PS 167 Assessment of Keyhole Limpet Hemocyanin-Specific T Cell-Dependent Antibody Responses in Beagle Dogs

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T-cell-dependent antibody response (TDAR) assessment is implemented in non-clinical safety testing to evaluate potential test article-mediated effects on immune function. Robust ELISA-based assays to analyze keyhole limpet hemocyanin (KLH)-specific antibodies (Abs) in serum from beagle dogs immunized with KLH were developed. Assay optimization included determination of sample collection times (up to 28 days), capture reagent concentrations, initial reference serum (pooled serum from dogs immunized with 5 mg KLH) and detection Ab dilutions, incubation times, reference serum stability, assay precision and acceptance criteria, and method robustness. In brief, KLH (0.4 μg/well) was adsorbed overnight onto a microtiter plate followed by incubation with an initial reference serum dilution of 1:200 which was titrated (3x) to a final dilution of 1:11,809,800. Bound KLH specific-Abs were detected using alkaline phosphatase-conjugated goat anti-dog IgM or IgG. After substrate addition and colormetric analysis, an endpoint titer (EPT) was calculated using a linear interpolation method. For assay precision and acceptance criteria calculation, four 11-point dilution curves of reference serum were assayed on 3 plates per day over a 3 day period by 2 analysts. Precision estimates demonstrated that the assay is precise and robust. Flexibility of the assay was demonstrated by acceptable incubation ranges of 1-1.5 hours for blocking, 2-2.5 hours for serum binding, and 1-1.5 hours for secondary-antibody incubation steps. Stability of reference serum was throughout 6 freeze/thaw cycles. Implementation of the assay procedure demonstrated that intramuscular immunization with 5 mg of KLH was sufficient to produce KLH-specific IgM and IgG responses. High KLH-specific IgM responses were observed 1 week post immunization which declined after 1 week. Peak KLH-specific IgG responses were observed 3 weeks post immunization.

PS 168 *In Vitro* Whole Blood Assay in Preclinical Safety: Correlation with IRR in Clinics

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Infusion related reactions (IRR) are adverse events potentially triggered by the immune system during IV administration of therapeutics. Difficult to predict, they require prompt and accurate clinical management to avoid severe complications and /or treatment discontinuation. Monoclonal antibodies (mAbs) have a potential for IRR caused by cytokine release but the TGN1412 case showed the limitations of animal models for prospective risk assessment due to lack of translatability to humans. To fulfill regulatory expectations, *in vitro* human cell-based assays such as the whole blood assay (WBA) have since become hazard identification tools for screening novel biotherapeutics. We established our WBA with 11 commercialized mAbs and routinely used it for Roche-developed mAb. After several years, we compiled sufficient clinical data to evaluate its predictivity for IRR. Fresh undiluted human blood from 30 healthy individuals was incubated for 24h with 0.1 to100μg/ml test mAb and subsequent release of TNF, IL-6, IL-8 and IFN was measured. Erbitux® was used as a negative comparator to establish cut-off values discriminating "positive" vs. "negative" response. MabCampath® known to induce IRR in greater than 90% recipients, was included as positive control. A risk score relating to cytokine release upon first dosing in humans for the tested mAb was calculated from the incidence and magnitude of cytokine levels relative to Erbitux. Once in clinical phases, the effective rate of IRR was used to define the assay performance. Its predictivity for clinical IRR was established based on data from 15 Roche mAbs in Ph.0/1, showing 82% accuracy, 100% sensitivity and 75% specificity. We concluded that our WBA provided safety-relevant data, enhancing decision making for clinical risk mitigation. Analyzed with immune expertise the assay successfully predicted the IRR potential of mAbs, allowing clinical sites to better prepare and thereby improve treatment outcome. Therefore we will continuously update this promising first evaluation with new project data.

PS 169 Macrophage Fusion into Multinucleated Giant Cells *In Vitro*

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Multinucleated giant cells (MGC) have been observed in a variety of granulomatous conditions, including microbial infections (e.g., tuberculosis), foreign body reactions to implants (e.g., medical devices), foreign body reaction to inhaled particles (e.g., engineered nanomaterials), and disorders of unknown etiology (e.g., sarcoidosis). Generally, MGC are morphologically classified based on the number and arrangement of nuclei. The two major types of MGC are foreign body giant cells and Langhans giant cells. These MGC are formed by the fusion of macrophages, often in response to persistent, foreign microorganisms or materials. Although MGC are known to be associated with granulomas, their involvement in the development of these conditions has not been well described. This is in part due to a lack of well-characterized models of MGC populations. The objective of this study is to develop an *in vitro* model of macrophage fusion in order to study MGC function. Previous reports have shown that MGC formation is induced by interleukin-4 (IL-4). Therefore, we investigated a model of IL-4-induced fusion in murine bone marrow-derived macrophages (BMdM). As expected, IL-4 treatment resulted in increased percent fusion of BMdM. The formation of MGC was optimized by modification of culture conditions, including alteration of the growth surface and treatment with either macrophage colony-stimulating factor (M-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Ongoing studies involve identification of molecules that regulate MGC formation. An increased understanding of this mechanism will provide additional targets to control fusion. Further development of this controlled *in vitro* model will facilitate future investigation of MGC inflammatory activity and contribution to pathogenesis of granulomas.

PS 170 A Comparison of Cell-Counting Methods in Rodent Pulmonary Inhalation Toxicity Studies

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Pulmonary toxicity studies use bronchoalveolar lavage (BAL) to assess lung responses to particulates. The BAL cellular fraction BAL is counted, using an automated (i.e., Coulter Counter® or flow cytometer) or manual (i.e., hemocytometer) method, to determine inflammatory cell influx. The goal is to compare different, commonly used, counting methods to determine which is optimal for examining cell influxes after particle inhalation. Inhalation exposures consisted of carbon nanotubes (CNT) at 5 and 0.5 mg/m3 x 5h/d x 19d in mice and mild and stainless steel welding fume (WF) at 30 mg/m3 x 3h/d x 4d in rats. High dose CNT inhalation resulted in marked lung cytotoxicity (3.5-fold increase in BAL fluid lactate dehydrogenase [LDH]) 1d post-inhalation. BAL cell counts by the automated counter (Coulter Counter®) indicated a 3.2-fold increase in cellular influx. Conversely, the hemocytometer method showed a slight decrease in total cells (0.84) compared to sham, which agreed with flow cytometry results (0.81 decrease). Similar changes were observed 28 and 84d post at time points associated with significant increased LDH levels (2.5 and 2.0-fold, respectively). At the lower CNT dose, LDH increased minimally (1.4 fold) and the three methods generally agreed at 0.93, 1.20, and 1.00 versus sham for flow cytometry, automated counter, and hemocytometer, respectively. Similarly, a highly cytotoxic (>5-fold increase in LDH) stainless steel WF exposure produced fold changes over sham for the automated (4.0) and manual (1.2) methods at 1d post that were also seen at 7d. By 28d, however, when cytotoxicity was near baseline (1.4 fold change), the two methods gave similar results, with fold changes of 1.6 and 1.8 for the automated and manual methods, respectively. After exposure to non-cytotoxic mild steel WF, there was agreement between the automated counter and manual methods with a fold change of 1.1 over sham for both. The results suggest a threshold of cytotoxicity magnitude may be important in determining whether an automated or manual method of cell counting is best suited for BAL studies.

PS 171 Development of a Flow Cytometry-Based Method to Measure Neutrophil Activation in the Cynomolgus Monkey

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During pre-clinical safety assessment of human pharmaceuticals, the data generated from standard toxicity studies may prompt the need for further immunotoxicity testing. When changes to the immune system such as white blood counts or structural changes to lymphoid tissue are observed, it is not always obvious which component has been targeted. This can make selection of the most appropriate follow on immunotoxicology studies very difficult. Often the innate immune system is the first line of defence when the body is challenged with foreign pathogens. However, standard sub-acute and sub-chronic toxicity studies are often not of sufficient duration to detect changes in innate immune function. In this study we have developed a method that can provide an indication of potential changes in neutrophil function that is easily incorporated into a standard toxicity study without the need for extra blood sampling and requires minimal additional analyses over and above what is normally performed in toxicology studies. When neutrophils from a healthy subject are stimulated, they undergo a shape change which is easily identified using flow cytometry and looking for increased cell size on the forward scatter plot. If

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