Demonstrating the feasibility ADA titer determination by singlicate analysis:

Case study based on a proposed tocilizumab biosimilar

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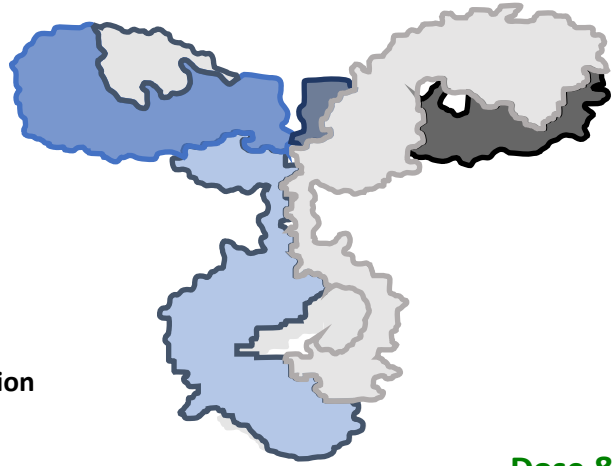
EBF – Challenging the Current Paradigm for ADA Testing

21-22 September, NH Malaga, Spain

Tocilizumab – Overview

Biochemical Features

- Recombinant humanized IgG1κ monoclonal antibody
- Produced in genetically engineered mammalian cells



Function

- Binds and neutralizes IL-6 Receptor
- Blocks IL-6 signaling & prevents T-cell activation
- Reduces local and systemic inflammation

Approved Indications

EU and US

- Moderate-to-severe RA (alone or in combination with MTX)
- Systemic juvenile idiopathic arthritis (sJIA),
- polyarticular juvenile idiopathic arthritis (pJIA),
- Cytokine release syndrome (CRS)
- Coronavirus disease 2019 (Covid-19)
- Giant cell arthritis (GCA)

US

- Systemic sclerosis associated interstitial lung disease (SSc – ILD)

Adverse Events

Most Common:

- Upper respiratory tract infections, nasopharyngitis, headache, hypertension, increased alanine aminotransferase, and injection-site reactions

Dose & Regimen

General Indications

- 4-8 mg /kg 60 min IV infusion every 4 weeks
- 162 mg subcutaneous injection every week

CRS:

- 60-minute IV infusion once
- 3 infusions ≥8 hours between consecutive doses

MSB11456 Clinical Development Program

Retrospective analysis

Singlicate analysis applied for ADA titration

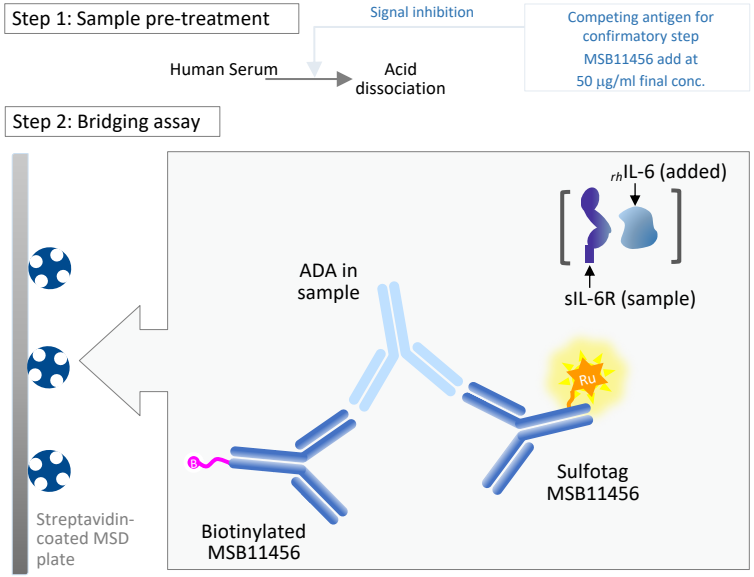
Study No/ Start-Completion	Phase / Description	Subjects	Study Arms	Dose / Treatment period
MS200740-0001 **(NCT03282851)** Nov 2017 – Oct 2019	Ph1 3-way comparative PK/PD, safety & immunogenicity	Healthy volunteers	• MSB11456 • US-Actemra • EU-RoActemra	Single 162 mg, s.c. injection 48-day assessment period
FKS456-002 (APTURA II) **(EudraCT 2019–003484-22)** Sep 2020 – Jan 2021	Ph1 2-way comparative PK, safety & immunogenicity	Healthy volunteers	• MSB11456 • US-Actemra	Single IV infusion of 8 mg/kg for 1 hour 48-day assessment period
FKS456-001 (APTURA I) **(NCT04512001, EudraCT Number: 2019-004369-42)** Aug 2020 – Jun 2022	Ph3 comparative efficacy & safety & immunogenicity	Moderately to severely active Rheumatoid Arthritis	• MSB11456 • EU-RoActemra → EU-RoActemra • EU-RoActemra → MSB11456 "→" = W24 re-randomization	Once-weekly (QW) 162 mg s.c. for 24 weeks to 52 weeks
FKS456-003 **(EudraCT 2020-003419-86)** Feb 2021 – Jun 2021	Ph1 2-way comparative PK & safety	Healthy volunteers	• → MSB11456 (PFS → washout → AI) • → MSB11456 (AI → washout → PFS) "→" Day 1 Randomization to sequence & injection site: abdomen, thigh or upper arm	Cross-over single Sc dose of 162 mg MSB11456 given as PFS or AI

All studies met their primary, secondary and exploratory endpoints supporting similarity between MSB11456 US-Actemra and EU-RoActemra

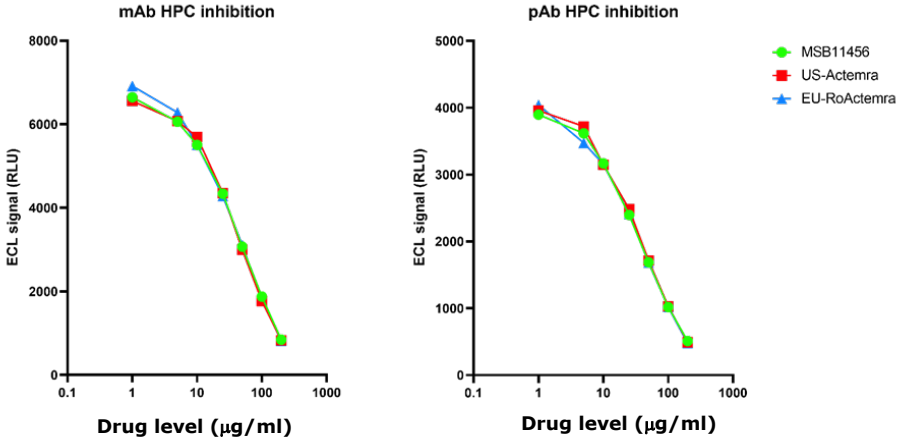
AI: autoinjector; **PFS**: pre-filled syringe

ADA Assay format and suitability

ADA assay format



Competitive inhibition curves (varying tocilizumab conc.) for mAb and pAb positive controls



A homogeneous ECL bridging ADA assay developed and validated

Assay has high sensitivity and drug tolerance

Antigenic equivalence was demonstrated, indicating suitability of the "One-Assay Approach"

Titer: Availability of ADA Magnitude Data Enables

- Subset analysis of ADA impact on PK, safety, and efficacy
- Monitoring of immune response progression, especially in subjects with pre-existing antibodies
- Comparison of ADA response characteristics between biosimilar and innovator

Immunogenicity testing in MSB11456 program

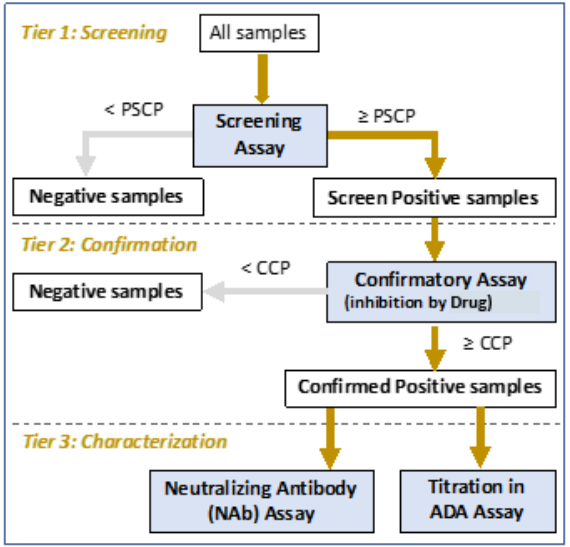
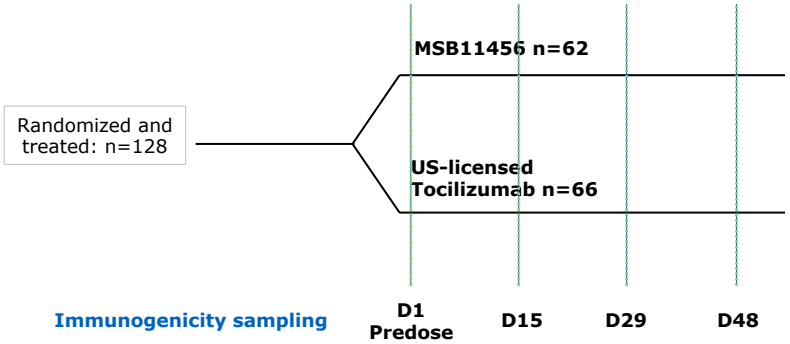


Figure P. Chamberlain (2022)

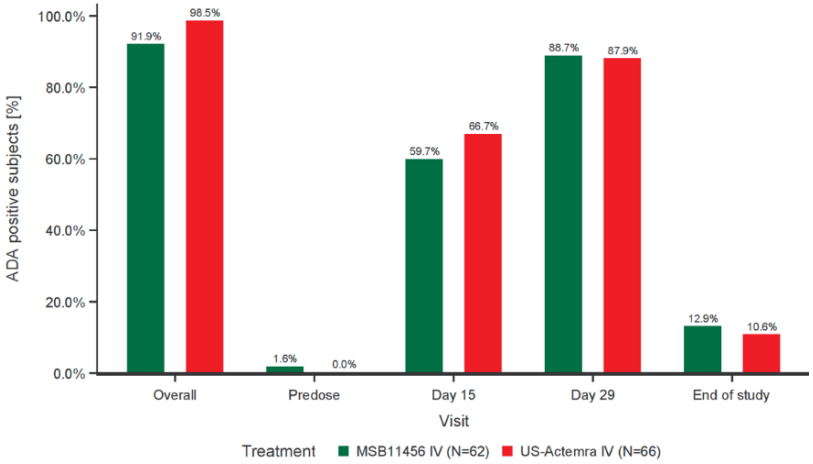
Titer is most currently used variable to assess the magnitude of the ADA response

Samples from the FKS456-002 study used in the retrospective analysis

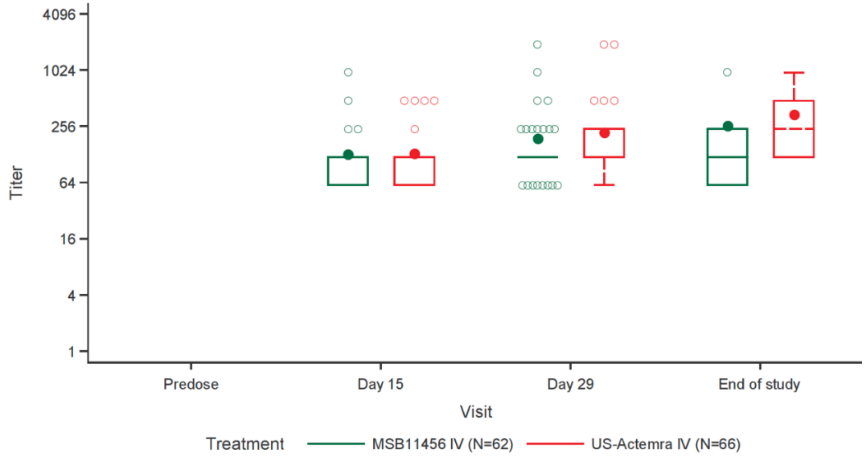


Immunogenicity Results: FKS456-002

ADA incidence



Titration

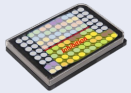
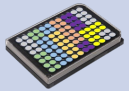


Similarly high ADA incidence (low titers) was detected in the MSB11456 and US-Actemra groups

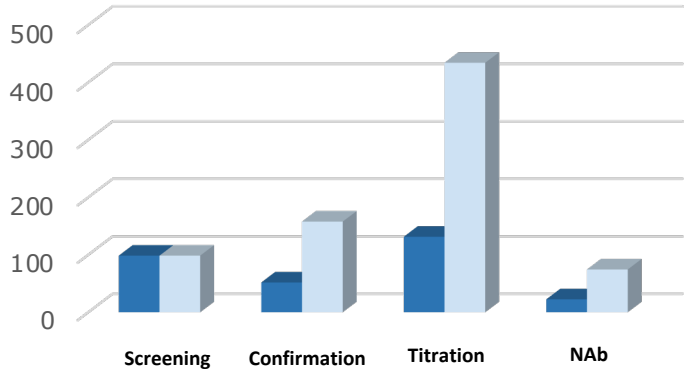
Overall NAb incidence was similarly low across treatment groups (7.0% for MSB11456 and 12.3% for US-Actemra)

No impact on PK and Safety

Microtiter Plate comparison per ADA Tier

Tier	Samples per plate	Cost per plate (USD)	Cost per sample (USD)
Screening 	40	2,000	50
Confirmatory 	19		105
Titration 	9		333
NAb 	35	2,500	70

Number of Analytical Runs
For two different scenarios of ADA positivity



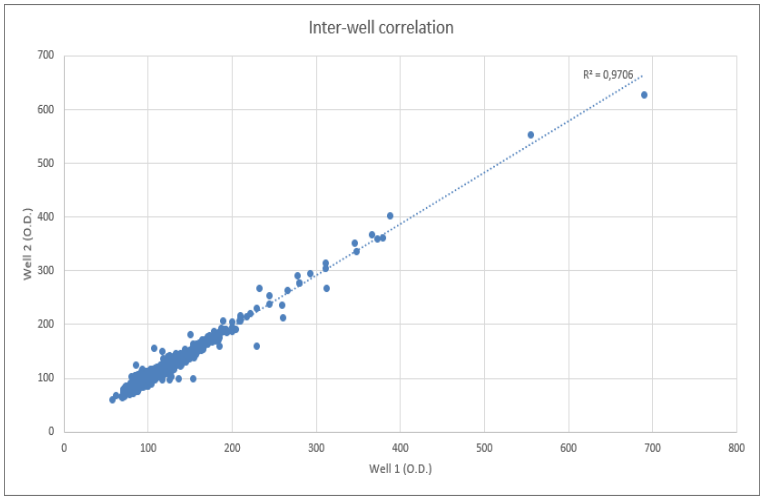
	# IMG samples	Positivity Screening	Positivity Confirmed
Scenario 1	4000	25%	20%
Scenario 2	4000	76%	66%

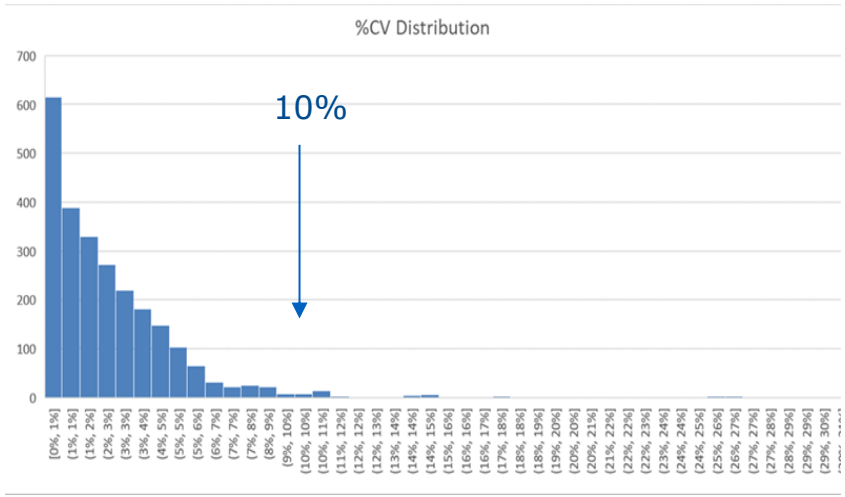
During ADA testing, the confirmatory, and especially the titration assay significantly impact sample throughput and costs

Can the data from the Ph1 study (FKS456-002) inform whether titration for the Ph3 study (FKS456-001) could be performed in singlicate, without any impact on data quality?

Preliminary assessment: FKS456-002 Study Data

Comparison of the degree of concordance between performance for the first and second well in terms of **instrument signal**

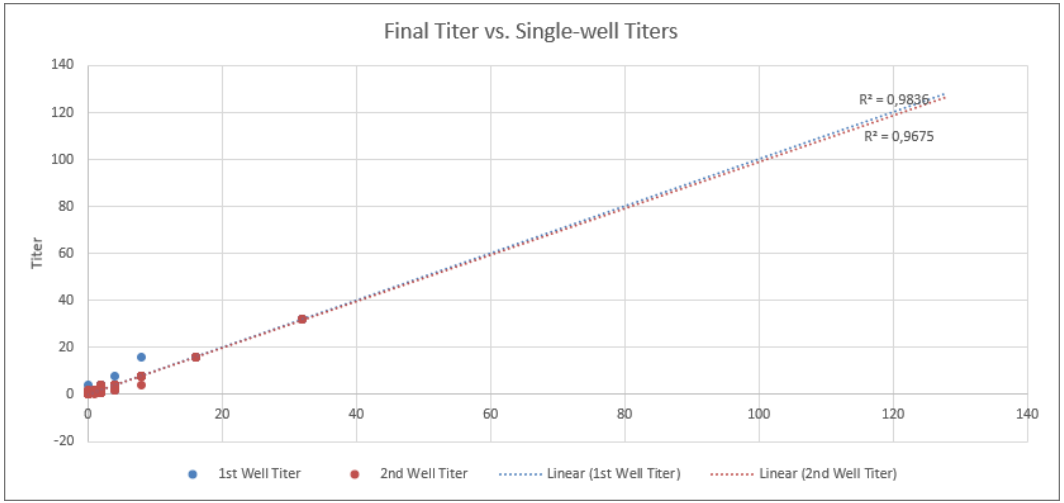




Average %CV = 2.8%

Preliminary assessment: FKS456-002 Study Data

Comparison of the degree of concordance between performance for the first and second well in terms of **titer**



Both analyses did show good concordance.

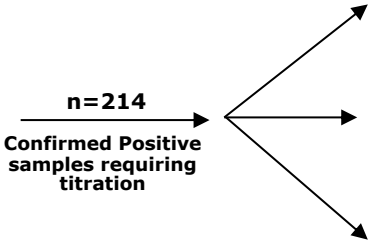
Follow up exploratory analysis

Objective: To compare Aptura 2 titer results obtained using a single well approach (singlicate) with titer results obtained using a duplicated well approach. For the purpose of this exploratory analysis, the following single well analyses will be considered:

1. Use of titer results obtained from single well (well-1) for all samples
2. Use of titer results obtained from single well (well-2) for all samples
3. For each sample, derivation of "worst" titer defined as the titer obtained from the well (well-1 or well-2) associated with the largest difference as compared to titer obtained based on the duplicated analysis.

The results obtained form the 3 different approaches will be compared to titer results obtained using duplicated approach by means of summary statistics. No formal statistical comparison will be performed.

Singlicate analysis and potentially missed repeats (from otherwise inconclusive tests)

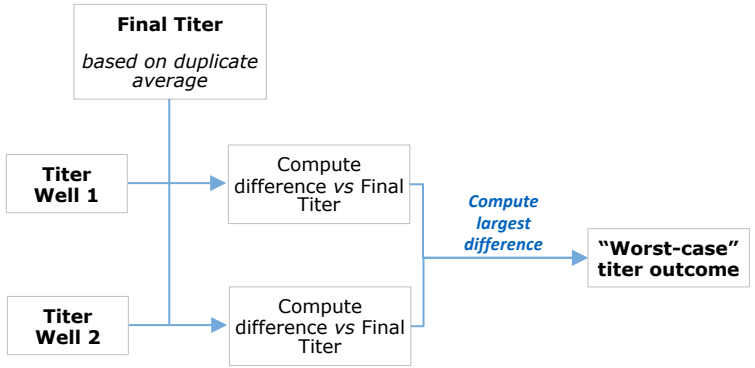


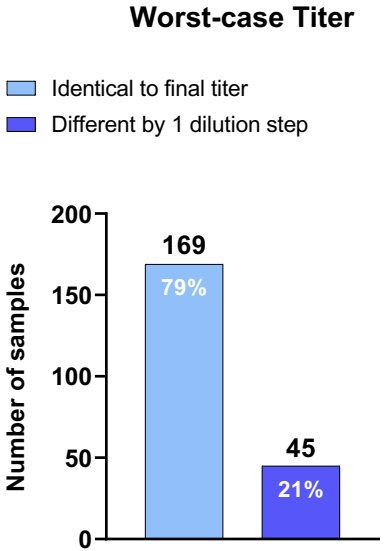
Outcome 1st test	Repeat necessary?	Impact of singlicate on test repeat or absence of repeat
Conclusive results for both wells **n=198**	No	**Test not to be repeated even if performed in singlicate**
Inconclusive results for both wells **n=14**	Yes	**Test would have to be repeated in both duplicate and singlicate formats**
Inconclusive results for either well-1 or well-2 **n=2**	Yes	**Titer* results for sample 000192a0** Test / Well 1 / Well 2 / Final Original / Inclsv. / 2 / **Inclsv.** Repeat / 2 / 2 / **2** **Titer* results for sample 000192a0** Test / Well 1 / Well 2 / Final Original / 0 / Inclsv. / **Inclsv.** Repeat / 2 / 1 / **1**

***not including MRD**

1. **Where inconclusive results were observed, those were generally seen for both wells**
2. **In the 2 only cases where an inconclusive result was observed for only one well, the repeat results from were either identical, or within ±1 dilution step when compared to the final titer**
3. **It is adequate to exclude assay results qualified as "inconclusive" from this exploratory retrospective analysis**

Retrospective assessment of “worst-case” outcome of singlicate analysis



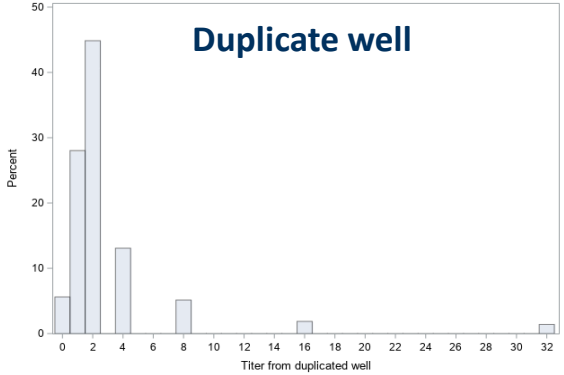


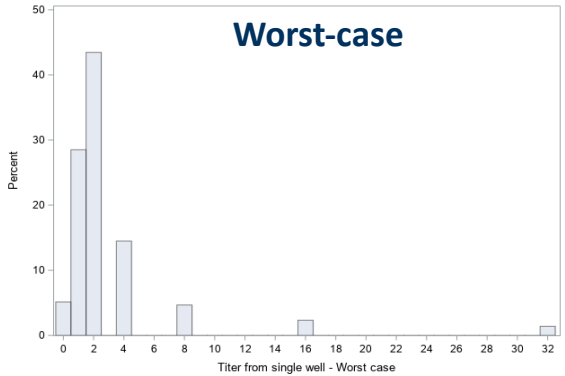
In the current retrospective analysis, there were no samples that would have been associated with a titer result different by ≥2 dilution steps

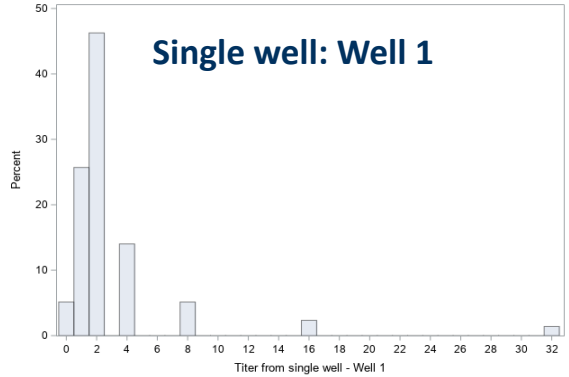
Had the assay been performed in singlicate, the maximal difference would have been 1 dilution step (2-fold* difference).

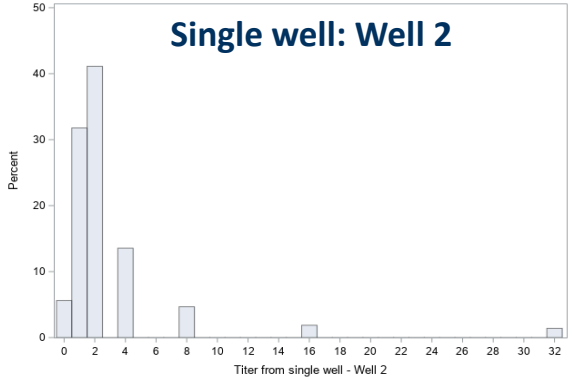
***The MSR (minimum significant ratio, i.e. the smallest fold change between the titers of any two anti-drug antibody positive samples that is considered significant) is around 2 for this assay (1.8 for polyclonal and 2.1 for the monoclonal antibody).**

Titer histograms on different approaches







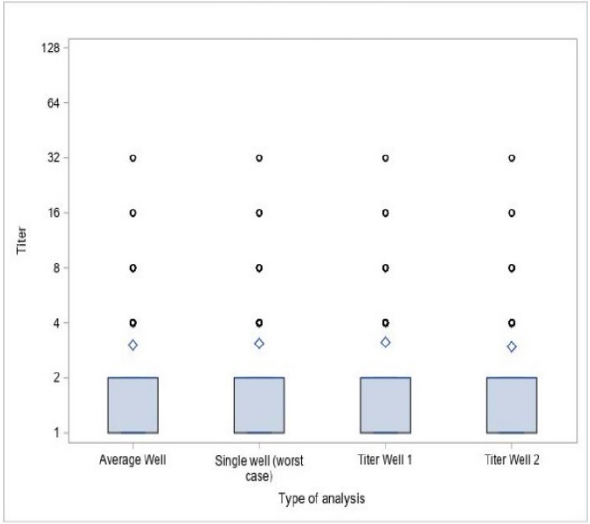


Similar titer result distribution observed among the 4 approaches

Summary Statistics and Box Plots

Table 4- Summary statistics of titer results according to type of analysis (Duplicated wells, singlicate - Well-1, singlicate – Well-2, singlicate (worst case))

Summary statistics	Duplicate Average of wells	Singlicate Well-1	Singlicate Well-2	Singlicate Worst case
N	214	214	214	214
Mean (SD)	2.86 (4.302)	2.98 (4.382)	2.80 (4.300)	2.93 (4.379)
Min	0	0	0	0
Q1	1	1	1	1
Median	2	2	2	2
Q3	2	2	2	2
Max	32	32	32	32
% increase in SD as compared to duplicated well analysis	.	1.9%	0%	1.8%



Similar titer distribution is observed among the 4 approaches suggesting that singlicate analysis may be acceptable for titer determination purposes

Discussion

- Our retrospective evaluation of the duplicate ADA titration data from a Ph1 study demonstrated that, for the method applied, titration could have been performed in singlicate, with no impact on the quality of results.
- Our conclusions are supported by results from singlicate re-evaluation and/or simulation models published in literature in different contexts

ADA

Feasibility of singlicate-based analysis in bridging ADA assay on Meso-Scale Discovery platform: comparison with duplicate analysis

Zhihua Jiang*[,1], John Kamerud[1], Zhiping You[2], Soma Basak[1], Elena Seletskaia[1], Gregory S Steeno[2] & Boris Gorovits[1]

Bioanalysis (2021) 13(14), 1123–1134

BioM

Singlicate analysis: should this be the default for biomarker measurements using ligand-binding assays?

Zhuqiu Ye*[,1], Jing Tu[1], Krishna Midde[1], Mike Edwards[1] & Patrick Bennett[1]

Bioanalysis (2018) 10(12), 909–912

PK

Well-developed ligand-binding assays demonstrate robust performance using singlet analysis

Douglas Donaldson[1], Shobha Purushothama[1,2], Eric David[1], Kristopher King[1], Shuguang Huang[3], Devangi S Mehta[1,4] & Lauren F Stevenson*[,1,4]

Bioanalysis (2019) 11(22), 2075–2086

European Bioanalysis Forum recommendation on singlicate analysis for ligand binding assays: time for a new mindset

Matthew Barfield[1], Joanne Goodman[2], John Hood[3] & Philip Timmerman*[,4]

Bioanalysis (2020) 12(5), 273–284

Implications for our candidate Tocilizumab Biosimilar program

Phase 3 study: ~3000 ADA samples to run in Tier 3

- Singlicate titer analysis brought significant cost-efficiency to the study (~6 weeks and ~USD 300K) without compromising the data quality and maintaining the familiar variable of “ADA Titer”.
- Ph3 titer results were in line with Ph1 studies (low titer, no impact on PK, safety and efficacy)

Perspectives on future programs

- Reflect on & discuss which development and validation assessments are required to demonstrate suitability of the singlicate approach
- Implement the singlicate approach to the immunogenicity and PK assays
- One step further: could instrument signal lead to similar conclusions as the titer?

Acknowledgements

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Martin Ullmann	VP Medical Affairs, Clinical Pharmacology & Immunology

Cytel

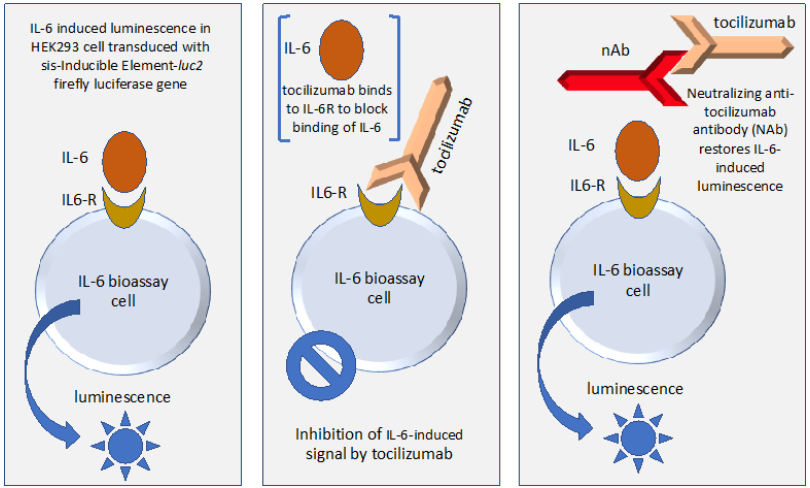
Nicola Lama	Associate Director, Biostatistics
Virginie Jego	Principal Biostatistician

Bioanalytical Laboratories

Syneos Health (Princeton, NJ, USA)

Back-up slides

Immunogenicity Approach to demonstrate biosimilarity: NAb assay



Applicable Clinical study

Phase I, Single-dose SC
Comparative PK/PD
Study MS200740-0001

NAb assay
IL-6 cell-based assay
No sample pre-treatment
MVR: Covance No. 8365-590
SOP: Covance No. CELL-0053

MRD = 80
Sensitivity (pAb) = 24500 ng/mL
LPC (pAb) = 25000 ng/mL
Drug tolerance @ LPC = 8 µg/mL

Method transfer & re-validation

Further optimization of method to improve assay sensitivity by approx. 10-fold

Phase I, Single-dose IV
Comparative PK
Study FKS456-002

Phase III, chronic SC
Rheumatoid arthritis
Study FKS456-001

NAb assay
IL-6 cell-based assay, with Affinity-Capture-Elution sample pre-treatment
MVR: Syneos Health No. 14445.230419
SOP: Syneos Health No. TM.2504

MRD = 2.94
Sensitivity (pAb) = 2200 ng/mL
LPC (pAb) = 3000 ng/mL
Drug tolerance @ LPC = 10 µg/mL

As per FDA request, a cell based assay was implemented for NAb determination. The assay was optimized to improve sensitivity and Drug Tolerance.

Immunogenicity: Impact on PK and sIL-6 Receptor: MS200740-0001 (NCT03282851)

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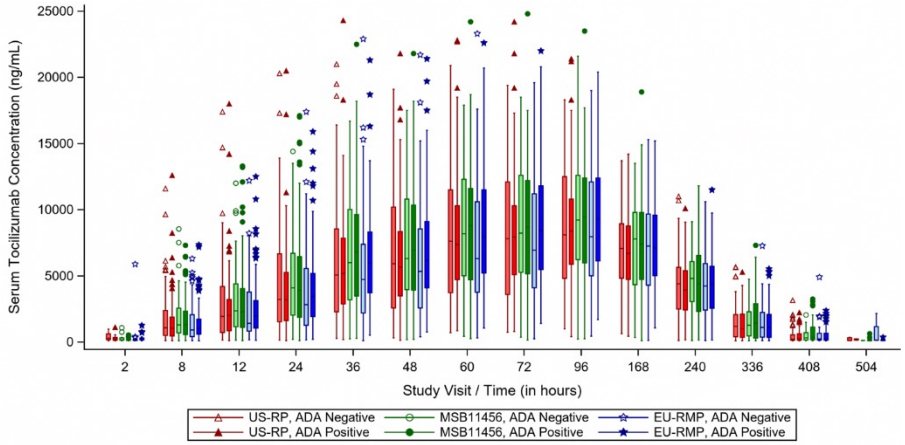
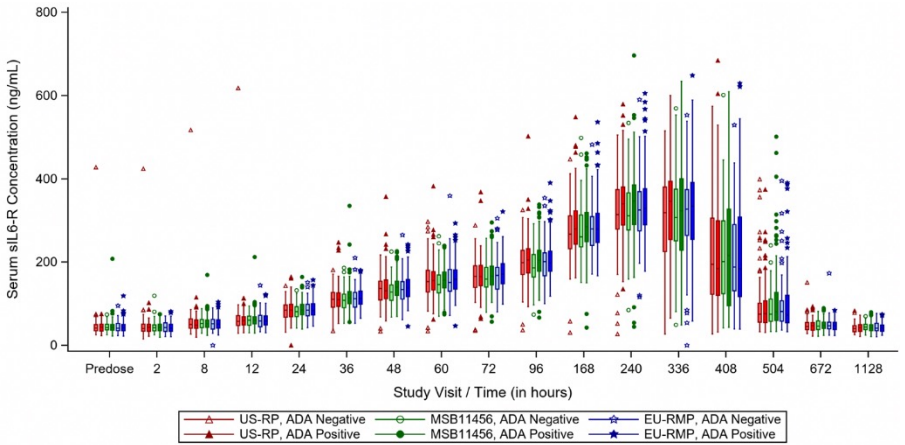
caring for life

Serum Tocilizumab concentrations by ADA status



Serum sIL-6R concentrations by ADA status



No impact of ADA status on PK or PD (sIL-6R)