

Review of the doctoral thesis '*Mechanistic studies on selected novel anticancer compounds modulating the function of telomeric proteins*'

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Overall assessment: this is an excellent, substantial doctoral thesis that makes a valuable experimental contribution to the study of telomere-associated cancer vulnerabilities. Its strengths are the breadth of the biological investigation, the use of complementary methods and the careful comparison of compounds and cellular responses. The results provide a strong foundation for further mechanistic and translational work.

I am commenting on the thesis section by section and provide comments intended to strengthen presentation and interpretation, while recognizing the considerable achievements of the thesis.

1) Introduction

Scientific rationale and presentation

The introduction establishes a convincing rationale for exploring telomere protection as a cancer vulnerability. A particularly strong feature is the distinction between maintaining telomere length and protecting chromosome ends. This gives the reader a clear explanation of why disruption of shelterin function could affect cancer cells before extensive telomere shortening becomes detectable. The account of TRF1, TIN2 and shelterin organization provides an appropriate biological foundation for the experimental outline.

The literature review demonstrates substantial engagement with the field, drawing on foundational work and recent structural studies. It connects telomere replication, DNA-damage signaling and cellular outcomes in a way that supports the research question. Recognition that normal proliferating cells may also be affected is welcome and helps place the therapeutic rationale in a realistic context.

The main opportunity for improvement is editorial focus. Some explanations of shelterin connectivity, cancer persistence and the TRF1–TIN2 rationale recur across the opening chapters. Reducing repetition would make the central research gap and objectives more prominent. A brief introduction to the biological differences between HeLa and MCF7 would also help prepare the reader for the contrasting responses observed later.

References, grammar and readability

The extensive bibliography is a strength, although a targeted citation check would improve accuracy. In Section 3.4.3, reference 113 concerns telomerase recruitment rather than the stated TRF1 recruitment to damage sites; reference 112 appears to be the relevant study. In Section 3.8.2, reference 170 should be replaced with the appropriate Mukherjee et al. study for the TRF2–IL1R1 claim. References 53 and 73 appear to be preprint and published versions of the same work, for which the published version should normally be preferred. These are correctable points within an otherwise substantial literature foundation.

The thesis is generally understandable and communicates a complex project successfully. A final language edit would improve fluency and precision. Examples include the repeated wording about reduced TRF1 occupancy in Section 3.4.1, and an incomplete sentence beginning “began to frame RAP1” in Section 3.8.1. Consistent gene and protein notation and replacement of conversational wording such as “Anyway” would further polish the text. These issues concern presentation and do not diminish the overall value of the experimental work.

2) Results

Breadth and coherence of the experimental work

The results are the strongest part of the thesis. The study moves from screening across 24 cell lines to a detailed biological investigation using complementary approaches. Binding experiments, microscopy, flow cytometry, DNA fiber preparation and analysis, telomere FISH, protein analysis, RNA sequencing, spheroids and embryo-based models together provide a substantial body of evidence. This breadth allows the candidate to evaluate several dimensions of compound activity instead of relying on a single viability assay.

The comparison of HeLa and MCF7, together with extended time courses, is particularly useful. It shows that responses develop over time and depend on cellular context. The work also demonstrates that the derivatives have distinct activity profiles: TRFiN49 performs well in several long-term and complex-model assays, TRFiN72 shows favorable potency and selectivity in selected screening comparisons, and TRFiN41 produces pronounced cellular effects. These differences are scientifically informative and provide a sound basis for discussing lead selection. The inclusion of non-cancer comparators, less active derivatives and orthogonal measurements strengthens the interpretation. MTT results are supported by other growth and cell-fate assays, which is especially helpful when compounds also affect mitochondrial function. The thesis appropriately recognizes that selectivity is context dependent. TRFiN72’s favorable comparisons in selected breast and colon models are encouraging, while sensitivity in other non-cancer models means that a broader therapeutic window still needs to be established. This is a sensible direction for further investigation.

Evidence for telomere involvement

Binding to purified TRF1, increased DNA-damage signals and structural telomere abnormalities form a coherent basis for investigating a shelterin-related mechanism. The metaphase telomere findings in Figure 5.23 are especially valuable because they provide chromosome-level evidence that complements the cellular stress measurements. The combination of these readouts supports the biological relevance of the project and makes the proposed mechanism worthy of further study.

The next step is to distinguish TRF1 binding from direct inhibition of the TRF1–TIN2 interaction in cells. The available evidence supports the proposed model but does not yet establish all of its causal steps. The discrepancies between kinetic and steady-state SPR estimates in Table 5.1 also deserve a clearer explanation of fitting quality and uncertainty. The thesis already recognizes possible surface-density and rebinding effects. Presenting these limitations alongside the

binding results would make the mechanistic argument more persuasive without reducing the importance of the findings.

TIN2- γ H2AX overlap is useful supporting evidence, but a whole-nucleus correlation coefficient is different from a count of DNA-defined telomere dysfunction-induced foci. Future colocalization of telomeric DNA with γ H2AX or 53BP1 would strengthen localization independently of shelterin protein organization. Similarly, the combined TRF1/2 antibody limits protein-specific interpretation. These considerations help define how the present results can be extended rather than undermine the evidence of telomere abnormalities.

Replication measurements

The DNA-fiber experiments add an important functional dimension, although one interpretation needs clarification. Sections 4.9 and 5.7 describe IdU followed by CldU with equal pulse durations. A reduced IdU/CldU ratio alone does not establish a decrease in absolute fork speed. The pulse order, ratio labels and conclusion should therefore be reconciled, ideally using the individual tract lengths. Because the measurements follow prolonged exposure and are not telomere-specific, they support altered genome-wide replication behavior rather than independently proving an early telomeric initiating event.

Cell fate and biological consequences

The characterization of cell fate is another substantial achievement. Senescence-associated β -galactosidase activity, enlarged morphology, Lamin B1 changes and reduced proliferation provide several complementary indicators of a senescence-like response. Apoptosis-related measurements and mitochondrial analyses further show that the compounds can produce different outcomes across time and cellular context. Together, these experiments give a much richer account of activity than growth inhibition alone.

The distinction between durable senescence and reversible stress arrest could be developed further through compound washout and recovery experiments. This would clarify the persistence of the phenotype and its relationship to clonogenic suppression. The colony assay is valuable evidence of reduced reproductive capacity; specifying the exact exposure duration and distinguishing colony number from colony size would improve its reproducibility and interpretation.

The mitochondrial experiments are strengthened by the use of multiple approaches. They establish treatment-associated mitochondrial injury, while its position in the causal sequence remains an interesting question. Early mitochondrial changes mean that a parallel contribution should remain possible alongside a downstream response to telomere damage. In MCF7, the absence of caspase-3 does not exclude a role for caspase-7, so the explanation for omitting the combined caspase-3/7 assay could be refined. Likewise, AIF-dependent death would require direct localization and functional evidence. These are useful mechanistic distinctions for the defense.

The interpretation of acidic-vesicle staining is appropriately cautious: the thesis does not equate this readout with proof of autophagic flux. This illustrates a good awareness of assay limitations. Similar care would benefit the cell-cycle analysis. The 12-hour BrdU interval measures DNA

synthesis over time, so it should be interpreted together with DNA content rather than as a simple instantaneous S-phase fraction. The unusually low reported untreated HeLa S-phase value deserves clarification through representative gating and culture conditions.

Complex models and molecular profiling

Spheroids and embryo-based microtumour experiments extend the findings beyond conventional two-dimensional culture and increase their biological interest. The antiangiogenic observations and reduction of tumour-related endpoints are promising exploratory results. The use of mitomycin C in wound-closure experiments shows attention to proliferation as a confounding factor, although its own effects should also be considered. These assays are most convincing when described according to what they directly measure: wound closure, spheroid growth, developing vasculature and short-term tumour-model responses.

The RNA-sequencing analysis adds depth to the comparison of compounds and cell lines. It offers useful hypotheses about stress responses and changes in proliferation-related programmes. Since sampling occurs at 168 hours, these signatures are best interpreted as features of the established response rather than direct evidence of the initiating mechanism. Earlier sampling would be a valuable extension. Clear reporting of replicate numbers, quality control and validation would further strengthen this part of the thesis.

A final consistency check would improve the presentation of this extensive dataset. The general statement of three independent experiments should be reconciled with figures reporting $n = 4$, and the meaning of n should be explicit for cells, fibers and biological replicates. Differences in control baselines between γ H2AX experiments merit explanation. Some densely populated figures would benefit from larger labels and a clearer emphasis on the principal effect sizes (e.g. Fig. 5.26). These practical improvements would make the results easier to evaluate and the thesis easier to defend.

3) Discussion

Scientific contribution and integration

The discussion brings together a large and technically varied dataset and demonstrates a good understanding of the biological questions. Its principal contribution is the integrated characterization of a series of compounds with effects on cancer-cell growth, DNA-damage responses, telomere integrity and cell fate. The comparison across derivatives and cellular contexts helps identify both shared responses and meaningful differences. This provides a useful platform for subsequent work on shelterin-associated therapeutic strategies.

Several aspects show constructive critical awareness. The thesis acknowledges context-dependent selectivity, limitations of the combined TRF1/2 antibody, the late timing of transcriptomic measurements and differences between SPR fitting approaches. Recognition that wound closure depends on more than migration, and that Cyclin D1 protein and CCND1 transcript changes do not necessarily agree, is welcome. Such observations make the discussion more credible and show that the candidate can engage with complexity rather than simply summarize favorable results.

The discussion would be even stronger if it organized the mechanistic argument into three levels: what was directly observed, what the observations support and what still requires experimental confirmation. Purified-protein association and structural telomere abnormalities are important observations. They support a plausible role for shelterin perturbation. Direct cellular TRF1–TIN2 inhibition and its necessity for the full phenotype remain questions for targeted validation. Wording such as “consistent with” or “supports the hypothesis” would accurately reflect this distinction.

Alternative explanations can be presented as productive avenues for discriminating experiments. General replication stress, mitochondrial effects or other cellular targets could contribute to some of the observed responses. Establishing their relative roles would refine the model. The use of each compound’s 72-hour IC50 provides a practical basis for comparing phenotypes, while acknowledging that exposure and population composition may differ later. A shorter discussion focused on these key questions would also reduce repetition of numerical results.

Conclusions

The conclusions are strongest when they emphasize the demonstrated biological achievements: selected derivatives associate with purified TRF1, inhibit cancer-cell growth, produce DNA-damage responses and are linked to structural telomere abnormalities and altered cell fate. These findings are substantial and justify further investigation. The observation that functional abnormalities occur without extensive bulk telomere shortening is consistent with the rationale for targeting telomere protection and helps connect the experimental results to the research question. Overall, my assessment is positive. The thesis represents a substantial research effort, demonstrates competence across a wide range of biological methods and delivers interesting findings with clear potential for follow-up. Its principal limitations concern the degree of mechanistic certainty and some aspects of assay interpretation and presentation. These are important points to clarify, but they do not outweigh the strengths of the work. The thesis provides a good basis for a constructive scientific defense, particularly if the candidate clearly distinguishes established findings from the proposed causal model.

4) Outlook

Promising directions for further research

The thesis opens several worthwhile directions. The most informative next step would be direct validation of TRF1–TIN2 interaction inhibition, followed by testing its contribution to the cellular phenotype. Purified interaction assays, cellular target-engagement measurements, endogenous tagging and a suitable rescue strategy offer complementary approaches. This would build directly on the existing binding and biological evidence and could substantially strengthen the mechanistic contribution.

Earlier measurements at sublethal exposures would help establish the sequence of events. DNA-based telomere damage assays, clarified fiber measurements and early molecular profiling could distinguish initiating changes from the consequences of prolonged stress and growth arrest. Recovery after washout would add an important functional test of the persistence of

senescence-like phenotypes. Comparisons with other cytostatic or genotoxic treatments would help identify features specific to the compound series.

The proposed organoid studies, dose-response experiments and combination treatments are sensible extensions. Lead selection should integrate potency, normal-cell responses, binding confidence and performance in complex models. The different strengths of TRFiN41, TRFiN49 and TRFiN72 make this a useful comparative exercise rather than a simple ranking by IC50. Broader tissue-relevant models and exposure measurements would help determine which derivative offers the best balance for further development.

The embryo experiments provide encouraging preliminary observations, while longer-term and broader models would be needed to characterize safety and therapeutic potential. Testing an ALT model would also explore whether the mechanism extends beyond the two main telomerase-positive cell lines. These directions follow naturally from the thesis and underline its value as a foundation for further research.

Five general questions for defense preparation

- 1. What is the main original contribution of your thesis and why is targeting telomere protection an attractive cancer strategy?** Explain the research gap, the contribution of this compound series and the distinction between telomere protection and length maintenance.
- 2. Which results provide the strongest support for your proposed mechanism and what experiment would most strengthen it?** Distinguish direct observations from inference and explain how cellular interaction or rescue experiments could test the model.
- 3. How do you interpret the different responses of HeLa and MCF7?** Discuss cellular context, timing and how the combined assays distinguish growth arrest, senescence-like responses and apoptosis.
- 4. What are the main strengths and limitations of your experimental approach?** Explain why complementary assays were useful, what the replication and telomere measurements establish, and how key uncertainties could be resolved.
- 5. Which compound would you prioritize for further development, and what would your next research steps be?** Balance potency, selectivity, binding evidence and complex-model activity, and identify the most useful mechanistic and translational experiments.

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