

Evaluation of Soluble- and Solid-Phase Cytokine Release Assays

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The goal of the studies was to evaluate the soluble- and solid-phase cytokine release assay (CRA) format using positive control monoclonal antibodies (mAbs) known to produce infusion reactions in humans and negative control mAbs that have a low incidence of reported infusion reactions. The soluble-phase assay involves incubating human whole blood (6-8 healthy donors) with the test mAb for 4 hours and harvesting plasma for cytokine analysis. The solid-phase assay is similar to the dry coating method described by Stebbings et al. (J. Immunology, 2007). Test mAbs were added to 96-well plates and allowed to dry overnight in the laminar flow hood. Human PBMC were added to the wells, incubated for 48 hours and cell culture supernatant was harvested. Cytokines, interferon- γ (IFN γ), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α), were measured in plasma and the supernatants by a multiplex human cytokine electrochemiluminescence immunoassay.

Anti-CD3 and anti-CD28 superagonist (SA) antibodies were used as positive controls. The human IgG1, IgG2 and IgG4 subclasses of anti-CD28 SA were prepared internally using the published sequence of TGN1412. The following mAbs with different incidence rates of clinical infusion reactions and different mechanism for these reactions were tested: Campath (anti-CD52), Rituxan (anti-CD20), Erbitux (anti-EGFR), Herceptin (anti-HER2), Vectibix (anti-EGFR), Avastin (anti-VEGF), Humira (anti-TNF) and Zenapax (anti-IL-2R). In addition, 2 therapeutic mAbs in clinical development from Pfizer were tested, mAb X which was associated with mild clinical infusion reactions and mAb Y which did not produce infusion reactions. Anti-KLH mAbs of different IgG subclasses were used as negative controls.

With the soluble-phase CRA, positive cytokine responses were observed with anti-CD3, Campath and Erbitux (increases in all 3 cytokines). Positive responses with the solid-phase CRA were observed with anti-CD3, anti-CD28 SA, Campath, Erbitux, Herceptin, Zenapax and mAb X. The anti-CD28 SA cytokine data with the solid-phase CRA are consistent with that reported by Stebbings et al. (2007). The cytokine response with Campath in the soluble- and solid-phase assays are also consistent with previous reports and are likely mediated by ADCC. The positive responses observed with Herceptin and Zenapax need to be further evaluated since only a weak IL-6 response was observed with Zenapax in a few donors and the herceptin response was limited to IL-6 and TNF. The positive responses with anti-CD28 SA, anti-CD3, Campath and mAb-X in the solid-phase format were consistent with reported clinical infusion reactions with these mAbs. Negative responses with Vectibix, Avastin and Humira are also consistent with the low rate of clinical infusion reactions reported. The negative response with Rituximab may be related to the low number of CD20 positive B cells in whole blood and PBMC from healthy human donors. Further understanding of the mechanisms of the clinical infusion reactions for Erbitux and Herceptin are needed to determine the sensitivity and specificity of the in vitro assays.