The manuscript, “Prognostic relevance of ALT-associated markers in liposarcoma: a comparative analysis,” by Venturini et al describes work assessing the prognostic significance of non-telomerase dependent telomere maintenance in human liposarcomas.

Background: Telomere maintenance appears to be required to support the large proliferative capacity of cancer cell populations. The majority of human cancers appear to maintain their telomeres through activation of the telomere synthetic enzyme telomerase, activity that, in contrast, is stringently repressed in normal tissues. Subsets of cancers exhibit a non-telomerase dependent telomere maintenance mechanism termed alternative lengthening of telomeres (ALT). ALT appears to involve telomere-telomere recombination, typically yielding an unusually broad distribution of telomere lengths which may be discerned in Southern blot assays of tumor genomic DNA. Another reported hallmark of the ALT phenotype is the presence of unique sub-nuclear structures termed ALT-associated PML bodies (APB), which are detectable in situ by combined staining for telomeric DNA by FISH, and PML protein by immunofluorescence. APBs are characterized by abnormally large, bright telomere DNA FISH signals surrounded by a shell of PML protein. Both of these methods have been used to classify tumors as either ALT+ or ALT- in previously published studies. The ALT phenotype is almost entirely restricted to cancers of mesenchymal lineage and prior reports have shown that telomere maintenance status has prognostic significance for patient survival in some settings.

Summary of findings: In the current work, surgical tissue specimens obtained from 85 consecutive adult liposarcoma patients were assessed for presence of the ALT phenotype, either by in situ staining for APB or by telomere-specific Southern blotting. Telomerase enzymatic activity was also assayed. The ability of telomere maintenance mechanism status to predict disease-specific survival was then assessed in this patient cohort having a median follow up of ~10 years.

Overall, ALT as defined by APB was present in 32% of cases, in good agreement with prior publications on the prevalence of ALT phenotype in liposarcomas. 28% were classified as ALT+ by Southern blot. 19% of cases considered ALT+ were scored as such by both methods, while 59% were scored as ALT- by both methods. Telomerase activity was detected in 35% of cases, nearly half of which were also classified as ALT+, a combined phenotype that has previously been
reported in other tumors, including liposarcomas.

Kaplan-Meier and Cox proportional hazards analysis indicated that cases displaying the ALT+ phenotype (scored by either method) exhibited worse outcome, with hazard ratios in the 2-3 range compared to ALT- cases. A similar relationship held for tumors classified as ALT+ by APB score alone, for both 10 year and 15 year survival; however, for cases classified by Southern blot alone this relationship was not statistically significant at 10 years, although it became so at the 15 year point.

Critique: Overall, the manuscript is well written, with the exception of a few relatively minor points as outlined below. The authors pose a well defined question and use appropriate methods to address it. The conclusions are supported by the data, which appears sound.

Minor Essential Revisions:

1. In the second paragraph on page 6, the sentence beginning, “To avoid false positives…” is incomplete.
2. At the top of page 7. “1,000 kb2” should read “1 Kb?”

Discretionary Revisions:

1. Inclusion of a table listing patient data and clinico-pathologic characteristics would be beneficial. This could also include the ALT status, positive or negative assessment by APB and TRF, estimated % tumor cells harboring APB (as applicable) and estimated fraction of sample composed of tumor cells.
2. Two other previously published papers examining telomerase and/or telomeres in liposarcomas should be cited (Schneider-Stock et al, Mol Carcinog. 1999 24(2): 144-, Johnson et al, Clin Ca Res. 2005 11(15):5347-).
3. In APB analysis, cases were scored as ALT+ if APBs were seen in equal or greater than 0.5% of tumor cells. Please explain the justification for this cut-off value. The APB method appears to be more sensitive than the TRF method. Only 8 of the 27 cases characterized as ALT+ are listed as being defined by TRF alone. However, it seems that even these 8 did show some level of APB detection, they just did not reach the 0.5% threshold. Is it perhaps fair then to conclude that ALT positivity might be defined by detection of any APB in the tumor? Were any APBs ever detected in any case considered ALT-?
4. In figure 1, the inclusion of arrows to point out APBs to the reader would be useful.
5. How to explain cases which have been characterized as ALT+ by APB criteria but do not show telomere length heterogeneity on TRF Southern blot (such as lane 1 in the right hand gel image of figure 1B)? As the authors point out on page 8, TRF analysis could potentially miss ALT due to admixed normal cell components, therefore is ALT positivity missed by TRF in these cases due to a low proportion of tumor cells in the samples?
6. On page 9, it is stated that results confirm the possibility of two TMMs existing in the same lesion as previously reported for other tumor types (ref# 3); this has also been shown in liposarcomas by Johnson et al (see above) and this should be cited here.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.