RESULTS AND DISCUSSION

PD-L1 expression increases after macrophage stimulation. PD-L1 was found dramatically increased after PMA stimulation (n=33 patients, 94.2%) ranging from 2 to 650 times (Figure 1A). Figure 1B shows PD-L1 levels with and without stimulation of a non-responding patient to PMA stimulus (PD-L1 fold-change is 1) and a responding patient to PMA stimulus (PD-L1 fold-change is 10), with a fold-change value of 1 and 162, respectively. This heterogeneity could be associated with a differential response to immunotherapy among patients.

PD-L1 was not found at cytoplasmic level. Variations in PD-L1 fold-change among patients led us to consider the possibility that PD-L1 was expressed at cytoplasmic level, and after stimulation, translocated to the cell membrane, in the same manner as CD11b. PD-L1 and CD11b cytoplasmic levels were simultaneously studied in 11 patients. PD-L1 was found to be undetectable, in comparison with CD11b cytoplasmic reactive antigen (Figure 2).

PD-L1 is detected differentially depending on stimulation time. The fact that PD-L1 was not present at cytoplasmic level led us to investigate changes in PD-L1 expression over time. After stimulation, PD-L1 expression was found to be higher after 1 to 5 min, with a progressive decrease up to 1h (Figure 3).

Co-incubation with DuraLumina showed different PD-L1 immunofluorescent profiles. When adding increasing concentrations of DuraLumina, PD-L1 detection by the fluorescent antibody showed different profiles among concentrations and patients. Both drug and monoclonal antibody bound in a similar PD-L1 site when the molecule had the proper conformation (Figure 4). Differences among patients could be related to structural modifications in PD-L1 molecule related with genetic mutations.

CONCLUSIONS

PD-L1 reactivity appears to result from complex interactions that can only be detected with minimal sample perturbation. Since this molecule is not found at cytoplasmic level, PD-L1 may reveal some erratic changes in response to stimulation, even for a short period of time. This conformational change may be associated with a PD-L1 immunoregulatory mechanism that may affect therapies targeting the PD-1/PD-L1 checkpoint. Critical assessment of PD-L1 expression, as well as those targets having similar unexpected features, may help to develop a better treatment strategies or to predict therapy resistance. Noxyte immunohistochemistry in combination with functional assays serve premises as an emerging strategy to model conformational changes in the target site.