

Review Article

Alternative Lengthening of Telomeres as an RNA-Governed Homeostatic Circuit: Mechanism, Instability, and the Prognostic Paradox

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Alternative Lengthening of Telomeres (ALT) allows a substantial minority of human cancers to maintain their telomeres without reactivating telomerase, using a recombination-based mechanism that is mechanistically inseparable from the genomic instability it also generates. This review traces ALT from its structural hallmarks, ALT-associated PML bodies, C-circles, and G-quadruplexes through the regulatory circuit now understood to govern it: transcription of the telomeric long non-coding RNA TERRA, its conversion via RAD51AP1 from a transcription-associated R-loop into a recombination-competent D-loop, and the demonstration that this cycle behaves as a homeostat rather than a switch, self-destructive if left unrestrained. It then asks whether this dependence on strict RNA governance is unique to ALT or reflects a deeper constraint shared with telomerase, by comparing the decay machinery that polices TERC on the telomerase side (PARN, the nuclear RNA exosome) against the machinery that polices TERRA on the ALT side (XRN2, RNaseH1): structurally distinct systems converging on the same functional requirement, consistent with convergent evolution on a shared design problem rather than one pathway doing double duty. The review goes on to examine ALT's genomic consequences, its divergent, tissue-dependent prognostic associations, its emerging sensitivity to ATR inhibition, and a candidate explanation for that prognostic paradox rooted in cGAS-STING signalling and the tumour microenvironment. It closes by identifying the central gap between cell-autonomous molecular mechanism and tumour-level clinical behaviour, and proposing specific experiments that would close it.

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Introduction

Telomeres are the repetitive TTAGGG sequences that cap the ends of linear chromosomes, protecting coding DNA from the degradation and end-to-end fusion that would otherwise follow ordinary replication. Each round of cell division erodes a small stretch of this buffer, because conventional DNA polymerases cannot fully replicate a chromosome's terminal end. In germline and stem cells, the reverse transcriptase telomerase resets this loss by adding new repeats directly onto the chromosome terminus. In most differentiated somatic cells, telomerase is transcriptionally silenced, so telomeres shorten with each division until they become critically short and trigger senescence or apoptosis. This progressive shortening is one of the oldest and best-established tumour-suppressive barriers in human biology: a cell that divides indefinitely without some way of maintaining its telomeres will eventually stall.

Cancer cells are defined by their escape from this barrier, and the overwhelming majority escape it by reactivating telomerase. Roughly 85 to 90 percent of human cancers show detectable telomerase activity, most commonly through mutations in the *TERT* promoter or amplification of the gene encoding the catalytic subunit. The remaining fraction, however, achieves the same outcome by an entirely different route. These tumours maintain their telomeres without ever reactivating telomerase, using a recombination-based pathway known as the Alternative Lengthening of Telomeres, or ALT^[1]. ALT was first identified in immortalised human cell lines that had escaped senescence despite having no measurable telomerase activity, and it has since been documented across a wide range of tumour types, with particularly high prevalence in cancers of mesenchymal origin such as osteosarcoma and soft-tissue sarcoma, as well as in some neuroendocrine tumours and gliomas.

What makes ALT scientifically interesting is not simply that it is a second way of solving the same problem. Telomerase reactivation is, in a sense, a clean solution: the enzyme adds repeats processively and with relatively little collateral disruption to the rest of the genome. ALT is not clean in this way. It resolves the end-replication problem by borrowing sequence from other telomeres through homologous recombination, and in doing so it generates some of the same instability it is meant to prevent. ALT-positive cells carry extremely heterogeneous telomere lengths within a single nucleus, elevated rates of telomere sister-chromatid exchange, and a persistent burden of replication stress at their chromosome ends. The pathway that lets these cells divide indefinitely is, mechanistically, inseparable from the genomic instability that also defines much of their malignant behaviour. Understanding ALT therefore

means understanding a form of cancer cell dynamics in which telomere maintenance and genome instability are not separate processes running in parallel, but two faces of the same molecular machinery. The tumour-suppressive role of telomere shortening was recognised long before its molecular basis was understood, in the observation that normal human cells in culture divide only a finite number of times before stopping, a limit historically named after Leonard Hayflick. It took several more decades to connect that limit to telomere attrition and to the discovery of telomerase, and several more again to appreciate that a meaningful minority of cancers bypass the limit without telomerase at all. That minority is not evenly distributed across tumour types. ALT is comparatively rare in the common epithelial cancers that dominate cancer incidence statistics, such as breast and colorectal carcinoma, but it is disproportionately common in cancers of mesenchymal and neuroepithelial origin, including osteosarcoma, soft-tissue sarcomas, and a subset of gliomas and neuroendocrine tumours. This skewed distribution is itself an unresolved puzzle: why a recombination-based telomere maintenance pathway should be so much more accessible to, or so much more favoured in, some tissue lineages than others is not fully explained by anything in the mechanism described below, and it is one of several places where this review will note that the field's molecular precision has outpaced its explanatory reach.

This review is about a narrower question than telomere length and cancer risk in general. Telomere length itself is a heritable trait: large genome-wide association studies have identified dozens of common genetic variants that shape an individual's average telomere length across their tissues, and that inherited variation is itself associated with the risk of developing several cancers, including neuroblastoma and other childhood tumours^[2]. That is a genuine and clinically important line of telomere research, but it sits at a different level of causation from the question this review asks. It concerns why some people are, from birth, more or less predisposed toward certain cancers because of the telomere length they inherit. What follows here is instead about a tumour that already exists, and the tumour-intrinsic mechanism, ALT, by which some of those tumours maintain their telomeres once they need to divide indefinitely. The two questions sit close to one another but are not the same one, and this review is concerned only with the second.

This review traces that machinery from its structural hallmarks through to its most recently resolved regulatory circuit, and then out to its clinical consequences. My own research background sits closer to the telomerase side of this story than the ALT side: my MSc project examined how MPHOSPH6 shapes the processing of TERC, the RNA component of telomerase, in CRISPR-edited HCT116 colorectal cancer cells. Returning to telomere biology through ALT has been a way of asking a related question from the opposite

direction — if telomerase-competent cells depend on precise RNA-processing machinery to build a functional telomerase enzyme, what role does RNA processing play in a pathway that dispenses with telomerase altogether? The answer that has emerged over the last few years is that RNA is central to ALT as well, and the parallel runs deeper than a shared reliance on RNA in general: both routes to telomere maintenance turn out to depend on strict quality control of a specific telomere-associated non-coding RNA, TERC on the telomerase side and TERRA on the ALT side. Whether that reflects a single constraint both pathways have had to satisfy, using shared or separate machinery, or simply a coincidence of two independently evolved systems, is a question this review returns to directly once the TERRA/RAD51AP1 circuit has been described.

The molecular anatomy of ALT

The clearest structural signature of ALT is a set of nuclear bodies known as ALT-associated PML bodies, or APBs. These are aggregates built around promyelocytic leukaemia (PML) protein that recruit telomeric DNA, shelterin components, and a wide range of DNA damage response and homologous recombination factors into a single compartment^[1]. APBs are not simply markers that happen to correlate with ALT activity; they are widely understood to be the physical sites where telomeric recombination itself takes place, clustering multiple telomeres together so that one chromosome end can use another as a template for extension. Their presence is one of the most reliable diagnostic features distinguishing ALT-positive from telomerase-positive tumour cells.

A second, complementary hallmark is biochemical rather than cytological: the C-circle. Henson and colleagues showed that ALT-positive cells accumulate partially single-stranded, C-rich circular telomeric DNA that can be amplified and quantified even from small amounts of genomic material^[2]. The C-circle assay has become the standard biomarker for ALT activity in both cultured cells and clinical tumour samples, precisely because it captures a molecular by-product of the recombination process itself rather than a downstream or correlative feature. Where APBs tell a structural story about where ALT happens, C-circles tell a biochemical story about what ALT leaves behind.

Mechanistically, ALT is best understood as a form of break-induced replication, a homologous-recombination pathway normally used to repair one-ended DNA double-strand breaks^[4]. At an ALT telomere, one chromosome end invades the duplex of another telomere, using it as a template to copy new repeats, in a process that can extend for very long stretches and is intrinsically more error-prone and more replication-stress-inducing than telomerase-mediated extension. This explains several otherwise

puzzling features of ALT biology: the extreme heterogeneity of telomere length within a single ALT-positive cell, the elevated frequency of telomere sister-chromatid exchanges, and the tendency of ALT telomeres to generate extrachromosomal telomeric repeat DNA, including the C-circles described above. ALT is not a variant flavour of telomere maintenance so much as a chronic, low-level DNA damage response that has been repurposed to keep chromosome ends viable.

A third structural signature sits alongside APBs and C-circles: the G-quadruplex, a four-stranded DNA secondary structure that forms readily in the G-rich single-stranded overhangs and R-loops that accumulate at telomeres. Yang and colleagues showed that G-quadruplexes are markedly enriched at ALT telomeres relative to telomerase-maintained ones, tracking closely with other ALT hallmarks such as APBs and C-circles, which places G-quadruplex formation alongside recombination itself as a defining structural feature of the pathway rather than an incidental by-product^[5]. Because G-quadruplexes are also chemically tractable, stabilised or destabilised by small-molecule ligands, this structural signature turns out to matter for more than diagnosis; it resurfaces later in this review as a candidate therapeutic handle.

Resolving a break-induced replication event of this kind, rather than simply initiating one, requires the same broad toolkit of helicases and nucleases that cells use elsewhere in the genome to process recombination intermediates and restrain runaway strand invasion, including the BLM and WRN helicases and structure-specific nucleases that dismantle aberrant joint molecules before they can produce chromosome-scale rearrangements^[4]. ALT telomeres depend on this toolkit more heavily and more continuously than the rest of the genome does under ordinary conditions, because the recombination event at an ALT telomere is not an occasional repair response to accidental damage but a routine, repeated requirement for the cell simply to keep dividing. This is part of why ALT-positive cells are frequently described as existing in a state of chronic, low-level replication stress rather than experiencing DNA damage as a discrete, resolved event: the same recombination machinery is engaged, disengaged, and re-engaged at telomeres on an ongoing basis across the cell cycle, and any imbalance in the availability or activity of the resolving factors described above has the potential to convert a productive elongation event into a destructive one.

For some years, ALT was treated as functionally synonymous with loss of the chromatin remodelling factors ATRX and DAXX. This association was established properly by Lovejoy and colleagues, working through the ALT Starr Cancer Consortium, who surveyed a large panel of ALT-positive cell lines and tumours and found ATRX loss, genome instability, and an altered DNA damage response to be recurring

hallmarks of the pathway, cementing ATRX/DAXX loss as the field's working explanation for what permits ALT to occur^[6]. Their mutation is found in a substantial fraction of ALT-positive tumours, and their normal function is thought to suppress recombination at telomeric heterochromatin. De Nonneville and Reddel's 2021 analysis complicated this picture directly: a meaningful proportion of ALT-positive cancers retain wild-type ATRX and DAXX, and conversely, ATRX or DAXX loss does not by itself guarantee that a tumour will adopt the ALT pathway^[7]. ATRX/DAXX loss appears to make ALT easier to initiate, by relaxing the chromatin state that would otherwise restrict recombination at telomeres, but it is neither necessary nor sufficient. This distinction matters for the rest of this review, because it reframes the central question away from "what mutation causes ALT" and toward "what regulatory system controls whether recombination at telomeres is permitted, restrained, or actively used for elongation." That regulatory system, as the next section describes, turns out to be built substantially out of RNA.

TERRA, R-loops, and the RAD51AP1 axis

Telomeres are transcribed. The long non-coding RNA product of this transcription, TERRA (telomeric repeat-containing RNA), is transcribed by RNA polymerase II from promoters embedded in subtelomeric DNA, running inward toward the chromosome end, and is complementary to the G-rich telomeric strand. Once made, it can hybridise back onto that strand, displacing the C-rich strand and forming an RNA:DNA hybrid known as an R-loop^[8]. For a long time this was treated mainly as a curiosity of telomere transcription, a molecule whose existence was documented long before its function was clear, but work over the past several years has established that TERRA and the R-loops it forms sit close to the centre of how ALT actually works, and close to the centre of why ALT is dangerous to the rest of the genome at the same time.

Silva, Arora and Azzalin^[9] showed why the word "dangerous" is not an overstatement. Using cell lines engineered to switch ALT activity on and off, they demonstrated that the ALT pathway itself, if left unrestrained, is a direct threat to the telomeres it is supposed to be maintaining: unchecked TERRA-associated R-loops and the recombination intermediates they generate can destabilise the very chromosome ends undergoing elongation, leading to catastrophic telomere loss rather than telomere extension. ALT, in other words, is not a mechanism the cell simply tolerates as a side effect of losing telomerase; it is a mechanism that has to be actively restrained, because run to completion without restriction, it degrades the substrate it is trying to protect. This finding reframes ALT-positive cells as

continuously walking a narrow line between the genomic instability that generates opportunities for recombination-mediated elongation and the instability that would destroy telomeres outright.

Two studies published together in 2022 identified the protein that helps hold that line, and named the mechanism by which it does so. RAD51AP1, a known accessory factor for the recombinase RAD51, was already implicated in ALT-associated recombination, but Kaminski and colleagues showed that its role is inseparable from chromatin regulation of TERRA itself: RAD51AP1 helps maintain the correct chromatin-directed balance of TERRA transcription and localisation that ALT-mediated homology-directed repair requires, and disrupting this balance impairs ALT activity^[10]. Working on the same system, Yadav and colleagues resolved a further mechanistic step, showing that RAD51AP1 promotes a structural switch at ALT telomeres from an R-loop, in which TERRA is hybridised to the G-rich telomeric strand, to a D-loop, in which the RAD51-coated invading strand displaces one strand of the recombination template^[11]. Together, these two papers describe a single regulatory circuit: TERRA transcription creates the R-loop substrate, RAD51AP1 converts that substrate into a productive D-loop, and chromatin state around the telomere governs how much of this cycle is allowed to occur at any given time. Figure 1 summarises this circuit and its two possible outcomes.

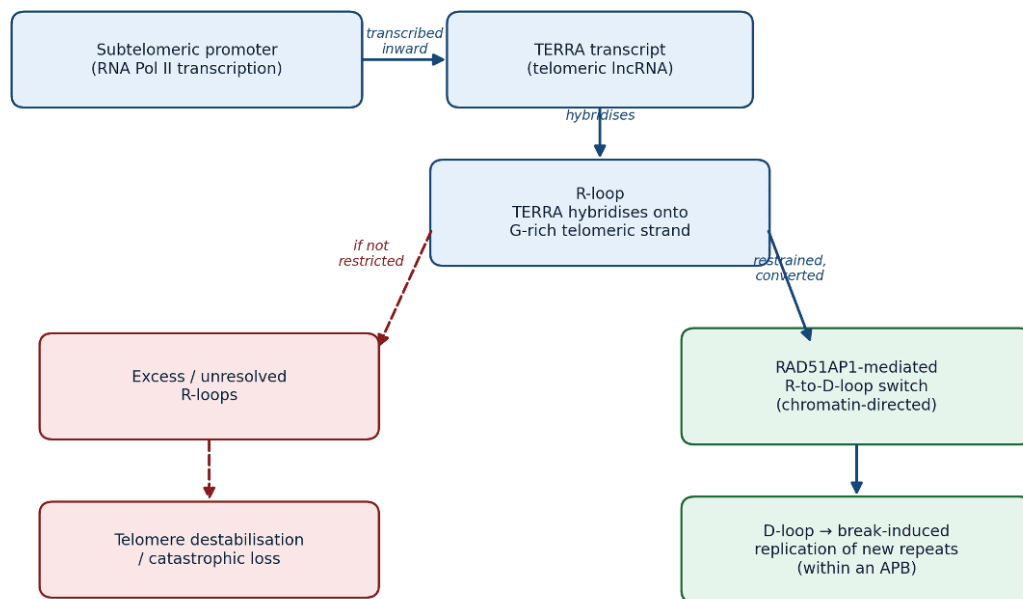


Figure 1. The TERRA-driven R-loop-to-D-loop switch that underlies ALT-mediated telomere elongation. Based on Azzalin (2025), Silva, Arora and Azzalin (2022), Kaminski et al. (2022) and Yadav et al. (2022).

Read alongside Silva, Arora and Azzalin's demonstration that unrestrained ALT activity is self-destructive, this circuit starts to look less like a single "ALT pathway" and more like a homeostat: a system whose output has to be kept within a functional range, too little activity leaving telomeres critically short, too much activity destabilising them by a different route. This is a genuinely different way of thinking about telomere maintenance in cancer than the telomerase side of the field has generally needed, because telomerase does not face an equivalent problem of its own product becoming toxic if produced in excess at the wrong time. This is a striking parallel to the telomerase-side RNA processing question raised in the introduction: MPHOSPH6, a component of the RNA exosome, shapes the 3' processing of TERC, the non-coding RNA that telomerase itself is built around, and disrupting that processing compromises the RNA component of the enzyme before telomerase ever reaches a chromosome end. ALT cells clearly have no TERC to process, since they have dispensed with telomerase altogether, but the RAD51AP1-TERRA circuit shows that they have inherited an equivalent problem in a different guise: a non-coding RNA, generated at the telomere itself, whose abundance, localisation and structural fate has to be tightly regulated for telomere maintenance to work at all. Two mechanistically unrelated pathways for solving the same underlying problem, telomerase reactivation and ALT, have each ended up depending on the careful control of a telomere-associated RNA. Whether this reflects a deep constraint on how eukaryotic cells are able to maintain chromosome ends, or simply a coincidence of two independently evolved systems, is a question worth asking directly rather than leaving as an aside, and the next section does exactly that.

TERC and TERRA: one governance problem or two?

The parallel raised above deserves to be tested rather than simply noted. If telomerase maintenance and ALT are both, at bottom, problems of policing a telomere-associated non-coding RNA, the strongest version of that claim would be that the two pathways lean on the same quality-control machinery to do it. The evidence assembled so far does not support that strong version, but it does support a real, if weaker, one.

On the telomerase side, TERC's maturation is tightly policed before the RNA is ever allowed to function. Moon and colleagues showed that the deadenylase PARN removes aberrant 3' oligo(A) extensions that would otherwise mark TERC for degradation and destabilise it; loss of PARN function causes exactly this failure and produces the short-telomere diseases dyskeratosis congenita and familial pulmonary fibrosis^[12]. Samajdar and colleagues have since resolved a further step in this quality-control checkpoint:

unstable, over-extended TERC precursors are recognised and degraded by the nuclear RNA exosome, but only once a second, independent modification, 5'-cap trimethylation carried out by TGS1, has occurred; precursors that escape trimethylation evade the exosome's MTR4-dependent recognition machinery and accumulate abnormally^[13]. Two separate post-transcriptional checkpoints, gating opposite ends of the same molecule, decide which TERC precursors survive to become part of a working telomerase enzyme.

On the ALT side, the equivalent turnover machinery has been identified only recently, and it is not the same machinery. Reiss and colleagues, working in the same laboratory that had already identified ALT's ATR-inhibitor vulnerability discussed later in this review^[14], showed that XRN2, a structurally distinct 5'-to-3' exoribonuclease rather than a component of the 3'-to-5' nuclear exosome that acts on TERC, is a key determinant of TERRA abundance specifically in ALT-dependent cancer cells, and that its activity shapes telomere stability in that context^[15]. This is a genuine quality-control system for TERRA, but it is built from a different enzymatic toolkit than the one that governs TERC.

A second point of control sits slightly later in TERRA's life cycle, once the transcript has already hybridised onto telomeric DNA rather than while it is still free in the nucleoplasm. Arora and colleagues showed that RNaseH1, an enzyme that resolves RNA:DNA hybrids by degrading the RNA strand within them, is itself a determinant of ALT telomere behaviour: depleting RNaseH1 lets TERRA-telomeric DNA hybrids accumulate, exposing single-stranded telomeric DNA and triggering abrupt telomere excision, while overexpressing it strips ALT telomeres of much of their recombinogenic character and shortens them^[16]. Where XRN2 turns over TERRA transcript directly, RNaseH1 acts on TERRA only once it is already base-paired to telomeric DNA, so ALT cells appear to hold TERRA's abundance and structural fate in check at more than one stage, echoing the two-checkpoint picture already described for TERC.

That distinction matters for how the original question should be answered. If the same nuclease complex handled TERC and TERRA, the parallel raised earlier in this review would point to a single, literally shared regulatory pathway repurposed by two different telomere maintenance routes. What the evidence instead shows is structurally separate decay systems on each side, the exosome and PARN policing TERC, XRN2 and RNaseH1 policing TERRA at two different stages of its life cycle, all converging on the same functional requirement: a chromosome-end-associated non-coding RNA whose levels and processing cannot be left unregulated without the RNA itself becoming a liability. This is closer to convergent evolution on a shared design problem than to one piece of machinery doing double duty. It does not resolve the deep-constraint-versus-coincidence question raised above, but it does sharpen it, from asking whether ALT and telomerase share an RNA-quality-control pathway to asking why eukaryotic

cells appear to have needed one, in some form, on both sides of the telomere maintenance divide, and whether the specific enzymatic solution differs by pathway for a functional reason or simply because different molecular parts were available to be repurposed in each case.

Whether the exosome itself has any role at ALT telomeres alongside XRN2 and RNaseH1, rather than none at all, remains untested to date; existing work has established the latter two as sufficient to regulate TERRA in ALT cells without yet ruling the exosome in or out as a further layer of control. Settling that question directly, for instance by testing whether exosome components or its MTR4/TGS1 recognition machinery physically associate with or process TERRA in ALT-positive cells, would determine whether the two pathways' RNA-governance systems are entirely separate inventions or share at least a partial toolkit beneath their different primary nucleases.

Genomic instability and cell dynamics

The consequences of this RNA-driven recombination circuit are not confined to the telomere itself. ALT telomeres are sites of chronic replication stress: their repetitive, G-rich sequence is intrinsically difficult to replicate, behaving like the fragile sites found elsewhere in the genome and requiring dedicated shelterin components such as TRF1 simply to complete replication without breaking, as Sfeir and colleagues demonstrated for telomeres generally^[17]. The recombination intermediates generated during ALT elongation specifically, R-loops, D-loops, and the extrachromosomal circles that spin off from them, add a further, ALT-specific layer of physical obstacles to replication fork progression on top of this baseline fragility, going well beyond what telomerase-maintained telomeres experience^[4]. This is one reason why ALT-positive cells show such pronounced telomere length heterogeneity even within a single nucleus: individual chromosome ends are being repaired, extended, or partially degraded on an ongoing, asynchronous basis rather than through the more uniform, processive addition that characterises telomerase activity.

This asynchrony has consequences that extend into mitosis. Recombination intermediates that are not fully resolved before a cell divides can persist as unresolved joint molecules or as fragile sites prone to breakage during chromosome segregation, contributing to the anaphase bridges and lagging chromosomes that are common cytological features of ALT-positive tumour cells. Because APBs cluster multiple telomeres from different chromosomes into a single recombination-competent compartment, errors in resolving this clustering can also generate telomere fusions between chromosomes that were never adjacent, a distinct route to structural rearrangement compared with the fusion events seen in

telomerase-driven crisis. These unresolved intermediates do not only threaten the telomere at which they arise; a chromosome entering mitosis with an unresolved recombination structure at one end carries a physical connection to another chromosome that ordinary segregation is not built to handle, and forcing that connection apart can itself generate breakage at a site distant from where the original recombination event occurred. Cell-cycle checkpoints that would ordinarily delay mitosis until DNA damage is resolved provide only partial protection here, because the signal generated by an ALT telomere in the middle of a slow, recombination-based extension is not always distinguishable from the routine signal generated by a telomere undergoing its ordinary, non-pathological maintenance. The net effect is that ALT-positive cancer cells tend to carry, and continue to generate, higher levels of structural genomic instability across their genome, not only at their telomeres, than most telomerase-positive cancer cells of comparable type. This is consistent with the broader framing of ALT as a chronic, low-grade DNA damage response rather than a discrete maintenance event: the same recombination machinery that elongates chromosome ends is, by its nature, a machinery that occasionally gets the wrong answer, and a cell dividing every day for years accumulates a great many attempts. This instability accounts well for the telomere-proximal phenomena that first defined ALT cytologically, extreme length heterogeneity, sister-chromatid exchange, extrachromosomal circles, and it plausibly extends to genome-wide structural variation through the mitotic segregation errors described above. It does not, on its own, explain why a cancer cell would select ALT over telomerase in the first place when a given tumour's genetic background permits either route, nor why the balance between the two routes differs so sharply by tissue of origin. That question sits upstream of the cell dynamics this section has focused on, in the domain of tumour initiation and lineage-specific chromatin state, and it remains open.

None of this instability is cost-free for the cell that carries it, which raises an obvious question: why does the ALT pathway persist as a viable route to immortalisation at all, given how much collateral damage it appears to generate? Part of the answer is simply that the alternative, telomerase reactivation, is not always available or not always selected for during tumour evolution, so a cell under selective pressure to escape senescence will use whatever route is mechanistically open to it, ALT included, even at a genomic cost. Part of the answer may also be that the instability ALT generates is not purely a liability. Genomic instability is itself a well-established driver of tumour heterogeneity and adaptive evolution, and a cell population that is continuously generating structural variation, at telomeres and, indirectly, elsewhere in the genome, may be better positioned to generate the rare variants that confer a further growth or survival advantage. Whether ALT is best understood as a tolerated cost of an otherwise necessary

maintenance pathway, or as a pathway whose instability is itself selected for because it accelerates tumour evolution, is a distinction the current literature has not cleanly resolved, and it is one of the questions this review returns to below.

A prognostic paradox

If ALT's genomic consequences were uniform across cancer types, its clinical significance would be straightforward: more instability, worse prognosis, in every tumour where it occurs. The clinical literature does not support that simple picture. Sung's 2025 review draws together outcome data across multiple tumour types and shows that ALT status carries genuinely divergent prognostic implications depending on where it occurs^[18]. In osteosarcoma, pancreatic neuroendocrine tumours, and high-grade sarcomas, ALT positivity is consistently associated with more aggressive disease and worse patient outcomes. This is not only a pattern in Sung's synthesis: a systematic review and meta-analysis by Lawlor and colleagues, pooling outcome data across soft-tissue sarcoma cohorts, found ALT positivity to be independently associated with worse survival^[19], and a large multi-institutional study of pancreatic neuroendocrine tumours by Hackeng and colleagues similarly found ALT status, alongside ATRX/DAXX loss, to be an independent predictor of poorer outcome in that tumour type^[20]. In glioblastoma and chondrosarcoma, by contrast, ALT positivity has been associated with more favourable outcomes relative to ALT-negative, typically telomerase-positive, tumours of the same type. Table 1 summarises this split.

Cancer type	Prognostic association with ALT positivity
Osteosarcoma	Worse prognosis / more aggressive disease
Pancreatic neuroendocrine tumours	Worse prognosis
High-grade sarcomas	Worse prognosis
Glioblastoma	More favourable prognosis
Chondrosarcoma	More favourable prognosis

Table 1. Alternative Lengthening of Telomeres carries opposite prognostic associations depending on tumour type^[18].

This divergence is difficult to explain from the ALT mechanism alone, because the core molecular machinery, APB formation, TERRA-driven recombination, break-induced replication, does not appear to differ fundamentally between an ALT-positive osteosarcoma and an ALT-positive glioblastoma. The more plausible explanation is that ALT's clinical impact depends heavily on the tissue and tumour context in which it occurs: the same underlying instability may accelerate progression in one cellular background and, in another, may push cells into replicative crisis or improve their response to particular treatments before they can expand into more aggressive clones. This is a genomic-instability-versus-selective-environment problem rather than a purely molecular one, and it points directly at the tumour microenvironment as a variable that the ALT literature has generally under-examined relative to how thoroughly it has mapped the pathway's biochemistry. A recombination-based telomere maintenance mechanism does not operate in isolation from the immune, stromal, and metabolic conditions surrounding the tumour, and there is little reason to expect that a chronic source of DNA damage and structural variation would have the same downstream consequences in every microenvironmental context. Resolving why ALT is a marker of aggression in some cancers and a marker of relatively better prognosis in others will likely require moving past cell-intrinsic mechanism and toward tumour-level and microenvironmental analysis. The next section takes up that question directly.

Targeting ALT: a therapeutic vulnerability

The same chronic replication stress that makes ALT-positive cells genomically unstable also makes them, in principle, more dependent than other cells on the DNA damage response pathways that manage replication stress. Flynn and colleagues tested this directly and found that ALT-positive cancer cells are markedly more sensitive than telomerase-positive cells to inhibitors of ATR, the primary kinase that coordinates the cellular response to replication stress and stalled replication forks^[14]. Because ALT telomeres generate an unusually high baseline load of replication stress through the mechanisms described above, ATR inhibition appears to tip these cells past a threshold that telomerase-positive cells, with a comparatively lower baseline burden, can tolerate. This is a form of synthetic lethality: not a specific genetic mutation being exploited, but a chronic physiological state, generated by the ALT pathway itself, being pushed to a point where the cell can no longer cope.

Mechanistically, this sensitivity follows fairly directly from the replication-stress biology described earlier in this review. ATR is the principal kinase that detects stalled or damaged replication forks and,

through its downstream effector CHK1, slows the cell cycle to allow those forks time to be resolved rather than collapsing into breaks. A telomerase-positive cell experiences this kind of stalling only occasionally, so losing ATR activity removes a safety mechanism that is rarely called upon. An ALT-positive cell, by contrast, is generating fork-stalling recombination intermediates at its telomeres continuously, simply by virtue of maintaining them, so the same loss of ATR activity removes a safety mechanism the cell is depending on essentially all the time. The appeal of this approach is that it turns ALT's central liability into a targetable feature rather than something oncology simply has to work around. Where the field still has ground to cover is in understanding how consistent this vulnerability is across the range of tumours that use ALT. The prognostic paradox described in the previous section is a warning sign here: if ALT's downstream consequences differ this much by tissue context, there is no strong reason to assume that its sensitivity to a given therapeutic strategy will be uniform either. A treatment approach validated in one ALT-positive cell line or tumour type cannot be assumed to generalise cleanly to another without direct testing, and the mechanistic circuit described in the TERRA/RAD51AP1 section above, in particular the idea that ALT activity has to be kept within a functional range rather than simply maximised or minimised, suggests that resistance mechanisms might plausibly emerge through cells shifting the balance of that circuit rather than through a conventional mutation. This remains, for now, a more open question than the initial synthetic-lethality finding might suggest.

ALT and the tumour microenvironment

The prognostic paradox described above is, at its core, a claim that the same molecular pathway behaves differently depending on where it occurs. That claim only becomes explicable once ALT's genomic instability is considered alongside the immune and stromal environment it occurs within, rather than as a purely cell-intrinsic property. The starting point for that connection is a general feature of genomic instability, not one specific to ALT: fragments of chromatin that fail to be properly incorporated into daughter nuclei during division form micronuclei, and Mackenzie and colleagues showed that these micronuclei are directly surveilled by cGAS, a cytosolic DNA sensor that, once triggered, activates the STING pathway and downstream innate immune signalling^[21]. A cell generating chromosomal instability is not doing so invisibly; it is producing a molecular signal that the innate immune system is built to detect.

ALT telomeres are a plausible, continuous source of exactly this kind of signal. Ebata, Loo and Takahashi's review of telomere maintenance and the cGAS-STING pathway lays out the mechanistic case directly: the

chronic replication stress, unresolved recombination intermediates, and micronuclei formation associated with telomere dysfunction, ALT-driven or otherwise, are well-positioned to activate cGAS-STING signalling, and the pathway is increasingly recognised as a node connecting telomere biology to the tumour immune response rather than a bystander to it^[22]. If ALT-positive cells are, as earlier sections of this review have argued, in a state of near-continuous low-level genomic instability, they should be expected to generate cGAS-STING activity more or less continuously as well, rather than in the discrete, resolved bursts that a telomerase-positive cell would produce only occasionally.

Whether that continuous signal helps or harms the tumour is not a settled question, and this is precisely where the tumour microenvironment stops being a side detail and becomes central to interpreting ALT's clinical behaviour. Bakhoun and colleagues showed that chromosomal instability does not straightforwardly trigger productive immune clearance; instead, chronic, low-level cGAS-STING activation can be rerouted through non-canonical signalling toward an inflammatory, pro-metastatic state, actively assisting tumour spread rather than provoking rejection^[23]. This is the mechanistic seed of an explanation for the prognostic paradox: the same chronic innate-immune signal that a well-surveilled tissue environment might use to help clear an unstable, ALT-positive clone could, in a different tissue context, be co-opted to promote the aggressive behaviour seen in ALT-positive osteosarcoma and high-grade sarcoma. Consistent with this, Sung and Kim's recent analysis of the telomere-metabolism-immunity axis in sarcoma describes specific immune evasion mechanisms, and correspondingly specific therapeutic strategies, that operate in that tissue context, treating telomere maintenance status as inseparable from the metabolic and immune conditions of the sarcoma microenvironment rather than as an independent variable layered on top of it^[24]. Tellingly, this is the same tissue type, high-grade sarcoma, where ALT positivity carries the worst prognosis in Sung's earlier cross-cancer synthesis, which is at least consistent with a microenvironment that channels ALT's instability toward aggression rather than clearance.

None of this amounts to a demonstrated, ALT-specific causal chain running from TERRA-driven recombination through cGAS-STING activation to a particular clinical outcome; the papers reviewed in this section establish the general mechanism (cGAS-STING senses genomic instability), a plausible telomere-specific bridge (that mechanism plausibly engages with ALT telomeres), a reason the same signal could cut either way (immune clearance versus inflammation-assisted spread), and one tissue-specific case study consistent with the aggressive side of that split (sarcoma). What would settle this is a direct, testable comparison rather than a further plausibility argument: measuring cGAS-STING pathway

activity and the immune infiltrate it is associated with, directly in ALT-positive tumours from tissue types on both sides of the prognostic paradox. If ALT-positive osteosarcoma and high-grade sarcoma show evidence of the inflammatory, non-canonical STING signalling that Bakhoun and colleagues associate with metastasis-promoting instability, while ALT-positive glioblastoma does not, that would be direct tumour-level evidence for the tissue-context explanation proposed here, rather than the plausibility argument it currently is. That gap, alongside the population-averaging problem raised in the next section, defines much of what remains to be done in this field.

The central gap

Bringing these threads together, the ALT literature has made substantial and genuinely fast-moving progress on the molecular biochemistry of the pathway. The R-loop-to-D-loop switch governed by RAD51AP1, and the demonstration that this switch has to be restrained rather than simply activated to avoid destabilising the telomeres it elongates, represent a real advance in mechanistic resolution over where the field stood even five years ago, when ALT was still substantially defined by its association with ATRX/DAXX loss. What has not kept pace with this molecular progress is an equally resolved account of how this fine-grained, largely cell-autonomous circuitry maps onto the clinical and tumour-level behaviour of ALT-positive cancers. The prognostic paradox is the clearest symptom of this gap: a single molecular pathway producing opposite clinical associations in different tissue contexts is not something the current mechanistic literature, built almost entirely on engineered cell lines rather than tumour-context comparisons, is positioned to explain.

A second, related dimension of the gap concerns timing and heterogeneity within a single tumour rather than only between tumour types. Almost everything known about the TERRA/R-loop/RAD51AP1 circuit comes from population-level biochemistry, averaging across many cells and, often, across an unsynchronised cell cycle. But the mechanism itself is explicitly framed as a balance that has to be held within a functional range at each individual telomere, at each division. Whether that balance is held with equal success across all the cells in a real, growing tumour, or whether some sub-population of cells is tipping toward destructive over-recombination while others sit closer to the productive centre of the range, and what determines which cells end up where, is essentially unaddressed by the current literature. This is not a minor gap: if ALT activity is heterogeneous within a tumour in this way, it would help explain both the tumour-level prognostic paradox and the uncertain generalisability of ATR-inhibitor sensitivity, because a therapy targeting the pathway's chronic replication stress would be

expected to act unevenly on a population that is not uniformly stressed to begin with. Closing this gap will require the field to move from the engineered, largely clonal cell-line systems that have generated the mechanistic insight described in this review, toward tools capable of resolving ALT activity at the level of individual cells within intact, heterogeneous tumours.

A third dimension of the gap, raised in the previous section, is that the field has general mechanistic pieces, cGAS-STING as a sensor of genomic instability, its capacity to be redirected toward an inflammatory, pro-metastatic state rather than clean immune clearance, and at least one tissue-specific account of telomere-immune-metabolic crosstalk in sarcoma, without yet having connected them into a demonstrated, ALT-specific causal account. The prognostic paradox, the within-tumour heterogeneity problem, and the tumour microenvironment may not be three separate gaps but one: a single unresolved question about whether a given ALT-positive tumour's local environment converts chronic innate-immune signalling into clearance or into aggression, and whether that conversion differs systematically enough by tissue and by cell-to-cell variation in ALT activity to produce the clinical picture this review has described.

Future directions

Several concrete lines of investigation follow directly from the gap described above. Live-cell imaging of APB formation and resolution, tracked against cell cycle stage in real time rather than inferred from fixed-cell snapshots, would establish directly whether the R-loop-to-D-loop switch and its associated risk of over-recombination are tied to specific windows of the cell cycle, and whether cells that mistime this switch are more likely to undergo the catastrophic telomere loss described by Silva, Arora and Azzalin. This would connect the biochemistry of the RAD51AP1 circuit to the cytological hallmark that has defined ALT since it was first described.

Single-cell approaches applied directly to ALT-positive tumour material, rather than to cultured cell lines, represent the most direct route to testing whether within-tumour heterogeneity in ALT activity is real and clinically meaningful. Combining single-cell measurements of TERRA abundance or R-loop burden with matched clinical outcome data, across the tumour types where Sung's review identifies opposing prognostic associations, would help establish whether the paradox reflects genuinely different molecular behaviour of the pathway by tissue, or a shared molecular behaviour whose consequences are simply filtered differently by different microenvironments and immune contexts.

On the therapeutic side, systematically testing ATR-inhibitor sensitivity across a genetically and tissue-diverse panel of ALT-positive lines, rather than relying on the original demonstration in a narrower set of models, would clarify how general the vulnerability Flynn and colleagues identified actually is, and would start to reveal whether resistance arises through the kind of circuit-rebalancing this review has proposed as a plausible mechanism. The G-quadruplex enrichment at ALT telomeres described earlier is a second structural vulnerability worth testing alongside ATR inhibition rather than instead of it, given that G-quadruplex-stabilising ligands and ATR inhibitors act on related but not identical sources of replication stress; whether combining the two produces an additive or synergistic effect specifically in ALT-positive cells has not, to my knowledge, been directly tested.

Finally, the RNA-governance question raised earlier in this review points to a specific, tractable experiment: testing directly whether nuclear exosome components, or its MTR4/TGS1 recognition machinery, physically associate with or process TERRA in ALT-positive cells alongside XRN2, and whether interfering with that additional layer, if it exists, shifts the R-loop/D-loop balance in a way that could be therapeutically exploited. A further line worth pursuing directly is the uneven tissue distribution of ALT noted in the introduction: comparing the chromatin state and transcriptional regulation of subtelomeric regions across the tissue types where ALT is common against those where it is rare might clarify whether some lineages simply start closer to the chromatin conditions the pathway requires, which would turn an observation currently treated as background epidemiology into a testable mechanistic question in its own right.

Conclusion

Alternative Lengthening of Telomeres is not simply a rarer, recombination-based substitute for telomerase. It is a pathway in which the maintenance of chromosome ends and the generation of genomic instability are mechanistically fused, coordinated by a telomere-associated RNA, TERRA, whose transcription, R-loop formation, and RAD51AP1-mediated conversion into a productive D-loop must be held within a narrow functional range to avoid destroying the very telomeres it is meant to protect. This molecular picture has become considerably sharper over the past several years, to the point that ALT is now better understood as a homeostatic circuit than as a simple consequence of ATRX or DAXX loss. Read alongside the telomerase side of telomere biology, this also reframes what maintaining a telomere requires in general: both of the cell's two routes to indefinite division depend on dedicated, if mechanistically distinct, machinery for policing a chromosome-end-associated non-coding RNA, TERC

on one side and TERRA on the other, and neither pathway is safe to run without that policing in place. What remains far less resolved is how this circuit's behaviour in an engineered cell line relates to its behaviour across the genuinely divergent clinical outcomes ALT produces in different tumour types, and to the therapeutic strategies, such as ATR inhibition, that aim to exploit it. Part of that resolution will have to come from outside the telomere itself: the same instability that drives ALT's genomic consequences is also, plausibly, a continuous source of innate-immune signalling through the cGAS-STING pathway, and whether a given tumour's microenvironment channels that signalling toward clearance or toward the kind of inflammation-assisted spread seen in other genomically unstable cancers may be a large part of why ALT's clinical face differs so sharply by tissue. Closing that gap between molecular precision and clinical, cellular, and microenvironmental heterogeneity is likely to be where the next substantial advances in this field are made.

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