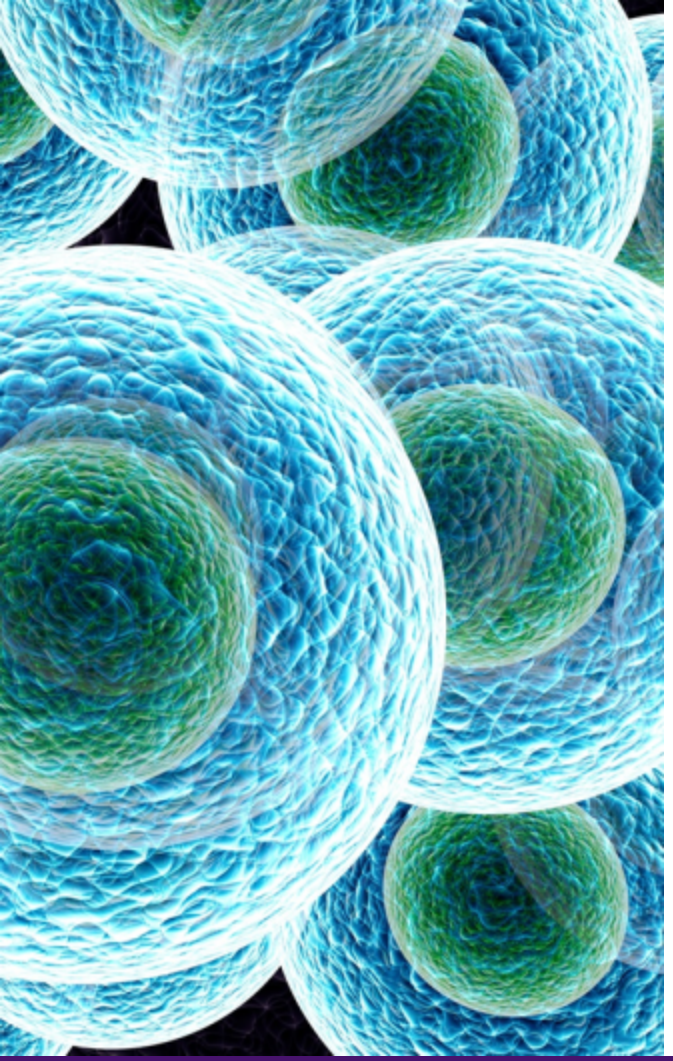


Pre-Existing Antibodies: Considerations for Cell & Gene Therapy Products

Kelli Phillips
PPD

HELPING DELIVER LIFE CHANGING THERAPIES

PPD®

Agenda

1. Introduction
2. PEG & AAV PEAs
3. PEA impact on the assay cut point and mitigation strategies
4. PEA as inclusion/exclusion criteria
5. Summary

Introduction

Pre Existing Antibodies (PEAs) are an immune response that an animal or patient may have following previous exposure to a non-endogenous molecule.

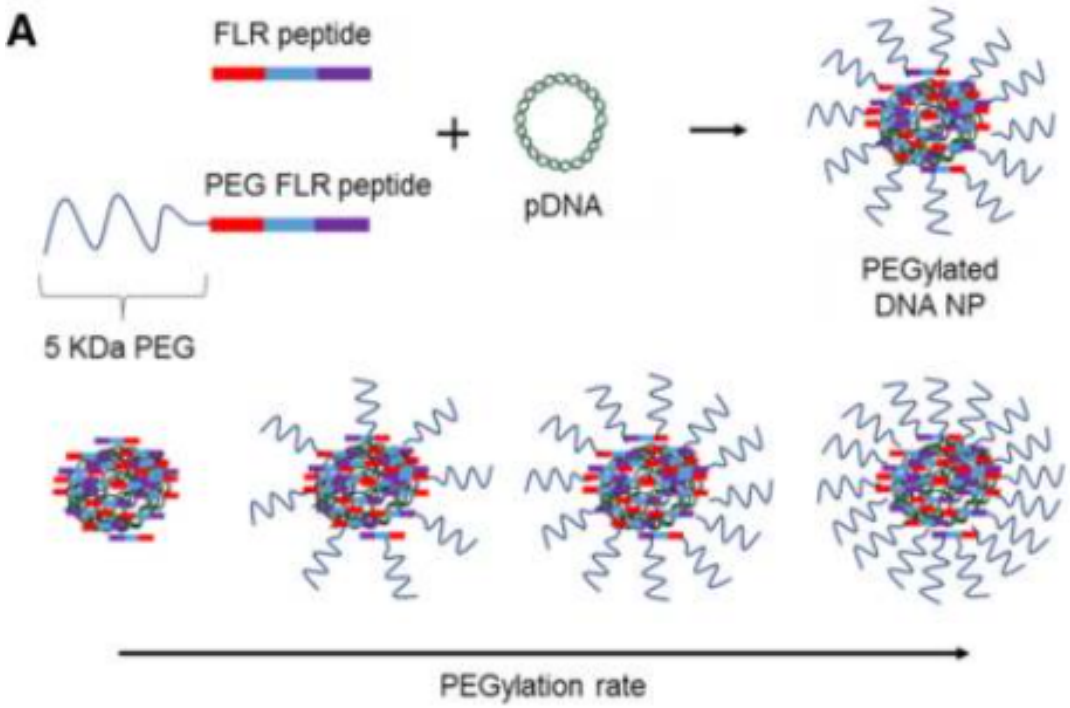
As CGT therapies become increasingly popular, and the vectors of such treatments become more diverse, we have found that populations of animals and patients have PEAs to CGT components.

PEAs are most concerning in two areas of BioA:

- PEAs may interact with a therapeutic reducing treatment efficacy
- PEAs may be detected in an ADA assay and make it difficult to establish a negative control or meaningful cut point.

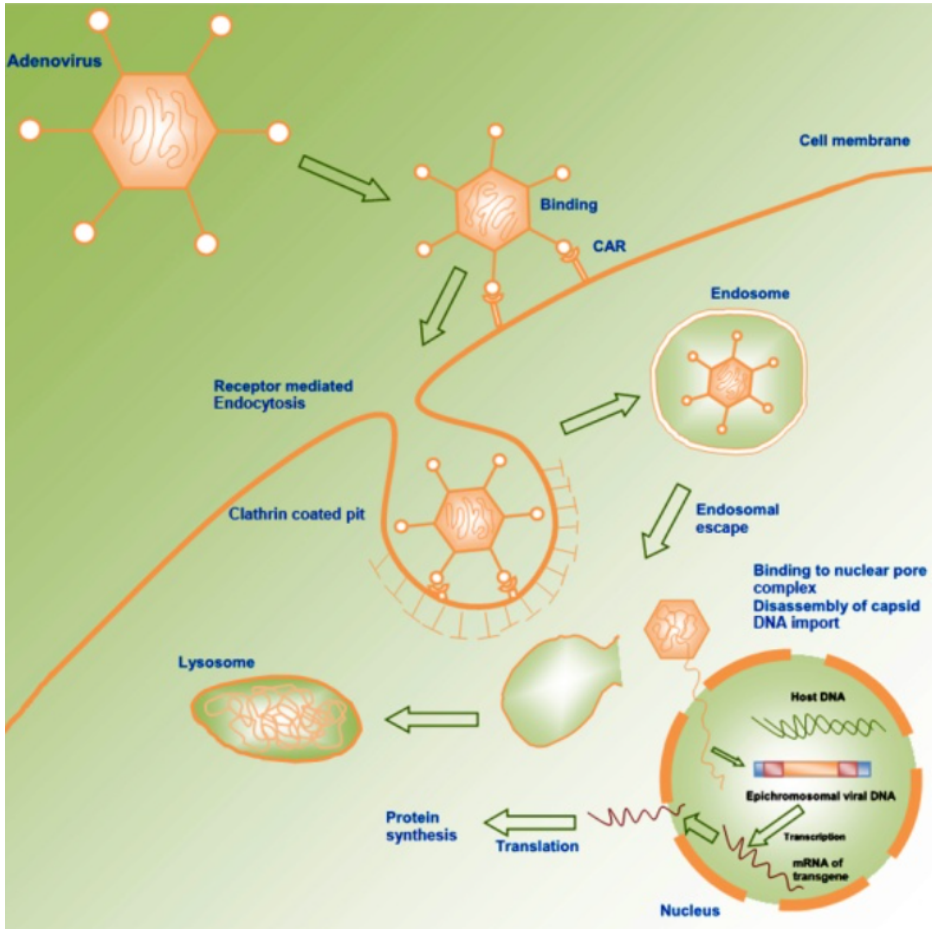
Delivery Mechanism for CGT: PEG

- PEG is a commonly used to deliver CGT treatments to targeted cells.
- Most individuals have high exposure to items containing PEG, making the propensity of preexisting anti-PEG antibodies in patient samples a common issue.
- It can be expected that 20-80% of samples may harbor pre-existing antibodies using most assay formats.



Delivery Mechanism for CGT: AVV

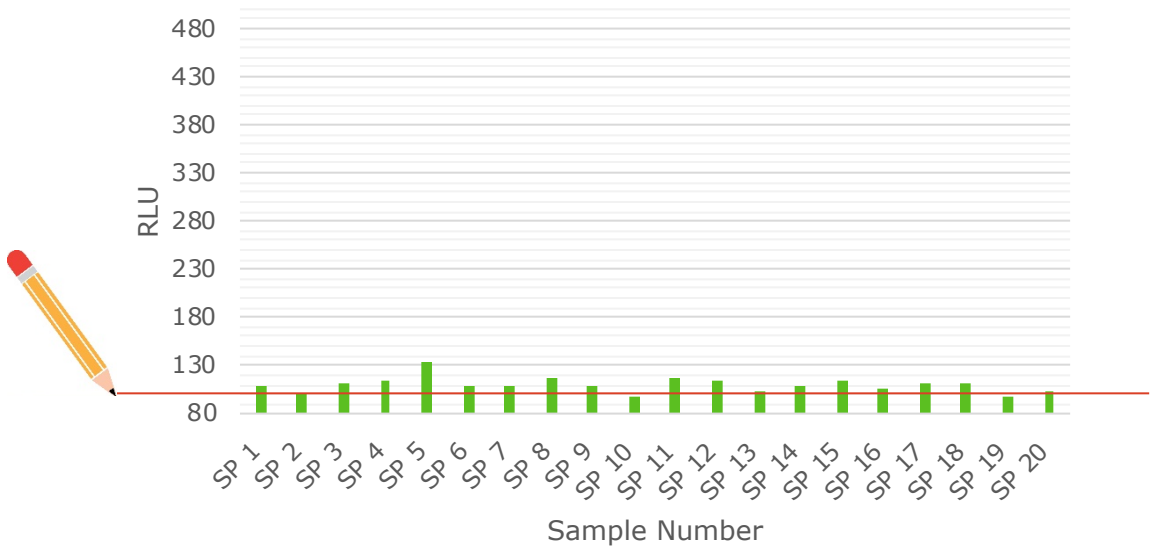
- CGT commonly uses AAV serotypes 1 to 9.
- That there is a high level of cross reactivity between AAV serotypes.
- Because some studies have shown that AAV therapies are distributed differently in individuals with preexisting antibodies, some protocols use the presence of preexisting antibodies as exclusion criteria.
- NAbs to AAVs have the potential to block delivery of virus cargo to target cells

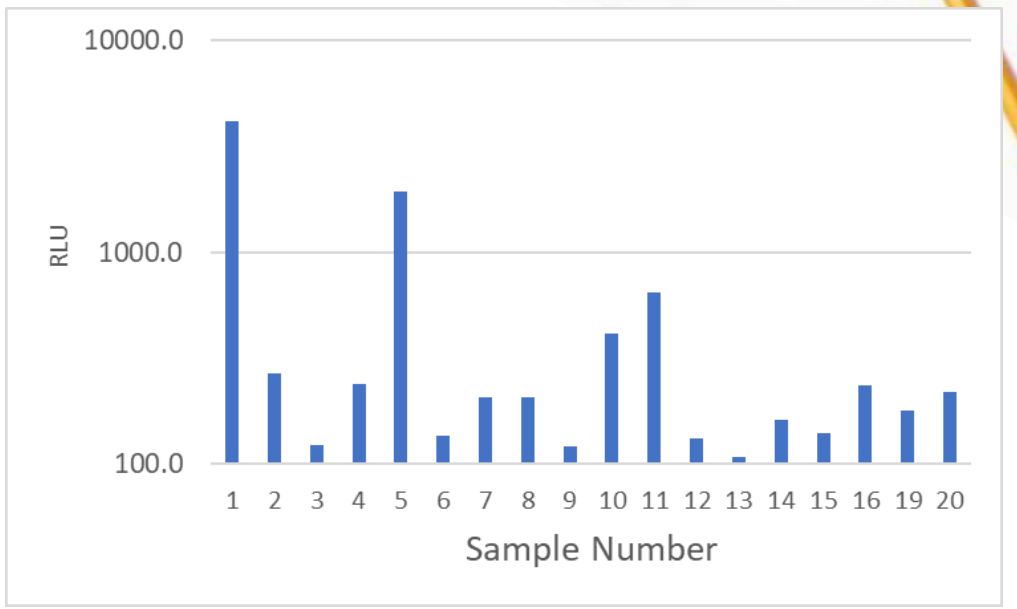


PEAs impact on Cut Point

PEAs may impact the accurate assessment of sample reactivity

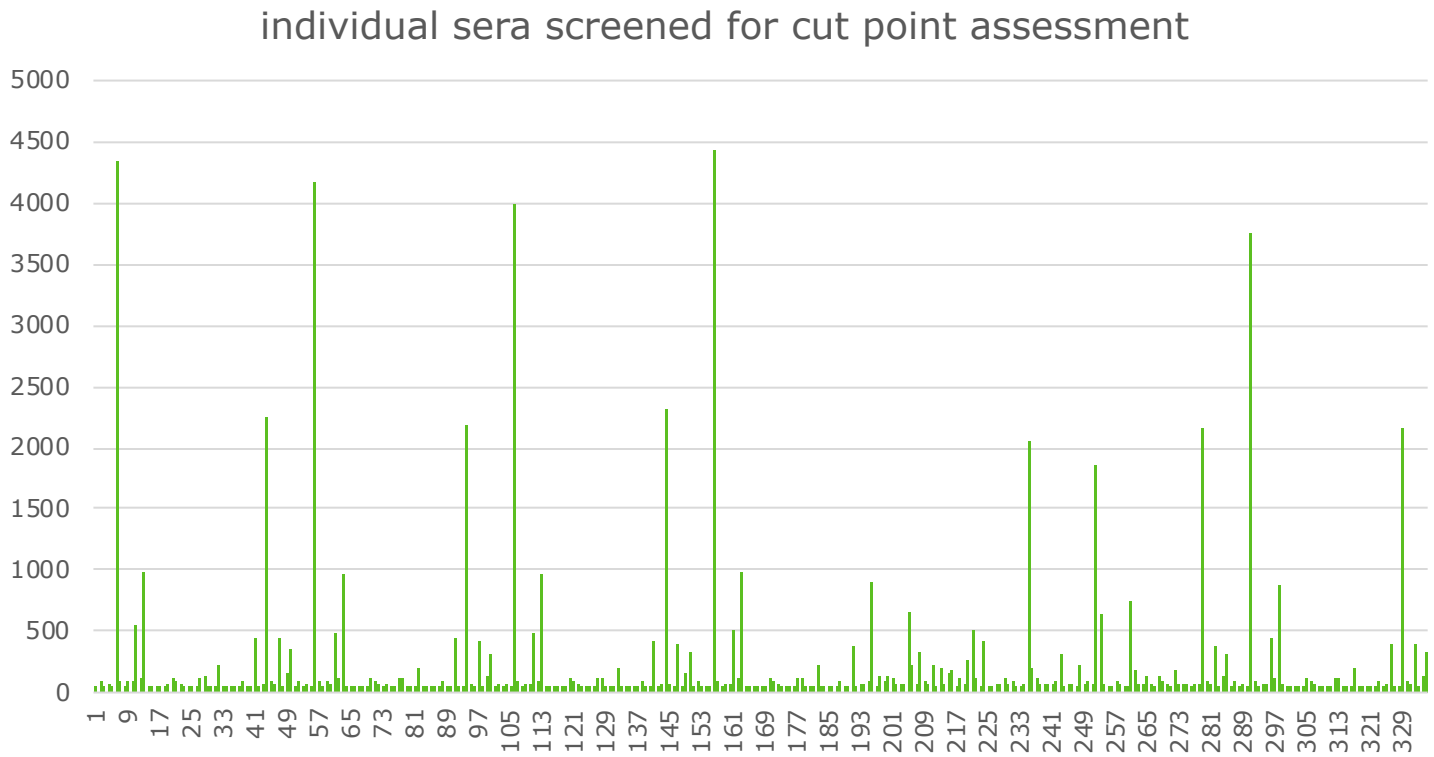
- High variability of naïve sample responses can inflate a statistically derived cut point and result in false negative designations for unknown samples
- A high incidence of PEAs may make it difficult to establish (or re-establish) a negative control





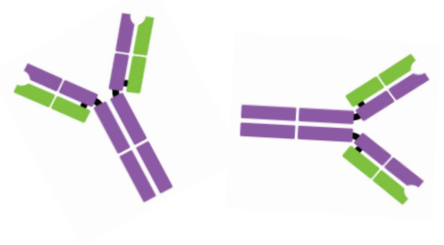
An screen of 335 individuals shows high incidence of PEAs to AAV

+ **Cut Point will be dependent on which samples we chose to run for cut point panel.**



	Screen RLU	S/N	Confirm	%Inhibition	NC
n	335	335	335	335	335
avg	225	3.88	160	10.7	59
Median	65	1.10	63	9.23	62
Min	41	0.74	39	-26.1	52
Max	4433	83.6	2868	57.4	65

Mitigation Strategies



There are standard approaches to mitigate PEA impact on cut point assessment:

- Screen MORE samples in development!
- Use standard approaches to identify and remove outliers and positive samples from cut point assessment.
- Establishing the cut point using inhibited individuals, bring the responses of even samples with PEA to a reactivity similar to that of a truly negative sample.
- Comparing the pre-dose sample response to post-dose response and using a cut point factor to establish a cut point for each sample.

Cut Point Estimation #1

+ 100 samples, outliers greater than ± 20% from the median, removed

	N-Factor	SNR	Ln(SNR)	%Inh
5% Parametric Cut Point	52.22	1.85	2.04	28.9%
False Positives	10	8	4	8
False Positive Rate	11.9%	9.5%	5%	8.3%
1% Parametric Cut Point	69.82	2.14	2.60	38.3%
False Positives	2	2	3	2
False Positive Rate	2.4%	2.4%	3%	2.1%
0.1% Parametric Cut Point	89.33	2.46	3.41	48.7%
False Positives	1	2	2	0
False Positive Rate	1.2%	2.4%	2%	0.0%
5% Non-parametric Cut Point	64.91	1.99	2.03	31.6%
False Positives	0	5	5	5
False Positive Rate	0.0%	6.0%	6%	5.2%
1% Non-parametric Cut Point	65.03	2.56	3.54	39.4%
False Positives	0	1	1	1
False Positive Rate	0.0%	1.2%	1%	1.0%
0.1% Non-parametric Cut Point	65.92	2.66	3.95	39.9%
False Positives	0	1	1	1
False Positive Rate	0.0%	1.2%	1%	1.0%

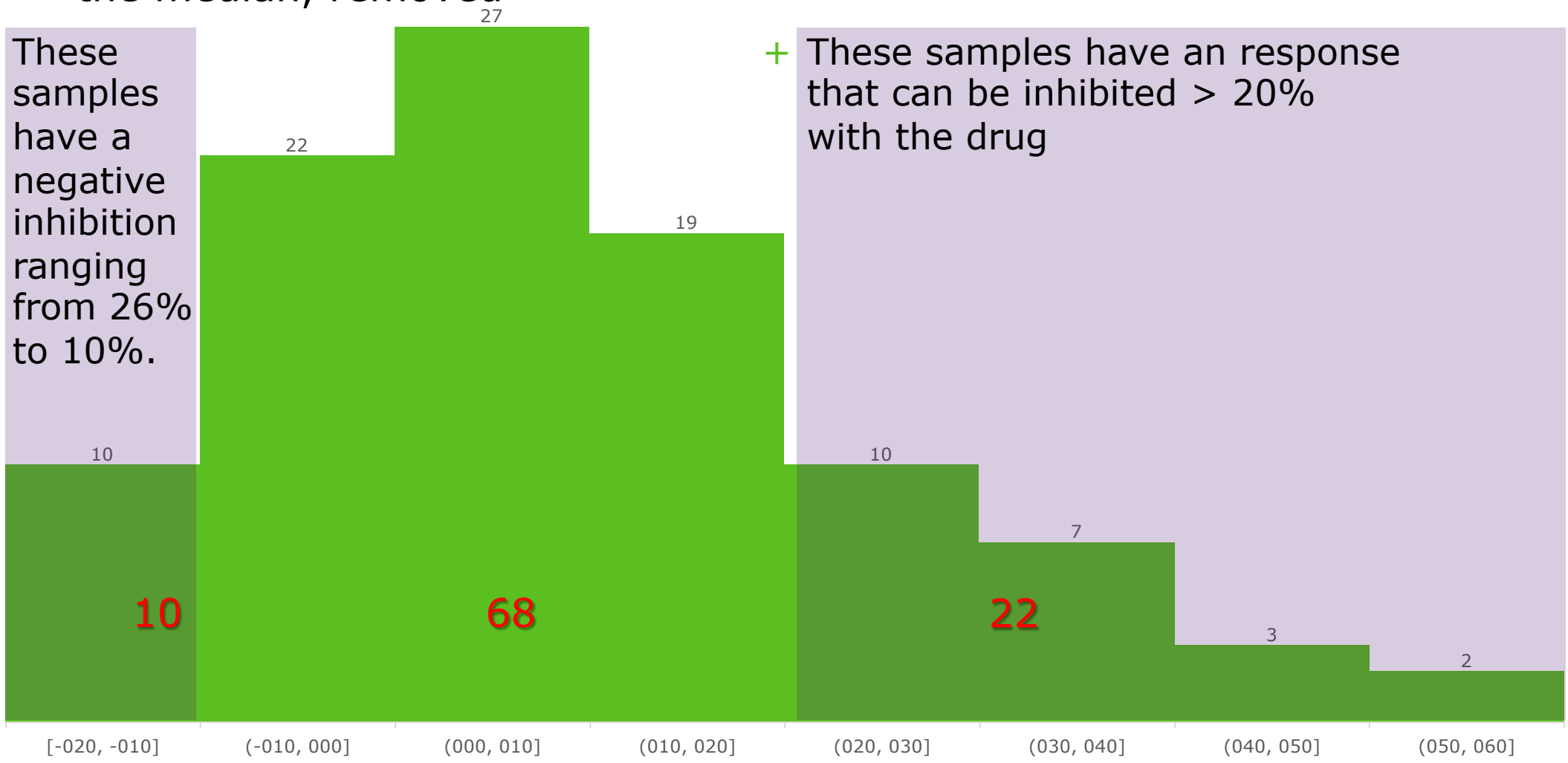
	N-factor	SNR	Ln(SNR)	%Inh
n	84	84	86	96
Mean	70.6	1.16	0.129	6.4%
Stdev	25.7	0.42	0.355	13.7%
%CV	36.4%	35.9%	275.6%	213.9%
Median	58.5	0.95	-0.049	5.5%
1st Quartile	54.0	0.89	-0.118	-3.9%
3rd Quartile	84.0	1.37	0.355	13.8%
IQR	30.0	0.48	0.472	17.7%
XIQR	45.0	0.72	0.709	26.6%
Fence Low	9.0	0.17	-0.826	-30.5%
Fence High	129.0	2.08	1.063	40.4%
# Too High	2	2	2	0
# Too Low	0	0	0	0

Cut Point Estimation #2

+ 100 samples, samples with %INH > 20% and outliers greater than ± 20% from the median, removed

+ These samples have a negative inhibition ranging from 26% to 10%.

+ These samples have an response that can be inhibited > 20% with the drug



Cut Point Estimation #2

+ 100 samples, samples with %INH > 20% and outliers greater than ± 20% from the median, removed

	N-Factor	SNR	Ln(SNR)	%Inh
5% Parametric Cut Point	47.06	1.65	1.73	16.5%
False Positives	7	6	7	2
False Positive Rate	10.1%	9.0%	10%	2.9%
1% Parametric Cut Point	62.84	1.87	2.09	21.8%
False Positives	2	3	0	0
False Positive Rate	2.9%	4.5%	0%	0.0%
0.1% Parametric Cut Point	80.34	2.12	2.57	27.7%
False Positives	0	0	0	0
False Positive Rate	0.0%	0.0%	0%	0.0%
5% Non-parametric Cut Point	58.89	1.83	1.93	14.9%
False Positives	0	4	4	4
False Positive Rate	0.0%	6.0%	6%	5.8%
1% Non-parametric Cut Point	63.00	1.95	2.02	18.4%
False Positives	0	1	1	1
False Positive Rate	0.0%	1.5%	1%	1.4%
0.1% Non-parametric Cut Point	63.89	1.95	2.03	19.7%
False Positives	0	1	1	1
False Positive Rate	0.0%	1.5%	1%	1.4%

	N-factor	SNR	Ln(SNR)	%Inh
n	69	67	69	69
Mean	69.7	1.12	0.097	3.8%
Stdev	23.0	0.32	0.275	7.7%
%CV	33.1%	28.9%	283.9%	205.4%
Median	59.0	0.95	-0.049	3.3%
1st Quartile	55.0	0.91	-0.084	-3.5%
3rd Quartile	84.0	1.30	0.288	10.4%
IQR	29.0	0.39	0.372	13.9%
XIQR	43.5	0.58	0.558	20.9%
Fence Low	11.5	0.33	-0.642	-24.4%
Fence High	127.5	1.88	0.845	31.3%
# Too High	0	3	0	0
# Too Low	0	0	0	0

Cut Point Estimation #3

Contents lists available at ScienceDirect

Journal of Immunological Methods

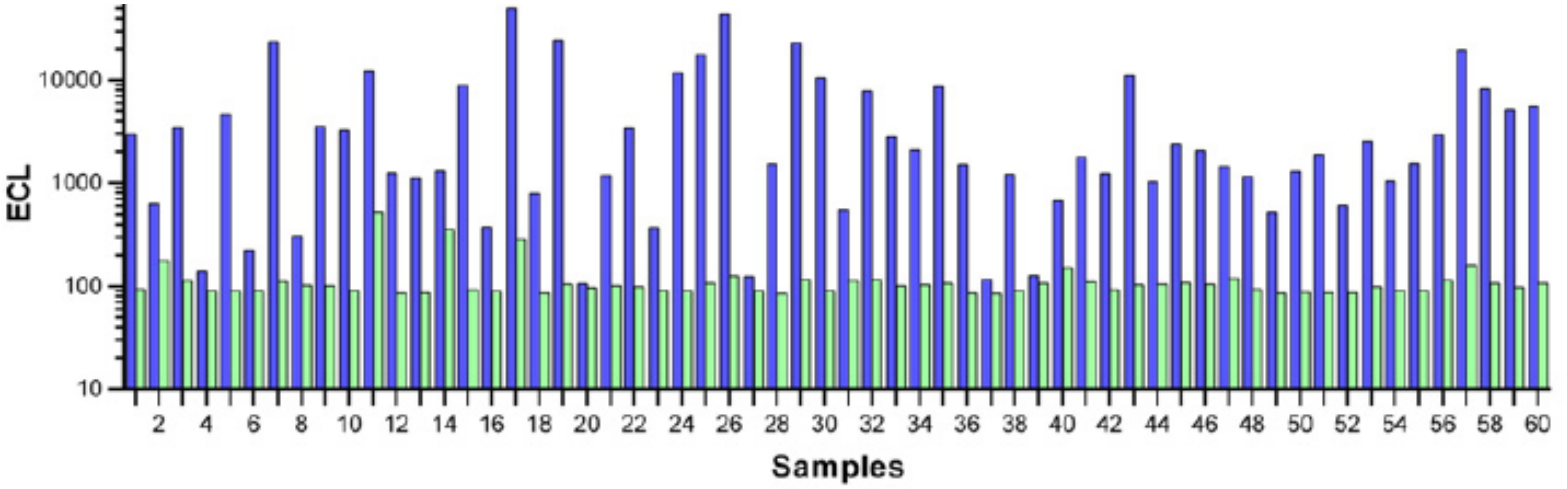
journal homepage: www.elsevier.com/locate/jim

Research paper

An immunoinhibition approach to overcome the impact of pre-existing antibodies on cut point establishment for immunogenicity assessment of moxetumomab pasudotox

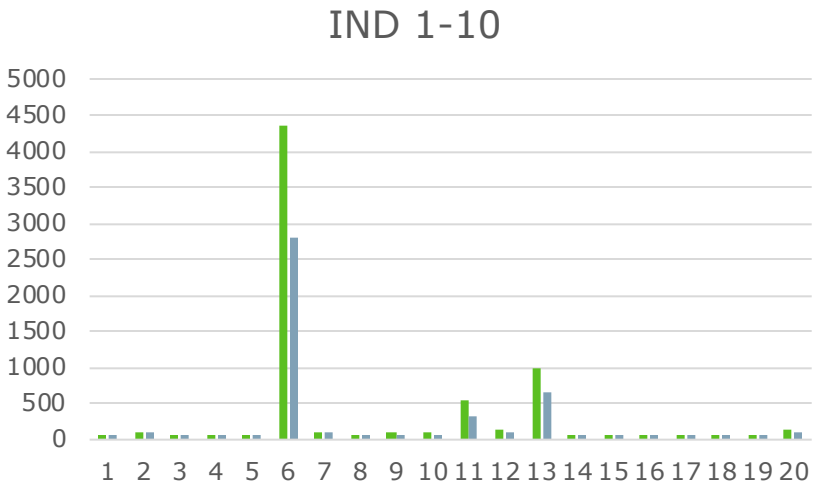
Amy K. Schneider [1], Inna Vainshtein [*,1], Lorin K. Roskos, Carlos Chavez, Bo Sun, Meina Liang [*]

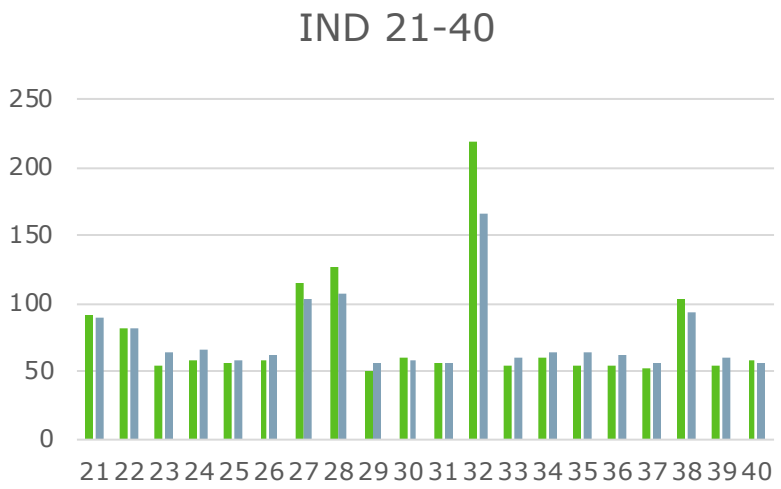
Clinical Pharmacology & DMPK, Medimmune, LLC, 319 North Bernardo Avenue, Mountain View, CA 94043, USA

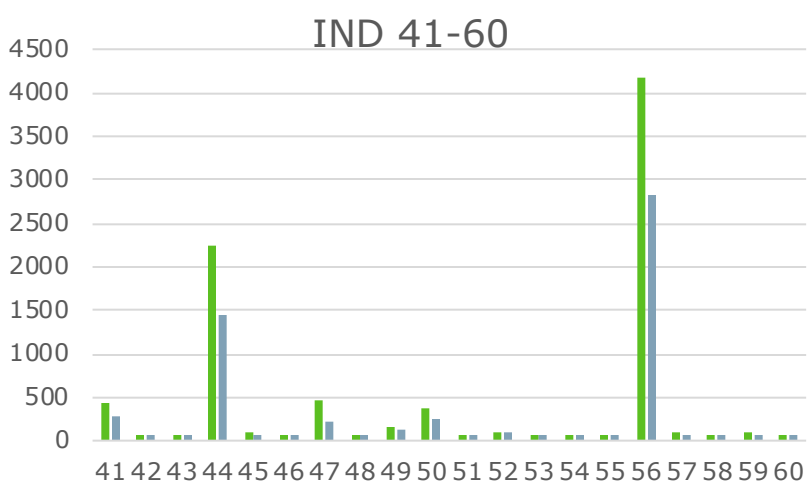


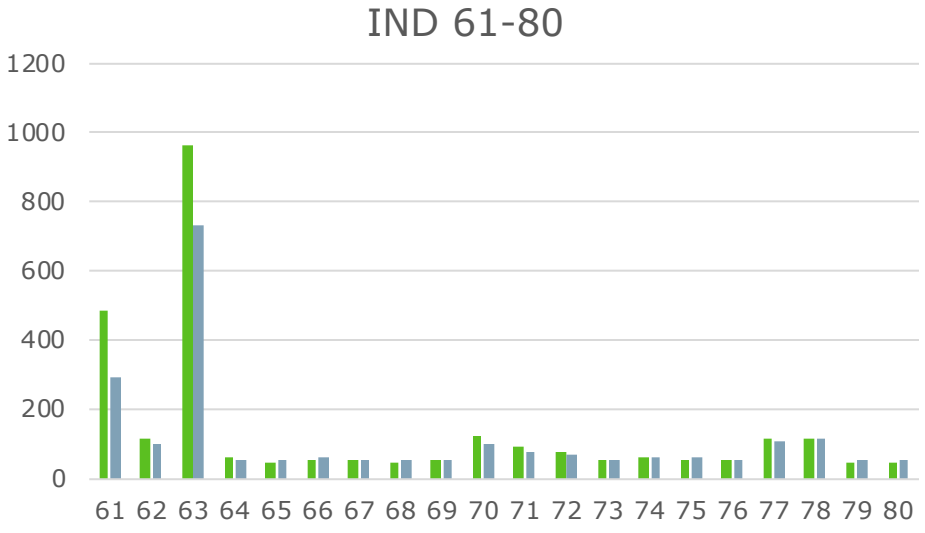
- In the absence of a truly negative sample population, an immunoinhibition approach was used to create a pseudo-ADA-negative sample population.
- The concentration of drug used for the immunodepletion step decreased assay signals in greater than 80% of samples to approximately 2–3 fold of the assay buffer signal without affecting the assay signal for the NC sample.

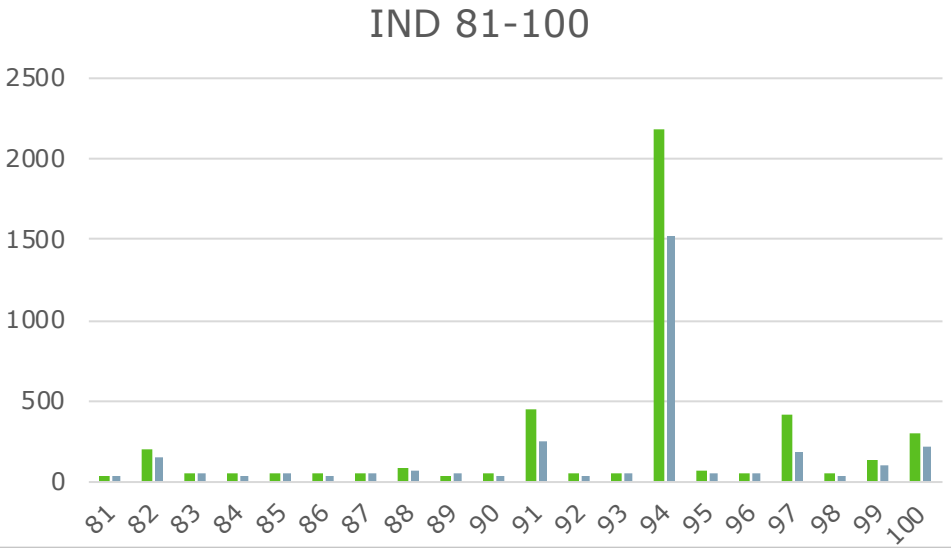
Cut Point Estimation #3











+ 18 of 100 samples have responses > 2 fold the NC value after inhibition.

Cut Point Estimation #3

Cutpoints Without Outliers (first iteration)				
	N-factor	SNR	Ln(SNR)	%Inh
n	94	93	95	0
Mean	58.1	1.02	0.018	
Stdev	11.4	0.17	0.170	
%CV	19.7%	16.8%	959.2%	
Median	57.5	0.96	-0.031	
1st Quartile	48.3	0.91	-0.098	
3rd Quartile	64.0	1.09	0.091	
IQR	15.8	0.19	0.189	
XIQR	23.6	0.28	0.283	
Fence Low	24.6	0.62	-0.381	
Fence High	87.6	1.38	0.374	
# Too High	1	4	4	0
# Too Low	0	0	0	0

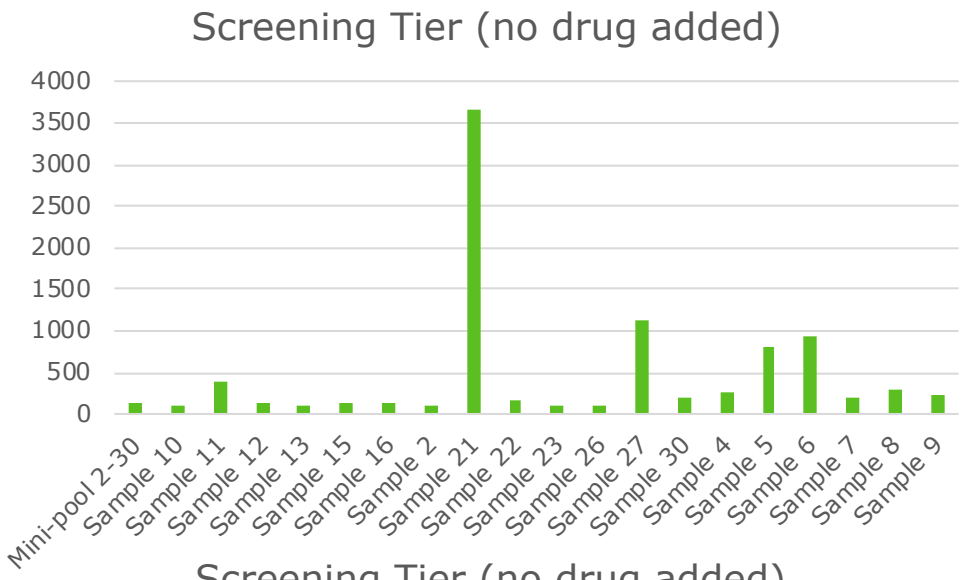
	N-Factor	SNR	Ln(SNR)	#1 SNR	#2 SNR
5% Parametric Cut Point	20.37	1.30	1.35	1.85	1.65
False Positives	8	8	9	8	6
False Positive Rate	8.5%	8.6%	9%	9.5%	9.0%
1% Parametric Cut Point	28.22	1.42	1.51	2.14	1.87
False Positives	3	3	3	2	3
False Positive Rate	3.2%	3.2%	3%	2.4%	4.5%
0.1% Parametric Cut Point	36.92	1.55	1.72	2.46	2.12
False Positives	0	1	0	2	0
False Positive Rate	0.0%	1.1%	0%	2.4%	0.0%
5% Non-parametric Cut Point	26.09	1.36	1.41	1.99	1.83
False Positives	18	5	5	5	4
False Positive Rate	18.0%	5.4%	5%	6.0%	6.0%
1% Non-parametric Cut Point	62.04	1.48	1.59	2.56	1.95
False Positives	0	1	1	1	1
False Positive Rate	0.0%	1.1%	1%	1.2%	1.5%
0.1% Non-parametric Cut Point	62.93	1.55	1.66	2.66	1.95
False Positives	0	1	1	1	1
False Positive Rate	0.0%	1.1%	1%	1.2%	1.5%

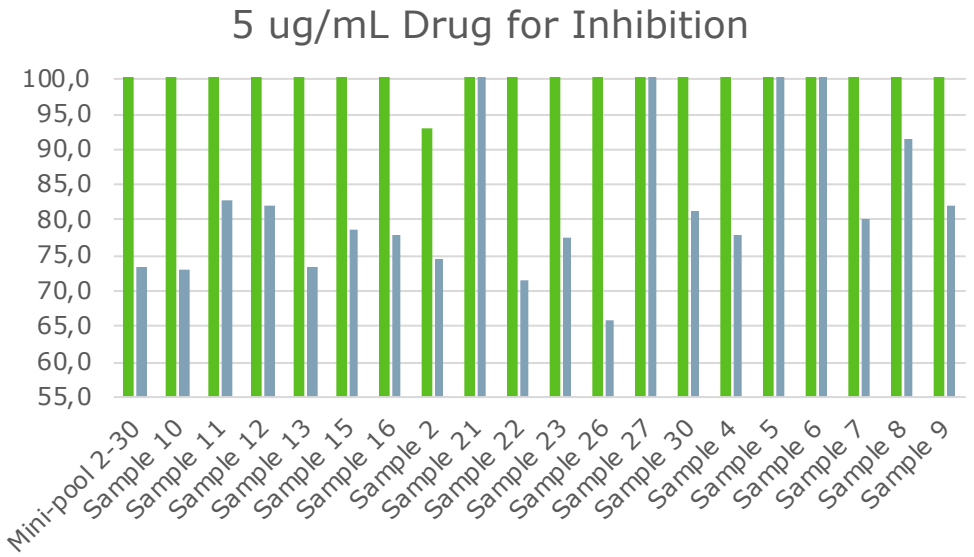
Mean NC = 60 RLU
CUT POINT #1 : 111
CUT POINT #2 : 99
CUT POINT #3 : 78

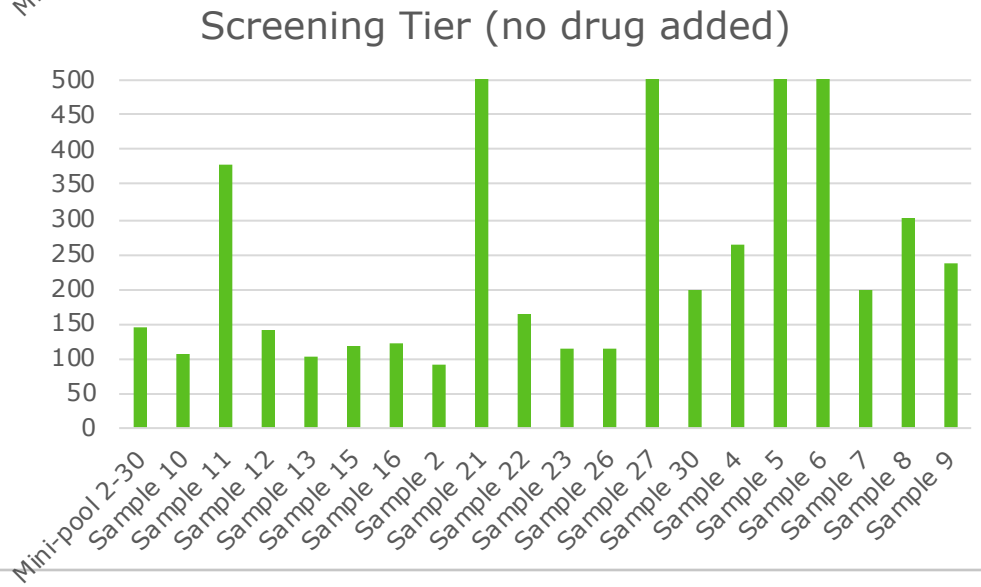
Cut Point Estimation #4

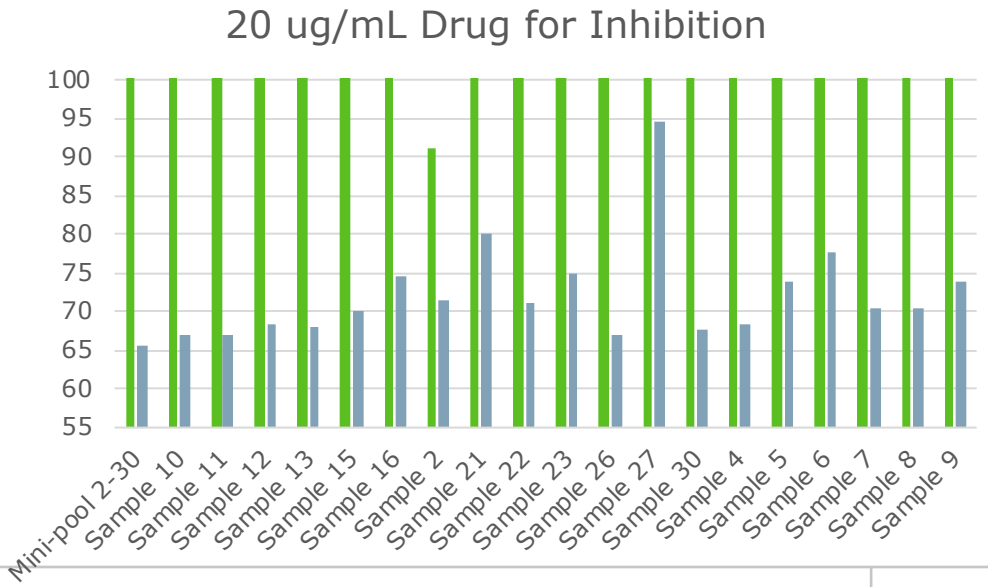
+ The population screened during the development of this assay had a 70% prevalence of pre-existing antibodies.
+ There were limitations on drug supply (making the Schneider et al method not feasible).

Cut Point Estimation #4









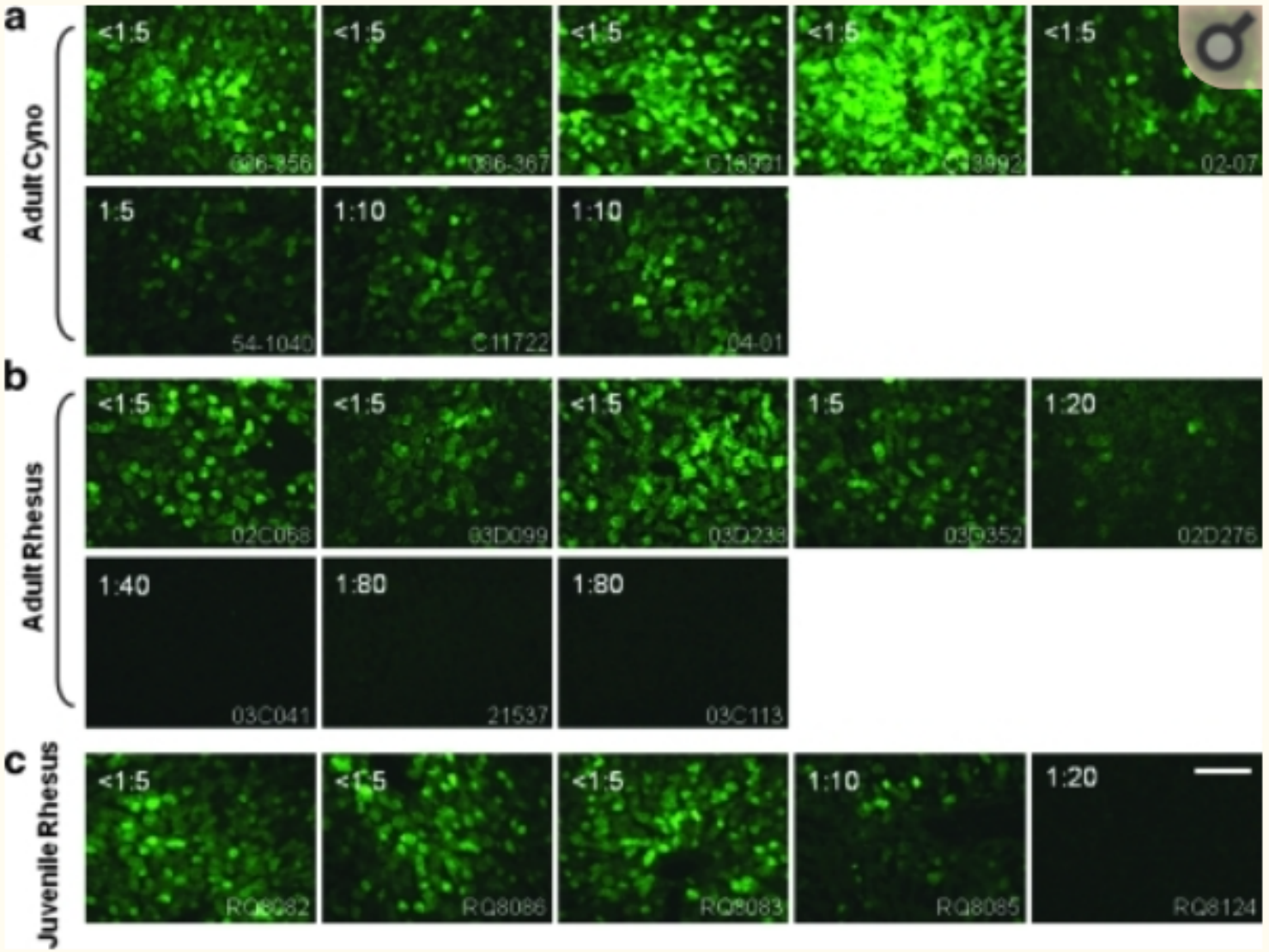
Cut Point Estimation #4

- The variability of titer responses for negative samples were evaluated during the validation and a cut point factor was calculated (similar to a standard cut point approach)
- The pre-dose titer is then multiplied by the cut point factor to determine a cut point for each sample.
- Samples are then evaluated individually, using the ratio of titer value from pre- to post-dose.

PEAs as Exclusion Criteria

- Early CGT studies indicated that PEAs may have significant impact on the efficacy of the treatment.
- As a result, many studies have pre-screened and excluded those patients that have PEAs.
- This approach has created a situation where the impact of PEAs on treatment efficacy is sometimes unknown.
- Because PEAs are prevalent in many populations, this negatively impacts many patients who may benefit from novel CGT treatments.

Early studies indicated that AAV NAbs may impact treatment…



- Notably, a study using AAV8 and primate liver demonstrated that AAV titers as low as **1:5** can impede transduction! (Wang et al., 2011)

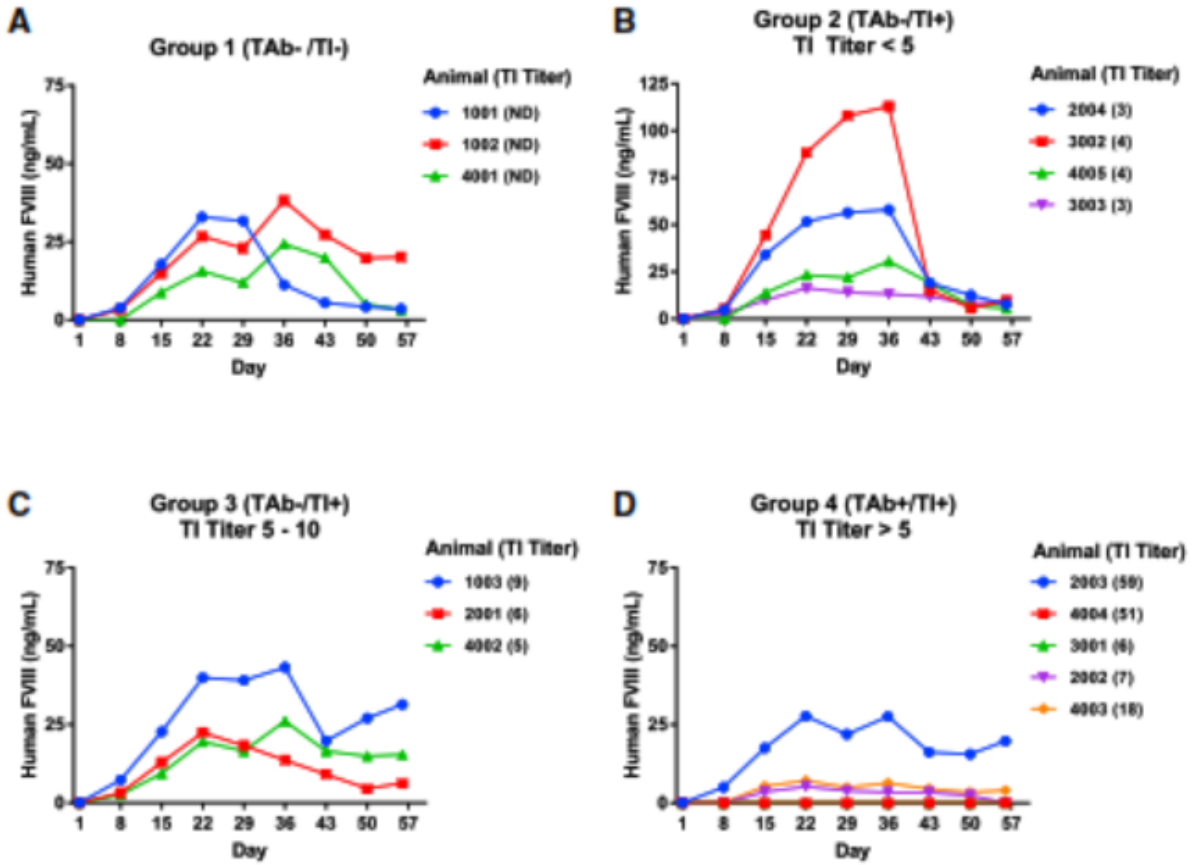
Molecular Therapy
Methods & Clinical Development
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

The Impact of Pre-existing Immunity on the Non-clinical Pharmacodynamics of AAV5-Based Gene Therapy

Brian R. Long,[1] Krystal Sandza,[1] Jennifer Holcomb,[1] Lucy Crockett,[1] Gregory M. Hayes,[1] Jeremy Arens,[1] Carlos Fonck,[1] Laurie S. Tsuruda,[1,2] Becky Schweighardt,[1] Charles A. O'Neill,[1] Stephen Zoog,[1] and Christian Vettermann[1]

[1]BioMarin Pharmaceutical Inc., Novato, CA, USA



+ BMN 270 is an AAV5-based vector for treating hemophilia A that encodes human B domain-deleted factor VIII (FVIII-SQ)
+ Cynomolgus monkeys with varying pre-dose levels of neutralizing anti-AAV antibodies

+ Group 1: No immunity factors for AAV5
+ Group 2: Low levels of inhibitory plasma components
+ Group 3: Higher levels of inhibitory plasma components
+ Group 4: NAbs for AAV5

June 2019

HUMAN GENE THERAPY
Mary Ann Liebert, Inc. publishers
Journals | Search | Alerts

Hum Gene Ther. 2019 Oct 1; 30(10): 1297–1305.
Published online 2019 Sep 24. doi: 10.1089/hum.2019.143
PMCID: PMC6763963
PMID: 31502485

Prevalence and Relevance of Pre-Existing Anti-Adeno-Associated Virus Immunity in the Context of Gene Therapy for Crigler–Najjar Syndrome

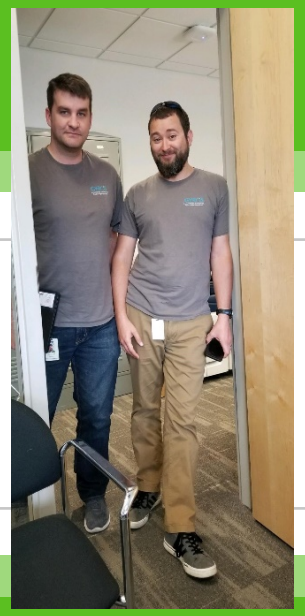
Sem J. Aronson,[1] Philippe Veron,[2] Fanny Collaud,[2] Aurélie Hubert,[3] Virginie Delahais,[2] Géraldine Honnet,[2] Robert J. de Knegt,[4] Norman Junge,[5,6] Ulrich Baumann,[5,6] Angelo Di Giorgio,[7] Lorenzo D'Antiga,[7] Virginia M. Ginocchio,[8,9] Nicola Brunetti-Pierri,[8,9] Philippe Labrune,[3] Ulrich Beuers,[1] Piter J. Bosma,[1] and Federico Mingozzi[2,*]

- Pre-existing anti-AAV immunity was found in ~30% of disease state subjects, which currently restricts enrollment of a significant proportion of patients in ongoing gene therapy trials.
- Low NAb titers, found in the context of natural immunity to AAV, can be overcome by administrating AAV preparations containing both full and **empty capsids**, offering a potential approach to treat a subgroup of borderline seropositive patients.

October 2019

Summary

- PEA for gene therapy vectors can be impactful to both the efficacy of a therapeutic *and* the development of a roust ADA assay for the therapeutic vector.
- Many mitigation strategies have been used to circumvent PEAs impact on the development of a meaningful ADA assay cut point.
- Because early studies have indicated that PEAs may interfere with a gene therapy vectors successful targeting, many clinical trials have used the existence of PEAs as exclusion criteria. As the field advances, and more protocols include patients with PEAs, we are learning mitigation strategies for this as well.
  - Increasing the amount of capsid that the patient is dosed with, to out compete PEAs
  - Plasmapheresis procedures that deplete PEAs from patient sera.

+ Laura Kelly
+ Cathy Vrentas
+ Nick Hoke
+ Molly Crowe
+ Atiya Taqui
+ Heather Myler
+ Inna Vainshtien
+ Jim McNally