**Supplemental material**

**Supplemental methods**

**ALK and c-MET cut slide age**

For ALK and c-MET (SP44) cut slide age assessment, two multi-tissue blocks (MTBs) were prepared from 8 unique cases of NSLC of different c-MET staining intensities (0-3) representing the dynamic range of staining for the assay. All test slides used for this study were sectioned at 4 microns onto positively charged slides, and stored at 30˚C until their respective test date. Stained specimens from the study start representing the initial time point (Day 0) were used at each time point tested as reference slides for comparison purposes. For each time point, the test slides were stained in triplicate with two slides stained with the antibody under test, and one Negative Rabbit Monoclonal control slide run at each time point. Stain intensity scores were assessed by a qualified reader (see section on IHC scoring) to determine if a significant drop in staining intensity was observed for each respective time point. Time points were assessed for day 0 (the day slides were cut), up to six months of slide storage.

**Supplemental results**

**Cut Slide Age (antigen stability) for ALK and c-MET studies**

For ALK cut slide age, the intensity of the staining maintained up to 4 months of slide storage when slides were stored at 30˚C (Supplemental Table 1). On the analytical scale ALK failed after 6 months due to a drop in staining intensity of 1 point; however, for ALK when scoring for the clinical scoring algorithm the cutoff point is 3 months of slide storage for NSCLC (data not shown).

For c-MET, slides stored under ambient 15-30ºC conditions (room temperature), no change in staining intensity (greater than 1 point for staining intensity) compared to day 0 was observed for up to 5 months of slide storage (Supplemental Table 2). For theambient 15-30ºC nitrogen flow desiccator storage condition, no change in staining intensity (greater than 1point for staining intensity) was observed for up to 7 months of slide storage. Finally, for the slides stored in the -20ºC storage condition, no change (greater than 1 point for staining intensity) in the staining intensity was observed for up to 7 months.

**Supplemental Discussion**

TTF1 and EGFR, which were used as comparator biomarkers expressed in lung cancer, were unaffected by fixation type and duration of fixation (Supplemental Figures 1 & 2). These data indicate that the ALK staining intensity by IHC is impacted to a much greater extent by fixation conditions compared with other lung biomarkers (EGFR and TTF1).

The impact of cut-slide age was evaluated as it is an important consideration in a clinical research setting when slides may not be evaluated immediately after sectioning. For ALK (D5F3), acceptable staining was observed for cut slides stored at 30˚C for up to 3 months. After 3 months of storage, a loss in staining intensity was observed, indicating that target antigens were potentially compromised due to cut slide age. For c-MET (SP44) acceptable staining was observed for cut slides stored at 30˚C for up to 5 months. On the analytical scale both ALK and c-MET fail at 6 months due to a drop in staining intensity of 1 point; however, for ALK when scoring for the clinical scoring algorithm the cutoff point is 3 months (data not shown). The data presented here for cut slide age are only for the ALK and c-MET antigens as detected by the ALK (D5F3) and c-MET (SP44) assays. Different lung biomarkers may have different performances for antigen stability within cut tissue sections. Finally, the impact of cut slide age combined with different fixatives was not evaluated within this study; therefore, 3 months may not be the optimal stability for fixatives other than 10% NBF.