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Review Article

Cytokine release assays: Current practices and future directions

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ABSTRACT

As a result of the CD28 superagonist biotherapeutic monoclonal antibody (TGN 1412) "cytokine storm" incident, cytokine release assays (CRA) have become hazard identification and prospective risk assessment tools for screening novel biotherapeutics directed against targets having a potential risk for eliciting adverse pro-inflammatory clinical infusion reactions. Different laboratories may have different strategies, assay formats, and approaches to the reporting, interpretation, and use of data for either decision making or risk assessment. Additionally, many independent contract research organizations (CROs), academic and government laboratories are involved in some aspect of CRA work. As a result, while some pharmaceutical companies are providing CRA data as part of the regulatory submissions when necessary, technical and regulatory practices are still evolving to provide data predictive of cytokine release in humans and that are relevant to safety. This manuscript provides an overview of different approaches employed by the pharmaceutical industry and CROs, for the use and application of CRA based upon a survey and post survey follow up conducted by ILSI–Health and Environmental Sciences Institute (HESI) Immunotoxicology Committee CRA Working Group. Also discussed is ongoing research in the academic sector, the regulatory environment, current limitations of the assays, and future directions and recommendations for cytokine release assays.

1. Introduction

Cytokine release syndrome (CRS) is a potentially adverse clinical event where proinflammatory cytokines (e.g. TNF-α, IL-6, IL-8, IFN-γ, etc.) are released from immune cells. Cytokine release assays (CRA) are in vitro assays using human cells in the preclinical setting as a means to predict the potential for a new biotherapeutic (also referred to as drug in this manuscript) to induce significant cytokine release from immune cells in vivo. CRS may occur on initial, and sometimes subsequent, intravenous infusion of some types of protein biotherapeutics. A recent dramatic example of severe CRS (i.e. "cytokine storm") in a first in human (FIH) trial occurred within hours of single dose administration of the CD28 superagonist IgG4 monoclonal antibody (mAb) TGN 1412. Six healthy male volunteers developed life-threatening multi-organ failure as a consequence of the harmful systemic effects evoked by release of high levels of pro-inflammatory cytokines. These effects, which included fever, chills, back pain, hypotension and organ failure, required weeks of hospitalization [1,2]. This highly unfortunate episode not only captured the attention of governments, regulators, pharmaceutical companies and patients globally but also highlighted the potential limitations of pre-clinical toxicology species for safety assessment in this area and the need for thorough review of the data supporting the dose selection and initiation of human testing of biotherapeutics and the conditions for administration and monitoring of subjects in FIH trials.

Due to the incident with TGN 1412 and a subsequent report issued by The Medicines and Healthcare Products Regulatory Agency [3], efforts were initiated by multiple groups to further develop and improve CRA assays to identify the potential hazard for

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cytokine release. Furthermore, the European Medicines Agency (EMA) also issued a guideline on risk management of FIH trials conducted with higher risk therapeutics [4], discussing the importance of science based preclinical strategies to identify potential risk. Although the testing of TGN 1412 in a variety of in vitro CRA formats was discussed extensively in the final report of the Expert Scientific Group [2], no specific recommendations were offered regarding the conduct or interpretation of these assays, nor was guidance on this subject included in the EMA guideline. Subsequently, the EMA sponsored a workshop in 2009 to discuss the state-of-the-science of CRAs and their use [5]. Although formal regulatory guidelines on CRAs have not been published, these efforts endorsed continued use of CRA in predicting the risk of pro-inflammatory clinical infusion reactions following administration of novel biotherapeutics and supported efforts toward further assay development and refinements.

Various approaches to CRAs have been published [6–13] and pharmaceutical companies and CROs are using a variety of approaches. CRA formats include assays where the biotherapeutic is presented in solution (drug in solution or aqueous phase), immobilized directly on plastic using 'dry-coat' or 'wet-coat' methods (solid phase), immobilized indirectly via Fc-capture, or presented in a 'co-culture' setting which includes test antibody presentation in the context of immune cells and other cell types (i.e. target expressing cells, non-target expressing cells, endothelial cells).

Many of the assays used TGN 1412 or TGN 1412 homolog(s) as a positive control/benchmark for optimization, but it has become clearer that the diversity of mechanisms (e.g. CD3-mediated vs. CD28-mediated) of specific drugs in the induction of cytokine release and the variety of disease indications targeted, requires multiple formats and adaptations to produce safety-relevant data for hazard prediction [5]. Moreover, with the assays that have been developed so far, it is not possible to define a threshold or exposure level in which cytokine release may be a concern during administration of therapeutic monoclonal antibodies (mAbs) to humans. The current assays were designed primarily to identify compounds that produce severe cytokine release (cytokine storm) and not necessarily to identify those that provide mild to moderate release [5]. Also, there are times when using the CRA may not be appropriate, due to absence of target cells/ligand in blood or involvement of blood cell populations and soluble factors missing in a test format using peripheral blood mononuclear cells (PBMCs). This paper presents a summary of responses to an on-line survey (Table 1) distributed to academic, government and industry scientists who participated anonymously to gain a better understanding of approaches to cytokine release assays. This manuscript further describes information collected from 16 pharmaceutical companies and independent CROs to enhance to understanding of assay formats, donor variability and other technical factors related to the conduct and interpretation of cytokine release assays. The data from the initial survey, along with additional detailed input from survey respondents was used to create this manuscript which is intended to provide an overview on current strategies and in vitro methods used for performing cytokine release assays, and use of data to convey whether the test article poses a hazard for human use. Furthermore, current shortcomings of the assays and various approaches to address them in the future are discussed. The manuscript is not intended to provide a "best practices" but rather to provide an overview of current practices, shortcomings and considerations for the future. The survey was conducted as part the ILSI HESI Immunotoxicology Technical Committee (ITC) Cytokine Release Assay Working Group. An additional contributor to the manuscript is from the National Institute for Biological Standards and Control (NIBSC).

2. Cytokine release assays: approaches

Among the independent testing facilities and pharmaceutical companies surveyed, there are various formats to conduct CRA, differing approaches on selection of types of molecules to be tested, timing of CRA along the drug development process, selection of appropriate controls, and data interpretation. Some laboratories take a tiered approach to hazard identification, with an initial screen in either solution phase or solid phase assay followed by testing positives in other formats as described in the Section 2.3 on case by case modifications. Other laboratories do potential profiling of cytokine release occurrence in vivo (e.g. non-human primate) although the relevance to humans may be limited.

Some laboratories test all antibody-derived biotherapeutics while others test only certain types of biologics based upon considerations such as anticipated mechanism of action and expected pharmacological activity, whether the molecules are agonists or mediate Fc effector functions, potential for cross-linking of receptors and subsequent cellular activation, and the nature of the target. One laboratory indicated they do not test antibody-derived therapeutics that target soluble cytokines or proteins, others only apply the assay to molecules with anticipated agonist potential for receptors expressed on circulating human immune cells. Depending upon biologic properties, mode of action and the drug target, different laboratories use different criteria to determine whether solution and/or solid phase formats are most appropriate. Some laboratories only test molecules in the solution phase while others select the solid phase method if cross-linking might be an in vivo relevant factor triggering cytokine release or if drug targets a receptor on immune cells.

While this is consistent with the EMA Workshop recommendations which state "that various approaches can be chosen, from simple test systems to more complex models including co-cultures, or other systems aimed at mimicking as far as possible the in vivo situation", the state-of-the-science is not adequate at present to allow specific recommendations regarding which assays/formats are most appropriate for different types of target and mechanisms" [5]. Some laboratories have not tested any of their biotherapeutics in the CRA although they have validated the assay with commercial antibodies; other laboratories test molecules early in development to support compound selection while other laboratories wait to test a clinical candidate prior to FIH. The results of the survey suggest a lack of consensus regarding the conduct of the assays and strategies for use.

In most instances, 62% of laboratories responding to the survey performed routine testing using a solution phase assay either with healthy donor whole blood or PBMCs, while 38% used a dry coat approach. At the time of the survey, the wet coat approach was not commonly being used amongst respondent labs. Whole blood or PBMCs derived from diseased patient populations has been used by some laboratories due to differences in target expression in disease state donors. Some laboratories use blood from donors with specific polymorphisms (i.e. FcγRIIA 131 H/H for IgG2 molecules). Additional assessments may be conducted based on the target biology, anticipated mechanism of action of the drug, and specific feedback from regulatory agencies, if available. Additional in vitro evaluations may include analysis of cytokine release using specialized blood cell types/subpopulations or intracellular cytokine labelling for identification of involved cell populations. It is clear that there is considerable variation across organizations with respect to strategies for testing and CRA assays reflect an evolution of this field which is likely to continue unless additional guidance is provided by regulatory agencies.

Depending upon the laboratory that responded to the survey, as few as 3 and as many as 12 different cytokines were measured. The