The 24-hour Whole Blood Assay as potential in vitro tool to predict in vivo cytokine release following treatment with monoclonal antibodies.

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Background: Cytokine release syndrome and infusion related reactions are potentially life-threatening side effects upon infusion of therapeutic mab. The TGN1412 trial in 2006, emphasized the need for pre-clinical in vitro models to predict massive cytokine release upon EIH. Ideally, such an in vitro test system model should be easy to perform and deliver consistent results with clinically relevant monoclonal antibodies (mab). We herein describe our experience with a 24-hour human whole blood assay (WBA) testing several mab with either low or high risk to lead to cytokine release in vivo.

Methods: Lithium-heparanized whole blood samples were processed within 3 hours after drawing. Spanning the concentration range of 0.1□g/ml to 200□g/ml a given mab was incubated with whole blood for 24 hours at 37°C and 5% CO_2. Thereafter, plasma concentrations of IL-6, IL-8 and TNF-□ were measured using a MSD multiplex platform. Tested monoclonal comparator antibodies included Synagis, Erbitux, Vectibix, Campath-1H and an αCD28 superagonist mab. In addition, PBS and LPS were included as controls. Subsequently, statistical analysis was performed, including inter-study comparison, outlier removal, Lc and cut-point establishment. Samples were analyzed by three complementary methods: (i) the +/- analysis according to the established cut-points; (ii) cytokine profile according to whether one, two or all three cytokines were induced by a compound; (iii) ratios between comparator mab-induced cytokine values were established and expressed as "–fold induction".

Results: Cytokine values in the presence of PBS values followed a normal distribution and Lc values were established by normal distribution model with 95% confidence interval. Outliers were identified based on significantly elevated PBS cytokine values. Cytokine release in the presence of Vectibix, Synagis and Erbitux did not follow a normal distribution and increased in a dose-dependent manner. Therefore, significance cut-points were set with 95% confidence using a quantile approach and for each compound concentration separately.

100% of samples secreted significant amounts of all three cytokines following stimulation with Campath-1H. A mean of 88.5% of donor samples had significant cytokine responses to αCD28 mab. A mean of 84% of samples showed significant levels of all three cytokines. By contrast, Synagis, Vectibix and Erbitux with low reported frequencies of severe IRR (<1% to 4.7%), showed significantly positive samples in 21% (Synagis, n=58), 26% (Vectibix, n=48) and 20% (Erbitux, n=30) of samples, respectively. However, in contrast

to Campath-1H and αCD28, significant levels of all three cytokines were detected in only 7%, 4% and 3% of samples, respectively. Since the severity of the cytokine release syndrome seems to be related to both the constitution and the absolute yields of cytokine responses, we created ratios between high and low-risk comparator compounds. Following stimulation with Campath, IL-6 levels were 172-fold induced, whereas IL-8 and TNF-a was 21- and 49-fold higher than those after Vectibix stimulation. As for

αCD28 mab IL-6 was induced 168-fold over levels induced by Vectibix, whereas IL-8 and TNF-a levels were only 17 and 15-fold higher, respectively.

Conclusions: The potential of several mabs to induce cytokines in the proposed 24-hour Whole Blood Assay seems to correlate with their in vivo risk to cause Cytokine Release Syndrome. Therefore, although the kinetics and absolute yields of cytokines differ between in vitro and in vivo, the 24-hour WBA may serve as a risk-assessment tool for in vivo infusion related cytokine release.