Cytokine release assay using human PBMCs enables CRS risk stratification of therapeutic antibodies

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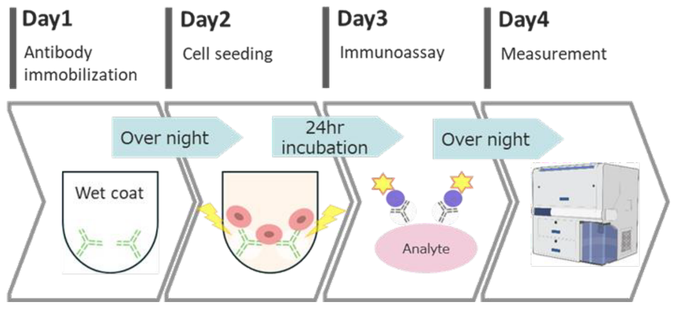
Background and Objective

Cytokine release syndrome (CRS) is classified into a spectrum ranging from mild cases characterized solely by fever to severe cases leading to multi-organ failure. In the development of antibody therapeutics, accurately predicting the risk of CRS onset remains a critical challenge. Advances in new approach methodologies (NAMs) have increased the use of cytokine release assays (CRAs) with human immune cells to assess immune-mediated safety risks, including CRS.

PBMCs from 13 donors were incubated with therapeutic antibodies with known CRS risk for 24 h. Cytokines in supernatants were quantified. CRS classification criteria were established using reference low- and high-risk antibodies. Clinical CRS risk was evaluated against CRS potential classification to assess the utility of the CRA assay. In conclusion, our CRA assay can classify the underlying mechanisms of antibody-induced cytokine release and reproducibly evaluate the clinical CRS risk of antibody therapeutics, thereby supporting the safety assessment of antibody therapeutics.

Materials and Methods



Assay flow

Table 1 Assay conditions

Cytokine release assay	
Cell	Human PBMC (Charles River Laboratories Cell Solutions, Inc.)
Donor number	13 (N=3)
Cell number	200,000 cells/well
Culture format	96-well plate
Antibody treatment	Solid phase (wet-coat)
Measurement System	Luminex ® 200 system (Luminex Corporation)
Analyte	IFN-Y, IL-2, IL-6, IL-10, TNF-α

Table 2 Reference antibodies

Reference antibodies	Targets	Isotypes	Manufacturers	Final concentrations	Clinical CRS_risk categories
Panitumumab	EGFR	hIgG2	HY-P99041, MedChemExpress LLC	1 μg/well	**Low** (IRs* incidence: 3% [1])
Trastuzumab	HER2	hIgG1	HY-P9907, MedChemExpress LLC	1 μg/well	**Intermediate** (IRs* incidence: 40% [1])
Rituximab	CD20	hIgG1	HY-P9913, MedChemExpress LLC	1 μg/well	**Intermediate** (IRs* incidence: Majority [1])
Alemtuzumab	CD52	hIgG1	HY-P9948, MedChemExpress LLC	1 μg/well	**Intermediate** (IRs* incidence: 89% [1])
Muromonab	CD3	mIgG2	HY-P99152, MedChemExpress LLC	0.1 μg/well	**High** Most patients develop CRS [2](Withdrawn from use in 2010)
TGN1412	CD28	hIgG4	HY-P9975, MedChemExpress LLC	1 μg/well	**High** In the Phase I clinical trial, all healthy volunteers occured a cytokine storm [3]

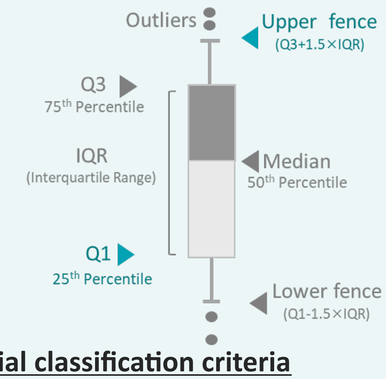
* IRs: Infusion reactions

Results

Establishment of risk classification criteria

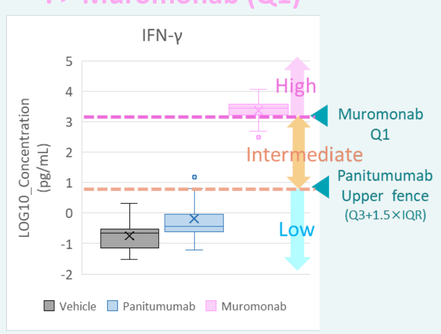
To minimize the impact of inter-donor variability, classification criteria for CRS risk potential were established using the interquartile range (IQR), a robust measure of the variability of the central 50% of the data. Low-risk responses were defined based on the background response of panitumumab (Q3 + 1.5×IQR), whereas high-risk responses were defined based on the background response of muromonab (Q1). Donors were classified into three risk categories (low, intermediate, and high). If cytokine responses from a donor spanned multiple risk categories, the highest risk category was assigned as the donor's final classification.

Explanation of the interquartile range (IQR)



CRS risk potential classification criteria

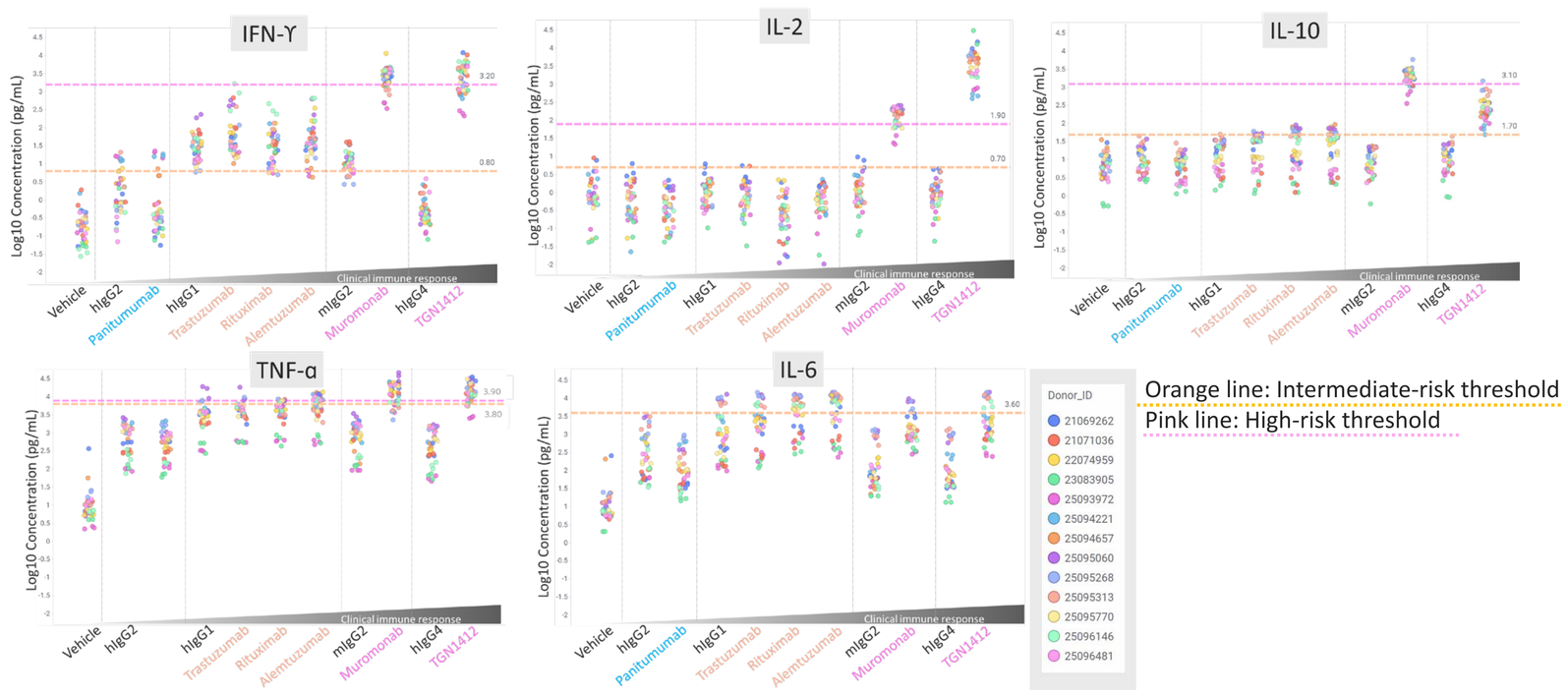
Low : < Panitumumab (Q3 + 1.5 × IQR)
Intermediate : Between low and high
High : > Muromonab (Q1)



Orange line: Intermediate-risk threshold
Pink line: High-risk threshold

Fig. 1 Box plot of cytokine levels in 13 donors stimulated with reference antibodies

Background IFN-γ data are shown as a representative example to illustrate the risk classification thresholds.



Orange line: Intermediate-risk threshold
Pink line: High-risk threshold

Fig. 2 Cytokine release profiles following stimulation with reference antibodies

The cytokine levels were measured after stimulation with six therapeutic antibodies, their corresponding isotype controls, and the vehicle control (PBS). Each point represents an individual value from 13 donors. Based on the cytokine release data of the reference antibodies, only two risk categories (low and intermediate) were established for IL-6.

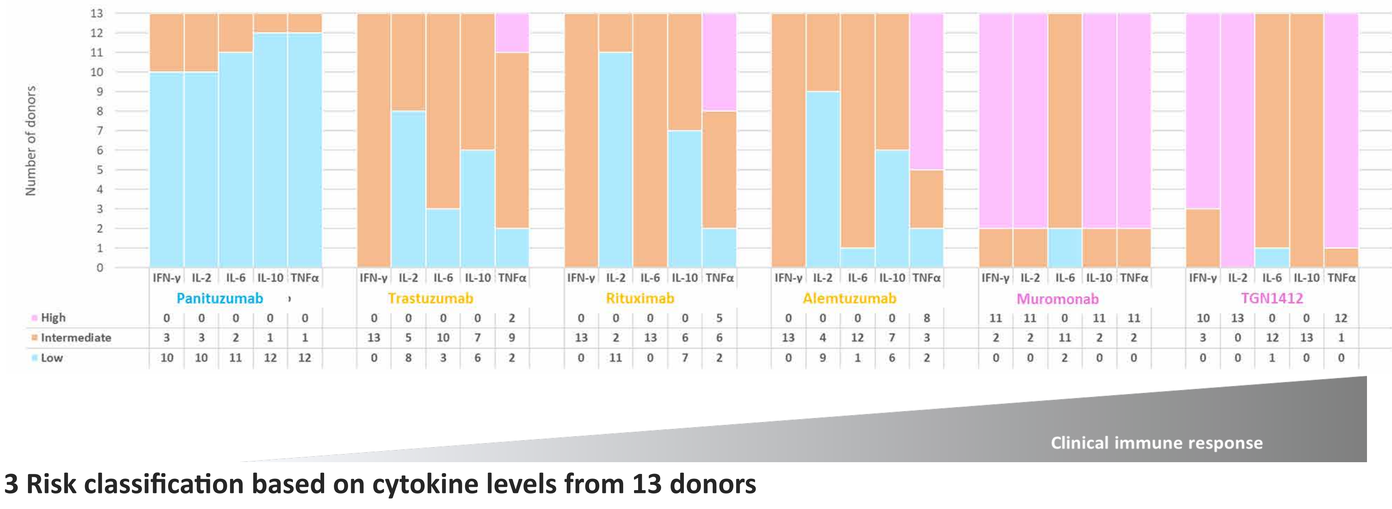


Fig. 3 Risk classification based on cytokine levels from 13 donors

The bar and color show the number of donors and CRS risk classifications, respectively (blue; low-risk, orange; intermediate-risk, pink; high-risk).

Conclusion

- The IQR-based CRS classification criteria accurately reflected the clinical CRS risk, supporting the validity of this CRAs.
- TNF-α and IL-6 increases induced by intermediate-risk antibodies may reflect Fc receptor-mediated and driven responses.
- In contrast, high-risk antibodies induced IFN-γ and IL-2, suggesting direct T-cell activation.
- This CRA assay enable mechanistic classification of cytokine release and support reproducible CRS risk assessment in antibody therapeutics.

COI disclosure information

Lead Presenter/Responsible Researcher:
Yuka Hasegawa
I have no financial relationships to disclose.

References

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