The comprehensive landscape of *TTMV::RARA* fusion-driven acute myeloid leukemia: from viral integration mechanisms to clinical outcomes

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Abstract

Acute myeloid leukemia (AML) with *TTMV*::*RARA* fusion represents a novel subtype driven by torque teno mini virus (TTMV) integration into the retinoic acid receptor α (*RARA*) locus, while current understanding of its molecular features and clinical presentation relies predominantly on isolated case observations. Here, we characterize a large and independent cohort (N=25) through integrative analysis of clinical-omics data, uncovering unique features that distinguish it from classic acute promyelocytic leukemia (APL) and other AML subtypes. Our findings reveal that TTMV integrates exclusively within intron 2 of the *RARA* gene via microhomology-mediated end joining, forming functional *TTMV*::*RARA* transcripts. Clinically, patients harboring this fusion were predominantly pediatric (72%, age <18 years) and often presented with extramedullary diseases (24% with myeloid sarcoma, 16% with central nervous system infiltration). Blasts displayed APL-like morphology and immunophenotype but lacked *PML*::*RARA*, instead harboring *TTMV*::*RARA* with recurrent i(17)(q10) abnormalities (24%). Unsupervised clustering revealed it as a molecularly distinct subgroup. Transcriptomic profiling identified a Wnt-activated/extracellular matrix-dysregulated signature, driving leukemogenesis via dual mechanisms of clonal expansion and metastatic pathways. Despite achieving a 96% complete remission rate with induction therapy, long-term outcomes were significantly inferior, with 2-year event-free survival and relapse-free survival rates of 53.6% and 53.8%, respectively. Hematopoietic stem cell transplantation achieved durable remission in nine of 11 patients, particularly those with extramedullary disease or i(17)(q10) abnormalities. Conclusively, this work establishes *TTMV*::*RARA* as a novel AML

subtype, highlighting the need for viral screening in APL-like cases and hematopoietic stem cell transplantation prioritization for this subset.

Introduction

Acute myeloid leukemia (AML) represents a group of molecularly heterogeneous malignancies, characterized by chromosomal rearrangements and genetic aberrations.[1-2] Approximately 40% of AML cases harbor clinically validated pathogenic fusion genes arising from these rearrangements.[3] Key fusion genes, such as *PML*::*RARA*, have been established as diagnostic markers and therapeutic targets in AML, owing to their distinct clinicopathological features and prognostic relevance.[4-6] The World Health Organization (WHO) has incorporated these molecular alterations into the classification of hematolymphoid tumors, with their clinical application significantly improving patient management and outcomes.[7] However, a subset of AML patients remain without definitive molecular markers, creating a critical gap in targeted therapeutic strategies and underscoring the urgent need to identify novel oncogenic drivers for precision medicine in leukemia.

Recent studies have revealed that torque teno mini virus (TTMV), conventionally considered as non-pathogenic, can aberrantly integrate into the human genome into the retinoic acid receptor α (*RARA*) locus, resulting in the formation of a cross-species "virus-host" fusion gene, *TTMV*::*RARA*.[8-17] This distinctive genomic rearrangement directly contributes to the development of AML with features resembling acute promyelocytic leukemia (APL), thereby challenging the traditional paradigm of leukemogenesis mediated by human-human gene fusion and broadening our understanding of AML pathogenesis.

While prior case reports have delineated the basic structural features of this fusion, the precise mechanisms of viral integration remain poorly characterized. Similarly, although clinical observations have documented certain phenotypic traits, an evidence-based treatment consensus and therapeutic guidelines await systematic validation through large-scale studies.

To address these critical knowledge gaps, we have assembled a large multicenter cohort of *TTMV*::*RARA* cases to date (N=25). This expanded dataset enables us to construct a comprehensive molecular profile of viral integration patterns, establish genotype-phenotype correlations across clinical presentations, and evaluate treatment response patterns to inform evidence-based therapeutic guidelines.

Methods

Case identification and study design

This observational, retrospective cohort study (approval number 2024-7-31-2) was approved by the Institutional Review Board of Beijing Chaoyang Hospital, Capital Medical University, China, and conducted in compliance with the Declaration of Helsinki.

The study cohort for initial *TTMV*::*RARA* screening comprised 2,553 AML patients, including 2,543 cases retrieved from published databases (*Online Supplementary Table S1*) and an additional ten cases recruited from a multicenter study spanning July 2014 to August 2024. These ten patients exhibited morphological and immunophenotypic features strongly resembling classical APL, but none carried known APL-defining genetic drivers (including classic *PML*::*RARA* and other *RARA*, *RARG*, or *RARB* fused with human genes), nor the hotspot *NPM1* mutations that have been implicated in APL-like presentations. Subsequently, through the application of a stringent filtering strategy that incorporated viral genome data, we ultimately identified four cases harboring the *TTMV*::*RARA* fusion event. This high frequency emphasizes the potential diagnostic relevance of TTMV in resolving unclassified APL-like cases.

Diagnostic and follow-up information for newly identified *TTMV*::*RARA*-positive patients were submitted by participating centers, while reported case data were extracted from the literature. Collected data included patient demographics, diagnostic/clinical laboratory results, the morphology, immunophenotype, cytogenetics, and molecular genetics (MICM) profiling, induction/consolidation chemotherapy regimens, and hematopoietic stem cell transplantation (HSCT) details. Treatment decisions were systematically recorded from medical records. All patients were followed until death or the data cutoff date (August 2024).

Expression-based comparisons

Transcript-level expressions were quantified by Salmon (v1.9.0)[18] and gene-level read counts were aggregated by the tximport R package. To minimize batch effects, an integrated dataset of *TTMV*::*RARA* samples and a separate dataset[19] (GSA-Human database, accession ID HRA002693) were analyzed for differential expression using DESeq2 (v1.40.2).[20] The EnhancedVolcano R package was used for visualization of DE results. Variance-stabilizing transformation of gene expression levels was conducted with DESeq2. Unsupervised hierarchical clustering was performed using the Ward.D2 algorithm. Gene set enrichment analysis was performed using the clusterProfiler[21] R package with gene sets from MSigDB and visualized by the GseaVis R package.

Definition of outcomes

Complete remission (CR) and overall response remission were defined according to the recommended criteria.[22]

Overall survival (OS) was defined as the period from initial diagnosis to the last follow-up with a status of either death or alive assigned. Event-free survival (EFS) was calculated from the time of initial diagnosis to treatment failure (the patient did not have CR by month 6), relapse, death or the last follow-up in CR. Relapse-free survival (RFS) was calculated from the time of first CR to the date of first relapse, death or the last follow-up still in CR.

Statistical analyses

Statistical analyses were performed using R software (v4.4.0) and GraphPad Prism (v10.1.2). Differential expression analysis was conducted using Wald's test, survival analysis included Kaplan-Meier curves with log-rank tests for group comparisons and Cox proportional hazards regression for multivariable analysis. $P<0.05$ was considered statistically significant. The benjamini and Hochberg method was applied for multiple test correction.

Results

Establishment of the *TTMV*::*RARA* clinical and omics dataset

To systematically identify TTMV integration at the *RARA* locus, we screened 2,543 publicly available AML RNA-sequencing (RNA-seq) datasets and ten APL-like cases from collaborative cohorts (Figure 1). Following a rigorous multi-step pipeline screening (*Online Supplementary Figure S1*), we identified ten cases harboring *TTMV*::*RARA* (Figure 1). By incorporating 15 cases from previous studies,[8-17] we established a comprehensive dataset comprising 25 patients in total. This dataset includes clinical profiles for all 25 cases, RNA-seq data for 15 cases, and whole genome sequencing (WGS) data for two cases (Figure 1).

General patterns of TTMV integration in acute myeloid leukemia

An integrative analysis of RNA-seq and WGS data from AML patients with *TTMV*::*RARA* revealed the presence of seven distinct TTMV strains involved in fusion gene formation. Among these, TTMV strain MN769771.1 was the most prevalent, accounting for 46.7% (7/15) of all fusion events (Figure 2A, B).

Localization analysis confirmed that all TTMV integration events were exclusively confined to intron 2 of the *RARA* gene, with a pronounced preference for the 3' breakpoint region (Figure 2A; *Online Supplementary Figure S2A*). This finding aligns with previous observations in the literature.[8-17] Notably, the insertion sites exhibited significant clustering, suggesting the presence of potential hotspot integration loci within this region ($P=0.00178$; Figure 2A). Sequence analysis of 15 integration junctions revealed the presence of two to four base pairs of microhomology in 12 cases (80%) at the integration sites (Figure 2A), microhomology-mediated end joining (MMEJ)[23] likely drives TTMV-host

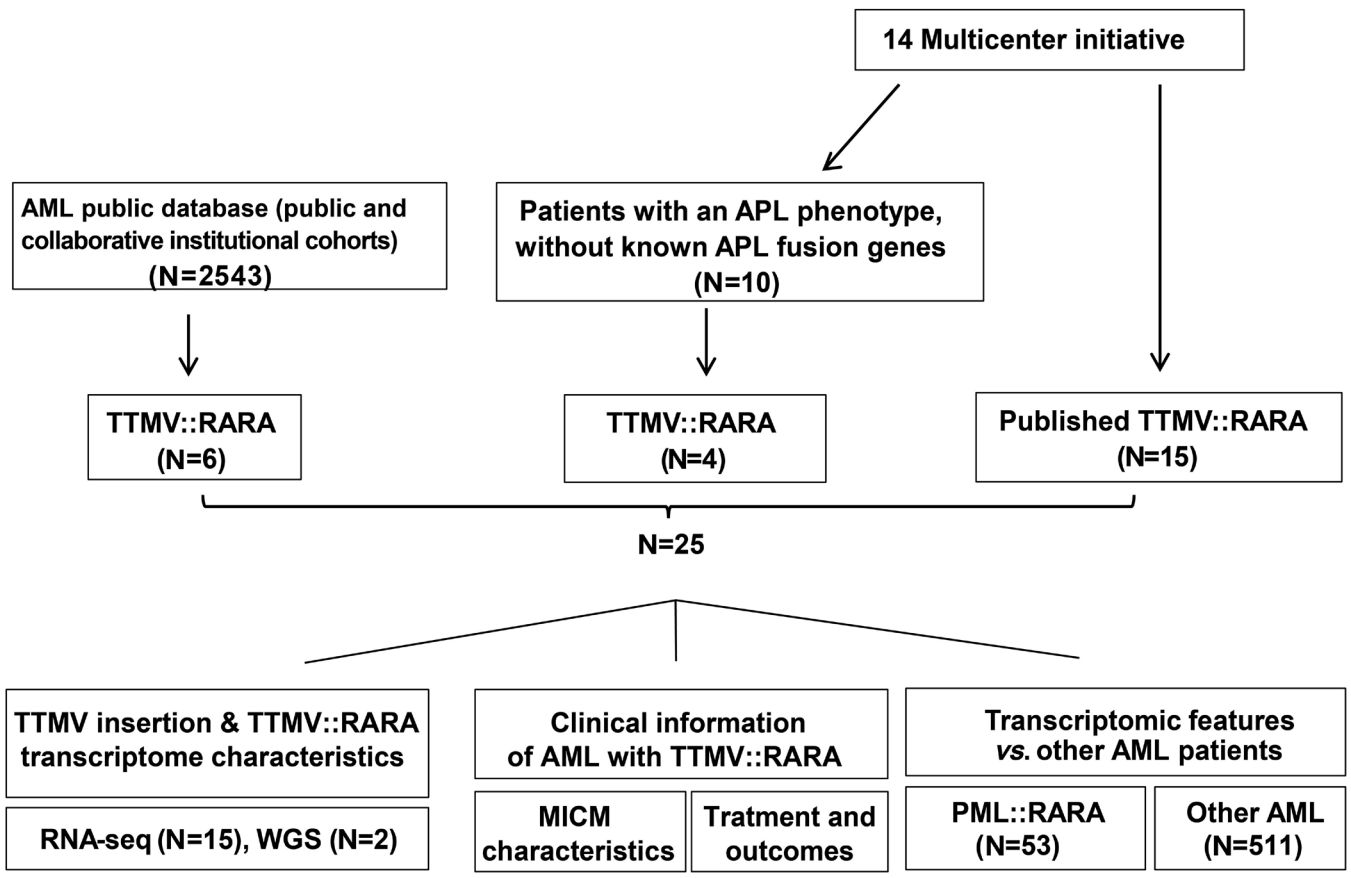

Figure 1. Study design for TTMV screening and characterization of *TTMV*::*RARA* in acute myeloid leukemia patients. This schematic illustrates the analytical pipeline for torque teno mini virus-retinoic acid receptor α (*TTMV*::*RARA*) investigation in acute myeloid leukemia (AML). Systematic analysis of 25 AML cases with *TTMV*::*RARA* fusions (10 newly identified from 2,553 screened patients and 15 from published reports) revealed distinct integration patterns of TTMV, along with unique transcriptional profiles and clinical manifestations of AML patients with *TTMV*::*RARA*.

integration in the majority of patients.

Structural characteristics of the *TTMV::RARA* transcripts

The profiling of fusion transcripts revealed that 13 of 15 cases (86.7%) harbored chimeric *RARA*::*TTMV*::*RARA* transcripts, with TTMV flanked by 5' and 3' RARA segments (*Online Supplementary Figures S2B* and *S3A, B; Online Supplementary Table S2*). These findings align with previous reports[14] and provide evidence for the existence of full length *RARA*::*TTMV*::*RARA* precursor transcripts (Figure 2C). In-depth sequence analysis of the fusion transcripts identified three distinctive structural features. Adjacent to the TTMV start codon, a highly conserved motif was detected in all 13 cases (*Online Supplementary Figure S4A*), while the downstream *RARA* open reading frames remained intact. Additionally, variable retention of *RARA* intron 2 sequences (ranging from 0 to 45 base pairs) was observed (Figure 2D). This tripartite structure suggests a functional hierarchy. The 5' *RARA* segment of *RARA*::*TTMV*::*RARA* probably functions as a regulatory untranslated region (UTR), enabling the conserved TTMV motif and intact *RARA* open reading frame (ORF) to maintain translational efficiency. The variable retention of intron 2 sequences further implies splicing-mediated regulation of this oncogenic fusion transcript, underscoring a complex mechanism by which viral integration dysregulates gene expression to drive leukemogenesis.

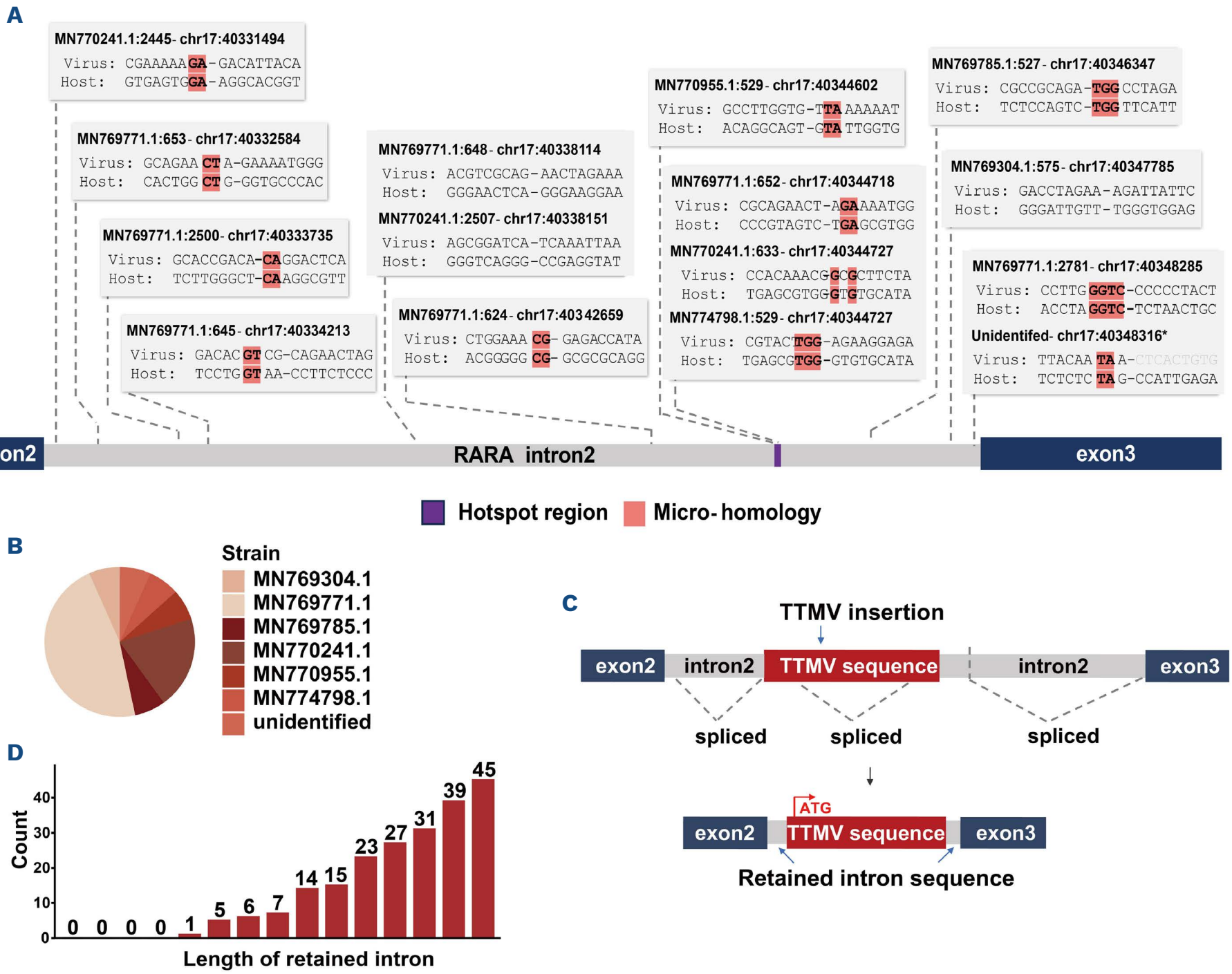

Figure 2. Characteristics of TTMV virus insertion into the acute myeloid leukemia genome. (A) Torque teno mini virus (TTMV) inserts into the retinoic acid receptor α (*RARA*) intron 2 at different positions. The red shades indicate sequence homology between viral and host DNA at the insertion site, whereas the hotspot regions denote short genomic segments including insertion sites across multiple samples. (B) Types and proportions of TTMV virus substrains inserted into the *RARA* gene among 15 *TTMV*::*RARA* patients. (C) The diagram illustrates the potential alternative splicing pattern of the *RARA*::*TTMV*::*RARA* fusion gene. (D) The length (base pairs) distribution of retained intron 2 sequences before *RARA* exon 3 in the spliced *TTMV*::*RARA* fusion transcripts. Each column represents a *TTMV*::*RARA* sample.

Collectively, these findings provide a comprehensive characterization of chimeric *RARA*::*TTMV*::*RARA* transcript structures that maintain a functional *TTMV*::*RARA* ORF, formed by TTMV viral integration into the human genome. These observations prompt inquiry into the clinical and phenotypic correlations mediated by these fusion genes.

Clinical characteristics of acute myeloid leukemia patients with *TTMV*::*RARA*

To elucidate the clinical and molecular characteristics of AML patients with *TTMV*::*RARA* fusion genes, we comprehensively analyzed 25 cases. The cohort predominantly comprised individuals aged ≤18 years (72%, 18/25), with an approximately equal sex distribution (male:female = 13:12) (Table 1). At the time of diagnosis, fever (36%, 9/25) and bleeding (32%, 8/25) were the most prevalent clinical manifestations (Table 1). Hematological parameters showed median values for white blood cell (WBC) counts, hemoglobin, and platelet counts of 7.88×10^9/L (range, 1-41.9×10^9/L), 88 g/L (range, 55-113 g/L), and 94×10^9/L (range, 13-334×10^9/L), respectively (Table 1). Coagulation studies revealed median prothrombin time and activated partial thromboplastin time of 13.55 and 32.6 seconds, respectively, with fibrinogen and D-dimer levels measured at 196 mg/dL and 10,660 μg/L, respectively (Table 1). Notably, 24% (6/25) of cases exhibited myeloid sarcoma and 16% (4/25) had central nervous system (CNS) infiltration, reflecting an aggressive disease phenotype (Table 1).

The MICM profiling of AML patients with *TTMV*::*RARA* fusion demonstrated both striking parallels and distinct divergences when compared to classic APL. Morphologically, 52% (13/25) of leukemic blasts displayed typical hypergranular APL morphology, while 8% (2/25) showed hypogranular variant APL characteristics. Additionally, Auer rods were observed in 32% (8/25) of the cases (Table 1; Figure 3A). Flow cytometric analysis revealed high expression levels of CD33 (100%), CD13 (100%), CD117 (79%), MPO (100%), CD99 (100%), and CD38 (87.5%) in the majority of leukemia cells, while CD34 (16.7%), HLA-DR (10%), and CD11b (15%) were rarely expressed (Figure 3B), consistent with APL immunophenotypic patterns.

Conversely, cytogenetic and molecular analyses uncovered distinct characteristics of AML patients with *TTMV*::*RARA*. Chromosomal abnormalities were detected in 60% (15/25) of patients, among which isochromosome i(17)(q10) (24%, 6/25) and trisomy 8 (8%, 2/25) emerged as the predominant aberrations (Table 1).

Furthermore, systematic screening for recurrent leukemia mutations using multimodal methods including real-time polymerase chain reaction (RT-PCR), next-generation sequencing (NGS)-targeted sequencing, and bulk RNA-seq analysis identified only one case with *FLT3* internal tandem duplication (*FLT3*-ITD), two cases harboring *NRAS* p.G12 codon mutations, and two cases with *WT1* mutations (Figure 3C). These mutations are lesions commonly observed in APL,[24] indicating a distinct mutational landscape for this subtype. Taken together, these results suggested that while AML patients with *TTMV*::*RARA* exhibit morphological and immunophenotypic similarities to classic APL, they also displayed significant differences in their cytogenetic, molecular, and clinical profiles.

Transcriptomic features of acute myeloid leukemia with *TTMV*::*RARA*

To determine whether AML patients with *TTMV*::*RARA* fusion represent a distinct subtype separate from conventional AML and classical APL, we performed a transcriptomic analysis involving 15 *TTMV*::*RARA* samples. These were compared against 53 *PML*::*RARA* (classic APL) samples and 511 non-APL AML samples (*Online Supplementary Table S3*). Unsupervised hierarchical clustering revealed that *TTMV*::*RARA* samples formed a transcriptionally distinct cluster. While they showed a close relationship with classical APL, a clear separation was observed (Figure 3C), indicating potential molecular differences between

Table 1. Clinical characteristic of *TTMV*::*RARA* acute myeloid leukemia patients (N=25).

Parameters	N=25
Characteristic, N (%)	
Age ≤18 years	18 (72)
Male	13 (52)
Clinical presentation, N (%)	
Fever	9 (36)
Bleeding	8 (32)
Blood tests, median (range)	
White blood cell count, $\times10^9$/L	7.88 (1-41.9)
Hemoglobin, g/L	88 (55-113)
Platelet count, $\times10^9$/L	94 (13-334)
PT, seconds	13.55 (11.6-19.6)
APTT, seconds	32.6 (23.6-48.2)
Fibrinogen, mg/dL	196 (52-357)
D-dimer, ug/L	10,660 (1,570-38,440)
Morphology, N (%)	
APL-like cells, median (range)	77.5 (18.8-99.2)
Hypergranular	13 (52)
Hypogranular	2 (8)
Auer body	8 (32)
Cytogenetics, N (%)	
Normal karyotype	9 (36)
idic(17)(p11.2)	1 (4)
i17(q10)	4 (16)
i17(q10), +8	2 (8)
Others karyotype	8 (32)
Unkonwn	1 (4)
Myeloid sarcoma, N (%)	6 (24)
At diagnosis	4 (67)
At relapse	2 (33)
Central nervous system leukemia, N (%)	4 (16)
At diagnosis	3 (75)
At relapse	1 (25)

PT: prothrombin time; APTT: activated partial thromboplastin time.

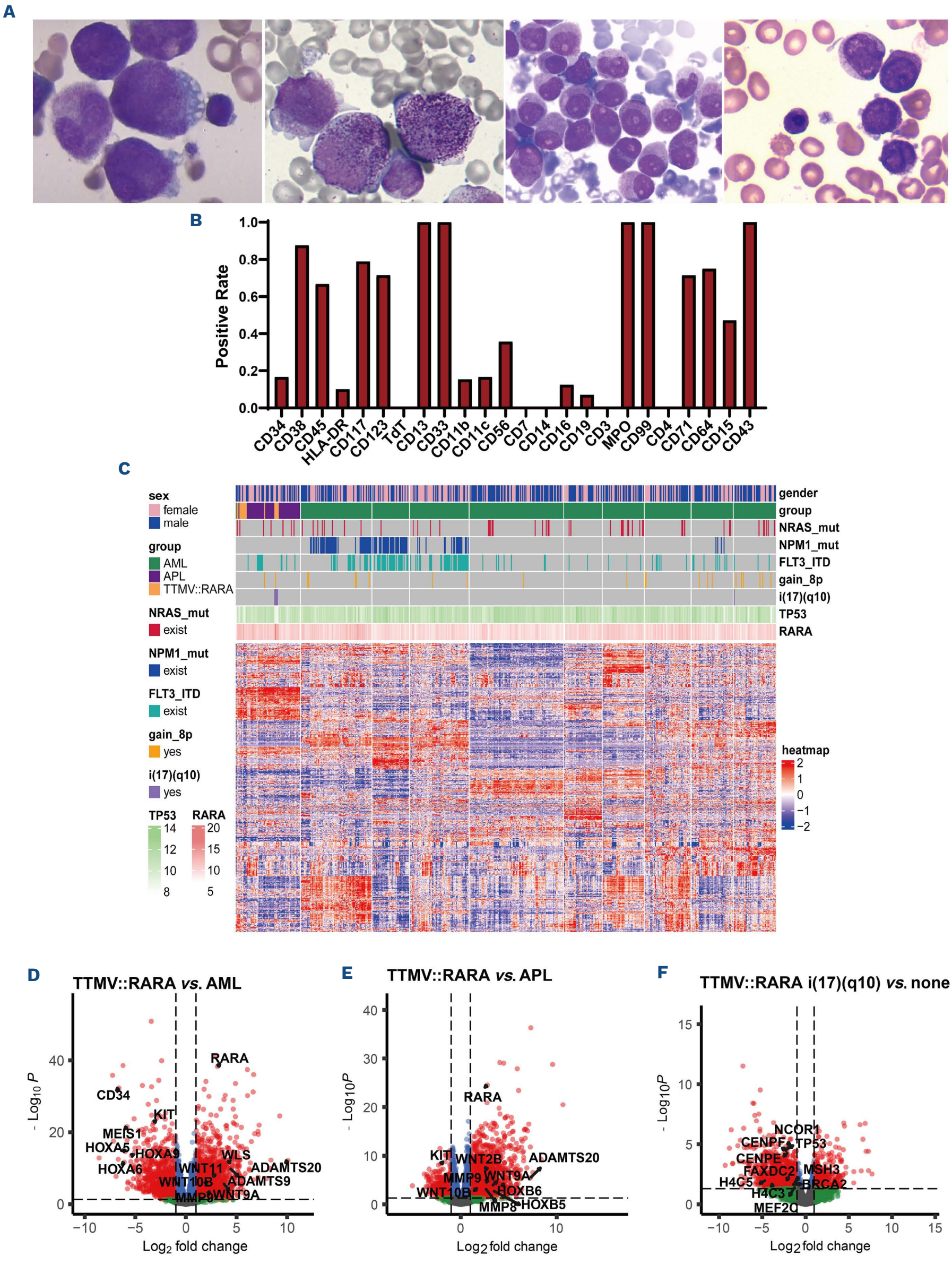

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Figure 3. The clinical characteristics and transcriptomic landscape of acute myeloid leukemia with *TTMV*::*RARA*. (A) The bone marrow aspirate morphology of patients with torque teno mini virus retinoic-acid receptor α (*TTMV*::*RARA*) showed promyelocytes with mono- to bilobated nuclear contours with dense cytoplasmic purple granules. (B) The immunophenotypic features of patients with *TTMV*::*RARA* showed that the majority of leukemia cells expressed CD38, CD117, CD13, CD33 and MPO. A minority of samples expressed CD34, HLA-DR, CD11b. (C) The heatmap displayed the results of unsupervised clustering from the transcriptomic data between 15 *TTMV*::*RARA* patients, 53 *PML*::*RARA* patients, and 511 acute myeloid leukemia (AML) patients without *TTMV*::*RARA* or *PML*::*RARA*. (D, E) Volcano plots showed differentially expressed genes (DEG) between *TTMV*::*RARA* patients with AML and classic acute promyelocytic leukemia (APL), respectively. (F) Volcano plots showed DEG between *TTMV*::*RARA* patients with i(17)(q10) and those without i(17)(q10).

these subtypes.

Differential gene expression and functional analysis revealed that AML patients with *TTMV*::*RARA* exhibited elevated *RARA* expression and robust enrichment of the Wnt signaling pathway (Figure 3D, E; *Online Supplementary Figure S5A, B*), which are associated with cell proliferation and self-renewal.[25] Concurrently, extracellular matrix (ECM) regulators such as *ADAMTS9* and *MMP8* showed marked overexpression (Figure 3D, E; *Online Supplementary Figure S5A, B*), in line with their functions in tissue remodeling and invasive migration.[26] These findings support a dual mechanism model: activation of the Wnt pathway drives clonal expansion, while ECM dysregulation facilitates metastatic dissemination in AML patients with *TTMV*::*RARA*. The further stratification of the 15 AML samples with *TTMV*::*RARA* utilizing the same unsupervised clustering approach revealed two distinct subgroups (Figure 3C). These subgroups were differentiated by the presence of isochromosome i(17)(q10), a chromosomal aberration detected in five patients (33.3%) through an integrated analysis of G-banding karyotyping and transcriptomic data. Comparative transcriptomic analysis revealed that the i(17)(q10)-positive subgroup (N=5) exhibited significant downregulation of 678 genes (fold change >2; adjusted *P* value <0.05) compared to the i(17)(q10)-negative subgroup (N=10), with *TP53* showing a 4.2-fold decrease (adjusted *P* value =0.003; Figure 3F). Pathway enrichment analysis (GSEA) demonstrated that the i(17)(q10)-positive subgroup was characterized by significant enrichment of pathways related to chromosome centromeric core domains (NES =-2.03; *P*<0.001) and DNA double strand break response (NES =-2.21; *P*<0.001) (*Online Supplementary Figure S5C*). These findings, combined with the known role of *TP53* in DNA repair, suggest a compromised DNA damage repair capacity in the i(17)(q10)-positive subgroup. Notably, the prevalence of i(17)(q10) in AML is less than 1%,[27] highlighting the unique molecular landscape of AML with *TTMV*::*RARA*. These unique features distinguish *TTMV*::*RARA* patients from those with classic APL and other AML subtypes, warranting further clinical and mechanistic investigation.

Treatments and outcomes

An analysis of treatment response in AML patients with *TTMV*::*RARA* revealed an impressive overall CR rate of 96% following induction therapy (Table 2). However, only 46% of patients achieved CR after the first treatment course. No early deaths occurred within 45 days after induction therapy, indicating a favorable tolerance to the initial therapy. AML patients with *TTMV*::*RARA* primarily received one of three initial induction regimens: all-*trans* retinoic acid combined with arsenic trioxide (ATRA+ATO, course duration >14 days, N=9), a short course of ATRA combined with chemotherapy

Table 2. Response to treatment (N=25).

Response	N=25
CR, N (%)	24 (96)
1 cycle to CR	11 (46)
2 cycles to CR	11 (46)
>3 cycles to CR	2 (8)
Response to the first induction treatment	
ATRA+ATO, N (%)	9
ORR	8 (88.9)
CR	6 (66.7)
PR	2 (22.2)
NR	1 (11. 1)
ATRA1+others, N (%)	7
ORR	5 (71.4)
CR	2 (28.6)
PR	3 (42.8)
NR	2 (28.6)
Standard AML induction chemotherapy, N (%)	8
ORR	5 (62.5)
CR	4 (50)
PR	1 (12.5)
NR	3 (37.5)
Patients with (17)(q10), N=6	
CR, N (%)	6 (100)
1 cycle to CR	1(16.7)
2 cycles to CR	3 (50)
>3 cycles to CR	2 (33.3)
Response to the first induction treatment, N (%)	
ORR	3 (50)
CR	1 (16.7)
PR	2 (33.3)
NR	3 (50)
Outcome at 2 years (%)	
OS rate	84.6
EFS rate	53.6
RFS rate	53.8

CR: complete response; ATRA: all-*trans* retinoic acid; ATO: arsenic trioxide; ORR: objective response rate; PR: partial response; NR: no response; OS: overall survival; EFS: event-free survival; RFS: relapse-free survival; AML: acute myeloid leukemia; ATRA1 indicated that 3 of the 6 patients had a duration of ATRA therapy of less than 8 days, whether continuous or intermittent.

(N=7), and standard AML induction chemotherapy (N=8). As shown in Table 2, the CR rates for the ATRA+ATO, short course ATRA with chemotherapy, and standard chemotherapy were 66.7%, 28.6%, and 50%, respectively, with overall response rates of 88.9%, 71.4%, and 62.5%. Although Fisher's exact test did not reveal statistically significant differences in CR rates among the three treatment groups (P=0.319), the ATRA+ATO group exhibited a relatively higher CR rate and OS compared to the other two groups (*Online Supplementary Figure S6A-C*). The observed trends in treatment response indicate that the ATRA+ATO combination therapy might have a beneficial effect on inducing remission, thereby warranting further investigation with larger sample sizes. Notably, patients with isochromosome i(17)(q10) exhibited significant treatment resistance, with a CR rate of 16.7% (1/6) and overall response rate of 50% (3/6) (Table 2).

A total of 11 patients underwent HSCT, including ten allogeneic and one autologous HSCT cases. Among them, five presented with myeloid sarcoma or CNS involvement, and one harbored the i(17q10) chromosomal abnormality, both of which represent high-risk prognostic markers. Clinical outcome analysis revealed that, of the 11 patients, one experienced relapse with multiorgan involvement and died 50 days post-transplantation, while another patient succumbed to disease progression 24 months post-transplantation. The remaining nine patients were still alive at the last follow-up, with a median follow-up period of 36 months (range, 7.7-115.5 months). Notably, two of these patients remained in remission for 8 and 9 years post-transplantation, respectively.

Survival analysis revealed 2-year OS, EFS, and RFS rates were 84.6% (95% confidence interval [CI]: 59-94.8%), 53.6% (95% CI: 30.8-71.8%), and 53.8% (95% CI: 31.1-72%), respectively (Figure 4A-C). When compared to classical APL patients treated with ATRA+ATO,[28-29] AML with *TTMV*::*RARA* showed significantly lower EFS and RFS despite similar OS rates.

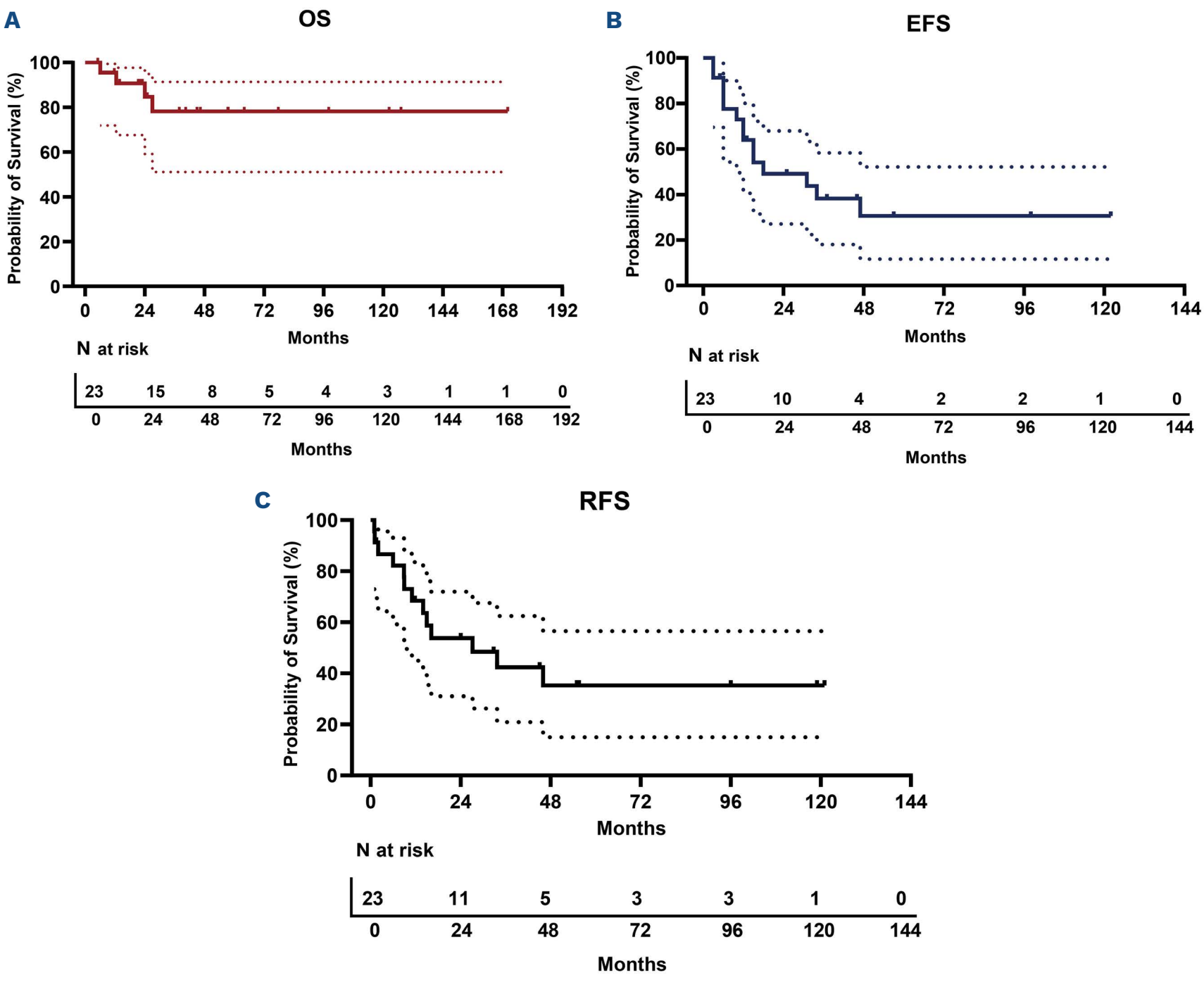

Figure 4. Clinical outcomes and treatment responses of acute myeloid leukemia patients with *TTMV*::*RARA* fusion gene. (A-C) The overall survival (OS), event-free survival (EFS) and relapse-free survival (RFS) of acute myeloid leukemia (AML) patients with torque teno mini virus-retinoic acid receptor α (*TTMV*::*RARA*) fusion gene.

Discussion

This study systematically screened 2,553 cases of AML to provide evidence for the specific integration of TTMV into the human *RARA* gene within a large patient cohort. By integrating clinical and multi-omics data through an international multicenter collaboration, we achieved a thorough characterization of the structural features and clinical manifestations associated with the *TTMV*::*RARA* fusion gene, thereby providing critical evidence for the establishment of a precision medicine framework for this hematological malignancy

In contrast to the eight previously identified pathogenic viruses,[30] TTMV is the first single-stranded DNA virus shown to directly induce oncogenesis by creating a chimeric fusion oncogene. Its carcinogenic mechanism operates independently of classical pathways, such as the expression of viral oncoproteins, genomic instability, or dysregulation of the cell cycle,[31] thereby broadening the theoretical framework of viral oncogenesis and offering significant insights into the field of viral oncology.

Our analysis of TTMV integration patterns revealed two key characteristics. Firstly, TTMV can integrate into intron 2 of the *RARA* gene from any region of its own genome and exhibits significant heterogeneity in viral subtypes among different patients. This spatial randomness and diversity of substrains pose challenges for clinical detection, highlighting the imperative for comprehensive viral integration screening in AML, particularly in cases exhibiting APL-like morphology. Secondly, we observed a marked predilection of TTMV for intron 2 of *RARA*, where we identified a recurrent integration hotspot, designating TTMV MN769771.1 as the predominant pathogenic subtype. MMEJ may be a significant mechanism facilitating this site-specific integration. These findings contribute to a deeper understanding of the interactions between viral and host genomes in leukemogenesis and carry clinical relevance. The identified recurrent integration patterns serve as a specific molecular signature that could aid in the development of diagnostic panels.

While earlier research indicated similarities between AML with *TTMV*::*RARA* and classical APL,[8-17] our investigation has confirmed the distinct clinical and molecular features of this subtype, aligning with the findings of Zhou *et al*.[32] This subtype predominantly affects pediatric populations and is marked by a notable frequency of extramedullary involvement and recurrent i(17)(q10) abnormalities, which are infrequently observed in classical APL or other AML subtypes.[33] Transcriptomic analysis has further delineated a unique gene expression profile, substantiating its classification as a separate disease entity.

Currently, there is no established induction therapy for this condition. Previous studies have indicated that the TTMV::RARA fusion protein exhibits a dose-dependent response to ATRA,[34] however, mutations within the ligand binding domain of RARA readily confer treatment resistance.[32] Predictive protein structures of TTMV::RARA suggest that C55/C57/C59 residues in TTMV ORF may form arsenic binding sites, thereby conferring sensitivity to ATO.[16] Our research provides direct clinical evidence supporting the efficacy of the ATRA+ATO in these patients, with a discernible trend toward enhanced OS, thereby supporting its potential as a first-line therapeutic option.

Utilizing unsupervised clustering analysis, we further categorized AML patients with *TTMV*::*RARA* into two subgroups exhibiting significant molecular heterogeneity, closely associated with clinical outcomes. The high-risk subgroup, distinguished by a prevalent occurrence of i(17)(q10) abnormalities, exhibited an extremely poor prognosis and a limited response to conventional therapies, including ATRA+ATO. Mechanistic studies revealed that this high-risk cohort displayed downregulated *TP53* expression and activated DNA damage repair pathways, which may contribute to its chemoresistant phenotype. Notably, HSCT demonstrated promising efficacy within this subgroup, presenting a viable strategy to address treatment challenges.

Although the sample size of this study remains limited, it represents one of the largest global cohorts of *TTMV*::*RARA* cases to date. Our observations regarding clinical manifestations, integration mechanisms, and therapeutic responses align closely with those reported in a separate, substantial cohort of *TTMV*::*RARA* cases,[32] reinforcing the validity of our conclusions and underscoring the importance of multicenter collaboration in studying rare hematological malignancies. To date, nearly 40 cases have been documented across two independent cohorts, indicating that the actual incidence of this malignancy is likely underestimated, primarily due to the absence of routine viral integration screening in current diagnostic workflows.

It should be acknowledged that the limited sample size has constrained the statistical power available for subgroup analyses. The frequency of *FLT3*-ITD, *NRAS/KRAS*, and *WT1* mutations in our cohort was lower than that in previously published cohorts.[32] These variations may reflect underlying population heterogeneity, differences in enrollment criteria, or intrinsic molecular diversity of the disease. Consequently, future initiatives should aim to amalgamate data from multiple centers and increase sample sizes to systematically delineate the clinical-molecular spectrum of this condition and validate the risk stratification model proposed herein. Simultaneously, a thorough investigation of the leukemogenic mechanisms instigated by the TTMV::RARA fusion protein, the development of highly sensitive diagnostic modalities and targeted therapeutics are paramount objectives for future research.

In conclusion, this study, through a comprehensive examination of the molecular mechanisms and clinical phenotypes associated with the *TTMV*::*RARA*, establishes it as a novel subtype of AML and proposes an initial risk-stratified diagnostic and therapeutic strategy. Given the unique biological behavior and poor prognosis associated with *TTM-*

V::*RARA*-positive AML, we advocate for the inclusion of viral genomic testing within next-generation sequencing based clinical diagnostic protocols to enhance detection rates. We are currently conducting further mechanistic studies, with the goal of addressing these critical biological questions and providing more substantive evidence in the future.

Disclosures

No conflicts of interest to disclose.

Contributions

HHZ designed the study. SS collected clinical data and interpreted the data. YJL and JYH contributed to the processing of the analysis of omics data. HHZ, SS, YJL, and QYX wrote the paper. JYW, ZRC, BY, HBY, NL and FW modified the images. LC, WG, HLW, HYW, LJW, JX, JCL, YYX, JGW, XJW, HJX, KC, YW, LPZ, SHS, SNC, HYW, and KKW collected the clinical samples, compiled the clinical information and omics sequencing data. HHZ, JYH, HYW, and KL critically reviewed the article. All authors read and approved the final manuscript.

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Data-sharing statement

For access to the original data, please contact the corresponding authors.

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