Challenges to Demonstrating Comparability of Fully Human Therapeutic Antibodies in Nonhuman Primates

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Presentation Outline

- Clinical development of fully human antibody therapeutics
- Pre-marketing biocomparability studies
- Bioanalysis of therapeutic and anti-drug antibodies
- Impact of immunogenicity on antibody bioanalysis and pharmacokinetics
- Study Design requirements for fully human antibody therapeutics
- PK analysis and biocomparability strategies
- Example case studies
- Conclusions

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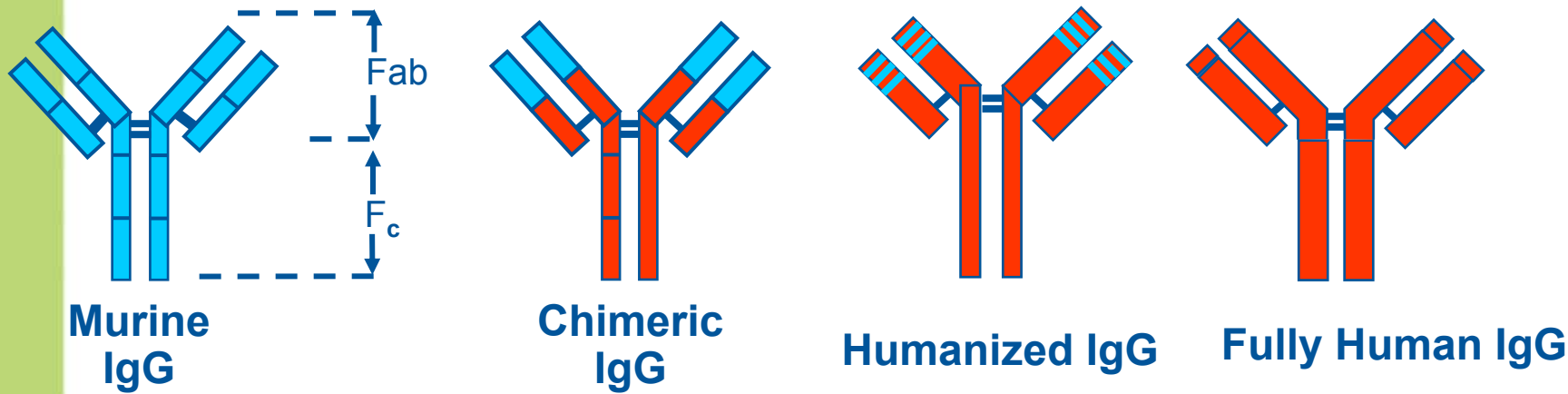
Progression in the Development of Molecule Antibody Products



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Advantages of Fully Human Antibodies

- Safety
  - Reduce hypersensitivity responses to initial treatment
  - Reduce immune responses following repeat treatment
- Efficacy
  - Long half-lives for chronic therapies
  - Highly specific

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MAbs vs Small Molecules Drugs Development

- Differences
  - MAb very long half-lives (21 days) vs. minutes to days
  - MAb limited species antigen cross-reactivity (primates) vs. ≥ or 2 species
  - Mab formulations in solution vs. various forms
  - MAb PK covariates for accelerated clearance: anti-drug antibodies vs. highly metabolized drug
  - MAb safety: predictive to loss of antigen/immune responses vs. often unpredictable
- Commonalities
  - MAb and small molecules manufacturing/formulation process changes thoughout development

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Hierarchy of Comparability Testing

- Analytical: Physical / Chemical /Biological *in vitro* characterization
- Bioassays
- Animal PK/PD studies (PD markers for safety/efficacy) can provide a good relative measure
- Human PK/PD studies (PD markers for safety/efficacy)
- Clinical Safety and Efficacy studies

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Nonclinical Biocomparability Testing in Clinical Development

- Regulatory requirements
- Sensitive indicator of relative differences in PK and immune response across formulations in the test system
- Risk reduction for clinical study participants
- Supportive to analytical and bioassay testing
- Nonclinical testing may not predict clinical response

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Bioanalysis of Fully Human Therapeutic and Anti-Drug Antibodies

- Typically immunoassay methods
  - Fully human therapeutic antibody assays
  - Anti-drug antibody = “Immune Response” assays
- Therapeutic antibody assay formats
  - “Sandwich” assays preferred over competition formats
  - Centocor employs non-cross reactive anti-idiotype reagent antibodies
- Assay performance is closely aligned with the “quality” of the reagent antibodies
- The presence of anti-drug antibodies will interfere with the detection of therapeutic antibodies

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Differences in Chemical and Immuno Drug Assays

	Chromatographic	Immunoassay
Assay basis	Physiochemical Props	Ag-Ab
Assay reagents	Characterized, accessible	Unique, less available
Analytes	Small molecule	Large molecule
Detection	Direct	Indirect
Sample pretreatment	Yes	No
Time req'd for development	Weeks	Months (reagents)
Inter-assay imprecision	Low (< 10%)	Moderate (< 20%)
Source of imprecision	Intra-assay	Inter-assay
Assay working range	Broad	Limited
Standard curves	Linear	Nonlinear

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Bioanalysis Sources of Variability

- Assay performance is largely aligned with the “quality” of the assay reagents
- Degree of antibody “human-ness” challenges
  - Reagents creation
  - Selectivity of measuring therapeutic antibody in a “sea” of blood antibody
- Inter-occasion variability with immunoassays
- Accuracy limitation: 4-6-30 total error

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Bioanalysis of Anti-Drug Antibodies (ADA)

- Anti-Drug Antibody formats
  - Drug – ADA - Drug-(*) “Bridging”
  - Others eg, Surface Plasmon Resonance
- Immune response assay issues
  - Assay sensitivity
  - Appropriate assay and control reagents
  - Assay cut-offs for positivity
  - Multistep testing: Screening, titering, specificity/neutralization
  - Interpretation
- Interference by therapeutic drug

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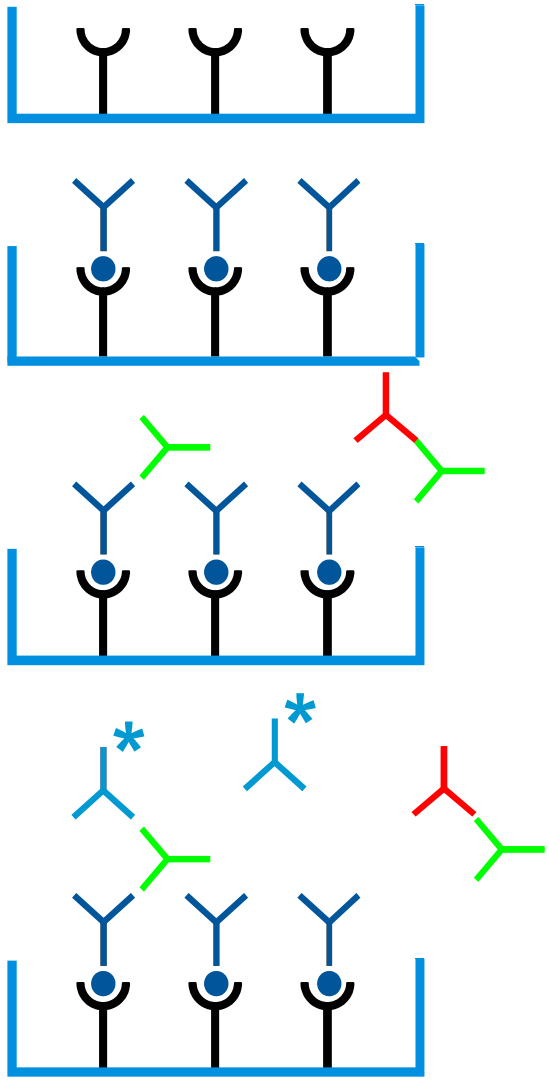
“Bridging” Anti-drug Antibody Assays Study Drug Interference



Streptavidin

Biotin-labeled drug

Anti-drug antibodies with study drug

Enzyme-labeled drug

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Effect of Anti-drug Antibodies on Antibody Pharmacokinetics in Nonclinical Studies

- Typically observe accelerated clearance of drug
  - More rapid blood clearance of drug - anti-drug antibody immune complexes
  - Interference of anti-drug antibodies in the bioanalysis of therapeutic drug
- Factors affecting accelerated clearance include the particular antibody, the test species, and the very long half-life of therapeutic antibodies
- The appearance of accelerated clearance may not depend upon re-exposure and is seen at predictable or non-predictable times following a single dose of drug

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Bioequivalence Study Designs Starting Point

- Test (T) and Reference (Ref) given in various parallel or cross-over group study designs
  - The absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.
  - 21 CFR 320.1 (e)
  - 90% Confidence interval testing for peak (Cmax) and total (AUCinf) drug exposures within 80 to 125%

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Nonclinical Biocomparability Study Designs Objectives:

- Evaluate relative differences in the test and reference products.
- Limit the impact of covariates that reduce sensitivity to detection of difference (ie, immune response).
- Support for human safety is only as useful as the predictability of the drug product in the test system.

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Typical Nonclinical Biocomparability Study Designs

- Typically in non-human primates
- Parallel group designs to limit drug re-exposure; no cross-overs
- Typically parenteral dosing
- Typically soluble protein product when administered
- Accelerated drug clearance is typically observed at some time following the generation of anti-drug antibodies

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Nonclinical Biocomparability Study Design Strategies: Limiting Immunogenicity

- Select a test system species most tolerant of human products
- Route of administration to minimize immunogenicity (I.V. > S.C. > I.M)
- High doses of therapeutic antibodies frequently induce tolerance
  - Clinically proposed therapeutic dose and route may not be preferred
  - High doses or repeated high doses
- For maximal immune response assay sensitivity collect samples until drug concentration is undetectable

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Other Nonclinical Biocomparability Study Design Strategies

- Bioanalytical methods to minimize assay variability
- Accelerated clearance is analogous to highly metabolized small molecule drugs; alternative limits eg, 75 - 133%
- Limit AUC estimates to a fixed time post-dose
- Limit AUC estimates to the time of the first incidence of accelerated clearance

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Powering Nonclinical Studies

- Cmax was more variable than AUC in the case study examples
- Availability of animals is a major limitation of study powering

CV	Power (%)		
	70	75	80
25	12	13	14
30	17	18	20
35	22	24	27
40	29	31	34
45	36	39	43
50	45	48	53

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Centocor Case Studies

- 3 Fully human therapeutic antibodies comparability studies
  - Single dose via I.V. and S.C. routes
  - Cynomolgus monkey test system
  - Examples of accelerated clearance related and un-related to treatment emergent immune responses

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CNTO Drug A: Drug with accelerated clearance unrelated to anti-drug antibodies

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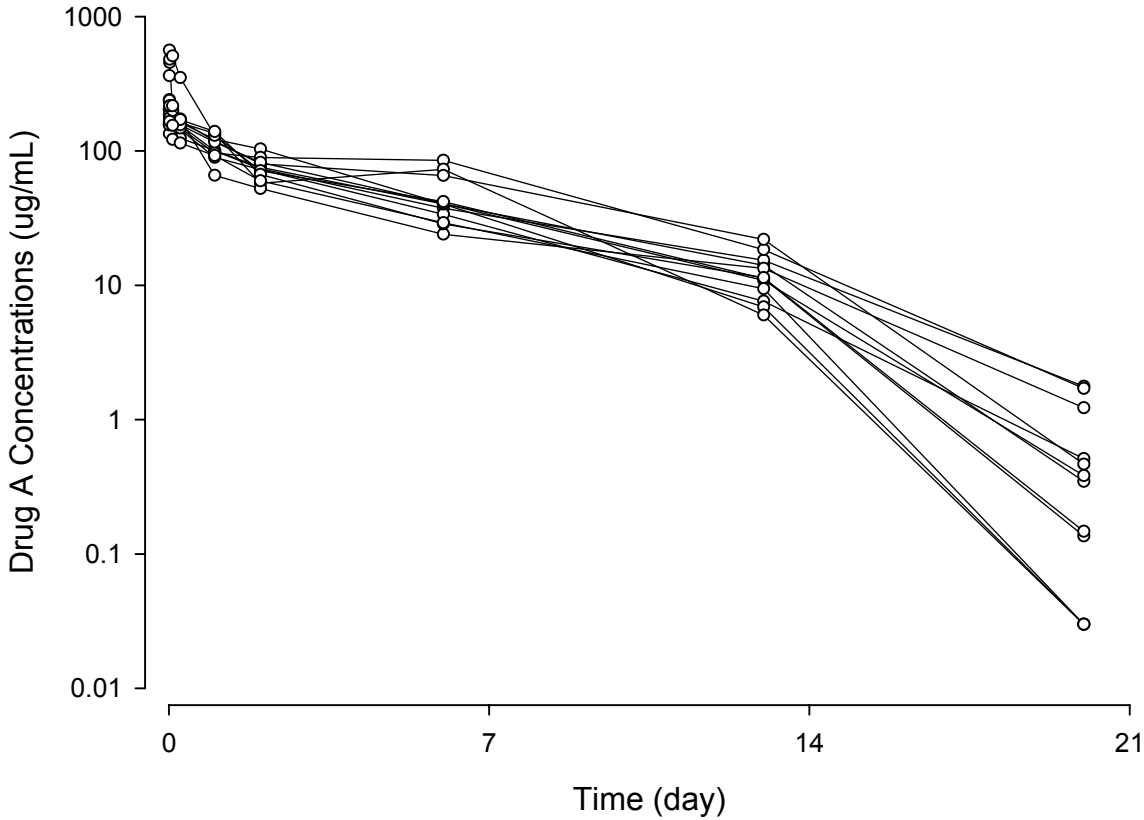
Individual Profiles Test Drug A



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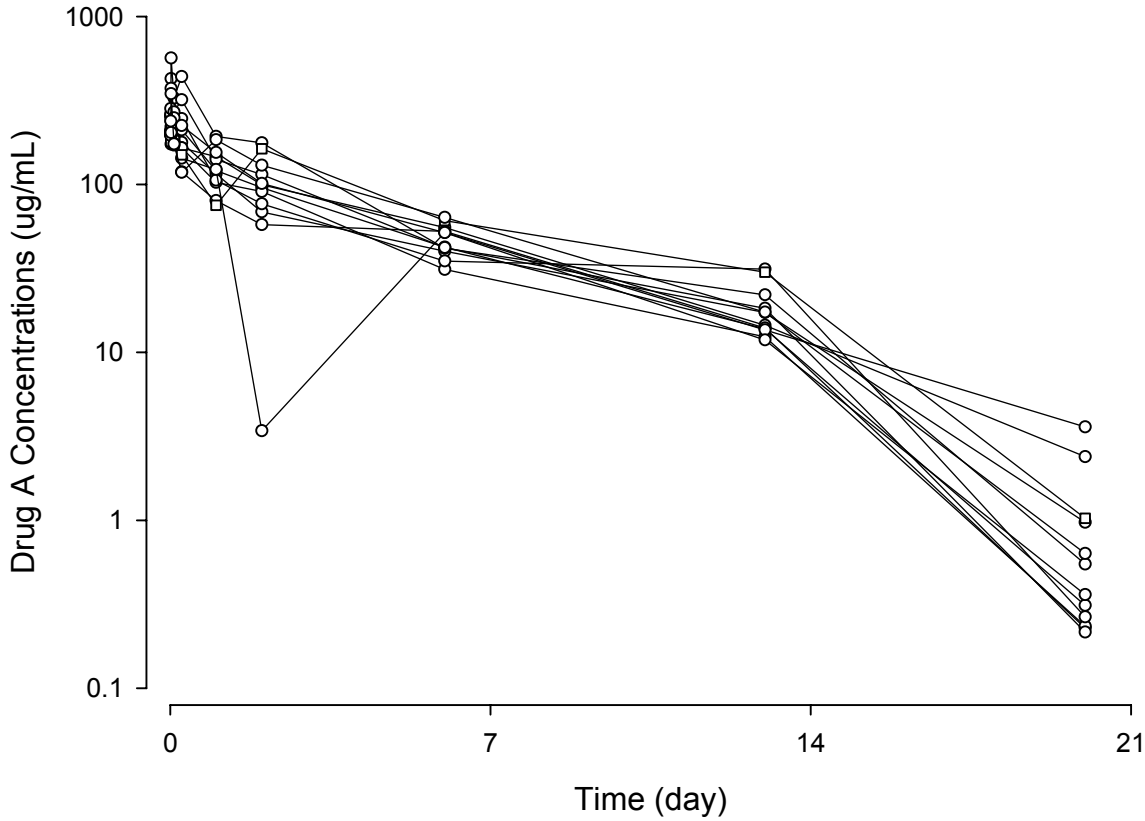
Individual Profiles Reference Drug A



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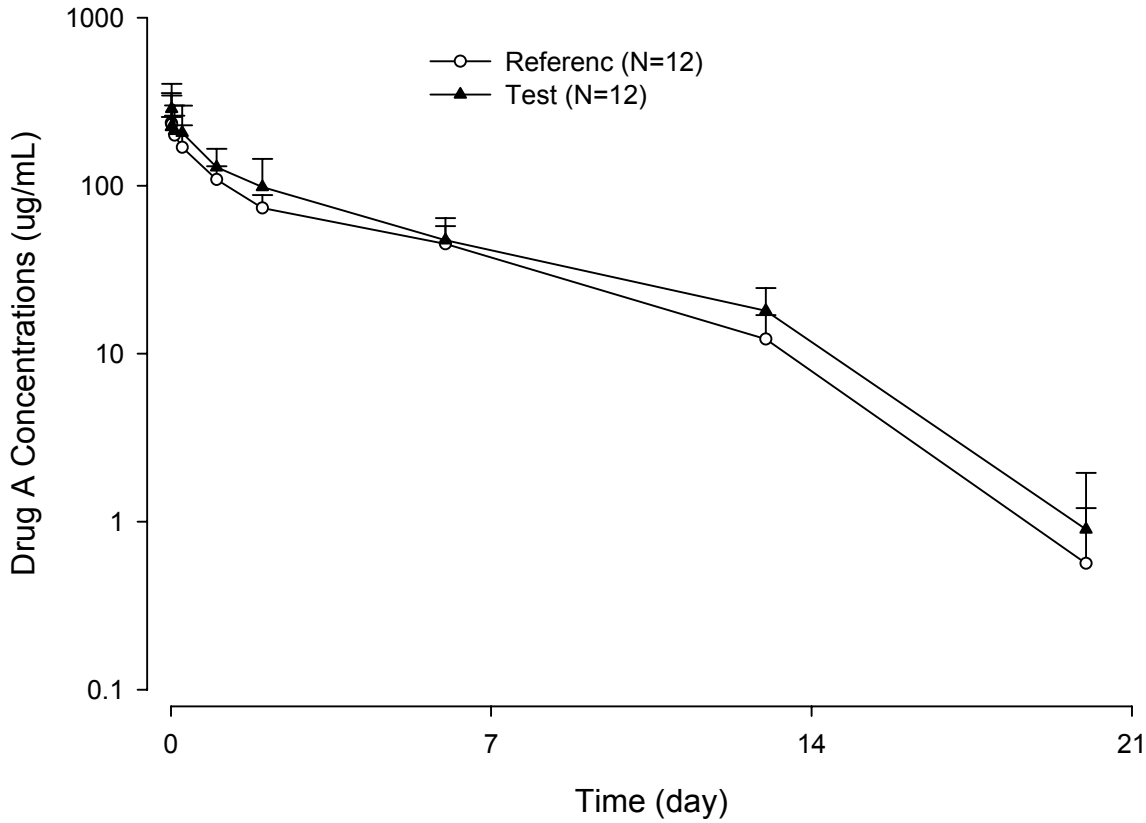
Mean Profiles of Test and Ref Drug A



Drug A Comparability

- No immune response was detected in samples from this study
- Accelerated drug clearance after Day 14 was not related to a detectable immune response to drug

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Two test formulations of CNTO Drug C: A greater incidence of anti-drug antibodies affecting drug profiles with a predictable timing

- $AUC_{0 – 10d}$
- Comparability limits 75 – 133%

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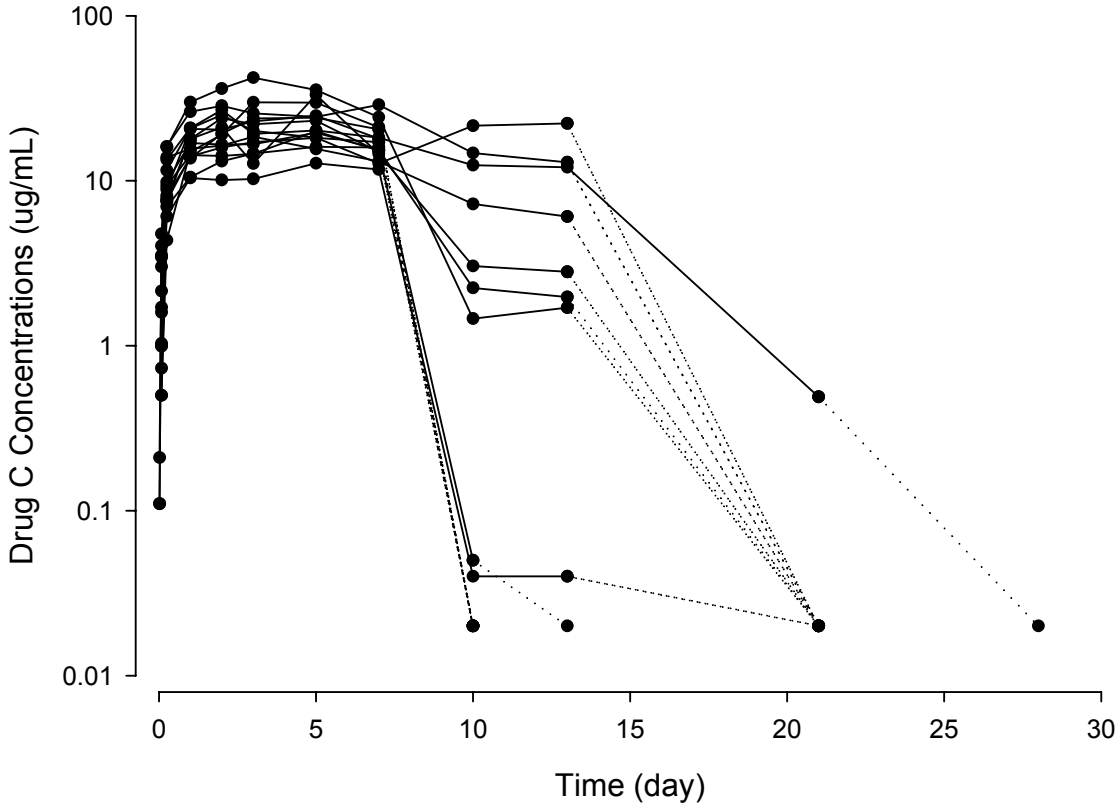
Individual Profiles of Ref Drug C



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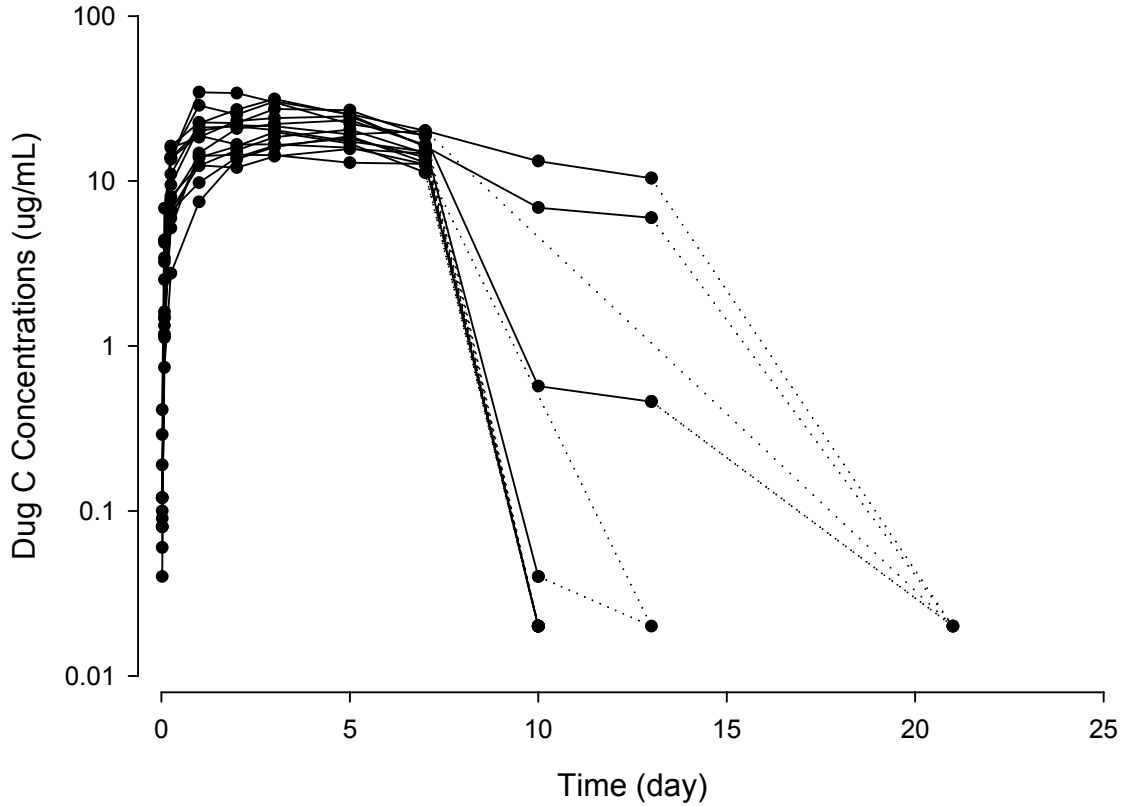
Individual Profiles of Test1 Drug C



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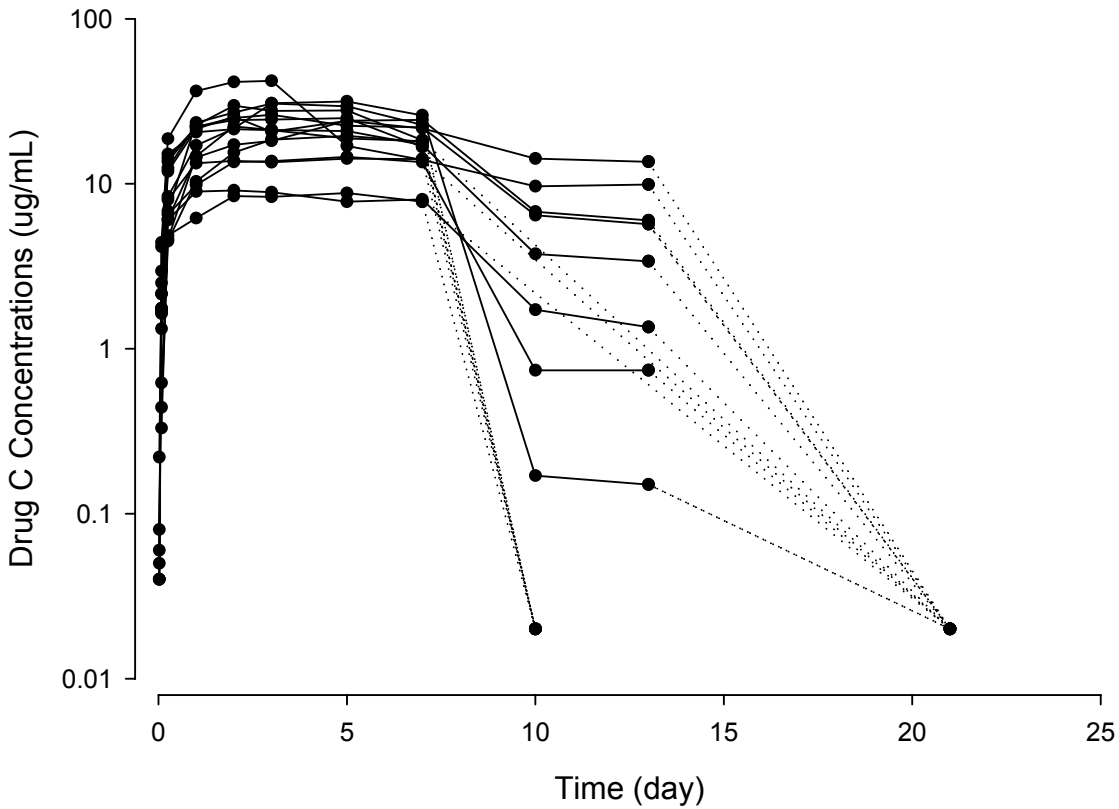
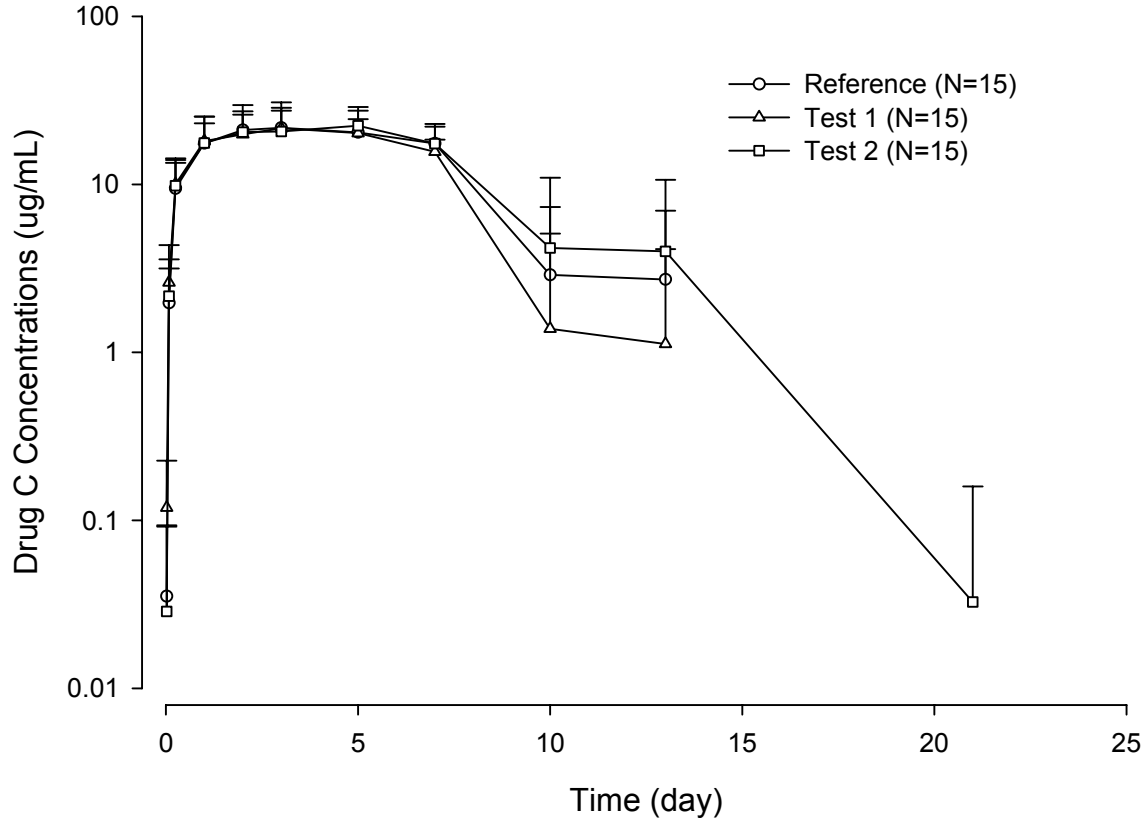
Individual Profiles of Test2 Drug C



Drug C Mean Profiles Test1, Test2, and Ref



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Drug C Test1

- Statistical Analysis of Cmax and AUC_{0-10} Following a Single 3 mg/kg S.C. dose in Cynomolgus Monkeys N = 15/group

PK Parameter	GeoMean of Test1	GeoMean of Ref	Ratio T/R	90% CI
Cmax	22.04	21.14	1.04	0.84-1.29
AUC(10d)	150.38	152.54	0.99	0.82-1.19

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Drug C Test2

- Statistical Analysis of Cmax and AUC_{0-10} Following a Single 3 mg/kg S.C. dose in Cynomolgus Monkeys N = 15/group

PK Parameter	GeoMean of Test2	GeoMean of Ref	Ratio T/R	90% CI
Cmax	23.23	21.14	1.10	0.89-1.36
AUC(0-10d)	160.80	152.54	1.05	0.87-1.27

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Drug C Comparability of Test1 and Test 2 with Ref Using Partial AUCs

- Apparent superimposible mean concentration profiles up to the time of accelerated clearance
- Point estimates of ratios of Cmax and AUC within 10%
- One test formulation was comparable by 75 – 133%, but not 80 -125%
- Immune responses detected in 44/45 animals
- The extent and timing of accelerated clearance have substantial effects on AUC estimates

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CNTO Drug D: A lesser incidence of accelerated clearance affecting profiles with an unpredictive timing

- AUC to the first appearance of accelerated clearance (AUC_{0-21d})
- Comparability limits 75 – 133%

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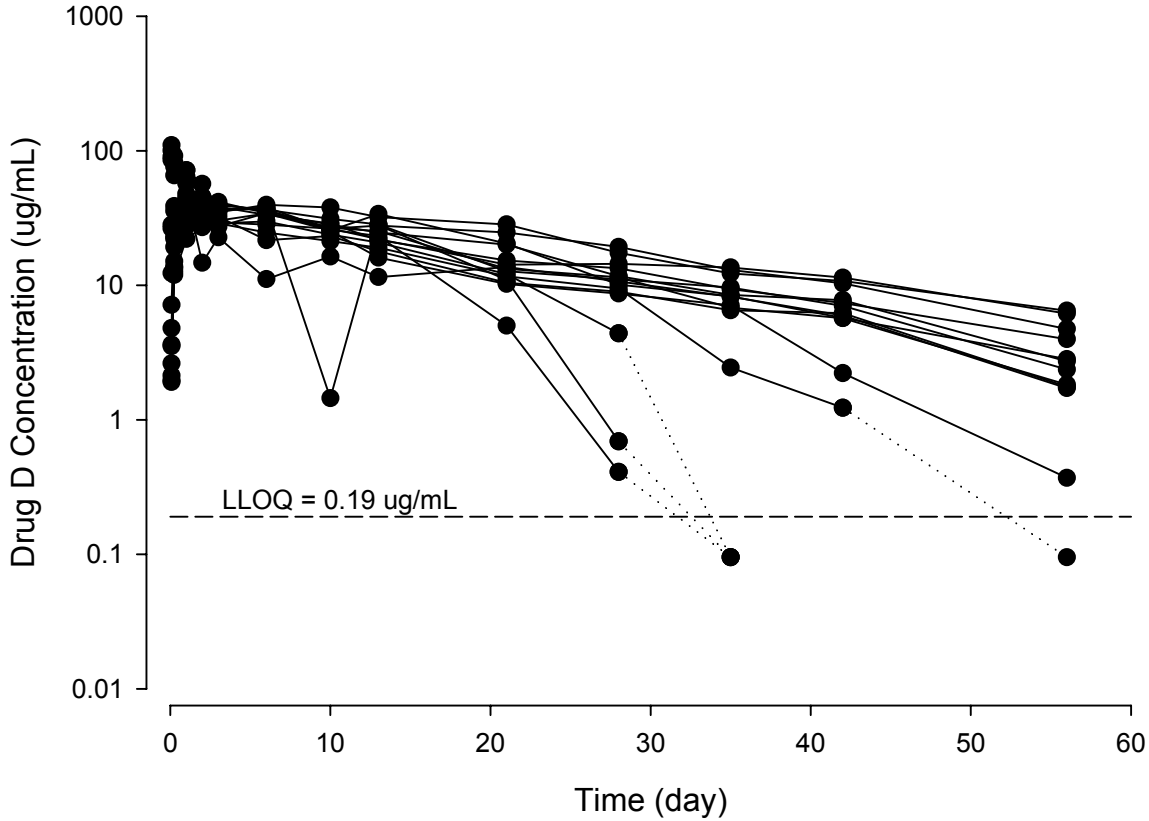
Individual Profiles of Test Drug D



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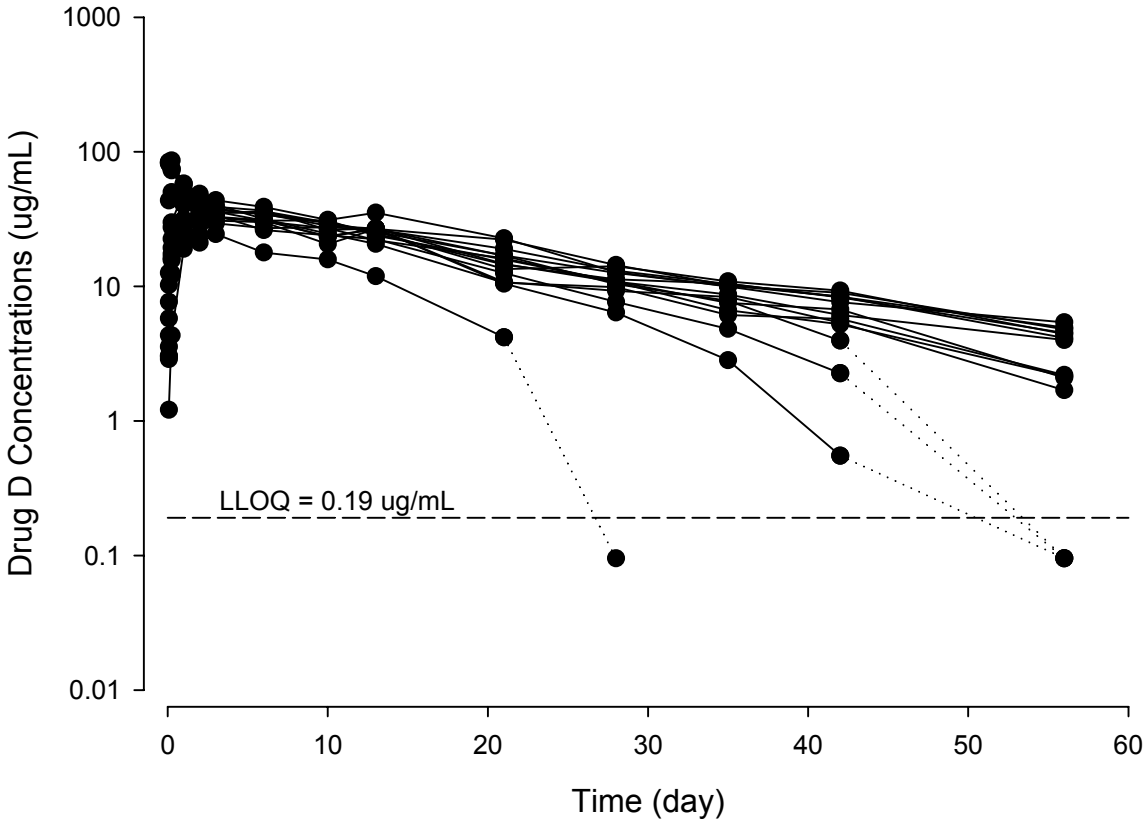
Individual Profiles of Reference Drug D



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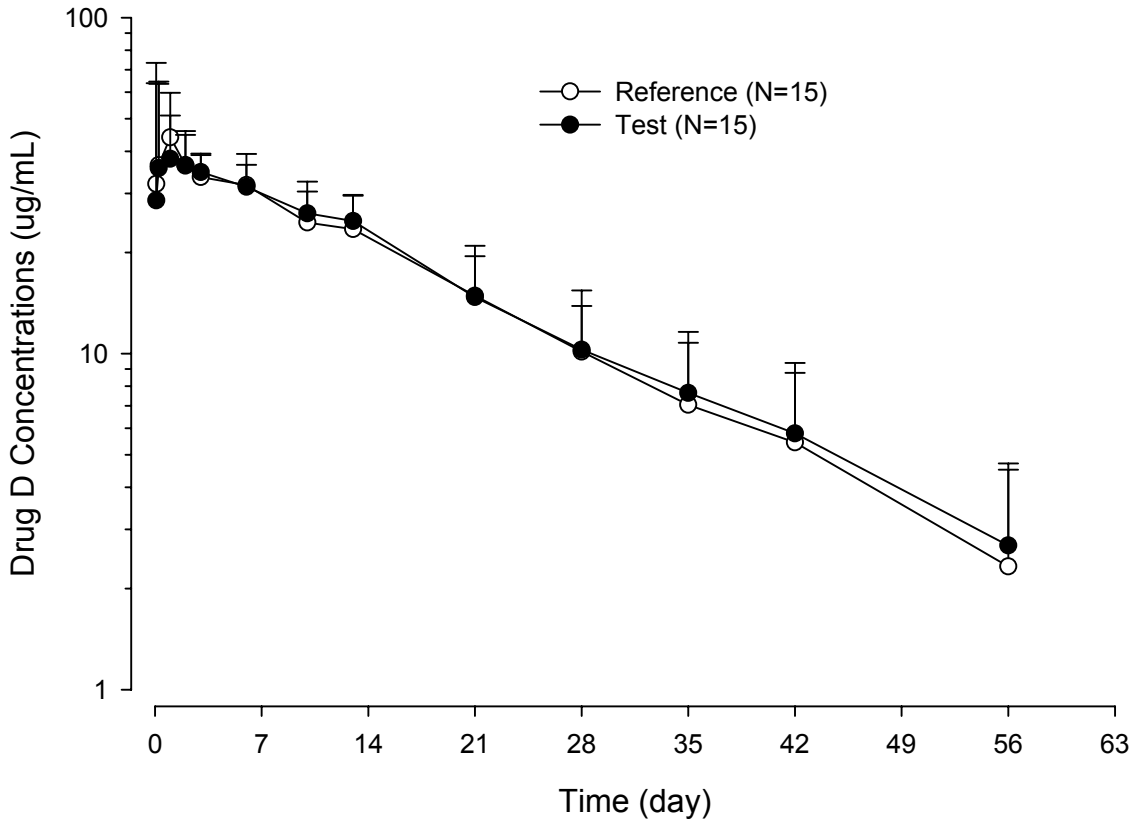
Drug D Mean Profiles Test and Ref



Drug D

- Statistical Analysis of Cmax, pAUC and AUC Following a Single 5 mg/kg IV dose in Cynomolgus Monkeys
  N = 15/group

PK Parameter	GeoMean of Test	GeoMean of Ref	Ratio T/R	90% CI
Cmax	46.28	49.61	0.93	0.72-1.21
AUC	845.58	813.29	1.04	0.85-1.27
AUC(21d)	546.74	538.80	1.01	0.90-1.15

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Drug D Comparability Test with Ref Using Total and Partial AUCs

- Superimposible mean concentration profiles through 28 days
- Point estimates of ratios of Cmax and AUC within 10%
- AUC comparable with 75 – 133%, while AUC $_{0-21d}$ was comparable with 80 -125%
- Immune responses detected in 4/15 Ref group animals, and 3/15 Test group animals

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Antibody Comparability Summary

- Nonclinical biocomparability studies are a necessary tool to enable detection of product formulation-related differences
- Immune response-related accelerated clearance is the major source of variability in drug concentration time profiles and study powering a major limitation
- Single doses of drug are sufficient to elicit immune responses to fully human therapeutic antibodies
- The incidence and timing for accelerated clearance are critical factors
- Good estimates of the variability prior to the study are crucial

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Antibody Comparability Questions

- Is the impact of immunogenicity like that of highly metabolized small drugs?
- Do assays accuracy / precision limitations and the impact of anti-drug antibodies justify different comparability acceptance requirements?
- Other strategies?

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Acknowledements

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