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Identification of an endogenous retroviral signature to predict anti-PD1 response in advanced clear cell renal cell carcinoma: an integrated analysis of three clinical trials

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Abstract

Background: Endogenous retrovirus (ERV) elements are genomic footprints of ancestral retroviral infections within the human genome. Previous studies have demonstrated that dysregulated ERV transcription level is associated with immune cell infiltration in cancers, but the association between ERV expression and programmed cell death protein 1 (PD-1) blockade response is currently unraveled for solid cancers, such as advanced clear cell renal cell carcinoma (ccRCC).

Methods: ERV mRNA profiles were obtained from three clinical trials of ccRCC where the patients were treated with anti-PD-1 (CM-009, CM-010, CM-025, and TCGA-KIRC data). Patients treated with nivolumab were divided into training and test cohort, while the TCGA-KIRC cohort was used as an external validation. Univariate Cox regression analysis and least absolute shrinkage and selection operator regression were used to establish the signature. Immune cell infiltration analysis and gene set enrichment analysis were performed to explore potential biological mechanisms.

Results: An ERV signature was established based on nine ERV expression patterns. In the training cohort, the median overall survival in the low- and high-risk group was 45.2 and 19.6 months [hazard ratio (HR) = 0.49, 0.32–0.75, $p < 0.001$], respectively. The results were confirmed in the test (HR = 0.41, 0.20–0.83, $p = 0.013$), and in the TCGA-KIRC cohort (HR = 0.55, 0.34–0.90, $p = 0.017$). Moreover, in the CM-025 cohort, the low-risk group that received nivolumab had a more favorable survival compared with those that received the mTOR inhibitor everolimus, while no significant differences were observed in the high-risk group. CD8+ T cells were enriched in the low-risk group, while immune suppressive pathways were suppressed.

Conclusion: The newly identified ERV signature is not only a prognostic, but also a predictive biomarker for advanced ccRCC patients who received anti-PD-1 therapy, which can guide personalized treatment in cancer patients in the future.

Keywords: ccRCC, ERV, nivolumab, predictive, prognostic

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Introduction

Endogenous retroviruses (ERVs) are a specific type of transposable elements which are the remnants of exogenous retroviruses incorporated into the host genome over evolutionary time, characterized by 5′ and 3′ long terminal repeats (LTRs), a primer binding site, a polypurine tract, as well as Gag, Pro, Pol, and Env genes.[1,2] Human ERVs

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could be further divided into eight families based on the subtypes of LTRs.[3] Although ERVs constitute nearly 8% of the human genome, the majority of ERVs are epigenetic repressed and functionally inactivated,[4] while loss of epigenetic repression leads to dysregulated expression of a subset of ERVs, which could affect the splicing and expression of nearby genes that are involved in embryogenesis, immune cell maturation, and tumorigenesis.[5,6] Induced ERV expression by inhibition of DNA methylation could modulate T-cell action[7] and inhibit cancer-initiating cells in colorectal cancer.[8] Furthermore, latest evidence showed that ERVs are involved in antitumor response.[9]

As one of common malignancies of kidney, clear cell renal cell carcinoma (ccRCC) is innately resistant to traditional therapies, and recently immune checkpoint inhibitors (ICIs) have achieved remarkable success in metastatic ccRCC patients.[10] Nevertheless, only a minority of ccRCC patients respond to ICIs. Therefore, establishment of a predictor to identify the immunotherapy responders is a challenge and has a high priority. The Cancer Genome Atlas (TCGA) pan-cancer analysis found that ERV expression was significantly prognostic in ccRCC.[9] Another study stratified ccRCC patients into three groups based on immunogenic ERV expression profiles, indicating ERV expression profile may be a potential biomarker to predict response to ICI therapy.[11] In a more recent study, Braun and colleagues quantified the ERVs expression with RNA sequencing data from advanced ccRCC cohorts receiving anti-PD1 therapy and found two ERVs out of 3173 were weakly associated with response to PD1 blockade.[12] Nevertheless, there is still lack of validation of the predictive value of ERV in immunotherapy cancer patients.

Here, we analyzed the ERV expression data of patients from three different clinical trial cohorts that received anti-programmed cell death protein 1 (PD1) therapy. A prognostic ERV signature for overall survival (OS) was first established and then validated in an external cohort. The ERV signature could successfully stratify advanced ccRCC patients into two groups that differ in OS and response to anti-PD1. Our results not only provide solid evidence for ERV signature as both a prognostic and predictive marker for immunotherapy, but also bring new insight into the potential crosstalk between ERVs and the tumor immune microenvironment.

Methods

Patient data

ERV expression data and corresponding clinical data of CheckMate-009 (CM-009) ($n=16$), CheckMate-010 (CM-010) ($n=45$), CheckMate-025 (CM-025) ($n=250$), and TCGA-KIRC ($n=83$) cohorts were obtained from Braun's work[12] and Smith's work,[9] respectively. Patients, who received nivolumab in the CM-009, CM-010, and CM-025 trials, were divided into training and test cohort with the ratio of 7:3. The training cohort was used to establish the ERV signature. Among the three cohorts, CM-025 is a two-arm cohort, with subjects randomly assigned to nivolumab (PD-1 inhibition) arm or everolimus (mTOR inhibitor) arm. To validate the predictive value of the ERV signature, patients in the nivolumab arm and everolimus arm of the CM-025 cohort were divided into low- and high-risk group, respectively.

Cox proportional hazard regression and least absolute shrinkage and selection operator regression analysis

A univariate Cox proportional hazard regression analysis and least absolute shrinkage and selection operator (LASSO) regression analysis were subsequently performed in the training cohort on the ERV expression to establish the ERVs signature. Briefly, univariate Cox proportional hazard regression analysis was first applied to examine the association between ERVs expression and patients' OS, and ERVs with $p<0.2$ were selected as candidates for subsequent analysis. LASSO was then applied to establish an ERV-based prognostic signature for OS in the training cohort with the time steps set as 200,000. The model with best C-index was selected as the final ERVs signature. Based on the expression of ERVs and the correlation coefficient, the risk score was then calculated for each patient in training and validation cohorts, respectively, with the optimal cutoff value that was determined by *surv_cutpoint* function in R package *survminer* (v0.4.9). Survival curve was also plotted for both OS and progression-free survival (PFS) by *survminer*.

Gene set enrichment analysis

Differential expressed genes (DEGs) were analyzed between low- and high-risk group in patients received anti-PD1 therapy in Checkmate cohort using the R package *Limma* (v3.52.2). Gene set

enrichment analysis (GSEA) was then conducted using the R package *clusterProfiler* (v4.4.4) to explore the potential molecular mechanisms underlying the distinct immunotherapy response. Normalized enrichment score (NES) was calculated with gene set permutations set as 1000 times. Gene sets with $|NES| > 1$, adjusted $p < 0.05$, $q < 0.05$ were considered as significant enrichment.

Immune cell infiltration and immune checkpoint analysis

Immune cell infiltration was analyzed with the TIMER algorithm using the R package *Immunedeconv*.[13] The tumor purity, stromal score, and ESTIMATE score were calculated with the ESTIMATE package in R.[14] The mRNA expression of CD8A, a cytotoxic T-cell marker, and a set of immune checkpoints including PD1, programmed death-ligand 1 (PDL1), and cytotoxic T lymphocyte antigen 4 (CTLA-4) was also analyzed. All these parameters were compared between the subgroups using Wilcoxon signed-rank test.

Statistical analysis

All statistical analyses were performed using the software R (v4.2.0) with corresponding packages. Continuous variables were presented as means ± SD, and categorical variables were presented as percentage. Kaplan–Meier survival analysis and the log-rank test were conducted to compare OS and PFS between the low- and high-risk groups in the training and validation cohorts, respectively. The area under the curve (AUC) was calculated using the R package *pROC* [15]. $p < 0.05$ was considered as statistically significant.

All statistical analyses were carried out in R V.3.6.1 (R Foundation for Statistical Computing). $p < 0.05$ was considered as statistically significant. In multivariate analysis, the ERV signature, PD1, PDL1, and CTLA4 mRNA level were included.

Results

Clinical characteristics of patients

Advanced ccRCC patients treated with nivolumab from three prospective clinical trials (CheckMate (CM)-009, CM-010, and CM-025) were randomly divided into the training cohort ($n = 129$) and the test cohort ($n = 52$), while patients treated with everolimus (mTOR inhibitor) in CM-025 was administered to the control arm (Supplemental Table 1). The clinicopathological characteristics of training and test cohorts are shown in Table 1.

Derivation of the prognostic ERV signature

A schematic diagram of the analysis workflow of our study is shown in Figure 1. Univariate Cox proportional hazard regression was first applied to explore the OS-associated ERV in training cohorts. In all, 61 ERVs are selected as prognostic biomarker candidates ($p < 0.2$) for further analysis (Supplemental Table 2). The Cox-LASSO regression model was applied to develop an ERV-based prognostic signature for OS in the training cohort. After 200,000 time steps for LASSO, the one with the best C-index was selected as the prognostic ERV signature, and nine ERVs were selected into the final prognostic model (Supplemental Table 3). Subsequently, we analyzed the ERV signature in a test cohort for this model, to assess its feasibility and reliability in patients treated with nivolumab.

Using the optimal cutoff value of risk score, patients were divided into low- and high-risk group in the training and test cohorts, respectively (Figure 2(a) and (b)). Among the nine selected ERVs, higher herv_3771, herv_1992, herv_3511, and herv_806 expressions were observed in low-risk group, while high-risk group is characterized by a higher expression of herv_4755, herv_5346, and herv_6068 (Figure 2(c) and (d)).

For the training cohort, the median OS in low-risk group is 45.2 [95% confidence interval (CI): 31.3–NA] months, while the median OS in high-risk group is 19.6 months [95% CI: 13.3 months–28.3 months; hazard ratio (HR) = 0.49, 95% CI: 0.32–0.75, $p < 0.001$; Figure 3(a)]. The receiver operating characteristic (ROC) curve analysis showed that the ERV signature results in acceptable prediction values at 12-month (AUC = 0.721), 36-month (AUC = 0.722), and 60-month survival (AUC = 0.750) (Figure 3(b)). Similarly, for the test cohort, median OS was significantly longer in the low-risk group than the high-risk group [37.0 (22.9–NA) months *versus* 16.4 (10.2–31.2) months in low- and high-risk group, respectively; HR = 0.41, 95% CI: 0.20–0.83, $p = 0.013$; Figure 3(d)]. In the test cohort, the 12-month, 36-month, and 60-month AUC is 0.607, 0.638, and 0.606,

Table 1. Clinical characteristics of training, test and validation cohort.

Characteristics	Training cohort (*N* = 129)	Test cohort (*N* = 52)	Validation cohort (*N* = 83)	*p* Value
Sex				
Male	106	31	58	0.005
Female	23	21	25	
Age (year), mean ± SD	61.55 ± 10.98	60.77 ± 11.06	60.08 ± 10.09	0.619
MSKCC				0.983
Favorable	37	15	/	
Intermediate	55	22	/	
Poor	25	11	/	
NA	12	4	/	
Prior therapy				0.859
Yes	127	51	/	
No	2	1	/	
Metastasis				0.541
Yes	31	16	/	
No	97	36	/	
NA	1	0	/	
ORR				0.401
CR/PR	24	15	/	
SD	46	18	/	
PD	53	16	/	
NE	6	3	/	
Benefit				
CB	34	23	/	0.064
ICB	44	13	/	
NCB	51	16	/	
OS (months), mean ± SD	27.94±21.61	29.95±21.28	34.97±33.27	0.153
PFS (months), mean ± SD	7.95±11.92	9.80±13.47	17.48±22.58	<0.001

CB, clinical benefit; CR, complete response; ICB, intermediate clinical benefit; MSKCC, Memorial Sloan-Kettering Cancer Center (MSKCC/Motzer) Score; NA, not applicable; NCB, no clinical benefit; NE, not evaluable; ORR, objective repose rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

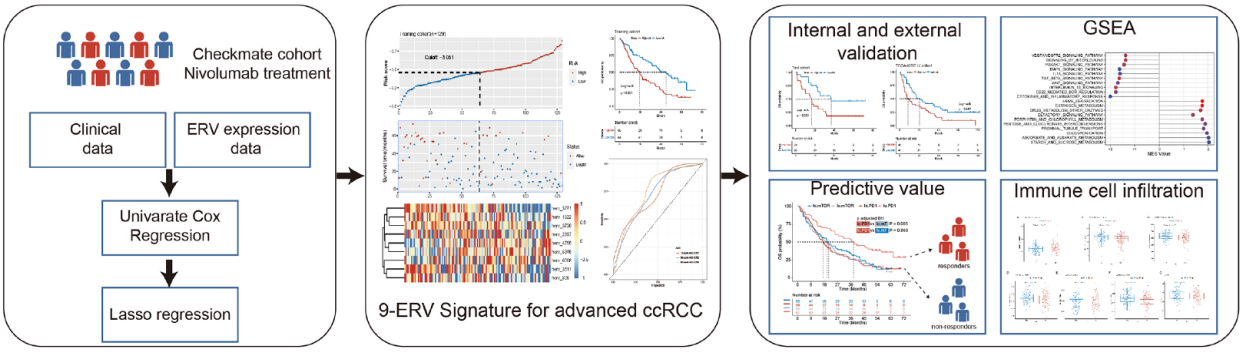

Figure 1. Schematic diagram of the analysis workflow of the study.

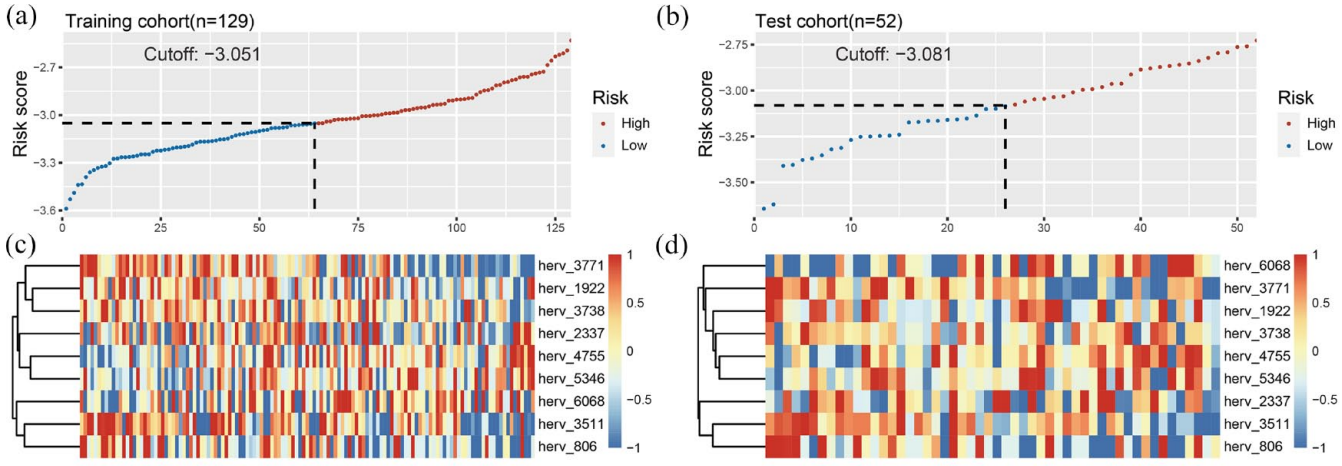

Figure 2. Establishment of a prognostic ERV signature in advanced ccRCC cohorts. (a) The ERV signature stratified training cohort ($n = 119$) into two groups with distinct survival status. (b) The ERV signature stratified test cohort ($n = 52$) into two groups with distinct survival status. (c) The expression pattern of the ERV signature in the training cohort. The expression pattern of the ERV signature in the test cohort.
ccRCC, clear cell renal cell carcinoma; ERV, endogenous retrovirus.

respectively (Figure 3(e)). Moreover, the low-risk group in both training and test cohort showed improved PFS (Supplemental Figure 1). Of note, more responders were observed in the low-risk group in both training and test cohorts (Figure 3(c) and (f)). In conclusion, the ERV signature is a prognostic biomarker for OS in advanced ccRCC patients.

External validation of the prognostic ERV signature

The TCGA Stage IV ccRCC patient cohort was further used to validate the robustness and the predictive ability of the ERV signature. For OS, significantly longer OS time was observed in the low-risk group [median OS is 30.6 (21.24–65.1) months *versus* 12.7 (7.96–39.5) months in low- and high-risk group, respectively; HR = 0.55, 95% CI: 0.34–0.90; $p = 0.017$; Figure 4(a)]. This ERV signature showed moderate prediction accuracy (Figure 4(b)). Nevertheless, no significant difference was observed considering PFS between low- and high-risk group (Supplemental Figure 2).

The ERV signature is a predictive marker for anti-PD1 response in advanced ccRCC patients

To validate whether the ERV signature could serve as a predictive biomarker for anti-PD1 therapy, we further performed survival analysis in the CM-025 cohort, in which nivolumab or everolimus was administered to two arms, respectively

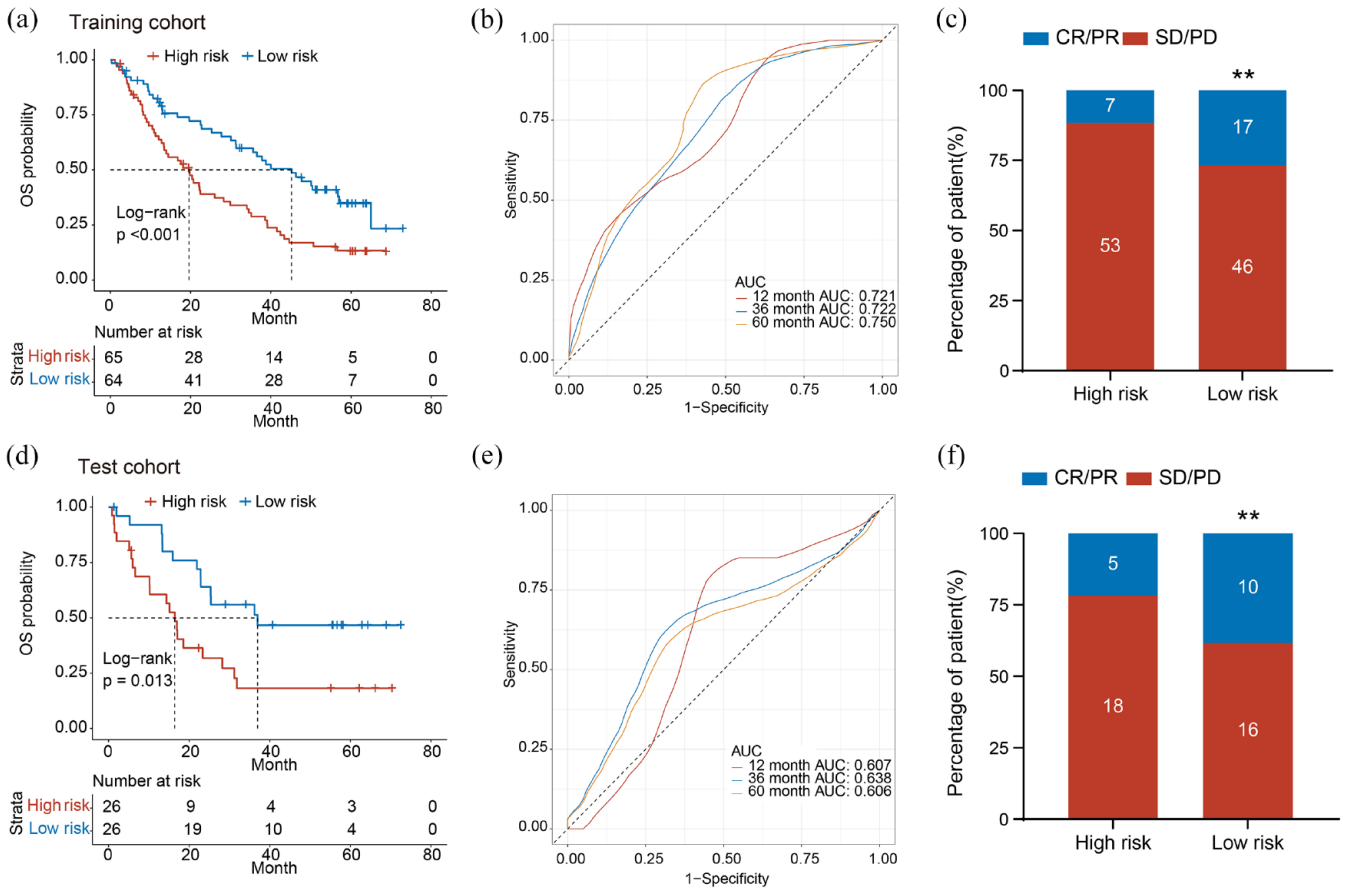

Figure 3. The ERV signature is a prognostic biomarker for OS in advanced ccRCC cohort. (a) Kaplan–Meier curve of OS between the ERV low- and high-risk advanced ccRCC patients in the training cohort. (b) ROC curve showed the performance of the ERV signature for OS in the training cohort. (c) Analysis of ORR in the training cohort. (d) Kaplan–Meier curve of OS between the ERV low- and high-risk advanced ccRCC patients in the test cohort. (e) ROC curve showed the performance of the ERV signature for OS in the test cohort. (f) Analysis of ORR in the test cohort.
ccRCC, clear cell renal cell carcinoma; ERV, endogenous retrovirus; ORR, objective response rate; OS, overall survival; ROC, receiver operating characteristic.

(Table 2). The same cutoff value was applied to stratify patients into low- and high-risk group in each arm, respectively. In the high-risk group, no significant benefit in either OS or PFS was observed in patients received nivolumab compared with those received everolimus [median OS, 17.1 (13.4–26.0) months *versus* 19.7 (14.9–34.8) months, $p = 0.87$; median PFS is 3.8 (1.91–5.85) months *versus* 5.4 (3.52–7.49) months, $p = 0.36$ in nivolumab and everolimus arm, respectively] (Figure 5(a) and (b)). However, in the low-risk group, patient who received nivolumab had a significantly longer survival compared with those who received everolimus [median OS is 37.8 (25.3–NA) months *versus* 21.0 (12.9–25.5) months, $p = 0.005$; median PFS is 5.4 (3.84–9.56) months *versus* 3.7 (2.14–5.65) months, $p = 0.01$ in nivolumab and everolimus arm, respectively; Figure 5(a) and (b)], indicating nivolumab could yield greater survival benefits compared with everolimus in advanced ccRCC patients with lower ERV-signature risk.

Considering objective response rate, while low-risk patients received nivolumab could achieve a complete response/partial response (CR/PR) rate of 30%, the CR/PR rate is only 5.56% in low-risk patients who received everolimus ($p < 0.001$, Figure 5(c)). A similar tendency was observed in the high-risk group, but no significant difference of CR/PR rate between patients who received nivolumab and everolimus was observed ($p = 0.063$, Figure 5(c)). Taken together, our results indicate that the ERV signature is a

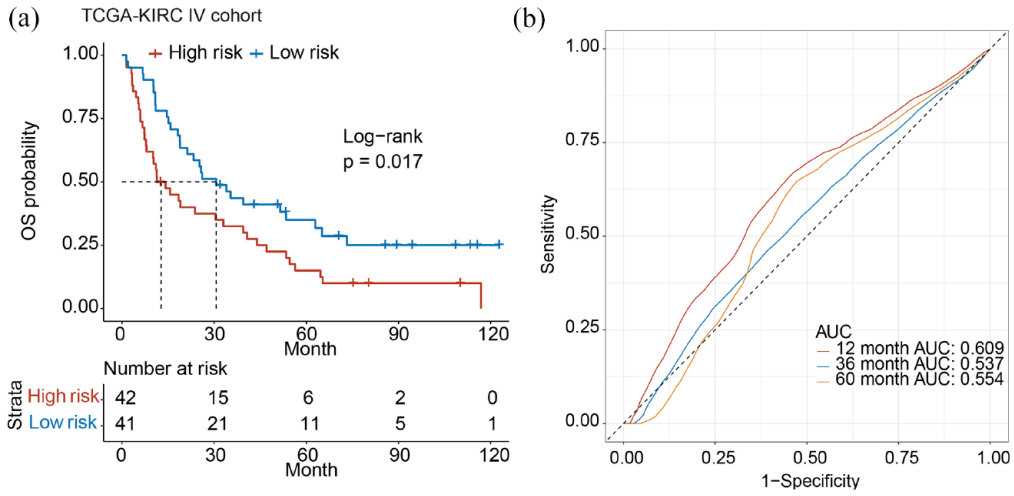

Figure 4. External validation of the prognostic ERV signature for OS in the TCGA-KIRC IV cohort. (a) Kaplan–Meier curve of OS between the low- and high-risk advanced ccRCC patients in the TCGA-KIRC IV cohort. (b) ROC curve showed the performance of the ERV signature for OS in the TCGA-KIRC IV cohort.
ccRCC, clear cell renal cell carcinoma; ERV, endogenous retrovirus; OS, overall survival; ROC, receiver operating characteristic; TGCA, The Cancer Genome Atlas.

well-performed predictive biomarker for anti-PD1 therapy response in advanced ccRCC patients.

Low ERV risk group had a higher immune cell infiltration

Immune cell infiltration analysis was performed to uncover the potential players in immune response that led to the different survival benefit observed between low- and high-risk group. Higher ESTIMATE and stromal score and lower tumor purity in low-risk group indicated that ccRCC tumors with low ERV risk may constitute of higher fraction of immune cells and stromal cells (Figure 6(a)–(c)). Specifically, a significantly higher fraction of CD8+ T cells was found in the low-risk group compared with the high-risk group, while no significant difference was found in neutrophils, CD4+ T cells, B cells, dendritic cells, and macrophages (Figure 6(d)–(i)). Consistently, the expression of the cytotoxic T-cell marker CD8A tended to be higher in the low-risk group compared with the high ERV risk group (Figure 6(j)).

GSEA to get first hints about the specific pathways involved in the identified ERV signature

To further explore the potential biological mechanisms underlying survival benefits from nivolumab observed in low-risk group, DEGs were analyzed between low- and high-risk group in patients received nivolumab, and GSEA was then performed to characterize the specific pathways that may be involved in the ERV signature. The most positively enriched gene sets in the low-risk group included starch and sucrose metabolism, ascorbate and aldarate metabolism, glucuronidation, pentose and glucuronate interconversions, porphyrin and chlorophyll metabolism, estrogen metabolism, and heme degradation (Figure 7). On the other hand, several immune suppressive pathways were negatively enriched in the low ERV risk group, including cytokines and inflammatory response, CD22-mediated B-cell receptor regulation, interleukin 10 (IL-10) signaling pathways, IL-18 signaling pathway, mitogen-activated protein kinase (MAPK) pathway, vascular endothelial growth factor A/vascular endothelial growth factor receptor 2 signaling, and WNT signaling pathway (Figure 7). The expression of immune checkpoint including PD1, PDL1, and CTLA4 was also examined, and no significant difference was found between the low- and high-risk group (Supplemental Figure 3). Moreover, multivariate Cox regression analysis showed that low ERV signature, not PD1, PDL1, or CTLA4, is the only independent predictor for OS and PFS in patients received nivolumab therapy, while all these four factors are not independent prognosis predictor in everolimus arm (Supplemental Figure 4).

Table 2. Clinical characteristics of Checkmate-025 cohort.

Characteristics	Nivolumab (*N* = 120)	Everolimus (*N* = 130)	*p* Value
Sex			
Male	94	92	0.22
Female	26	38	
Age (year), mean ± SD	60.94±12.02	62.54±9.51	0.24
MSKCC			0.51
Favorable	36	48	
Intermediate	60	58	
Poor	24	24	
IMDC			0.17
Favorable	20	25	
Intermediate	67	78	
Poor	29	27	
Not reported	4	0	
Metastasis			0.9
Yes	31	37	
No	88	92	
NA	1	1	
ORR			5.70E-05
CR/PR	25	5	
PD	41	37	
SD	45	67	
NE	9	21	
OS (months), mean ± SD	29.71±20.66	23.81±18.49	0.02
PFS (months), mean ± SD	8.89±12.69	6.15±7.12	0.04

CB, clinical benefit; CR, complete response; ICB, intermediate clinical benefit; IMDC, IMDC (International Metastatic RCC Database Consortium) Risk Model; NA, not applicable; NCB, no clinical benefit; NE, not evaluable; ORR, objective repose rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

Discussion

ccRCC represents 70–80% of malignancy in kidney, and it rarely responds to chemotherapy and is usually treated with radical nephrectomy.[16] However, recurrence and metastasis are rather common, which result in 5-year OS rate ranging from 0 to 20%.[17] As one of most common immunotherapies, immune checkpoint inhibition is a promising alternative and increases OS of advanced ccRCC patients.[18,19] However, only a small fraction of patients can benefit from immunotherapy, indicating the urgent need for an appropriate patient selection.[20] Recent studies have shown that ERV expression signature may be associated with the immune landscape and anti-PD1/PDL1 response in 24 advanced ccRCC

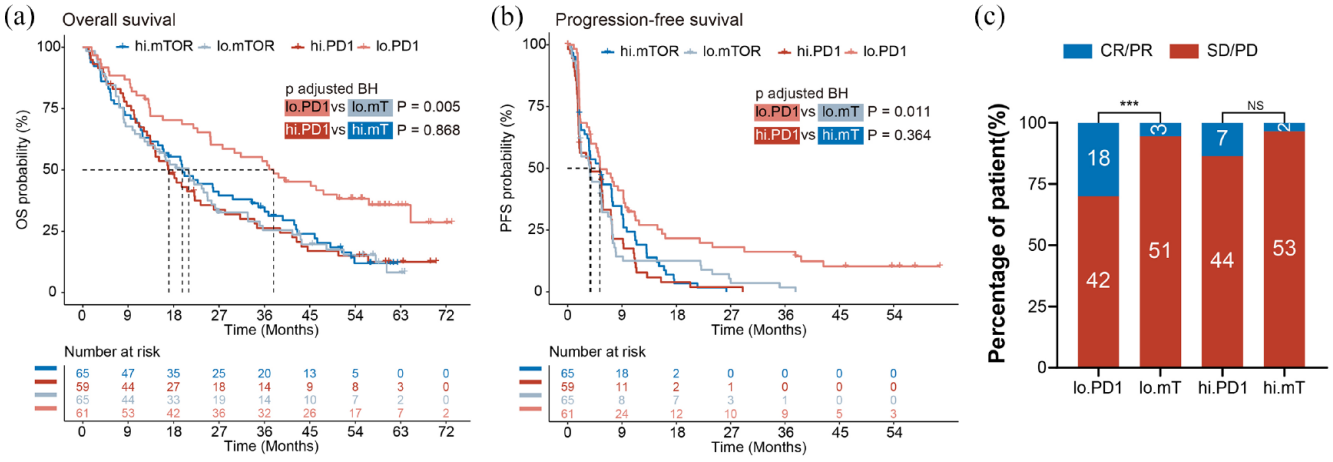

Figure 5. The ERV signature is a predictive biomarker for anti-PD1 therapy in advanced ccRCC cohort. (a) Kaplan–Meier curve of OS for advanced ccRCC patients received nivolumab against those received everolimus in the low- and high-risk group, respectively. (b) Kaplan–Meier curve of PFS for advanced ccRCC patients received nivolumab against those received everolimus in the low- and high-risk group, respectively. (c) Analysis of the ORR for advanced ccRCC patients received nivolumab against those received everolimus in the low- and high-risk group, respectively.

ccRCC, clear cell renal cell carcinoma; ERV, endogenous retrovirus; OS, overall survival; PD1, programmed cell death protein 1.

patients.[9,11] However, the association between ERV expression signature and anti-PD-1 therapy response has not yet been deeply investigated. Based on three recent clinical trials, our study established a prognostic and predictive ERV signature, which provided further evidence that advanced ccRCC patients with lower ERV-signature score present with favorable prognosis, while these patients could benefit more from ICB therapy compared to those with high-risk ERV. We also started to uncover the potential immune player and signaling pathways underlying this benefit and found that $CD8^+$ T cells were highly enriched in low-risk group, while no significant difference was found in neutrophils and $CD4^+$ T cells. Also, several immunosuppressive pathways were found to be negatively enriched in the low-risk group, while immune checkpoint levels were comparable in the low- and high-risk group, which partially explained that the survival benefit from immunotherapy observed in the low-risk group may be PD1/PD-L1 independent.

Though most ERVs are epigenetic silenced, dysregulated ERVs expression could involve in regulation in multiple cancers. For example, three tumor-specific ERVs including ERVH-5 (herv_3215), ERVH48-1 (herv_4906), and ERVE-4 (herv_2256) were hardly detectable in normal tissues but highly expressed in tumor tissues.[21] Though their precise role is unclear, they may yield tumor-specific antigens or activate local immunity as immunological adjuvants. Rathmell *et al.* confirmed that ERV3-2 (herv_2637) was associated with immune checkpoint activation in 11 solid tumors, and metastatic ccRCC patients with higher herv_2637 expression in tumor showed better response to PD-1/PD-L1 blockade compared with those with low expression.[11] Intriguingly, Braun *et al.*[12] failed to reliably infer herv_2637 expression in formalin-fixed tissues but found herv_2282 and herv_3382 to be weakly associated with clinical outcomes in Checkmate cohorts.[12] Nevertheless, only limited set of ERV loci was analyzed in previous study and comprehensive analysis may improve the prognostic performance. In the present study, a total of 1717 ERVs was involved in the analysis, and we established the nine-ERV signature. This is the first signature based on ERV which could successfully distinguish responders to nivolumab from advanced ccRCC patients. Thus, our results provide a distinct pattern of ERV association with the OS and response of patients with advanced ccRCC who received PD-1 blockade.

ccRCC represents a highly immune infiltrated tumor type. It is suggested that T cells are the dominant population of tumor-infiltrating lymphocytes (TILs) in most ccRCC cases.[22] Nevertheless, distinct $CD8^+$ subpopulations may be correlated with different prognosis in patients receiving checkpoint immunotherapy.[23] A study based on CM-025 cohort found that higher

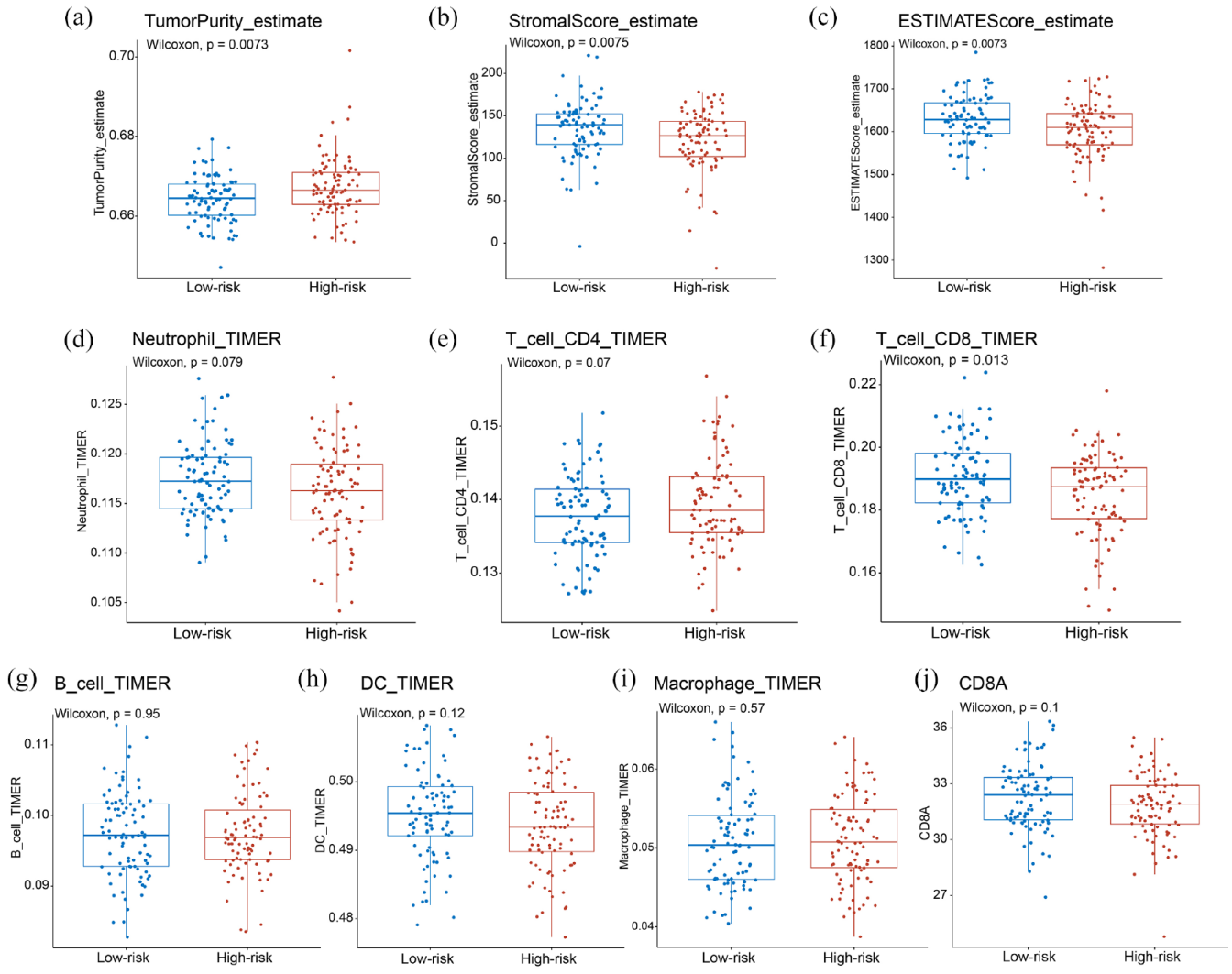

Figure 6. Low ERV risk group had a higher immune infiltration. (a–c) The tumor purity, stromal score, and ESTIMATE score were calculated in the low- and high-risk group, respectively. (d–i) The proportion of neutrophils, CD4+ T cells, CD8+ T cells, B cells, DC, and macrophages was analyzed in the low- and high-risk group, respectively. (j) The expression of CD8A was analyzed in the low- and high-risk group, respectively.
DC, dendritic cell; ERV, endogenous retrovirus.

infiltration of $CD8^+$ TILs expressing PD-1 could predict response to nivolumab, but no to everolimus in advanced ccRCC patients.[24] Moreover, in ADAPTeR, a recent phase II study of nivolumab in treatment-naive patients with advanced ccRCC, higher fraction of pre-treatment CD8+ T cells were found in responders.[25] Though T-cell infiltration increased on-treatment irrespective of nivolumab response, hyperexpanded nivolumab-bound CD8+ clones and upregulated granzyme B and CD8A in nivolumab-bound CD8+ T cells were only observed in responders.[25] Consistent with these findings, our results showed higher CD8+ T-cell infiltration along with higher CD8A mRNA expression in the low ERV risk group, indicating ERV expression patterns are associated with the immune microenvironment of ccRCC. This might lead to the distinct response to nivolumab in ccRCC patients.

Several immunosuppressive gene sets, including cytokines and inflammatory response,[26,27] IL-10,[28,29] MAPK,[30] and WNT signaling pathways,[31,32] were negatively enriched in the low-risk group. IL signaling pathways are critical regulator of immunotherapy response. For example, serum

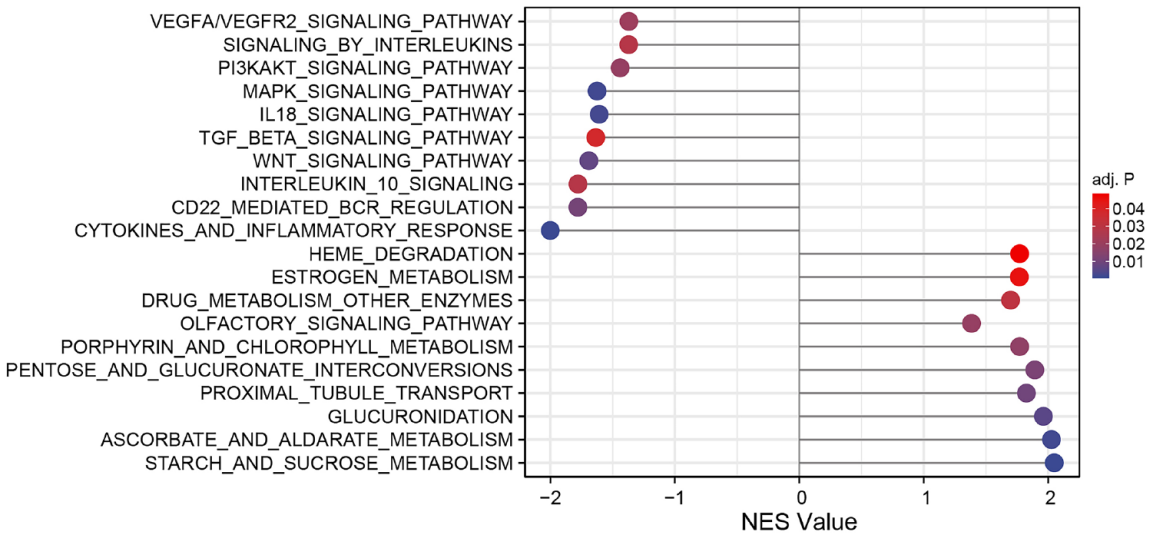

Figure 7. Result of GSEA showing the negatively and positively enriched gene sets in the low-risk group.
GSEA, gene set enrichment analysis.

level of IL-10 could significantly reduce after nivolumab treatment in advanced lung cancer patients,[33] and addition of IL-10 might even potentially suppress T-cell responses in some cases.[29] HERV-W family envelope protein could significantly increase both mRNA and protein levels of tumor necrosis factor α (TNF-α) and IL-10 in glioblastoma cells and human peripheral blood mononuclear cells (PBMCs).[34,35] Of note, in activated PBMCs, the envelope proteins of HERV-W and HERV-FDR have also been found to activate the MAPK pathway leading to reduced production of Th1 cytokines including IL-2, TNF-α, and interferon γ, supporting the potential immunosuppressive role of these two ERVs.[36] Moreover, Np9, an HERV-K-derived protein could interact with promyelocytic leukemia zinc finger protein and activate the immunosuppressive Wnt/β-catenin signaling pathway in chronic lymphocytic leukemia.[37] Intriguingly, in ADAPTeR cohort, upregulated genes found in nivolumab responders were significantly enriched in 'immune activation' and 'TCR signaling' gene sets.[25] Consistently, the low ERV risk group was also found to have a less immunosuppressive microenvironment, which may, to some extent, explain the survival benefit and response to nivolumab observed in these patients.

Of note, differences of treatment strategy among the cohorts should be taken into consideration. Most patients in TCGA-KIRC IV cohort have received combination of chemotherapy (gemcitabine or/and 5-fluorouracil) or/and targeted molecular therapy (temsirolimus, everolimus, sunitinib, sorafenib, pazopanib, vandetanib, bevacizumab, and perifosine); 21 patients have received immunotherapy (interferon alpha, oncophage vaccine, or IL-2). Nevertheless, ERV signature is still prognostic even in this population with different treatment strategy. Moreover, participants in the everolimus arm in CM-025 cohort could be assessed for a crossover to nivolumab treatment if they met all inclusion criteria. In this case, the OS of everolimus arm may be longer than it actually was. However, we still observed significantly longer survival in low ERV-risk patients received nivolumab compared with those received everolimus. Collectively, this evidence supported that ERV is a satisfactory tool to predict anti-PD1 therapy response in advanced ccRCC patients.

In summary, our study for the first time demonstrated that the ERV signature is a prognostic and predictive biomarker for advanced ccRCC patients treated with anti-PD1 therapy. The reliability of the signature was verified in two independent cohorts and the interpretability of the model was illustrated by exploring the correlation between the immune infiltration and immune-related pathways. Future prospective studies are warranted to validate the ERV signature in advanced ccRCC and other malignancies.

Declarations

Ethics approval and consent to participate

This series study was approved by the Institutional Review Board of the Second Affiliated Hospital, Zunyi Medical University (No.YXLL(KY-R)-2021-010).

Consent for publication

Not applicable.

Author contribution(s)

Jian-Guo Zhou: Conceptualization; Data curation; Funding acquisition; Software; Validation; Writing – original draft; Writing – review & editing.

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Su-Han Jin: Data curation; Funding acquisition; Investigation.

Yun-Jia Wang: Formal analysis; Methodology.

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Benjamin Frey: Conceptualization; Investigation; Writing – original draft.

Rainer Fietkau: Project administration; Supervision.

Markus Hecht: Investigation; Writing – review & editing.

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Competing interests

The authors declare no relevant conflict of interest regarding this manuscript. M.H. reports collaborations with Merck Serono (advisory role, speakers' bureau, honoraria, travel expenses, research funding); MSD (advisory role, speakers' bureau, honoraria, travel expenses, research funding); AstraZeneca (research funding); Novartis (research funding); BMS (advisory role, honoraria, speakers' bureau); Teva (travel expenses). U.S.G. and P.R.F. received support for presentation activities for Dr Sennewald Medizintechnik GmbH, have received support for investigator initiated clinical studies (IITs) from MSD and AstraZeneca and contributed at Advisory Boards Meetings of AstraZeneca and Bristol-Myers Squibb.

Availability of data and materials

Data are available in a public, open access repository. All data relevant to the study are included in the article or uploaded as supplemental information. The ERV expression data and clinical information of those studies were extracted from the supplemental materials of previously published studies as described in Methods section.

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Supplemental material

Supplemental material for this article is available online.

References

1. Lee HE, Jo A, Im J, *et al.* Characterization of the long terminal repeat of the endogenous retrovirus-derived micrornas in the olive flounder. *Sci Rep* 2019; 9: 14007.

2. Wang X, Wang B, Liu Z, *et al.* Genome-wide characterization of endogenous retroviruses in snub-nosed monkeys. *PeerJ* 2019; 7: e6602.
3. Thompson PJ, Macfarlan TS and Lorincz MC. Long terminal repeats: from parasitic elements to building blocks of the transcriptional regulatory repertoire. *Mol Cell* 2016; 62: 766–776.
4. Tokuyama M, Kong Y, Song E, *et al.* ERVmap analysis reveals genome-wide transcription of human endogenous retroviruses. *Proc Natl Acad Sci U S A* 2018; 115: 12565–12572.
5. Adoue V, Binet B, Malbec A, *et al.* The histone methyltransferase SETDB1 controls T helper cell lineage integrity by repressing endogenous retroviruses. *Immunity* 2019; 50: 629–644 e628.
6. Jansz N and Faulkner GJ. Endogenous retroviruses in the origins and treatment of cancer. *Genome Biol* 2021; 22: 147.
7. de Cubas AA, Dunker W, Zaninovich A, *et al.* DNA hypomethylation promotes transposable element expression and activation of immune signaling in renal cell cancer. *JCI Insight* 2020; 5: e137569.
8. Roulois D, Loo Yau H, Singhania R, *et al.* DNA-demethylating agents target colorectal cancer cells by inducing viral mimicry by endogenous transcripts. *Cell* 2015; 162: 961–973.
9. Smith CC, Beckermann KE, Bortone DS, *et al.* Endogenous retroviral signatures predict immunotherapy response in clear cell renal cell carcinoma. *J Clin Invest* 2018; 128: 4804–4820.
10. Sharma R, Kadife E, Myers M, *et al.* Determinants of resistance to VEGF-TKI and immune checkpoint inhibitors in metastatic renal cell carcinoma. *J Exp Clin Cancer Res* 2021; 40: 186.
11. Panda A, de Cubas AA, Stein M, *et al.* Endogenous retrovirus expression is associated with response to immune checkpoint blockade in clear cell renal cell carcinoma. *JCI Insight* 2020; 3(11):e137569. doi: 10.1172/jci.insight.137569.
12. Braun DA, Hou Y, Bakouny Z, *et al.* Interplay of somatic alterations and immune infiltration modulates response to PD-1 blockade in advanced clear cell renal cell carcinoma. *Nat Med* 2020; 26: 909–918.
13. Li B, Severson E, Pignon JC, *et al.* Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biol* 2016; 17: 174.
14. Yoshihara K, Shahmoradgoli M, Martinez E, *et al.* Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun* 2013; 4: 2612.
15. Robin X, Turck N, Hainard A, *et al.* pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics* 2011; 12: 77. https://doi.org/10.1186/1471-2105-12-77
16. Hoefflin R, Harlander S, Schafer S, *et al.* HIF-1alpha and HIF-2alpha differently regulate tumour development and inflammation of clear cell renal cell carcinoma in mice. *Nat Commun* 2020; 11: 4111.
17. Kalra S, Atkinson BJ, Matrana MR, *et al.* Prognosis of patients with metastatic renal cell carcinoma and pancreatic metastases. *BJU Int* 2016; 117: 761–765.
18. Motzer RJ, Escudier B, McDermott DF, *et al.* Nivolumab versus Everolimus in advanced renal-cell carcinoma. *N Engl J Med* 2015; 373: 1803–1813.
19. Motzer RJ, Tannir NM, McDermott DF, *et al.* Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med* 2018; 378: 1277–1290.
20. Ou D, Wang X, Wu M, *et al.* Prognostic value of post-radiotherapy neutrophil-to-lymphocyte ratio in locally advanced nasopharyngeal carcinoma. *Strahlenther Onkol* 2020; 196: 252–261.
21. Rooney MS, Shukla SA, Wu CJ, *et al.* Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* 2015; 160: 48–61.
22. Geissler K, Fornara P, Lautenschlager C, *et al.* Immune signature of tumor infiltrating immune cells in renal cancer. *Oncoimmunology* 2015; 4: e985082.
23. Sade-Feldman M, Yizhak K, Bjorgaard SL, *et al.* Defining T cell states associated with response to checkpoint immunotherapy in melanoma. *Cell* 2018; 175: 998–1013 e1020.
24. Ficial M, Jegede OA, Sant'Angelo M, *et al.* Expression of T-cell exhaustion molecules and human endogenous retroviruses as predictive biomarkers for response to Nivolumab in metastatic clear cell renal cell carcinoma. *Clin Cancer Res* 2021; 27: 1371–1380.
25. Au L, Hatipoglu E, Robert de Massy M, *et al.* Determinants of anti-PD-1 response and resistance in clear cell renal cell carcinoma. *Cancer Cell* 2021; 39: 1497–1518 e1411.
26. Mehta A, Kim YJ, Robert L, *et al.* Immunotherapy resistance by inflammation-induced dedifferentiation. *Cancer Discov* 2018; 8: 935–943.

27. Jonsson ME, Garza R, Sharma Y, *et al.* Activation of endogenous retroviruses during brain development causes an inflammatory response. *EMBO J* 2021; 40: e106423.

28. Ni G, Zhang L, Yang X, *et al.* Targeting interleukin-10 signalling for cancer immunotherapy, a promising and complicated task. *Hum Vaccin Immunother* 2020; 16: 2328–2332.

29. Harper TA, Bacot SM, Fennell CJ, *et al.* IL-10 Signaling elicited by nivolumab-induced activation of the map kinase pathway does not fully contribute to nivolumab-modulated heterogeneous T cell responses. *Int J Mol Sci* 2021; 22: 11848.

30. Su X, Xu Y, Fox GC, *et al.* Breast cancer-derived GM-CSF regulates arginase 1 in myeloid cells to promote an immunosuppressive microenvironment. *J Clin Invest* 2021; 131: e145296.

31. Yaguchi T, Goto Y, Kido K, *et al.* Immune suppression and resistance mediated by constitutive activation of Wnt/beta-catenin signaling in human melanoma cells. *J Immunol* 2012; 189: 2110–2117.

32. Li X, Xiang Y, Li F, *et al.* WNT/beta-catenin signaling pathway regulating T cell-inflammation in the tumor microenvironment. *Front Immunol* 2019; 10: 2293.

33. Ye H, Pang H, Shi X, *et al.* Nivolumab and hypofractionated radiotherapy in patients with advanced lung cancer: ABSCOPAL-1 clinical trial. *Front Oncol* 2021; 11: 657024.

34. Wang X, Wu X, Huang J, *et al.* Human endogenous retrovirus W family envelope protein (HERV-W env) facilitates the production of TNF-alpha and IL-10 by inhibiting MyD88s in glial cells. *Arch Virol* 2021; 166: 1035–1045.

35. Morozov VA, Dao Thi VL and Denner J. The transmembrane protein of the human endogenous retrovirus–K (HERV-K) modulates cytokine release and gene expression. *PLoS One* 2013; 8: e70399.

36. Lokossou AG, Toudic C, Nguyen PT, *et al.* Endogenous retrovirus-encoded Syncytin-2 contributes to exosome-mediated immunosuppression of T cellsdagger. *Biol Reprod* 2020; 102: 185–198.

37. Lin DY, Huang CC, Hsieh YT, *et al.* Analysis of the interaction between Zinc finger protein 179 (Znf179) and promyelocytic leukemia zinc finger (Plzf). *J Biomed Sci* 2013; 20: 98.