Cytokine Release Assays

Current practices and future directions

Submitted Manuscript

OVERVIEW

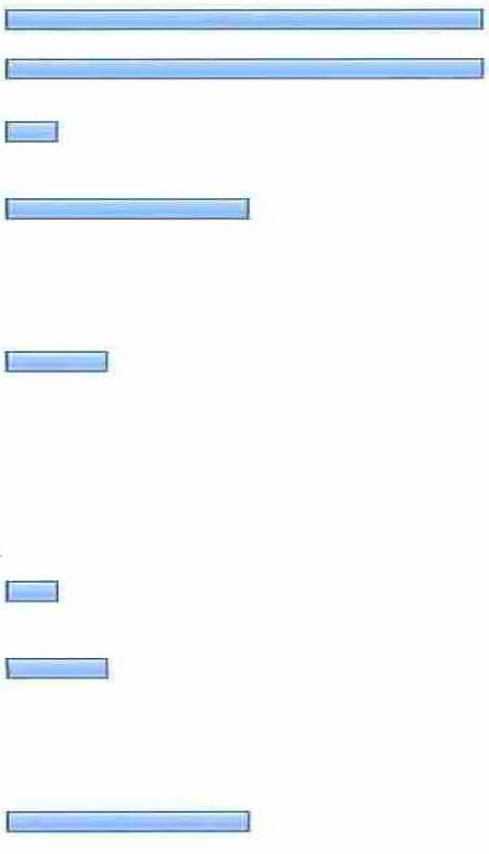
- Background
  - Survey
  - Other
- Manuscript Outline
  - Formats
  - Data
  - Regulatory
  - Gaps/Future
- Conclusions

BACKGROUND

- A survey of CRA practices (Table 1 in manuscript) along with group discussions was conducted amongst participating ILSI HESI ITC CRA working group members in 2011 on:
  - Methods used for CRA
  - Strategies for testing
  - Data capture
  - Potential use of data to inform the Minimum Anticipated Biological Effect Level (MABEL)
  - Current shortcomings of the assays, and various approaches to address them

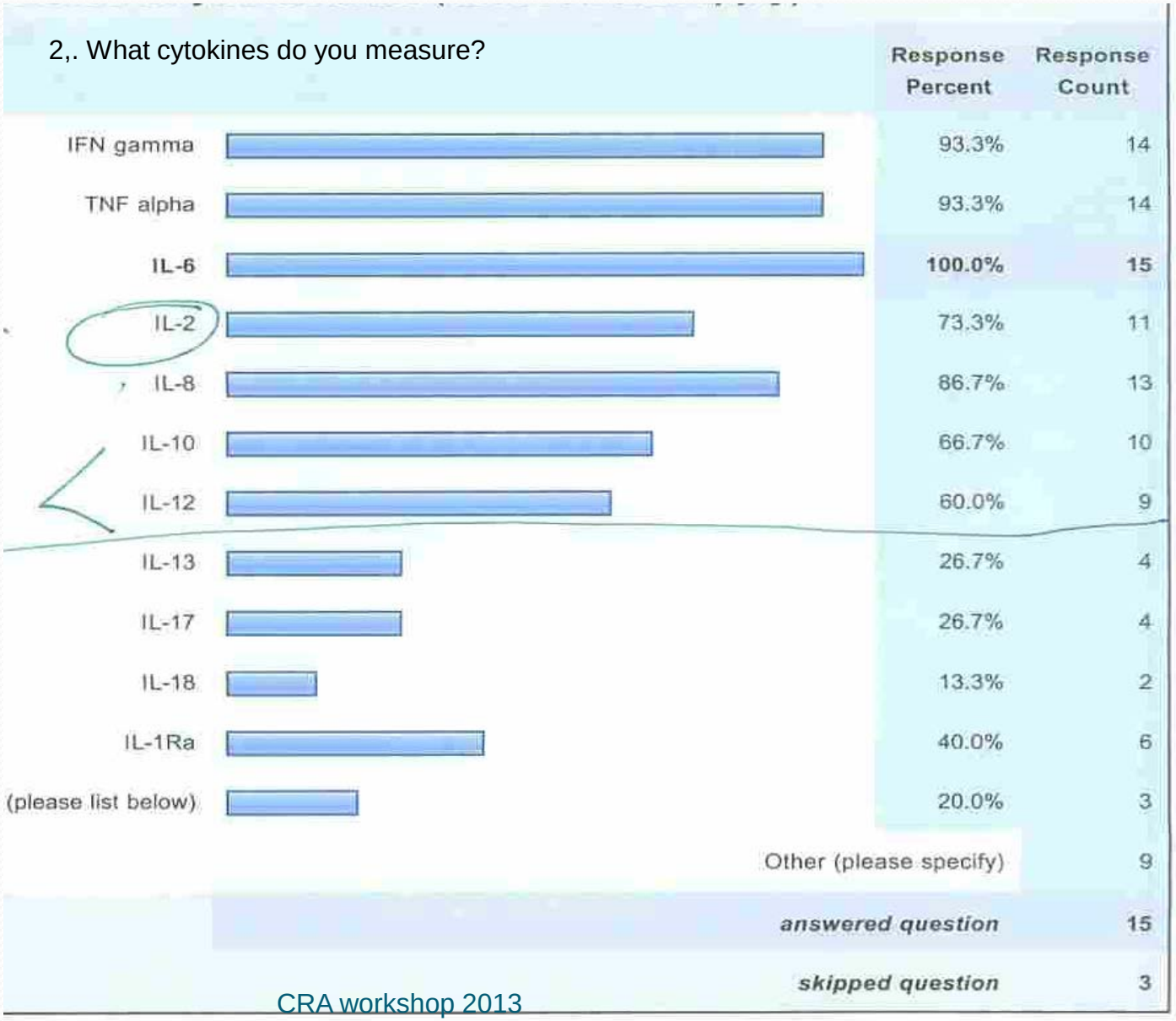
BACKGROUND: SURVEY

1. What assay formats are you currently using human cytokine release assays (check all that apply)?



	Response Percent	Response Count
Solution phase with blood	62.5%	10
Solution phase with PBMC's	62.5%	10
Dry coat with drug directly on plate with blood	6.3%	1
Dry coat with drug directly on plate with PBMC's	31.3%	5
Dry coat with anti human Fc on plate with blood	0.0%	0
Dry coat with anti-human Fc on plate with PBMCs	12.5%	2
Drug immobilized on protein-A coated beads with whole blood	0.0%	0
Solution phase with endothelial cells and PBMC's	0.0%	0
Solution phase with endothelial cells and blood	6.3%	1
Flow cytometric methods: intracellular staining	12.5%	2
Flow cytometric methods: other (please describe below)	0.0%	0
Other assay formats (please describe below)	31.3%	5
Other (please specify)		7
answered question		16
skipped question		2

BACKGROUND: SURVEY



2,. What cytokines do you measure?

	Response Percent	Response Count
IFN gamma	93.3%	14
TNF alpha	93.3%	14
IL-6	**100.0%**	**15**
IL-2	73.3%	11
IL-8	86.7%	13
IL-10	66.7%	10
IL-12	60.0%	9
IL-13	26.7%	4
IL-17	26.7%	4
IL-18	13.3%	2
IL-1Ra	40.0%	6
(please list below)	20.0%	3
Other (please specify)		9
answered question		15
skipped question		3

BACKGROUND: OTHER DATA

- Individual organizations (Pharma, Biotech, CROs) provided written input regarding their assay formats, strategies and data interpretation
- Input solicited from FDA but they were unable to provide input; NIBSC rep joined manuscript effort after a draft had been generated and provided input
- Organizations also were also invited to provide data regarding levels of cytokines they obtained for donors to demonstrate variability of donors
  - Reported as high/low responses amongst donors tested on a given day for a given company for their particular positive and negative controls. (8 participants provided data) (Tables 2 and 3 in manuscript)

BACKGROUND: FINAL PRODUCT

- The survey results, written input from respondents and ongoing research in the academic sector and the regulatory environment were used to produce a manuscript that was submitted to Cytokine.
  - Received comments from reviewers in 3rd Qtr
  - Revised manuscript to address reviewer comments and resubmitted in 3rd Qtr.
  - Reviewer comments in Oct 2013 from revised manuscript requesting we more clearly state "limitations" of whole blood assay in particular for molecules which may have MOA similar to TGN
    - We are currently revising manuscript to resubmit in Nov.

MANUSCRIPT OVERVIEW

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- Abstract and introduction
- Cytokine release assays formats
- Evaluation and interpretation of data
  - Variability across donors (Tables 2/3)
- Regulatory
- The current science and considerations in assay design
  - Assays and pros/cons (Table 4)
- Conclusion

ASSAY FORMATS & STRATEGY

- Little consistency of CRA assay formats, selection of controls, and data interpretation across respondents
  - More common formats are whole blood assays with soluble presentation, PBMC in combination with the biotherapeutics presented after overnight dry-coating on the plates (more recently wet coat presentation)
    - Different organizations may have different criteria for selection of type of assay to use
    - Panel of cytokines tested various among respondent labs
    - Controls vary considerably across labs
    - When using blood, dilutions of blood varies across labs
    - Number of donors tested varies considerably across labs

- Different approaches regarding criteria for molecules to be tested and at what point in the life-cycle of a compound the CRAs are conducted
  - Some companies test all biotherapeutics while others test on a case by case basis
  - Tiered testing/testing funnel (e.g first test in one format than in another if needed)
  - Some companies do cytokine profiling in vivo in tox species; although relevance to humans may be limited

ASSAY CONSIDERATIONS

- Different formats (solution or solid phase, bead-coating, co-culture systems, whole blood, PBMCs, isolated cell populations) may be relevant depending on the MoA and specific question(s) being asked(e.g.)
  - What is expected *in vivo* mechanism ?
  - What type of cells is the target on?
  - What is the target population(s)?
  - What is the relevance of cross-linking?
  - What is the contribution of Fc-related mechanisms?
  - Are there soluble factors that may be involved?
  - Are there potential interactions with endothelial cells?

DATA INTERPRETATION

- Factors to consider:
  - What is the target biology
  - What cytokines were produced?
  - What are the targeted disease population(s)
  - Is cytokine release a part of the MOA or an unwanted side effect?
  - Is the cytokine production a self-limiting mechanism?
  - What is the frequency of cytokine release detected when testing multiple donors?
  - Are there additional individual risk factors for cytokine release syndrome within a heterogeneous target population?
  - Are their potential contributions due to complement activation /aggregation of drug product in test system
  - Based upon results should reg tox species cells be tested in CRA?
    - If similar results to humans should additional endpoints be added to reg tox studies to monitor CRA findings?

DATA INTERPRETATION (cont.)

- Variability across donors presents challenges (some donors that are very low responders to anti CD3 PC due to a polymorphism)
- Data should be interpreted in context of other data (FcγR binding, ADCC, complement binding/activation etc.)
- Concentration, percent relative to PC, fold changes relative to negative control, stimulation indices, averages and SD for each test article results across donors tested, hierarchical cluster analysis, use of cut point to determine positivity, thresh holds relative to known drugs that cause infusion reactions due to cytokines
  - No consistent approach
- What constitutes a hazard?
  - There is no defined threshold of concern
  - Response 25, 50, 75 % of PC such as TGN homolog or anti-CD3 or other PC?
  - 25, 50, 75, 100% of donors respond at least with at least TBD level of cytokines?

IMPACT

- Re-engineer drug (rare as an outcome of CRA results)
- Dosing considerations for FIH (dose)
  - Implement data in the identification of a safe human starting dose by MABEL approach (in addition to pharmacological active dose)
- Exclusion of specific subpopulations at risk from the treatment
- Consideration of disease indication and safety margins
- Clinical site preparation for potential infusion reactions
- Stop (rare as an outcome of CRA results alone)

REGULATORY

- Testing in CRA not mandatory but there is a general expectation to perform testing
  - Science based evaluation (MOA, target cells)
- Some companies are testing all Mabs, fusion proteins and Ig derived molecules regardless of MOA. While others are testing based upon the science based evaluation
- If testing is done, companies are including results in IND and other regulatory submissions
- There have been regulatory queries to some organizations asking them to determine the mechanism(s) of cytokine release when it has been observed in the CRA

CURRENT SCIENCE

- State of the art:
  - Based on TGN 1412
    - Many organizations testing a large variety of molecule types (Mabs, nanobodies, Modified Ig molecules, fusion proteins, Mabs that are bound to peptides, payloads or other proteins for delivery, Mabs/modified Igs that bind multiple targets, etc.)
  - Does not allow for any specific recommendation regarding a single assay system for all types of molecules
  - Advantages and disadvantages of various assay formats (Table 4)

CURRENT SCIENCE

Assay Component		Considerations and Potential Limitations				
		Physiologic test agent presentation	Target cross-linking	Fc-binding	HI for TGN1412 reported [Ref]	Others
	Solution Phase	x			[9, 10,]	Less prone to aggregation of test agent The response magnitude and number of donors to TGN1412 is much lower and in fewer donors than the dry or wet coat methods.
	Solid Phase		x	x	[6, 7 ,12)]	Potential alteration of mAb presentation and conformation by artificial immobilization (influenced mAb-target interactions, exaggerated target cross-linking) Air-drying may induce stronger cytokine responses than wet coating Increased Fc mediated cytokine release Reveals immunosuppressive properties of therapeutic mAbs
	Bead Immobilization (either in solution or immobilized)	x	x		[14]	Limited utility for evaluation of potential Fc-mediated effects Risk of cytokine release by bacterial proteins thereby increasing background effects

CURRENT SCIENCE: GAPS

- Lack or low representation of target cells in blood
  - Spiking of target cells done but relevance to in vitro localized expression of target unclear
- Specific disease conditions are generally not reflected since usually normal human donors are used
- High variability of responses across donors
- Difficulty to define a threshold of concern
- Multiple ways of data evaluation and application
- Higher complexity of test systems may be associated with reduced reproducibility and transferability
- Limited comparability of multiplex cytokine detection kit results

CONCLUSIONS

- "Case-by Case" approach based on biology of target & pharmacology of the drug
  - A 'one-size-fits-all' approach may not be feasible
- Alignment is desired in:
  - Selection of assay controls
  - Approaches to data evaluation and reporting
  - Data interpretation and application esp. with respect to clinical programs
  - Regulatory expectations for CRA data and use of data

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