TCCIA: A Comprehensive Resource for Exploring CircRNA in Cancer Immunotherapy

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Abstract

Background Immunotherapies targeting immune checkpoints have gained increasing attention in cancer treatment, emphasizing the need for predictive biomarkers. Circular RNAs (circRNAs) have emerged as critical regulators of tumor immunity, particularly in the PD-1/PD-L1 pathway, and have shown potential in predicting the efficacy of immunotherapies. However, the precise roles of circRNAs in cancer immunotherapy remain incompletely understood. While existing databases focus on either circRNA profiles or immunotherapy cohorts, there is currently no platform that enables the exploration of the intricate interplay between circRNAs and anti-tumor immunotherapy. Therefore, the development of a comprehensive resource that integrates circRNA profiles, immunotherapy response data, and clinical benefits is crucial for advancing our understanding of circRNA-mediated tumor-immune interactions and developing effective immunotherapy biomarkers.

Methods To address these gaps, we constructed the Cancer CircRNA Immunome Atlas (TCCIA), the first database that combines circRNA profiles, immunotherapy response data, and clinical outcomes across multi-cancer types. The construction of TCCIA involved applying standardized preprocessing to the raw sequencing FASTQ files, characterizing circRNA profiles using CIRCexplorer2, analyzing tumor immunophenotypes through IOBR, and compiling immunotherapy response data from diverse cohorts treated with immune-checkpoint blockades (ICBs).

Results TCCIA encompasses over 3,700 clinical samples obtained from 18 cohorts treated with ICBs, including PD-1/PD-L1 and CTLA-4 inhibitors, along with other treatment modalities. The database provides researchers and clinicians with a cloud-based platform that enables interactive exploration of circRNA data in the context of ICB. The platform offers a range of analytical tools, including visualization of circRNA abundance and correlation, association analysis between circRNAs and clinical variables, assessment of the tumor immune microenvironment, exploration of tumor molecular signatures, evaluation of treatment response or prognosis, and identification of altered circRNAs in immunotherapy-sensitive and resistant tumors. To illustrate the utility of TCCIA, we performed a re-analysis on a melanoma cohort with TCCIA, and found that an isoform of circTMTC3, TMTC3:+:chr12:88148287:88176319, played a significant role in predicting unfavorable

survival outcomes and treatment nonresponse.

Conclusions TCCIA represents a significant advancement over existing resources, providing a comprehensive platform to investigate the role of circRNAs in immune oncology.

What is already known on this topic

Prior knowledge indicated that circRNAs are involved in tumor immunity and have potential as predictive biomarkers for immunotherapy efficacy. However, there lacked a comprehensive database that integrated circRNA profiles and immunotherapy response data, necessitating this study.

What this study adds

This study introduces TCCIA, a database that combines circRNA profiles, immunotherapy response data, and clinical outcomes. It provides a diverse collection of clinical samples and an interactive platform, enabling in-depth exploration of circRNAs in the context of checkpoint-blockade immunotherapy.

How this study might affect research, practice or policy

The findings of this study offer valuable insights into the roles of circRNAs in tumor-immune interactions and provide a resource for researchers and clinicians in the field of immune-oncology. TCCIA has the potential to guide personalized immunotherapeutic strategies and contribute to future research, clinical practice, and policy decisions in checkpoint-blockade immunotherapy and biomarker development.

Introduction

Immunotherapy has revolutionized the treatment of cancer over the past decade, emerging as a groundbreaking approach that harnesses the patient's own immune system to fight cancer. Therapies like immune checkpoint inhibitors, chimeric antigen receptor (CAR) T-cell therapy, and therapeutic vaccines aim to reinvigorate anti-tumor immunity against malignant cells [1–3]. Checkpoint inhibitors targeting programmed cell death protein-1 (PD-1), PD-1 ligand (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) in particular have demonstrated remarkable clinical efficacy across diverse cancer types, highlighting immunotherapy's potential for a durable and curative response [4]. Adoptive cell transfer using engineered T cells expressing CARs has also shown great promise for blood cancers [5]. However, significant challenges remain in extending immunotherapies to larger patient populations and solid tumors. Heterogeneity in response is a major limitation – while some patients achieve long-term remission, others exhibit intrinsic resistance or relapse after an initial response [6]. This variable efficacy likely stems from immunosuppressive mechanisms within the tumor microenvironment (TME) that enable cancer cells to evade immune attack [7]. Elucidating the complex cellular and molecular interactions underlying immunotherapy resistance will be critical to unlock the full potential of immune-based cancer treatments. Reliable predictive biomarkers are also imperative to guide patient selection and combination immunotherapies tailored to each patient’s TME [8].

Circular RNAs (circRNAs) have recently emerged as fascinating non-coding RNA regulators with unique covalently closed loop structures. Initially considered splicing byproducts, circRNAs are now recognized as important gene expression modulators with diverse functions [9,10]. In cancer, circRNAs have been implicated in proliferation, metastasis, and malignancy hallmarks [11]. Moreover, circRNAs are now recognized as critical regulators and potential biomarker of tumor immunity and immunotherapy response [7,12,13]. Accumulating evidence indicates circRNAs modulate TME and immunotherapy outcomes through various mechanisms in cancers like lung cancer, melanoma, colorectal cancer, and pancreatic cancer [14,15]. For example, circRNAs such as circFGFR1, circ-CPA4, and circ_0000284 facilitate immune evasion by modulating PD-L1 via sponging tumor-suppressive microRNAs [16–18]. Additionally, circRNAs including hsa_circ_0000190 [19],

circ_0020710 [20], CDR1-AS [16], and circ-UBAP2 [21] upregulate immune checkpoint proteins like PD-L1, CTLA-4 and PD-1, hampering T cell function and promoting immune evasion. Furthermore, cancer cell-derived circRNAs can reprogram intratumoral immune cells via exosomal transfer or cytokine signaling, thereby impacting facets like angiogenesis that affect immunotherapy efficacy [22–24]. CircRNAs influence various aspects of the TME, including vascularization [25], metabolism [26], hypoxia [27], macrophage polarization [28], natural killer cell cytotoxicity [17], and T cell exhaustion/apoptosis [29]. These factors can impede the efficacy of immunotherapy [15,30]. Dysregulation of circRNAs promotes immune destruction evasion and reduced immunotherapy efficacy. CircRNAs employ diverse regulatory mechanisms—from sponging miRNAs and proteins to scaffolding proteins and translating peptides [31]. While many circRNAs originate in tumors, others come from stromal and immune cells, underscoring complex multicellular regulation [32]. Exploring circRNA networks will be critical to unraveling this intricate cancer-immunity interplay. With emerging roles in tumor immunity, prognostic potential, and biomarker utility, circRNAs represent a promising new frontier in cancer immunotherapy.

Despite growing interest in circRNAs and their potential relevance in cancer immunotherapy, a comprehensive understanding of their precise functions and clinical implications remains incomplete. Existing databases have limitations in either profiling circRNAs, such as riboCIRC [33], CSCD [34] and CircNet [35] offering circRNA profiles across tissues or cancers, or curating immunotherapy cohorts, like ICBatlas [36] and TCIA [37] compiling immune infiltration and immunotherapy data across tumor types. Crucially, no resources systematically integrate comprehensive circRNA expression with multi-omics datasets including immune cell fractions, ICB types, and clinical outcomes for systematic exploration of the circRNA-immunotherapy interplay.

To address this unmet need, we developed the first-of-its-kind database, The Cancer CircRNA Immunome Atlas (TCCIA), a comprehensive database that integrates circRNA profiles, immunotherapy response data, and clinical outcomes for multiple cancer types, with the objective of providing a valuable resource for systematic exploration of the circRNA-immune axis, advancing our understanding of their functions and to facilitates discovery of potential biomarkers, therapeutic targets and clinical implications in cancer immunotherapy.

Results

Integrating circular RNAs in cancer immunotherapy

The development of the TCCIA database encompassed a comprehensive process involving data collection, preprocessing, and integration (Figure 1). In terms of data collection, we carefully curated research articles detailing cohorts treated with immune-checkpoint blockades (ICBs), utilizing the PubMed database (https://www.ncbi.nlm.nih.gov/pubmed/) for selection. We acquired raw RNA-seq datasets from several genome sequence archive repositories, including dbGaP, EGA, EMBL-EBI and GSA. We applied standardized preprocessing techniques to identify circRNAs, quantify TME/gene signatures, and incorporate clinical annotations and outcomes. This approach was aimed at improving the consistency and comparability of data across different datasets. Notably, TCCIA addresses a crucial gap by providing a unified platform with multiple tools that facilitates the exploration of circRNAs' impact on immunotherapy outcomes (Figure 1). This sets it apart from many existing databases [33–47] that predominantly focus on circRNA profiles, circRNA annotations, or immunotherapy cohorts (Table 1).

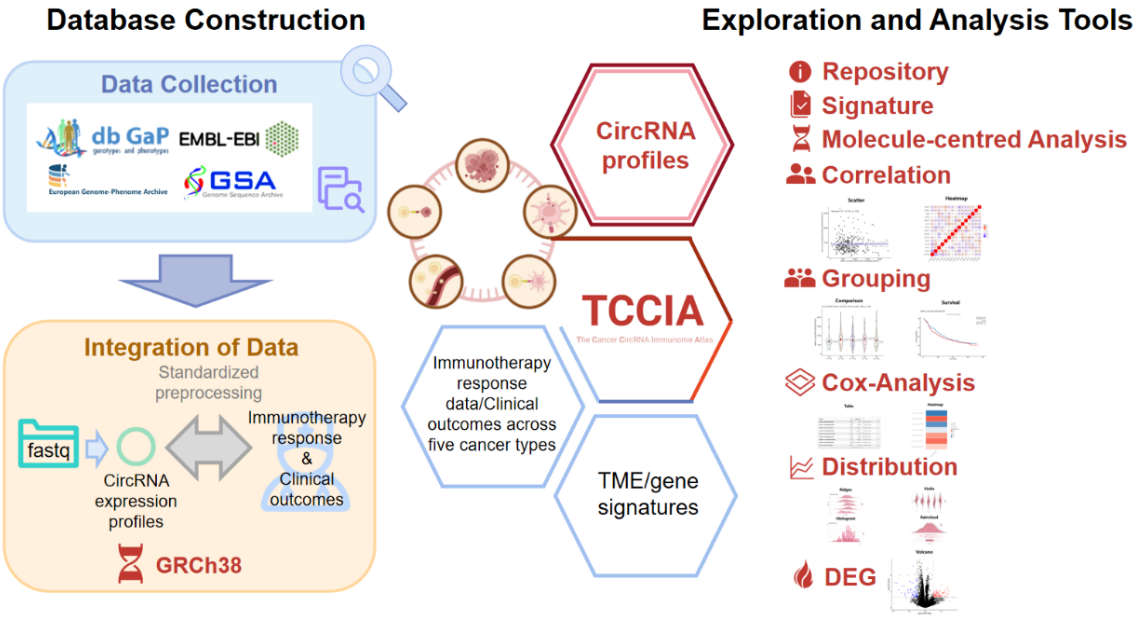

Figure 1. Overview of TCCIA.

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Table 1. Comparison between TCCIA and other similar database resources [33–47].

Type	Name	Publication	Last update	Support species	Sample types (N)	Number of samples	Data sources	Characteristics
Mixed	TCCIA	Submitted	2023	Homo sapiens	cancer (5)	> 3700 cancer samples	dbGaP, EMBL-EBI, EGA, GSA	The first online interface for exploring circRNA expression and analysis in 18 immunotherapy cohorts, supporting systematic comparisons between circRNAs, clinical phenotypes, and immune signatures/infiltration at both the cohort and molecular levels, and offering the ability to explore differential gene expression between responsive and non-responsive groups for biomarker discovery.
CircRNA related	CSCD2	NAR Database Issue (2022)	2022	Homo sapiens	cancer (23)	825 tissues + 288 cell lines	ENCODE, SRA	Includes a large number of circRNAs, predicts potential miRNA–circRNA and RBP–circRNA interactions, and the potential full-length and open reading frame sequence.
	circMine	NAR Database Issue (2022)	2022	Homo sapiens	disease (87)	1107 samples	GEO	Provides online analytical functions to comprehensively evaluate the clinical and biological significance of circRNA and discover the circRNA–miRNA interaction and circRNA translatability.
	CircR2Disease2	Genomics, Proteomics & Bioinformatics (2021)	2022	5 species	disease (313)	2449 studies	PubMed	Serves as a platform to systematically investigate the roles of dysregulated circRNAs in various diseases and further explore the posttranscriptional regulatory function in diseases.
	CircNet2	NAR Database Issue (2021)	2021	Homo sapiens	cancer (37)	2732 cancer samples	TCGA, GEO, CircAtlas, MiOncoCirc	Cancer tissue-specific circRNA expression profiles and circRNA-miRNA-gene regulatory network.
	riboCIRC	Genome Biology (2021)	2021	21 species	tissue/cell line (314)	1970 samples	GEO	Provides computationally predicted ribosome-associated circRNAs and experimentally verified translated circRNAs.
	CircAtlas2	Genome Biology (2020)	2020	7 species	tissue (20)	1070 samples	SRA, NGDC, GeneBank	Integrating the most comprehensive circRNAs and their expression and functional profiles in vertebrates, which provides a foundation for investigating their biological significance.
	CircInteractome	RNA Biology (2016)	2020	Homo sapiens	tissue/cell line (34)	34 samples	circBase	Predicts the interactions between miRNAs and circRNAs with 109 RBPs, and the database also focus on IRESs and ORFs.
	TransCirc	NAR Database Issue (2020)	2020	Homo sapiens	tissues (17)	17 tissues	CircAtlas	Provides comprehensive evidence supporting the translation potential of circRNAs.
	circRic	Genome Medicine (2019)	2019	Homo sapiens	cancer (22)	935 cancer celllines	CCLE	Characterizes circRNA expression profiles; analyzes the circRNA biogenesis regulators, the effect of circRNAs on drug response, the association of circRNAs with mRNAs, proteins, and mutations, etc.
	CIRCpedia2	Genomics, Proteomics & Bioinformatics (2018)	2018	6 species	tissue (13)	185 samples	GEO, ENCODE, EMBL-EBI	Comprehensive circRNA annotation from over 180 RNA-seq datasets across six different species. Conservation analysis of circRNAs between humans and mice.
	circBase	RNA (2014)	2014	6 species	tissues/cell line (77)	77 samples	GEO, ENCODE, EMBL-EBI	Explores merged and unified circRNA data sets and the evidence supporting their expression. Provides scripts to identify known and novel circRNAs in sequencing data.
Immunotherapy related	TISMO	NAR Database Issue (2022)	2022	Mouse	cancer (19)	1518 mouse samples	GEO and In-house data	Interactive interfaces for exploring gene expression and immune infiltration, and allowing systematic comparisons between different model characteristics, and treatment and response groups.
	ICBatlas	Cancer Immunol Res (2022)	2022	Homo sapiens	cancer (9)	1515 cancer samples	GEO,ArrayExpress, TCGA, dbGaP	Transcriptome features of ICB therapy through the analysis of 1,515 ICB-treated samples from 25 studies across nine cancer types.
	ImmuCellAI	Advanced Science (2020)	2020	Homo sapiens	cancer (37)	NA	GEO, TCGA, dbGaP	The abundance of 24 immune cell types including 18 T-cell subsets, from gene expression data from self-designed approach Immune Cell Abundance Identifier.
	TCIA	Cell Reports (2017)	2017	Homo sapiens	cancer (20)	9562 cancer samples	TCGA and two immunotherapy studies	Exploration of comprehensive immunogenomic analyses of next generation sequencing data for 20 solid cancers from TCGA and other datasources.

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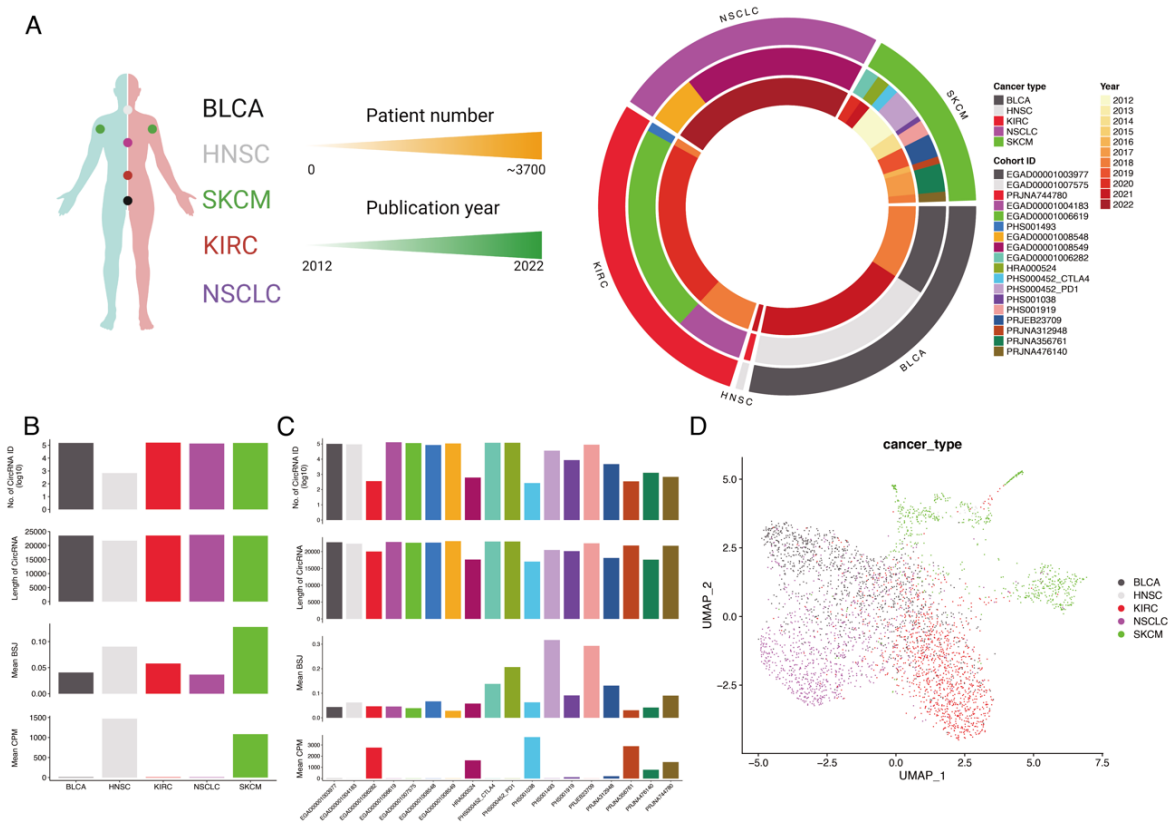

Figure 2. Content of TCCIA. (**A**) Inclusion of cancer types, cohort and publication years in this study. Abbr. BLCA, Bladder urothelial carcinoma; HNSC, Head and neck squamous cell carcinoma; KIRC, Kidney renal clear cell carcinoma; NSCLC, non-small lung cancer; SKCM, Skin cutaneous melanoma. (**B-C**) The number of detected circRNAs, length of circRNAs, mean BSJ (back-splicing junction count) and mean CPM (counts per million) in different cancer types (**B**) or cohort (**C**). (**D**) UMAP plot of all samples, colored by cancer types.

Data summary of TCCIA

In this study, a comprehensive compilation was made, involving approximately 3700 patients from 18 immune-checkpoint blockade (ICB) cohorts [48–63] with raw RNA-seq datasets published between 2012 and 2022, encompassing 5 distinct cancer types (Figure 2A). The circRNA profiling revealed the identification of an impressive total of 281,556 circRNAs. Among these, kidney renal clear cell carcinoma (KIRC) exhibited the highest count, encompassing 159,577 circRNAs (representing 56.7% of the total). Conversely, head and neck squamous cell carcinoma (HNSC) demonstrated the lowest count, with only 680 circRNAs, constituting a mere 0.2% of the total (Figure 2B). The average lengths of these circRNAs demonstrated notable consistency across various cancer types, ranging from 21,739.64 to 23,582.26, as well as within individual cohorts, ranging from 17,041.13 to

218 23,112.20 (Figure 2B, C). Intriguingly, skin cutaneous melanoma (SKCM) exhibited the
219 highest mean back-splice junction (BSJ) reads at 0.13, whereas non-small cell lung cancer
220 (NSCLC) had the lowest mean at 0.04. Additionally, HNSC displayed the highest mean
221 counts per million (CPM) at 1,470.6, while KIRC had the lowest mean CPM at 9.0. A
222 comprehensive analysis encompassing all the circRNAs from the sampled datasets was
223 visualized using a UMAP plot (Figure 2D). This visualization revealed discernible circRNA
224 clustering patterns specific to various cancer types, highlighting the nuanced circRNA
225 heterogeneity within human cancers and emphasizing the need for independent circRNA
226 analysis considerations.
227
228 **Web functionality of TCCIA**
229 TCCIA introduces an array of advanced analytical tools, encompasses multifaceted
230 functionalities to aid researchers in uncovering intricate connections and insights (Figure 1).
231 These functionalities empower the exploration of circRNA abundance, correlation,
232 associations with clinical variables, the tumor immune microenvironment, molecular
233 signatures, treatment responses, and prognosis predictions, along with identifying circRNAs
234 implicated in immunotherapy-sensitive and resistant tumor scenarios (Figure 3). The well-
235 established exploration and analysis pipeline within the TCCIA framework is described in
236 Figure 4. This schematic outlines the typical path that researchers follow when engaging with
237 the platform. A more comprehensive elucidation of all fundamental modules is provided below.
238
239 ***Cohort Selection and Data Access***. The TCCIA interface offers an intuitive approach for
240 cohort selection and data access. The Repository Page serves as a gateway, enabling users
241 to filter datasets based on crucial parameters such as cancer type, treatment modalities,
242 drugs administered, and cohort sizes. Essential details pertaining to each dataset are
243 presented in a comprehensive cohort table, facilitating informed decision-making regarding
244 cohort selection.
245
246 ***Cohort/Molecule-Centered Analysis Modules.*** At the heart of TCCIA's capabilities lie the
247 cohort-centered analysis modules and molecule-centred analysis modules (for analyzing

circRNAs across multiple cohorts), providing a profound lens into circRNA dynamics within specific immunotherapy cohorts. These modules encompass:

(1) *Scatter-Correlation and Heatmap-Correlation*: Researchers gain insights into circRNA correlations through scatter plots and heatmaps. These visualizations are pivotal in elucidating potential connections between circRNAs and other variables within the chosen cohort.

(2) *Group-Comparison (including simplified and comprehensive versions)*: TCCIA facilitates nuanced analysis of numeric differences in circRNA expression across multiple groups within a cohort. The dual modes of simplified and comprehensive group comparison empower researchers to unravel intricate circRNA expression patterns.

(3) *KM-Analysis and Cox-Analysis*: Survival analysis is made accessible through the KM-Analysis module, which generates Kaplan Meier survival curves among distinct variable groups. Additionally, the Cox-Analysis module allows for an in-depth examination of survival outcomes of any circRNA expression, opening avenues to prognostic evaluations.

***Signature and DEG Analysis*.** TCCIA introduces dedicated modules for signature analysis and differential expression circRNAs (DEG) assessment. The Signature Page facilitates the investigation of associations between circRNAs and tumor microenvironment metrics using eight prominent deconvolution methods. It also allows for the examination of connections between circRNAs and 255 cancer signatures categorized into three distinct groups: TME-associated, tumor-metabolism, and tumor-intrinsic signatures. These analyses encompass a wide range of cohorts, ensuring comprehensive exploration of these relationships. The DEG Page empowers researchers to pinpoint differentially expressed circRNAs between patients who respond and those who do not respond to immunotherapy, thus unraveling the intricate web of circRNA involvement in treatment outcomes.

***User Customized Configurations*.** Global settings within TCCIA add a layer of refinement to the user experience, allowing for customized exploration. These settings grant users control over data access and enable tailoring analyses to align with their specific research objectives. For example, by default, the platform prioritizes immunotherapy-related sample

data by filtering out samples without checkpoint immunotherapy treatment, streamlining analyses for coherent research goals. As users become acclimated to the platform, customization options foster enhanced flexibility, enabling researchers to uncover novel insights.

In essence, the web functionality of TCCIA embodies an advanced and user-centric avenue for investigating the complex roles of circRNAs in cancer immunotherapy. The integration of diverse analysis modules, coupled with a cohort-centered approach and adaptable settings, positions TCCIA as an indispensable tool for advancing our comprehension of circRNA-mediated immune responses and guiding the formulation of personalized immunotherapeutic strategies. This interactive platform stands poised to reshape the landscape of circRNA-immunotherapy research.

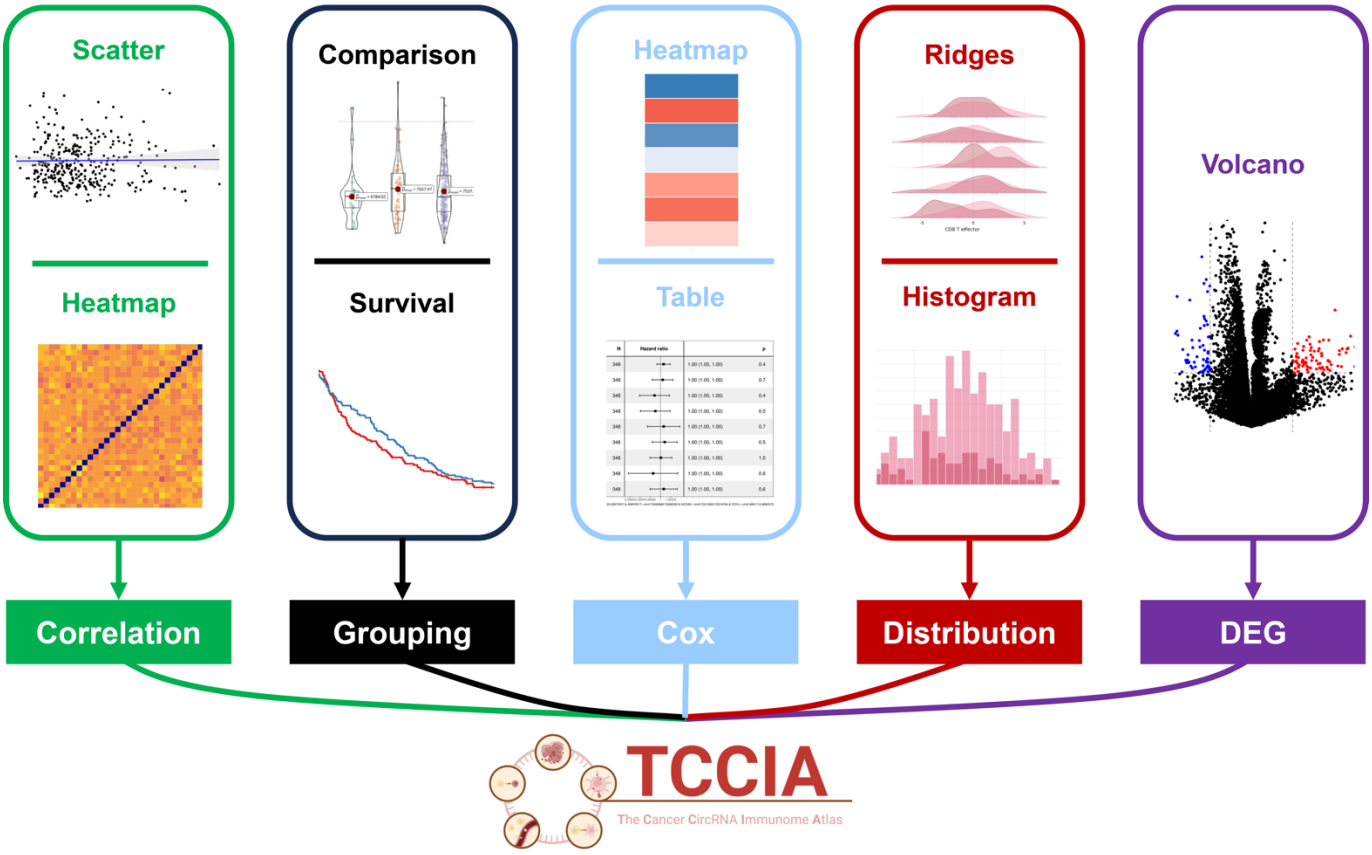

Figure 3. The core modules of TCCIA.

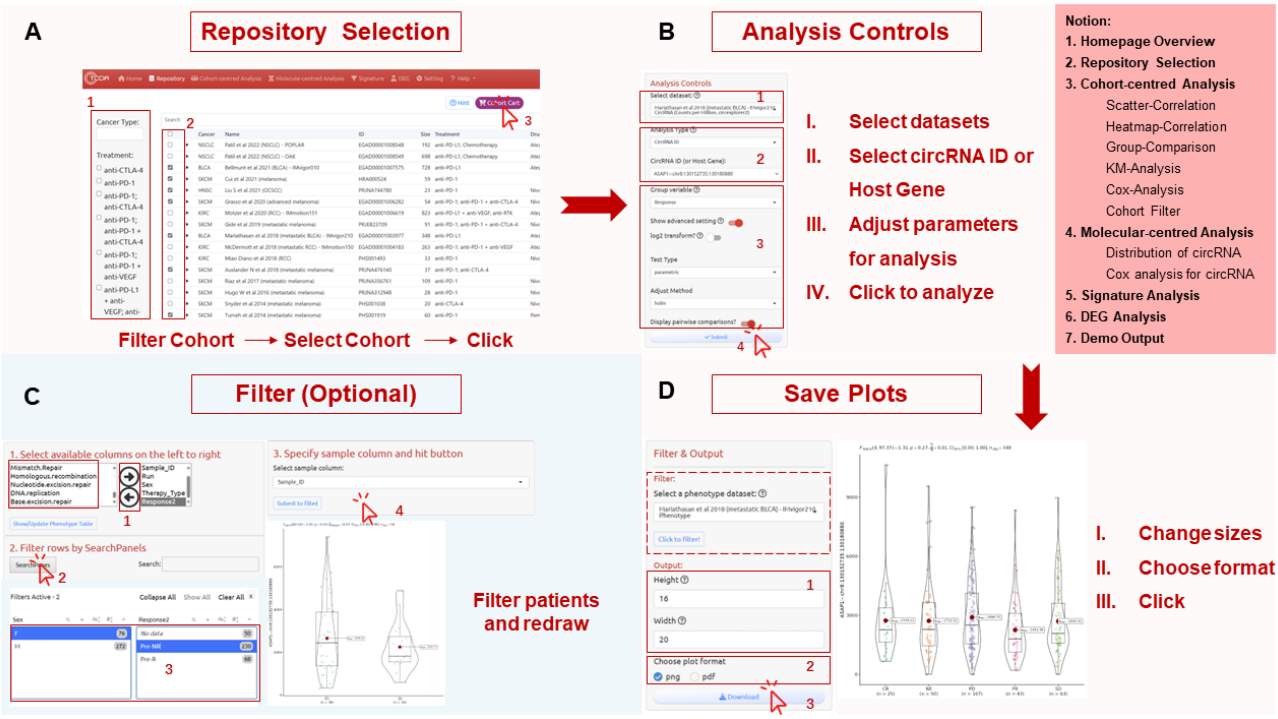

Figure 4. A standard exploration and analysis pipeline within TCCIA. (A) Users need to navigate to the Repository page and select the clinical trial dataset of their interest. On the leftmost panel, users can filter the dataset based on various parameters. **(B)** Users can choose an appropriate analysis strategy according to their needs (core analysis steps are listed on the rightmost panel). The analysis consists of four steps: I. Select the dataset. II. Choose the CircRNA ID or Host Gene of interest. III. Adjust various parameters such as Test Type, Color Selection, etc. IV. Click the Submit button to obtain the analysis results. **(C)** For users aiming for in-depth analysis, a more personalized clinical data filtering suite is provided. Users can perform patient selection based on different features such as Sex, Response, etc., and redraw the plots. **(D)** Users have the option to save the result images in PDF or PNG format, with the desired dimensions.

Case study: validating circTMTC3 prediction efficacy in Gide et al. melanoma cohort

ICB therapies targeting PD-1 and CTLA-4 have significantly transformed the field of oncology, particularly in the treatment of metastatic melanoma. However, it is important to note that only a limited number of melanoma patients experience positive outcomes from these immunotherapies. Consequently, there is a pressing need to identify predictive biomarkers that can guide precision oncology.

In a study conducted by Dong et al. [13], it was observed that melanoma patients from the study (cohort ID: PRJEB23709) by Gide et al. [54], who exhibited high expression of circTMTC3, experienced poorer survival outcomes and demonstrated reduced treatment responsiveness compared to those with low circTMTC3 expression. To illustrate the functionality of the TCCIA, here, we performed a re-analysis on the same cohort. Figure 5A

314 provides an overview of the analysis panel used to assess the association between a circRNA
315 and patient survival. Additionally, Figure 5B clearly demonstrates that high levels of
316 circTMTC3 are predictive of poor overall survival ($HR_{low\ vs\ high}$=0.47, 95% CI: 0.24-0.92,
317 *P*=0.02). Upon closer examination of the different isoforms of circRNAs derived from TMTC3,
318 it was found that only TMTC3:+:chr12:88148287:88176319 was abundant in this patient
319 cohort and played a significant role in predicting unfavorable survival outcomes (Figure 5C,
320 $HR_{low\ vs\ high}$=0.42, 95% CI: 0.2-0.9, *P*=0.008). The remaining isoforms, such as
321 TMTC3:+:chr12:88160113:88190622, did not exhibit the same predictive ability (Figure 5D).
322 Furthermore, employing three different approaches to assess differential expression of
323 circRNAs between checkpoint immunotherapy responders and non-responders, it was
324 consistently discovered that TMTC3:+:chr12:88148287:88176319 was the sole isoform of
325 circTMTC3 that displayed significant upregulation in non-responding patients (Figure 5E-G).
326

327 **Discussion**

328 Circular RNAs, emerging as pivotal gene expression regulators, exert diverse functions
329 across biological processes, with substantial clinical research potential [64]. However, the
330 existing landscape of circRNA-related tumor-immune checkpoint research falls short of
331 satisfying the burgeoning need for insights. This limitation is compounded by constraints
332 stemming from sample quantity and diversity, which subsequently curtail the generalizability
333 of research findings due to geographical, racial, and tumor-specific factors. Consequently,
334 given this evolving landscape, the urgency and significance of developing a CircRNA tool for
335 preliminary data mining have become increasingly pronounced. TCCIA (the Cancer CircRNA
336 Immunome Atlas) an integrated online platform, building upon datasets from four genome
337 sequence archive repositories, encompassing around 3,700 cancer samples, spanning five
338 cancer types and 18 checkpoint-blockade immunotherapy cohorts, incorporating over
339 280,000 circRNA expression profiles, 255 established cancer signatures, and TME
340 decomposition results from eight immune infiltration algorithms. TCCIA emphasizes user-
341 friendly visual presentations, eliminating the need for intricate programming skills. Moreover,
342 the platform offers a range of customizable visualization options, ensuring adaptability to user
343 needs. Notably, TCCIA is readily accessible without mandatory registration or login,

potentially rendering it an economical and efficient solution for both researchers and clinical practitioners.

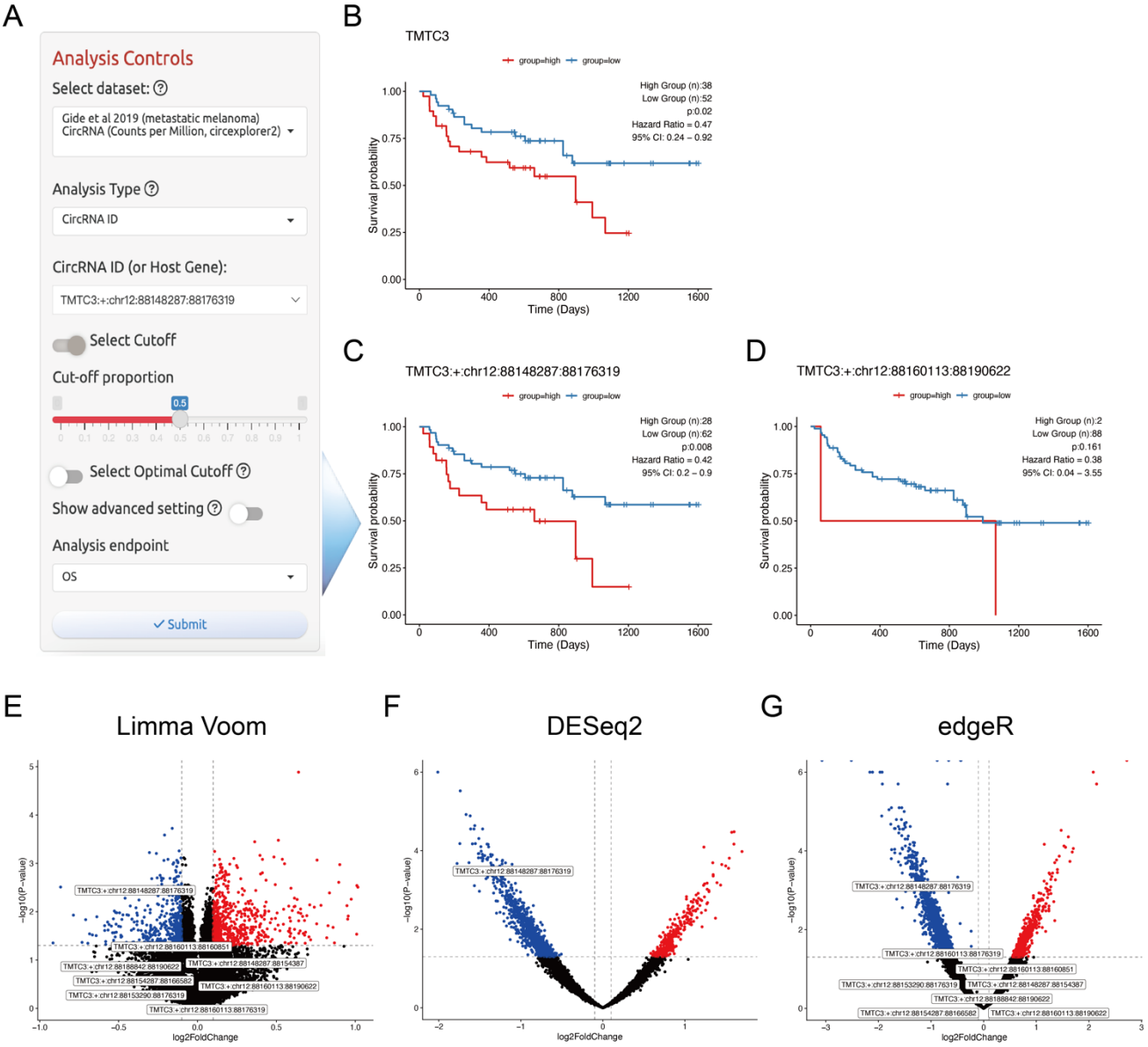

Figure 5. circTMTC3 predicts poor survival and non-response to checkpoint immunotherapy in the melanoma cohort of Gide et al. (**A**) Analysis panel for analyzing and visualizing associations between circRNAs and patient survival. Here, we present the data and analysis settings used to generate the plot shown in (**C**). (**B**) CircTMTC3 predicts poor survival. (**C**) A circRNA isoform of circTMTC3, TMTC3:+:chr12:88148287:88176319, predicts poor survival. (**D**) A circRNA isoform of circTMTC3, TMTC3:+:chr12:88160113:88190622, predicts poor survival. (**E-G**) Volcano plots showing differential expressed circRNAs between checkpoint immunotherapy response and nonresponse patients using the approaches: (**E**) Limma Voom, (**F**) DESeq2, and (**G**) edgeR.

In this manuscript, we delineate the data sources, collection and standardization processes, TCCIA's functionalities, website analysis modules, and provide a step-by-step guide to its operation. To further illustrate TCCIA, we present a concrete example of the

circTMTC3 as a molecular marker for melanoma and confirm its specific isoform, TMTC3:+:chr12:88148287:88176319, in playing a major role in prognosticating unfavorable survival outcomes and non-response to treatment.

While TCCIA has various advantages and uniqueness, there are still some limitations. In terms of data, although TCCIA includes data from five different types of cancer, the sample size for head and neck cancer remains sparse. Recently, the application of immunotherapy has been expanding to more cancer types, such as digestive system tumors. However, it is important to note that our TCCIA does not currently cover these types of tumors. The main reason for this is that current clinical genomics research of such cancer types primarily focuses on the DNA level, specifically on whole exome sequencing and targeted sequencing [65]. As a result, there are few RNA-seq datasets to infer the presence of circRNAs. Another reason is that some raw RNA-seq datasets are difficult to access due to restricted availability, e.g., Carroll et al. study [66]. In terms of circRNA abundance, there are significant differences between different cohorts, batch effects may exist in different cohorts, and there is also strong heterogeneity within the same tumor type, so users need to be cautious when performing cross-dataset analysis and comparison. In terms of functionality, some databases provide the characteristics of studying circRNA-miRNA-gene regulatory networks (such as CSCD2 [34], circMine [38], and CircNet2 [35]), which have not been considered in TCCIA with two reasons: First, further experimental validation is generally required to confirm the authenticity of detected circRNAs. In the recent large-scale circRNA detection benchmark study [67], Vromman et al. recommended using qPCR+ Ribonuclease R or qPCR+amplicon sequencing for circRNA validation. Therefore, it is recommended to incorporate these experimental validation methods to ensure the accuracy of circRNA detection. Second, the focus of our study is on integrating circRNA profiles and clinical outcomes in cancer patients treated with immunotherapy. However, instead of duplicating efforts, users can leverage already well-established and high-quality circRNA databases to complete other circRNA annotation and analysis explorations. By linking to these databases (a widget is provided in the footer of the TCCIA website), users can access comprehensive circRNA information and utilize existing tools for further analysis, optimizing the accuracy and efficiency of circRNA annotation and deep investigation.

Looking ahead, we aim to continually update TCCIA by incorporating more circRNA data from diverse immunotherapy cohorts and introducing new functionalities based on user feedback. In summary, the distinctive features, analytical capabilities, and potential for growth position it as a pivotal tool in advancing our understanding of circRNAs in tumor immunity and in shaping development of personalized immunotherapy strategies guided by circRNA.

Methods

Data collection

To conduct a systematic search, we utilized PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) to search for articles related to Bulk RNAseq data from solid cancer patients treated with immune checkpoint blockers (ICB). The search expression used was "(ICB [Title/Abstract] OR PD-1[Title/Abstract] OR PD-L1[Title/Abstract] OR CTLA-4[Title/Abstract]) AND (rnaseq[Title/Abstract] OR rna-seq[Title/Abstract] OR rna-sequencing[Title/Abstract] OR rna seq[Title/Abstract] OR rna sequencing[Title/Abstract])". No filters were applied, and there were no restrictions on language or geographic region. Peer-reviewed publications, preprints, and press releases were considered for inclusion. To obtain raw RNA sequencing data, we submitted requests to the Database of Genotypes and Phenotypes (dbGaP) (https://dbgap.ncbi.nlm.nih.gov/), the European Genome-phenome Archive (EGA) (https://ega-archive.org/), and the Genome Sequence Archive (GSA) (https://ngdc.cncb.ac.cn/gsa/) of the National Genomics Data Center (NGDC) after receiving approval from the Data Access Committee (DAC). However, it is important to note that not all raw RNAseq datasets were accessible and available for use. In total, we collected 16 studies [48–63] related to checkpoint immunotherapy that provided raw RNAseq datasets. We gathered relevant clinical data from publications and clinical meta documents associated with these RNAseq datasets. Additionally, we extracted information on the cohorts' fundamental characteristics, such as sample size, treatment methods, and specific drugs used, based on the abstracts. It should be noted that, for Patil et al. study, two clinical cohorts were included; and for the study identified as PHS000452, the two patient subgroups had distinct drug treatments and clinical annotations. Hence, we treated them as two separate cohorts during the analysis. For the TCCIA project, CircRNA profiles, immunotherapy response, and clinical

418 benefits were analyzed for five cancer types. This analysis included over 3,700 clinical
419 samples from 18 cohorts treated with immune-checkpoint blockades (ICBs) such as PD-
420 1/PD-L1 and CTLA-4 inhibitors, as well as other treatments. The analysis considered both
421 pre-treatment and on-treatment responses.
422
423 **CircRNA identification and differential expression analysis**
424 We aligned the raw RNA sequencing data to the human genome hg38 using STAR [68]. Next,
425 we utilized CIRCexplorer2 [69] to identify, parse, and annotate circRNA junctions within each
426 sample. These identified junctions were then analyzed for differential expression using
427 Limma Voom [70], edgeR [71], and DESeq2 [72], enabling a comparison between patients
428 who responded to checkpoint immunotherapy and those who did not.
429
430 **TME decomposition and cancer gene signature estimation**
431 We employed IOBR [73] for TME decomposition and the scoring of cancer gene signatures.
432 IOBR seamlessly integrates eight widely-used open-source deconvolution methods,
433 including CIBERSORT [74], ESTIMATE [75], quanTIseq [76], TIMER [77], IPS [37],
434 MCPCounter [78], xCell [79], and EPIC [80]. Furthermore, IOBR incorporates a
435 comprehensive compilation of 255 established cancer signatures. These diverse signatures
436 are organized into three distinct categories: TME-associated, tumor-metabolism, and tumor-
437 intrinsic signatures.
438
439 **TCCIA implementation**
440 The TCCIA database is developed as a Web application leveraging R Shiny
441 (https://shiny.posit.co/) and built using the golem framework (https://github.com/ThinkR-
442 open/golem) to achieve optimization. TCCIA, is developed solely for research purposes and
443 does not utilize any cookies or collect any personal identifiable information. TCCIA is free
444 available in https://tccia.zmu-zhoulab.com/ and https://shiny.hiplot.cn/TCCIA.
445
446 **Statistical analysis**
447 We performed Kaplan-Meier survival analysis to generate and compare survival curves. The

log-rank test was used for comparison. We also conducted multivariate survival analysis using the Cox regression model. All reported *P*-values are two-tailed, and a significance level of $p \leqslant 0.05$ was used unless otherwise specified. All statistical analyses and visualization were conducted using R v4.2.0.

Patient consent for publication

Not applicable.

Data availability

All relevant data reported in the study can be found in the article or on the TCCIA website. Please note that access to the raw RNA-seq datasets is not provided. For any other data requests, please contact the leader of this project, Jian-Guo Zhou.

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Contributions

SW: Conceptualization, software, methodology, formal analysis, writing original draft, review and editing. YX: Software, methodology, formal analysis, visualization, writing original draft. YZ: Software, formal analysis, visualization, writing original draft. HW: Conceptualization, writing original draft, review and editing. JL and USG: Resources, review and editing. MC: Visualization, review and editing. PL, YHL, MH and BF: Review and editing. XL, QZ and HM: Supervision, resources, funding acquisition. JGZ: Conceptualization, methodology,

resources, supervision, funding acquisition, project administration, writing original draft, writing–review and editing.

Conflict of interest

None were declared.

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Database Construction

Data Collection

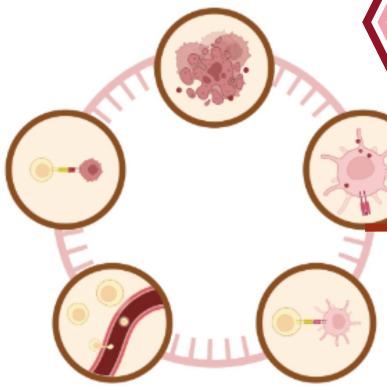
dbGaP genotypes and phenotypes
EMBL-EBI
European Genome-Phenome Archive
GSA Genome Sequence Archive

Integration of Data

Standardized preprocessing

fastq → CircRNA expression profiles ⇔ Immunotherapy response & Clinical outcomes

GRCh38



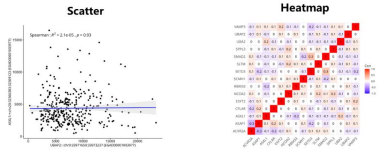
TCCIA

The Cancer CircRNA Immunome Atlas

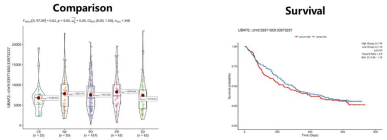
- CircRNA profiles
- Immunotherapy response data/Clinical outcomes across five cancer types
- TME/gene signatures

Exploration and Analysis Tools

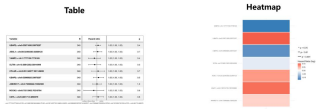
- Repository
- Signature
- Molecule-centred Analysis
- Correlation



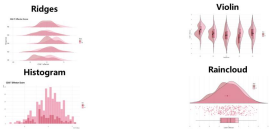
- Grouping



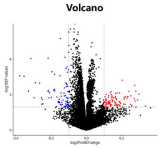
- Cox-Analysis

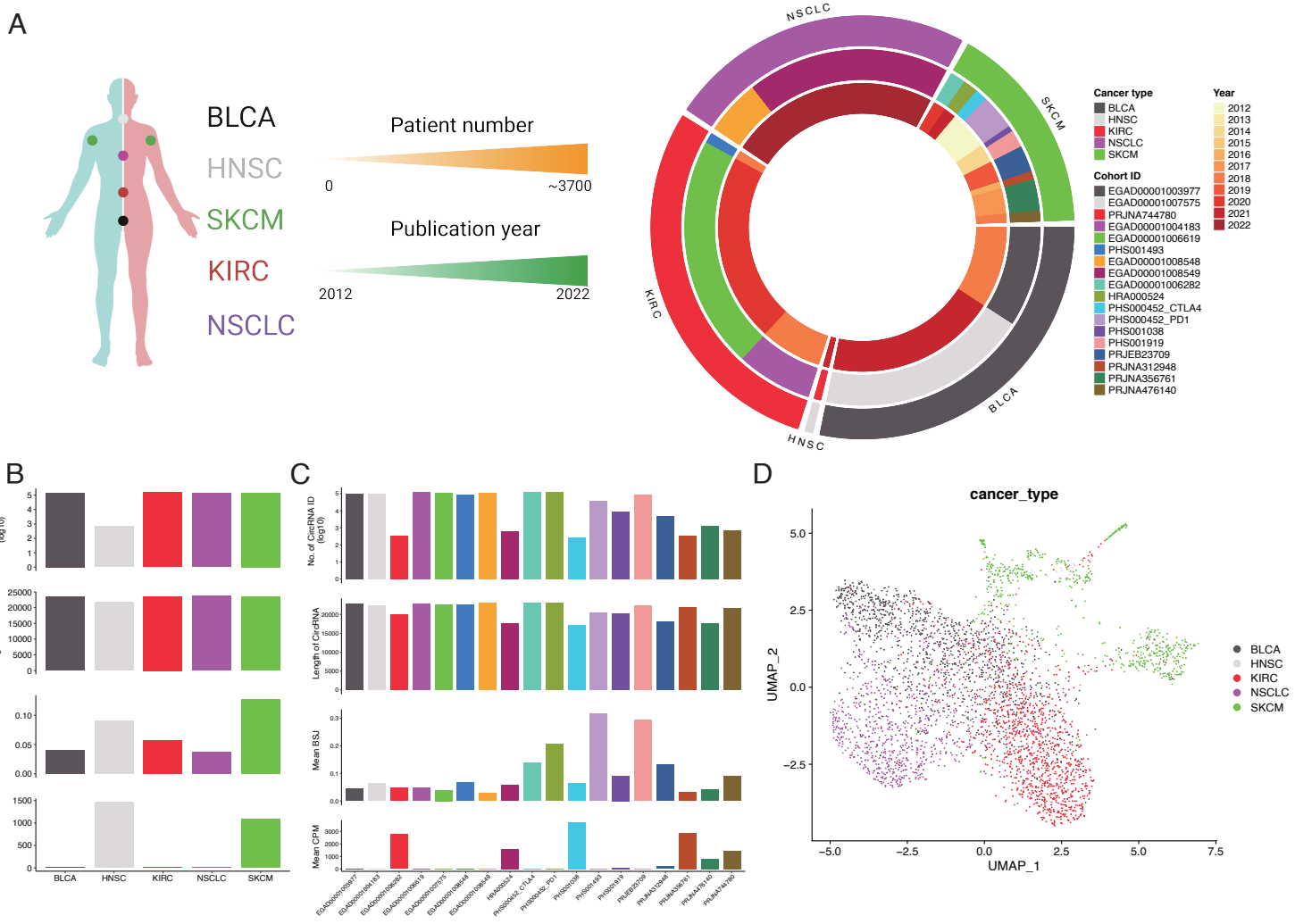


- Distribution



- DEG




A
BLCA
HNSC
SKCM
KIRC
NSCLC
Patient number
0
~3700
Publication year
2012
2022
NSCLC
SKCM
KIRC
HNSC
BLCA
Cancer type
BLCA
HNSC
KIRC
NSCLC
SKCM
Cohort ID
EGAD00001003977
EGAD00001007575
PRJNA744780
EGAD00001004183
EGAD00001006619
PHS001493
EGAD00001008548
EGAD00001008549
EGAD00001006282
HRA000524
PHS000452_CTLA4
PHS000452_PD1
PHS001038
PHS001919
PRJEB23709
PRJNA312948
PRJNA356761
PRJNA476140
Year
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
B
No. of CircRNA ID (log10)
Length of CircRNA
Mean BSJ
Mean CPM
BLCA
HNSC
KIRC
NSCLC
SKCM
C
No. of CircRNA ID (log10)
Length of CircRNA
Mean BSJ
Mean CPM
D
cancer_type
UMAP_2
UMAP_1
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KIRC
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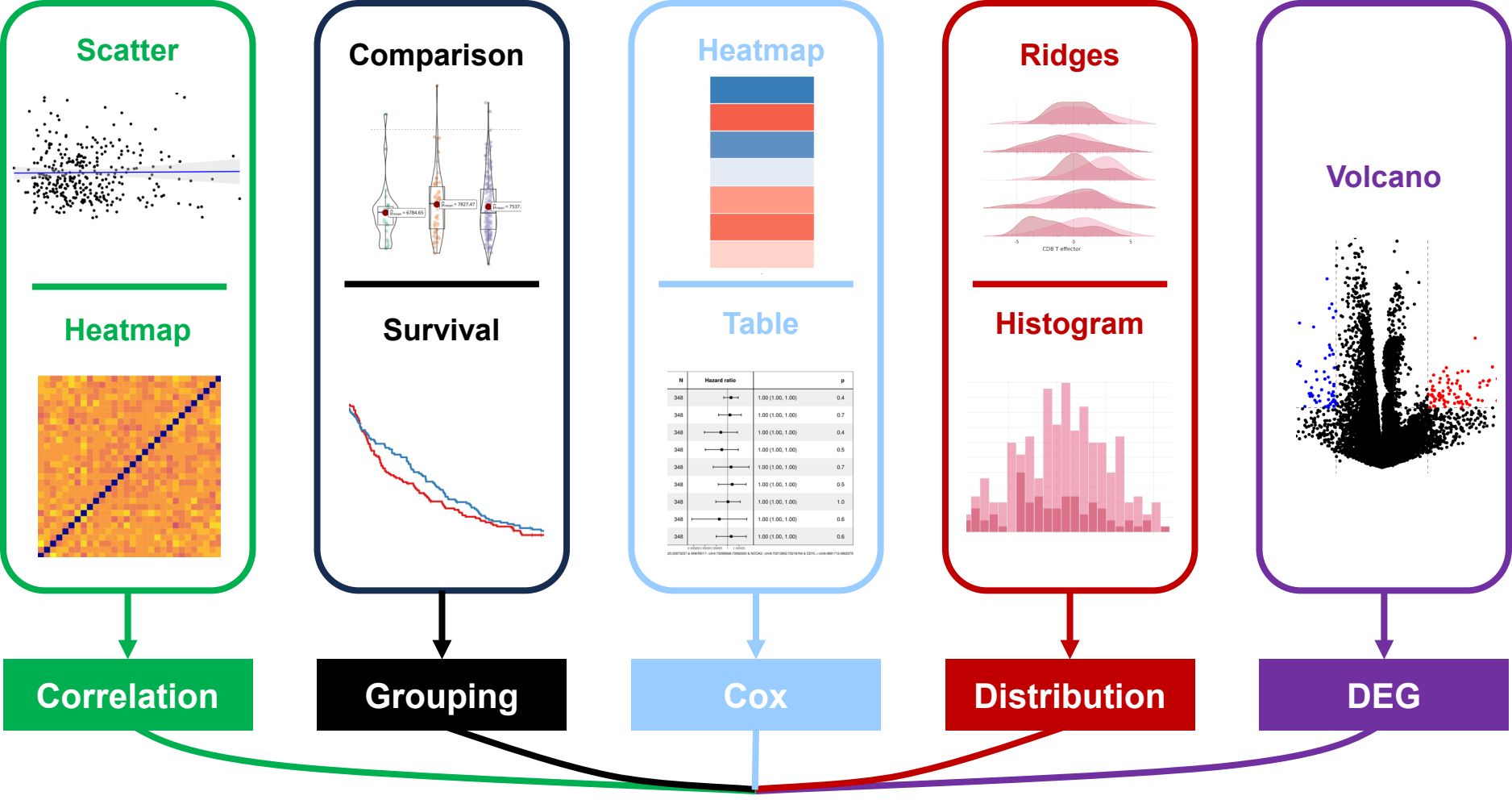
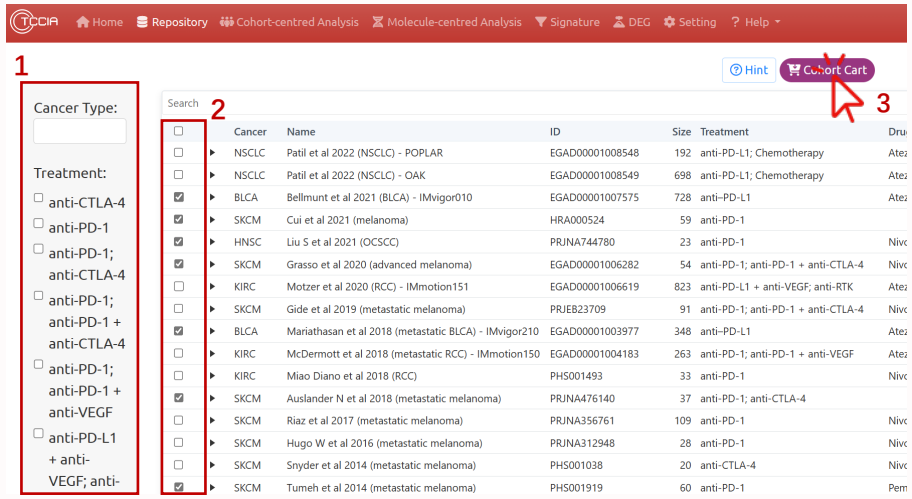


TCCIA

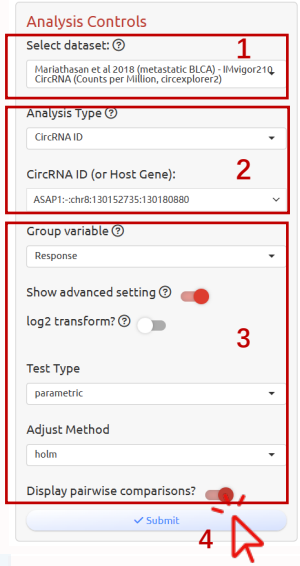
The Cancer CircRNA Immunome Atlas

A Repository Selection



Filter Cohort → Select Cohort → Click

B Analysis Controls



I. Select datasets

II. Select circRNA ID or Host Gene

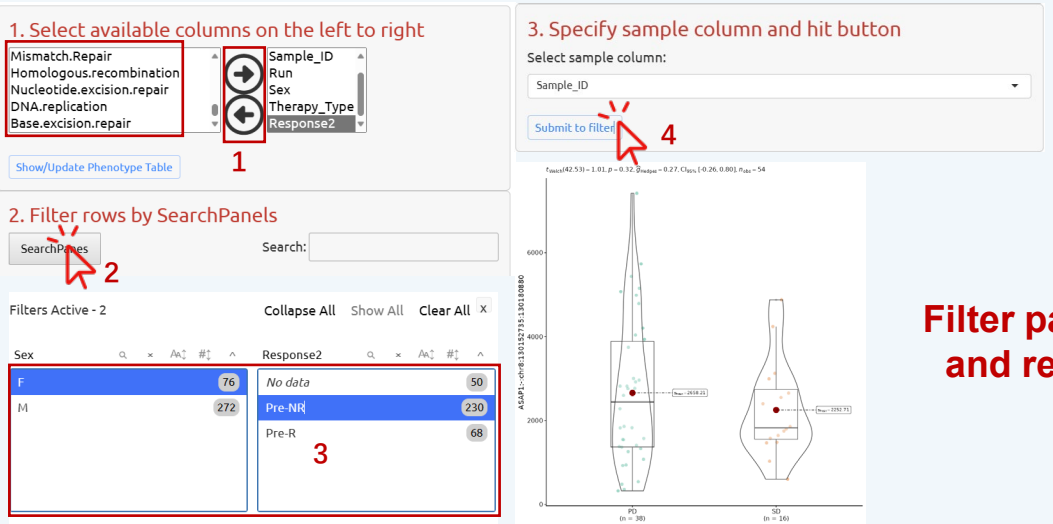
III. Adjust parameters for analysis

IV. Click to analyze

Notion:

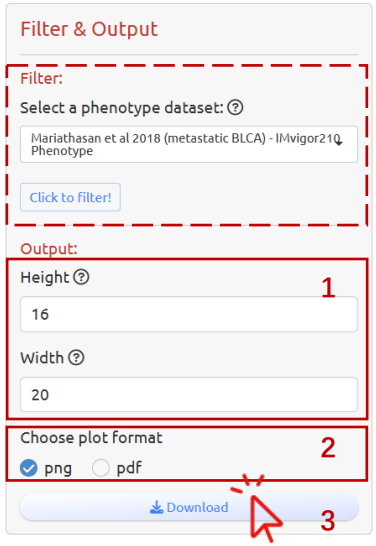
1. **Homepage Overview**
2. **Repository Selection**
3. **Cohort-centred Analysis**
   - Scatter-Correlation
   - Heatmap-Correlation
   - Group-Comparison
   - KM-Analysis
   - Cox-Analysis
   - Cohort Filter
4. **Molecular-centred Analysis**
   - Distribution of circRNA
   - Cox analysis for circRNA
5. **Signature Analysis**
6. **DEG Analysis**
7. **Demo Output**

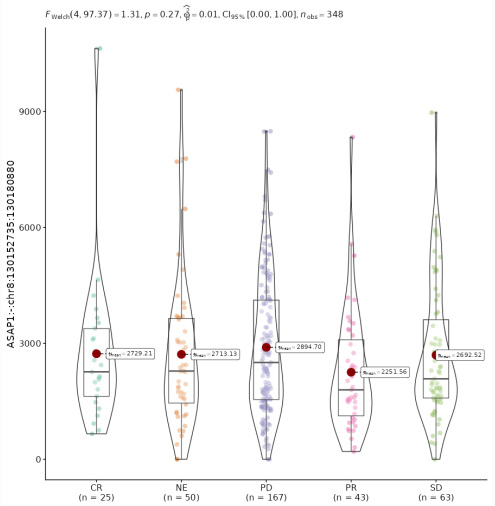
C Filter (Optional)



Filter patients and redraw

D Save Plots





I. Change sizes

II. Choose format

III. Click

A

Analysis Controls

Select dataset:

Gide et al 2019 (metastatic melanoma)
CircRNA (Counts per Million, circexplorer2)

Analysis Type

CircRNA ID

CircRNA ID (or Host Gene):

TMTC3:+:chr12:88148287:88176319

Select Cutoff

Cut-off proportion

0.5

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

Select Optimal Cutoff

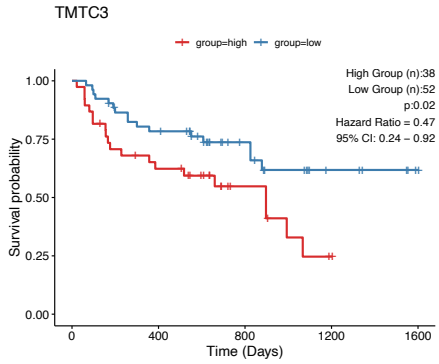
Show advanced setting

Analysis endpoint

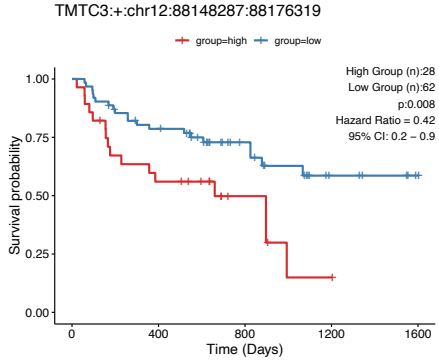
OS

✓ Submit

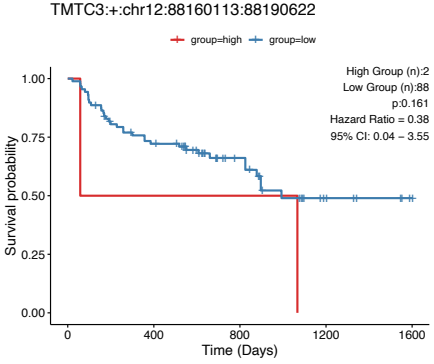
B



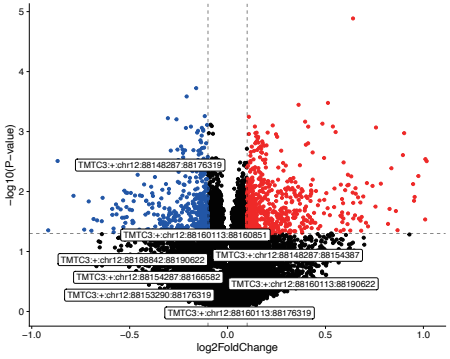
C



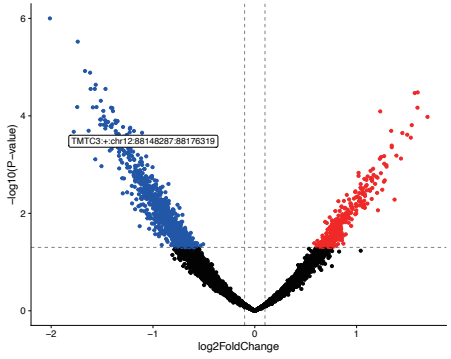
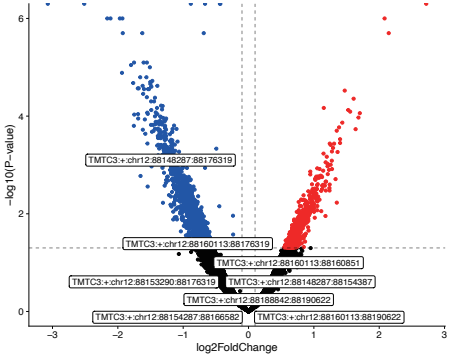
D



E



F



G