Cytokine 85 (2016) 101–108

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Cytokine release: A workshop proceedings on the state-of-the-science, current challenges and future directions

Christine Grimaldi [a], Deborah Finco [b], Madeline M. Fort [c], Daniel Gliddon [d,1], Kirsty Harper [d], Whitney S. Helms [e], Jane A. Mitchell [f], Raegan O'Lone [g], Stanley T. Parish [g,*], Marie-Soleil Piche [h], Daniel M. Reed [f], Gabriele Reichmann [i], Patricia C. Ryan [j], Richard Stebbings [k,2], Mindi Walker [l]

[a] Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877, USA
[b] Pfizer Inc., 1 Eastern Point Road, Groton, CT 06340, USA
[c] Amgen, Inc., Comparative Biology and Safety Sciences, USA
[d] Envigo, Woolley Road, Alconbury, Huntingdon, Cambridgeshire PE28 4HS, United Kingdom
[e] US Food and Drug Administration, 10903 New Hampshire Ave, Rockville, MD, USA
[f] Imperial College London, London SW3 6LY, United Kingdom
[g] ILSI Health and Environmental Sciences Institute, 1156 15th St NW, Suite 200, Washington, DC 20005, USA
[h] Charles River Laboratories, 20222 Transcanadien, Senneville, QC H9X 3R3, Canada
[i] Paul-Ehrlich-Institute, Federal Institute for Vaccines and Biomedicines, Paul-Ehrlich Str. 51-59, 63225 Langen, Germany
[j] Medimmune, Inc., One MedImmune Way, Gaithersburg, MD 20878, USA
[k] The National Institute of Biological Standards and Controls, Blanche Ln, South Mimms, Potters Bar EN6 3QG, United Kingdom
[l] Johnson and Johnson, Inc., 1 Johnson and Johnson Plaza, New Brunswick, NJ 08933, USA

ARTICLE INFO

Article history:
Received 1 June 2016
Accepted 6 June 2016
Available online 13 June 2016

Keywords:
Cytokine release assay
Cytokine release syndrome
Pro-inflammatory cytokines

ABSTRACT

In October 2013, the International Life Sciences Institute - Health and Environmental Sciences Institute Immunotoxicology Technical Committee (ILSI-HESI ITC) held a one-day workshop entitled, "Workshop on Cytokine Release: State-of-the-Science, Current Challenges and Future Directions". The workshop brought together scientists from pharmaceutical, academic, health authority, and contract research organizations to discuss novel approaches and current challenges for the use of in vitro cytokine release assays (CRAs) for the identification of cytokine release syndrome (CRS) potential of novel monoclonal antibody (mAb) therapeutics. Topics presented encompassed a regulatory perspective on cytokine release and assessment, case studies regarding the translatability of preclinical cytokine data to the clinic, and the latest state of the science of CRAs, including comparisons between mAb therapeutics within one platform and across several assay platforms, a novel physiological assay platform, and assay optimization approaches such as determination of FcR expression profiles and use of statistical tests. The data and approaches presented confirmed that multiple CRA platforms are in use for identification of CRS potential and that the choice of a particular CRA platform is highly dependent on the availability of resources for individual laboratories (e.g. positive and negative controls, number of human blood donors), the assay through-put required, and the mechanism-of-action of the therapeutic candidate to be tested. Workshop participants agreed that more data on the predictive performance of CRA platforms is needed, and current efforts to compare in vitro assay results with clinical cytokine assessments were discussed. In summary, many laboratories continue to focus research efforts on the improvement of the translatability of current CRA platforms as well explore novel approaches which may lead to more accurate, and potentially patient-specific, CRS prediction in the future.

1. Introduction

As a result of the CD28 superagonist TGN 1412 monoclonal antibody (mAb) cytokine storm incident in 2006, cytokine release assays (CRAs) have become more commonly used as hazard identification and risk assessment tools for therapeutic candidates, particularly mAbs with the potential to elicit adverse pro-inflammatory cytokine

* Corresponding author.
E-mail address: sparish@hesiglobal.org (S.T. Parish).
[1] Present address: F-star Biotechnology Limited, Eddeva B920 Babraham Research Campus, Cambridge CB22 3AT, United Kingdom.
[2] Present address: MedImmune, Aaron Klug Building, Granta Park, Cambridge CB21 6GH, United Kingdom.

http://dx.doi.org/10.1016/j.cyto.2016.06.006
1043-4666/

responses in patients [1–3]. Although cytokine release syndrome (CRS) is a relatively rare event in the clinic, evaluating the potential of certain novel therapeutic mAbs to cause CRS is now part of preclinical safety testing [4]. Severe CRS is reported to have occurred in approximately 50% of recipients administered muromonab-CD3 (OKT3, an anti-CD3 mAb), before the introduction of high-dose corticosteroid pre-treatment [5], although in subsequent protocols using a lower dose, pretreatment with anti-inflammatory agents and a slower infusion rate also reduced the risk. Moderate-to-severe CRS is reported in a small number of multiple sclerosis patients given alemtuzumab (Campath-1H®), an anti-CD52 mAb [6]. Other therapeutic mAbs currently in use such as the tumor necrosis factor α (TNFα) antagonists infliximab, adalimumab and certolizumab pegol (Remicade®, Humira® and Cimzia® respectively) and many others such as bevacizumab (Avastin®) and natalizumab (Tysabri®) are not associated with CRS [4,7]. Thus, in terms of predicting the safety of novel therapeutic mAbs in man, the CRA should ideally differentiate between mAbs with moderate-to-severe clinical risk (e.g. infliximab < alemtuzumab < muromonab-CD3 (Orthoclone OKT3) < TGN 1412).

Significant progress has been made in designing and developing improved methods for CRAs as a result of the CD28 superagonist TGN 1412 incident. In 2007, a solid-phase CRA, which involves the co-incubation of human peripheral blood mononuclear cells (PBMCs) with mAbs that have been dry-coated onto a tissue culture plate, was shown to be predictive for the cytokine release potential of TGN 1412 [8]. In 2009, the European Medicines Agency (EMA) held a workshop to discuss in vitro CRAs, with the conclusion that while a specific assay could not be endorsed at that time, CRAs have a place in predicting the effect of a product in humans [9]. Currently, a number of in vitro assay formats can be considered when evaluating the potential for cytokine release for hazard identification by a novel therapeutic. Various CRA platforms have been designed to identify mAbs that can be associated with CRS, however, not all CRA platforms can discriminate between mAbs inducing mild or moderate cytokine release, nor can they be used to determine a threshold where the levels of cytokines released may be associated with serious adverse events in humans. The diversity in the modes of action of specific drugs in the induction of cytokine release may require the availability of adapted or flexible CRA platforms to identify potential hazard in the clinic for a particular therapeutic candidate. As pharmaceutical companies become more familiar with the mechanisms related to mAb-induced cytokine release, new assays, platforms and data interpretation approaches are being adopted.

Considerable progress has been made in understanding mechanistic aspects of CRS as well as in developing CRA formats suitable for hazard identification. Thus, the International Life Sciences Institute - Health and Environmental Sciences Institute Immunotoxicology Technical Committee (ILSI-HESI ITC) Cytokine Release Assay Working Group set out to address the scientific issues pertaining to CRA conduct and CRS risk assessment in a multi-pronged approach. First, in 2013, ILSI-HESI ITC sponsored a survey of pharmaceutical companies, contract research organizations, and academic laboratories that demonstrated that a variety of in vitro assay approaches were used, including testing strategies, assay formats and reporting and interpretation of CRA data which was subsequently published [2]. The survey indicated that variations in assay design include solution and/or solid phase based assays, the use of either various dilutions of whole blood (WB), PBMCs, or peripheral blood leukocytes (PBLs) as responder cells, and in some cases, the capture of mAbs on plates or beads via Fc using protein A or antibodies to Fc. The survey also indicated that positive CRA controls vary across laboratories with many using anti-CD3 reagents, while others use anti-CD28 superagonist mAbs (such as TGN 1412 homologs) or LPS. Some laboratories also include other marketed mAbs as positive controls. Negative controls include phosphate buffered saline, tissue culture medium, isotype mAb controls or marketed mAbs not known to cause clinical cytokine release. Data readouts vary across laboratories from concentration of cytokines (e.g. pg/mL), ratios relative to negative controls and/or rank order comparison to other mAbs tested. Overall the results from the survey highlighted that there are no standard approaches, and the alignment of technical procedures for frequently used formats may pave the way for a more harmonized assay system.

Next, on October 22, 2013, in Silver Spring, Maryland, the ILSI-HESI ITC Cytokine Release Assay working group sponsored a 1-day workshop entitled, "Workshop on Cytokine Release: State-of-the-Science, Current Challenges and Future Directions". This workshop brought together 93 experts in the field from pharmaceutical, academic, health authority, and contract research organizations to discuss novel technologies, experimental designs, practices and scientific challenges. The workshop included both oral and poster presentations of the latest science concerning CRA design, use, and interpretation, and concluded with an open panel discussion featuring the speakers. Topics presented encompassed a regulatory perspective on cytokine release and assessment, 2 case studies regarding the translatability of preclinical cytokine data to the clinic, and the latest state of the science of CRAs, including comparisons between mAb therapeutics within one platform and across several assay platforms, a novel physiological assay platform, and assay optimization approaches such as determination of FcR expression profiles and use of statistical tests. This manuscript summarizes the scientific presentations and provides a current view on the approaches being adopted to identify the risk of CRS for novel therapeutic candidates.

2. Cytokine release and assessment: a regulatory perspective

Following the TGN 1412 incident, testing for cytokine release-inducing activity has been increasingly included in the nonclinical studies conducted to support clinical testing of mAbs [1]. Results of in vitro cytokine release testing are now frequently included in regulatory submissions when the therapeutic target is characterized as being involved in immune activation. Further, CRA results are also often submitted for mAbs with targets that have not been pharmacologically characterized as immune activators but are expressed on immune cells, or for products such as immune checkpoint inhibitors that are designed to disrupt immune inhibitory signals [2]. During the workshop, both Whitney Helms from the US Food and Drug Administration and Gabriele Reichmann from the Paul Ehrlich Institute described cytokine release and assessment from a regulatory perspective.

The conclusions from the EMA 2009 cytokine release workshop (that cytokine release assays have a place in predicting the potential of a product to trigger cytokine release in humans [9]) suggest that assays should be customized taking into account the degree of knowledge of the mechanism of action of the product. Data derived from these assays should be considered for hazard identification purposes rather than for accurate and reliable risk quantification purposes. Further regulatory guidance on cytokine release testing has not been developed in the EU and there are still open questions regarding the products for which investigators should perform cytokine release testing and which assay format(s) they should use. Similarly, the United States Food and Drug Administration (FDA) does not currently have a requirement for any particular assay that must be used for the assessment of cytokine release. When FDA has requested cytokine assessments, sponsors have commonly been referred to Stebbings et al. and Römer et al. for information on the design of in vitro testing methods [8,10].