock and patents) in Gritstone Oncology; and consultancy or advisory board membership for Gritstone Oncology, Illumina, and Bristol-Myers Squibb. RMo reports commercial research grants from Bristol-Myers Squibb, Pfizer, Novartis, Genentech (Roche), and Eisai; and consultancy or advisory board membership for Genentech, Pfizer, Merck, Novartis, and Incyte. MHV reports commercial research grants from Bristol-Myers Squibb, Pfizer, and Genentech; consultancy or advisory board membership for Alexion Pharmaceuticals, Calithera Biosciences, Corvus Pharmaceuticals, Exelixis, Eisai, Natera, Novartis, and Pfizer; travel remuneration from Eisai and Takeda; and honoraria from Novartis. All other authors declare no competing interests.

Data sharing

Novartis aims to share access to patient-level data and supporting clinical documents from eligible studies with qualified external researchers. Data requests are reviewed and approved by an independent review panel, based on scientific merit. All shared data are anonymised to respect the privacy of patients who have participated in the trial in accordance with applicable laws and regulations. The criteria and process involved in the availability of trial data is available online. Data will be made available with publication, via the same website.

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