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The dark side of immunity: From a byproduct of V(D)J recombination to a promoter of leukemic relapse

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Excised signal circles (ESCs) are a form of extrachromosomal DNA (ecDNA) generated during V(D)J recombination. They have been detected in a wide range of cancers and are associated with poor prognosis, yet are traditionally thought to be incapable of replication during cell division. A recent study published in Nature by Gao et al. fundamentally reshapes our understanding of the role of ESCs in acute lymphoblastic leukemia (ALL).[1] Previous studies have indicated that ALL relapse is primarily driven by clonal evolution, therapy-induced mutagenesis, and the expansion of resistant subclones.[2] However, Gao et al. advance our understanding of ALL relapse by demonstrating that ESCs, previously regarded as innocuous by-products of immune diversification, can persist across cell generations and interact with recombination-activating gene (RAG) proteins to promote genomic instability. This positions ESCs as strong candidate contributors to relapse, thereby providing potential therapeutic targets for ALL relapse.

Relapse in ALL is commonly interpreted within frameworks of clonal evolution and therapy-induced mutagenesis, in which treatment-associated selective pressure drives the expansion of resistant subclones.[2] Within these models, genomic instability is largely considered episodic and temporally linked to chemotherapy exposure. However, several clinically observed features of ALL relapse remain insufficiently explained, including relapse occurring in the absence of overt treatment pressure, minimal residual disease-negative relapse, and disease recurrence in genetically favorable subtypes. These observations reveal a critical gap in knowledge regarding whether cell-intrinsic sources of ongoing genomic instability may persist independently of therapy. As a result, prevailing models are limited in their ability to account for mechanisms that sustain mutational accumulation across cell generations and enable relapse beyond classical selection-driven paradigms.

ESCs are conceptually related to ecDNAs, which are increasingly recognized as drivers of tumor heterogeneity and evolution.[3] Traditionally, ESCs were thought to lack replication origins and therefore to be progressively diluted during cell division.[4] Although ESCs share similarities with ecDNAs, their functional consequences differ substantially: whereas ecDNAs typically promote tumor evolution through direct oncogene amplification, ESCs drive mutagenesis via a cut-and-run mechanism, forming complexes with recombination-activating gene (RAG) proteins that induce aberrant cleavage at cryptic recombination signal sequences (cRSSs),[5] Once cRSSs are released, the intact ESC-recombinase complex can persist in initiating additional DNA breaks. A high ESC burden predicts relapse across multiple ALL subtypes, including favorable entities such as ETV6-RUNX1 and hypodiploid ALL,[4] leading to relapse-associated mutations and structural variants that accelerate clonal evolution. This conclusion is supported by convergent lines of evidence derived from complementary detection approaches. Droplet digital PCR (ddPCR) enables sensitive and absolute quantification of ESC copy number from limited clinical material, making it particularly suitable for relapse risk stratification at diagnosis, although it provides limited structural information. In contrast, linear amplification-mediated ESC (LAM-ESC) sequencing selectively captures signal joint-containing circular DNA molecules, allowing direct validation of ESC circularity and higher specificity, albeit with increased technical complexity and reduced scalability for routine clinical use. Genome-wide and transcriptomic analyses further reveal mutational footprints and activation of replication and DNA repair pathways associated with ESC persistence, while murine transplantation experiments demonstrate their ability to persist across cell generations in vivo. Together, these complementary approaches underscore both the robustness of ESC detection and the context-dependent utility of each method in clinical and experimental settings.

Beyond its immediate clinical implications, the identification of ESCs as persistent mutagenic agents positions them as a previously unrecognized mechanism of leukemic relapse. Previous frameworks have largely attributed relapse to the selection of pre-existing resistant clones or therapy-induced mutagenesis, implicitly assuming that the immune recombination machinery becomes biologically inert after malignant transformation. The work by Gao et al. challenges this assumption by revealing that immune diversification may constitute an endogenous and sustained source of genomic instability in susceptible leukemic contexts. In this sense, ESCs expose a conceptual parallel to somatic hypermutation-driven lymphomagenesis, reframing V(D)J recombination not merely as a developmental necessity but as a latent driver of malignant evolution. This paradigm shift offers a compelling explanation for clinically puzzling phenomena such as MRD-negative relapse. Even in the apparent absence of detectable residual disease, persistent ESC-RAG activity may continue to seed new mutations across successive cell generations, gradually fostering therapy resistance and clonal re-emergence. From this perspective, relapse is not solely a consequence of residual leukemic burden but may also reflect ongoing, post-treatment mutagenesis driven by immune recombination by-products. These insights raise the provocative possibility that ESCs represent a leukemia-specific, endogenous mutational engine-and, importantly, a potential therapeutic vulnerability. While direct inhibition of RAG proteins is untenable due to their essential role in lymphocyte development, selectively targeting the stability, replication, or maintenance of ESCs could define a previously unrecognized therapeutic window.[4] More broadly, ESC biology may point toward a unifying mechanism linking immune recombination, extrachromosomal DNA dynamics, and cancer evolution, with implications extending beyond ALL. Testing whether ESC-targeted strategies can attenuate relapse risk will be a critical next step in translating this conceptual advance into clinical benefit.[2]

Despite the conceptual and clinical implications of this work, several important limitations and open questions remain. Notably, the precise molecular basis of ESC "replication" is not yet fully resolved. While the data presented by Gao et al. are consistent with ESC persistence across cell divisions, they do not conclusively demonstrate autonomous replication. Instead, current evidence more strongly supports a model of persistence coupled with replication, potentially through interactions with host somatic replication or repair machinery. Whether ESCs possess intrinsic replication capacity or hijack cellular replication pathways therefore remains a critical area for future investigation. Equally important, the causal relationship between ESC burden and leukemic relapse has yet to be definitively established. Although elevated ESC levels correlate with increased relapse risk, this association alone does not distinguish whether ESCs act as primary drivers of relapse or function as accelerators within a broader mutagenic landscape. An alternative possibility is that high ESC burden represents a phenotypic consequence of a RAG1-high leukemia subtype, rather than an independent oncogenic force. The observation that not all patients with elevated ESC levels ultimately relapse further underscores the likelihood that additional genetic, epigenetic, or microenvironmental factors modulate disease outcome. Functional studies, including gain- and loss-of-function approaches and longitudinal intervention models, will be essential to resolve whether ESCs operate as drivers or

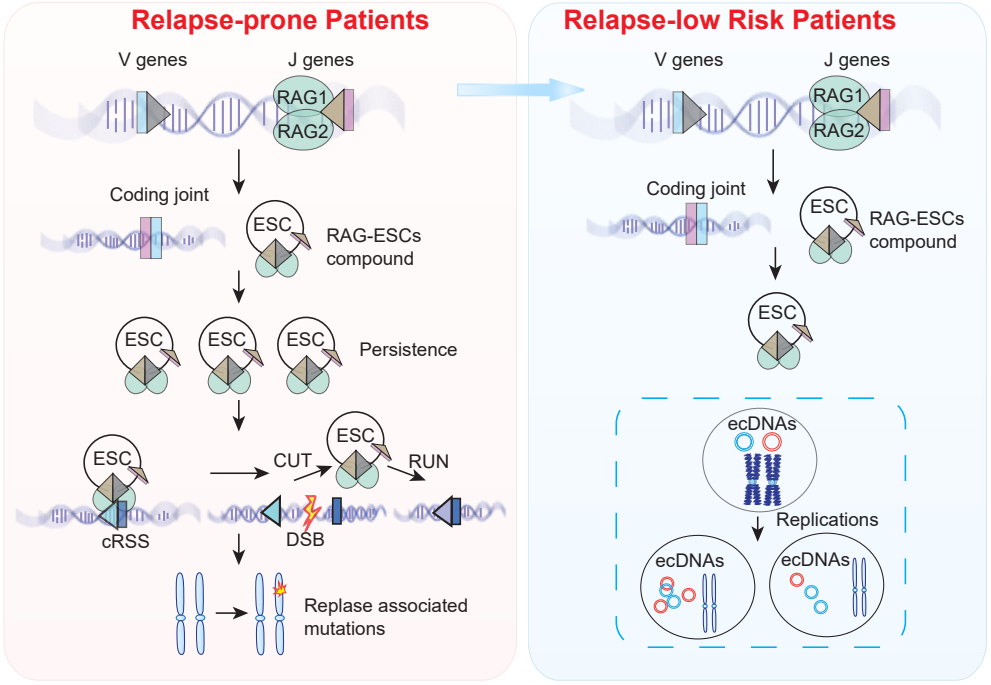

Figure 1. Mechanistic model of ESC-driven relapse in ALL In relapse-prone patients (left), elevated RAG1 promotes ESC amplification, leading to cut-and-run-mediated double-strand breaks at cRSSs and accumulation of relapse-associated mutations. In relapse-unlikely patients (right), inefficient ESC replication limits mutagenesis and supports sustained remission. ecDNA formation (bottom right) is distinct from ESCs, with replication inferred from persistence across cell divisions.

passengers in leukemic evolution. From a translational perspective, substantial challenges must be addressed before ESC analysis can be incorporated into routine clinical practice. Current ESC detection relies on technically demanding assays, limiting near-term scalability and widespread adoption, underscoring the need for standardized, reproducible, and cost-effective methodologies with validated reference standards and uniform cutoff thresholds. From a therapeutic standpoint, translating ESC biology into effective interventions also remains challenging. ESCs are not conventional drug targets, and perturbing pathways involved in their maintenance or replication may carry risks of unintended effects on normal lymphocyte development or genome stability. As such, ESC-directed strategies may be more realistically positioned as adjuncts to risk-adapted therapy rather than standalone treatments in the near term.

In conclusion, Gao et al.[1] redefine excision signal circles (ESCs)-long considered inert by-products of V(D)J recombination-as persistent, mutagenic contributors to leukemic relapse. This work reframes immune diversification as a double-edged sword, revealing an intrinsic cost of genome-editing processes essential for host defense. Recognizing and ultimately mitigating this cost may open new avenues for relapse prediction and intervention in ALL and potentially other malignancies shaped by immune recombination or extrachromosomal DNA dynamics (Figure 1).

REFERENCES

1. Gao Z., Scott J.N.F., Edwards M.P., et al. (2025). Excised DNA circles from V(D)J recombination promote relapsed leukaemia. *Nature* **645**:774–783. DOI:10.1038/s41586-025-09372-6
2. Mullighan C.G., Phillips L.A., Su X., et al. (2008). Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. *Science* **322**:1377–1380. DOI:10.1126/science.1164266
3. Turner K.M., Deshpande V., Beyter D., et al. (2017). Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. *Nature* **543**:122–125. DOI:10.1038/nature21356
4. Papaemmanuil E., Rapado I., Li Y., et al. (2014). RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. *Nat. Genet.* **46**:116–125. DOI:10.1038/ng.2874
5. Smith A.L., Scott J.N.F. and Boyes J. (2019). The ESC: The dangerous by-product of V(D)J recombination. *Front. Immunol.* **10**:1572. DOI:10.3389/fimmu.2019.01572

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

The authors declare no competing interests.