Safety and Immunogenicity of LY3415244, a Bispecific Antibody Against TIM-3 and PD-L1, in Patients With Advanced Solid Tumors

Supplemental Methods

Immunogenicity Testing and Epitope Specificity Methods

Immunogenicity testing was conducted using a validated affinity capture elution (ACE)-Bridge immunoassay (Supplemental Figure 2) (20). The ACE component of the assay provides optimum drug tolerance by allowing the removal of excess free drug, release and transfer of bound ADA, and subsequent detection using biotinylated and ruthenium-labeled drug (Bridge) (29). In this assay format, streptavidin-coated 96-well plates (Pierce, 15500) were coated using 100 µL per well of biotin-LY3415244. Samples were diluted 1:20 in buffer, and 100 µL of diluted samples were added to the coated plates and allowed to incubate overnight at 4^{o}C. The following day, the captured ADA were acid eluted to permit selective transfer of the ADA to a second plate for detection. The transferred sample was mixed with a neutralizing solution containing 0.5 µg/mL each of biotin-LY3415244 and ruthenium-LY3415244. Next, ADA were allowed to bridge the labeled antibodies and the resulting complexes were captured by streptavidin plates (Mesoscale, L15SA-1). Plates were then read on a Mesoscale reader to provide the screening ADA signal (Tier 1) expressed as electrochemiluminescent units (ECLU).

Addition of excess unlabeled LY3415244 or portions of it will result in an inhibited screening ADA signal if the ADA are specific for the competing agent being added (confirmatory signal). The confirmatory inhibition (Tier 2) is then calculated using screening and confirmatory ADA signal with the following formula:

$$\frac{Screening\ ADA\ signal - Confirmatory\ signal}{Screening\ ADA\ signal} \times 100.$$

Epitope specificity was assessed in Lilly Research Laboratories using the confirmatory assay and equimolar concentrations of unlabeled LY3415244, anti-TIM-3 monoclonal antibody (C22), or anti-PD-L1 monoclonal antibody (ABC110) to compete for the ADA signal generated by labeled LY3415244.