Tissue Agnostic TMB Clinical Cut-off Harmonization Initiative

Goal of the tissue agnostic TMB clinical cut-off harmonization initiative:
To align on a minimum, or lower-bound cut-off TMB value for a pan-tumor indication that will help harmonize clinical trial design and expedite drug development by aligning on a common strategy for patient recruitment and data collection in pan-tumor drug trials.
It is important to note that although a single cut-off or equivocal zone of TMB values for clinical trial enrolment and statistical planning is being pursued as part of this initiative, the determination of the cut-off in itself will have no bearing for regulatory purposes outside of the strategic alignment for clinical trials.

Background:
Tumor mutational burden (TMB), defined as the number of somatic mutations per megabase of interrogated genomic sequence, demonstrates predictive biomarker potential for the identification of cancer patients most likely to respond to immune checkpoint inhibitors.

As multiple sponsors work independently to optimize TMB measurement for their specific therapy, it is possible that each sponsor may set different cut points for a tissue agnostic TMB cut-point (e.g., TMB ≥ 10 mut/Mb, 16 mut/Mb, 20 mut/Mb, etc.) based on how a company defines it rather than being based on biology. This is especially problematic for tissue agnostic development because it is redefining the disease based on a biomarker rather than a site of origin or pathologic disease. Agreement on a tissue agnostic cut-point, or an equivocal zone of TMB values that indicate a strong association with a biologically defined state of immune checkpoint activation will facilitate different device companies being able to market in vitro diagnostic devices that can measure the same disease state as well as inform the development of current and future clinical trials incorporating TMB.

Methods:
Friends of Cancer Research convened a focused working group composed of members of pharmaceutical and diagnostic companies, and the US. Food and Drug Administration. This working group developed a project proposal outlining considerations and parameters for a proof of concept analysis, such as necessary data elements, uniform clinical trial components, and appropriate patient populations.

First, in collaboration with working group members, *Friends* conducted a comprehensive review of publicly available and/or published studies that reported TMB and patient response rates to immunotherapies. These studies reported outcomes from several cohorts investigating immunotherapies in lung cancer, melanoma, urothelial carcinoma and pan-cancer cohorts, among others. Moreover, these cohorts investigated different lines of therapy and different immune checkpoint inhibitors alone or in combination with other agents. TMB was assessed using whole exome sequencing (WES) or gene panels.

Second, participating pharma companies provided retrospective clinical trial data for more than 1700 patients diagnosed with several different cancer types treated with various immune checkpoint inhibitors and for which TMB scores had been estimated. The TMB values provided by the sponsors consisted of WES values that had been transformed to an "FMI equivalent TMB value" or TMB values estimated by the FoundationOne assay (F1CDx). *Friends* anonymized, pooled and analyzed the data to guide the identification of a rational cut-point for defining a disease that is independent of any one drug. The data were described by cohort, by immunotherapy, and combined.

Findings:

- The review of published studies found that most of these studies used 10 mut/Mb as their cut-off so that any sample above 10 mut/Mb was categorized as TMB high. The average response rates across all trials and diseases were 33% vs. 13% for TMB high vs. non-TMB high, respectively (Figure 1).

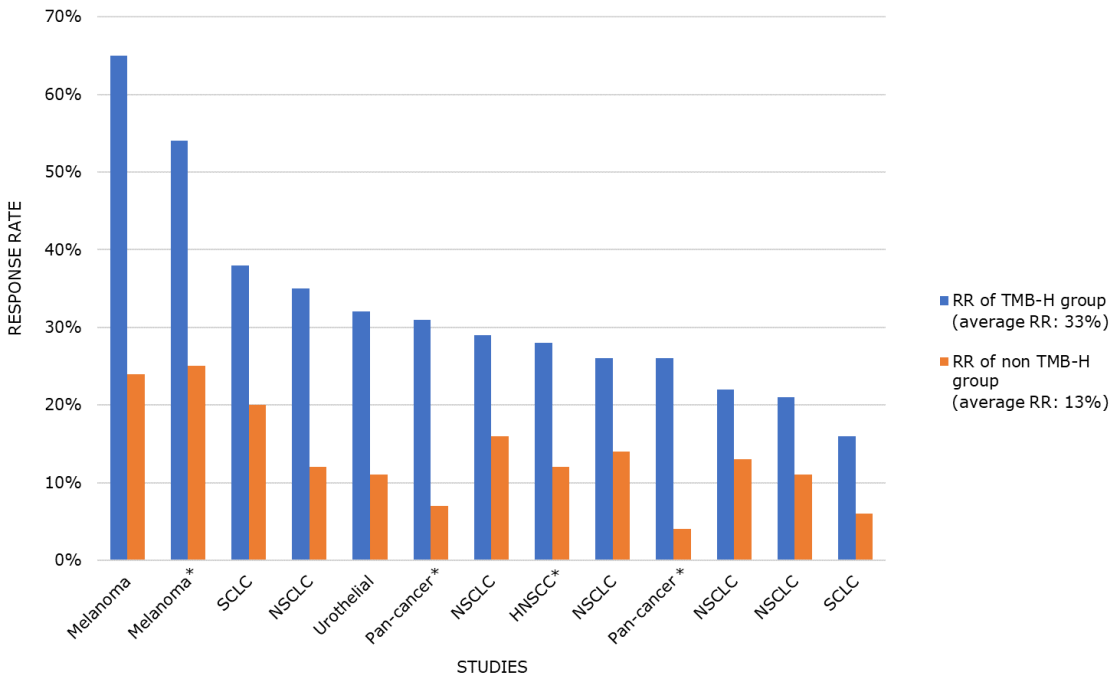

Figure 1: Response Rates for Published Studies Investigating Immunotherapies and Reporting TMB-based Response Rates.
** indicates studies that did not use TMB 10 mut/Mb as the study cut-off*

- The working group agreed on an equivocal zone of TMB values spanning 10 mut/Mb to 15 mut/Mb to further explore a pan-tumor cutoff that would be supported by biological evidence and retrospective published outcome data in several checkpoint blockade studies.
    - Biological evidence on the association between TMB and a state of immune checkpoint activation, supported by the presence of leukocyte infiltration and an inflamed tumor environment, was derived from Panda and colleagues (Panda et al., JCO Precision Oncology 2017).
- Based upon initial FDA feedback to examine additional response rates above and below the equivocal zone, *Friends* aggregated additional retrospective clinical trial data collected by pharmaceutical companies investigating PD-1/L1 targeting agents in 1732 patients diagnosed with more than 15 different cancers, ranging from the most prevalent (lung cancer, 29% of cases; metastatic urothelial carcinoma, 23%; cancer to the head and neck, 10%; and bladder cancer, 9%) to the least (prostate cancer, 1%; endometrial cancer, 1%; salivary gland cancer, 1%; and ovarian cancer, 0.35%).
- When focusing on the equivocal zone of the combined cohort, the response rate in patients with TMB ≥10 mut/Mb was 30.1%, while those with TMB <10 mut/Mb was 13.8%. Response rates in patients with TMB ≥15 mut/Mb was 37.4%, while those patients with TMB <15 mut/Mb had a response rate of 14.9% (Figure 2).

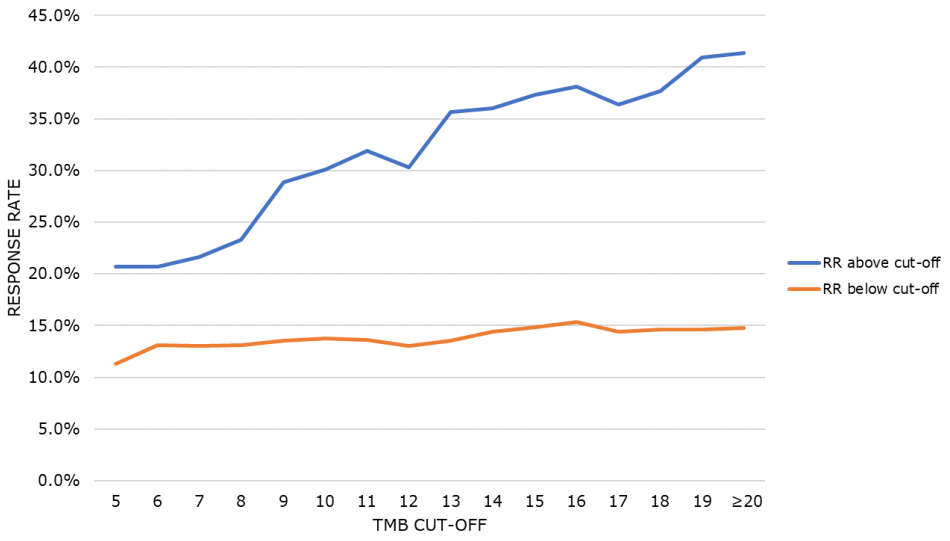

Figure 2. Average Response Rates for Patients Treated with IO Agents - Pooled Cohort

- When response rate data were evaluated by IO agent, different response rate patterns were observed. Nonetheless the response rates within the equivocal zone for all of three datasets also reflected what was observed in the published studies: generally, that response rate for TMB high (≥10 mut/Mb) ranged between 23.8%-

39.2%, while response rate for TMB low (<10 mut/Mb) ranged between 11.7%-15.3%.

- Although different assays may differ in the way TMB is estimated, results from the *Friends* TMB Harmonization Project have shown that the empirical variability in TMB values ranging between 10-15 mut/Mb is not as large as what is observed at lower or higher TMB values (Figure 3). For instance, the variability at TMB of 10 mut/Mb is ±3.4 mut/Mb.

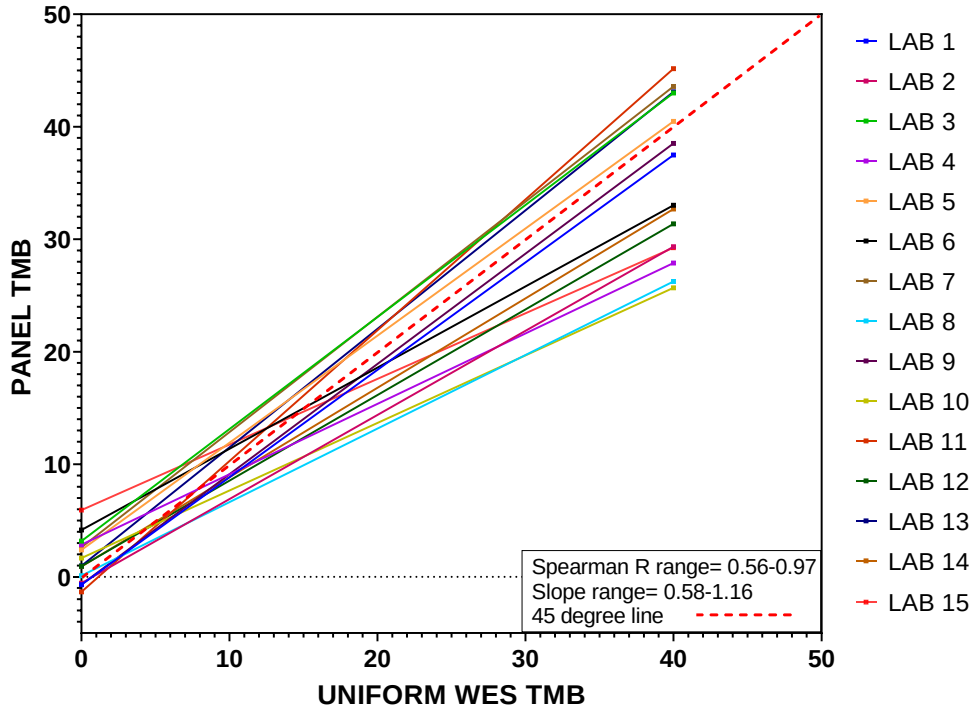

Figure 3. Association between WES-TMB and panel-TMB using human-derived matched tumor-normal cell lines. Results from the Friends of Cancer Research Harmonization Project- Phase 2A: Empirical Phase.

- After the working group observed the data, there was consistent agreement that 10 mut/Mb represented a reasonable lower bound for clinical trial enrolment for a study evaluating a pan-tumor indication. Moreover, the working group also discussed that for statistical planning purposes, this lower bound value is also reasonable for the selection of a primary endpoint.

- However, it was noted that although the group agreed that 10 mut/Mb should be defined as the minimum threshold to support clinical trial enrolment and statistical planning, this was not to have any bearing for regulatory purposes and prospectively designed clinical trial analyses would be required to validate a clinical claim.

Drug Development Strategies & Approaches

- Given the agreement on 10 mut/Mb as the lower bound of the equivocal zone for clinical pan-cancer drug trials, sponsors were encouraged to consider this value when designing trials and planning statistical analyses for their clinical trials.
- Having addressed this initial aspect of aligning on a common strategy for patient recruitment and data collection by identifying the lower bound of the TMB equivocal zone, it is expected that a large amount of clinical data will be collected, and as these data mature, further considerations should be explored, such as:
    - The need for future discussions on how the proposed equivocal zone could ultimately be included in diagnostic test labels.
    - The need for processes by which findings from other studies, such as the *Friends* TMB Harmonization Project, can be leveraged and implemented to promote alignment across different assays.