

SUPPLEMENTARY METHODS

Double IHC staining for CD68 and PD-L1 was performed on selected cases within the cohort. PD-L1 staining using the rabbit anti-PD-L1 monoclonal antibody was performed first using the same protocol as above, except counterstaining was omitted at this stage. The slides were taken off the autostainer and washed in soap water and distilled water, and antigen retrieval was performed with 10mM citrate buffer, pH6.0 (Invitrogen, Carlsbad, CA) in a steam pressure cooker (Decloaking Chamber, Biocare Medical, Concord, CA) according to the manufacturer's instruction. All further steps were carried out at room temperature in a hydrated chamber. Slides were incubated with monoclonal mouse anti-human CD68 (clone PG-M1, #M0876, 0.3 µg/ml final concentration, Dako) in diluent (Dako) for 1 hour. Slides were then washed in Tris buffer and treated with MACH2 Mouse AP-Polymer (#MALP521L, Biocare Medical) for 30 minutes. After further washing, slides were incubated with Vulcan Fast Red Chromogen (#FR805H, Biocare Medical) for 20 minutes. Slides were counterstained with hematoxylin, washed in distilled water, and dehydrated in 60°C oven for 20 minutes, and briefly washed in xylene, mounted and coverslipped.

Double IHC staining for PAX5 (BSAP) and PD-L1 was performed on selected cases within the cohort. PD-L1 staining using the mouse anti-PD-L1 monoclonal antibody was performed first using the same protocol described above, except counterstaining was omitted at this stage. The slides were taken off the autostainer and washed in soap water and distilled water, and antigen retrieval was performed with 1mM EDTA, pH8.0

(Invitrogen) in a steam pressure cooker. Slides were incubated with mouse anti-BSAP/PAX5 monoclonal antibody (clone 24/Pax-5, #610863, 2.5 μ g/ml final concentration, BD Transduction Laboratories, Franklin Lakes, NJ) in diluent (Dako, Carpinteria, CA) for 1 hour. Slides were then washed in Tris buffer and treated with MACH2 Mouse AP-Polymer for 30 minutes, followed by Vulcan Fast Red Chromogen for 20 minutes. Slides were counterstained with hematoxylin, washed in distilled water, and dehydrated in 60°C oven for 20 minutes, and briefly washed in xylene, mounted and coverslipped.