Peer Review File

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Reviewer comments:

Comment 1: This is an important preliminary study, though very limited in its scope. A strength of this study is that it was designed as a pre-planned correlative study as part of a larger prospective study. A clear limitation is the small number of patients included in the analysis – the breadth of this analysis is along the lines of what I would expect to see from a conference proceeding or abstract and not a publication. See my full comments to the author below.

Reply comment 1: We acknowledge the preliminary nature of our data, which is why we choose to share our experience in the format of "Letter to the Editor" rather than a full manuscript.
Changes in the text 1: none

Comment 2: In this manuscript, the authors nicely describe the problem, that is that novel approaches to monitoring treatment response and progression are needed for patients with T cell lymphomas. They utilize HTS/NGS of clonal TCRβ and TCRγ rearrangements in correlative samples collected prospectively as part of a larger prospective study. They demonstrate that clonal calibrating sequences can be detected in many patients with TCL and that those sequences could potentially be followed over time.

Reply comment 2: No response required
Changes in the text 2: none

Comment 3: One clear limitation of this study is the lack of discussion regarding their results. The authors fail to describe any significant limitations of their study, of which there are several. First, the authors do not comment on why the assay only detected calibrating sequences in 1 of 3 patients. Instead, they say that the assay detects calibrating sequences in "the majority" of TCL patients. This is an overstatement. An assay that only detects clonal sequences in ~65% of subjects is not clinically useful – I do acknowledge that this is a pilot study. What are the other technical limitations/challenges with this methodology? Why is there discordance in some of the cfDNA samples (some sequences increase in frequency while others decrease or remain stable over time; how can this be interpreted clinically)? With so few subjects in this study (N=6) and no substantive results in 1/3 of the subjects, this is very preliminary and in order for the

study to be useful, a greater emphasis would need to be placed on the discussion (limitations/technical challenges).

Reply comment 3: The comments are well received, and we agree that the discussion is limited in scope. However, we tried to address the main concerns as best as possible within the constraints of the limitations of a letter to the editor (1000 words; 10 references)

While immunosequencing has been utilized across a wide range of tissues and shown to be reproducible, it can be limited by the level of the TCR rearrangements in the given sample. Patients with lower disease burden may not have high enough frequency clones to identify a trackable disease clone and may have lower levels of cfDNA. Within this study the patients in with high disease burden we detected the relevant clones across multiple samples suggesting the utility to identify high frequency disease associated TCR rearrangements. We strongly agree that it will also be important to validate these findings in a larger study. Furthermore, the detection of a clonotype in 65% of patients is within the range of other methods to detect MRD using HTS for immunoglobulin gene segments in B-cell malignancies, as for example published by the NCI, where among 198 patients with untreated DLBCL, a tumor-specific clonotype was identified in 126 (64%) study cases (Roschewski M et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. Lancet Oncol. 2015 May;16(5):541-9).

In some patients for example, patient 1, there was a discordance between rearrangements. While we utilized an algorithm to identify high frequency potentially disease associated TCR rearrangements, it is possible that we identified rearrangements associated with multiple cells. Thus it is important to assess the pattern over time when assessing the clinical response of the patients. For example in patient 1, the highest frequency clone decreased coincident with a partial response while the two lower frequency clones were steady over time, but may reflect a potential non-disease associated rearrangements.

Changes in the text 3:
In the manuscript, we made the following revisions:

1. Our pilot project demonstrates that HTS assessment is able to identify a malignant clone that can be followed over time in ~~the majority of~~ patients with TCL.
2. However, our results are very preliminary and have several limitations. These include the failure to identify a trackable clonotype in all patients, which may be related to low frequency clones in patients with low tumor burden, as well as discordance of trackable TCR rearrangements, which may be related to presence of potential non-disease associated clones. While larger investigations will be needed ~~to validate the role of HTS in TCL~~, these preliminary data suggest that frequency of the tumor clone at baseline and reduction in frequency may be predictive of response to treatment.

Comment 4: It would be informative for the authors to include additional clinical information in Figure 1A (the table), including IPI, LDH, etc, and other potentially prognostic clinical variables.

Reply comment 4: Data on LDH, stage, and IPI (as applicable) were added to Fig 1A.
Changes in the text 4: none; however, added column for LDH, stage and IPI to Fig 1A.

Comment 5: The title of figure 1B is not accurate. This does not appear to be longitudinal data (that is figure 1C). Also, authors should comment on whether all data points represented on this figure. In other words, in the figure caption, they may want to clarify that if sequence was not detected, there is no data point for that sequence.

Reply comment 5: The title of Figure 1B was revised.
Changes in the text comment 5: We added additionally a statement to the Fig 1C caption that ..The graph only contains data points for TCR sequences that were detected.