Poster PB1178

Validation of a neutralizing antibody (NAb) assay with an extended reportable range for etranacogene dezaparvovec, an adeno-associated virus serotype 5 (AAV5)-based gene therapy for adult hemophilia B

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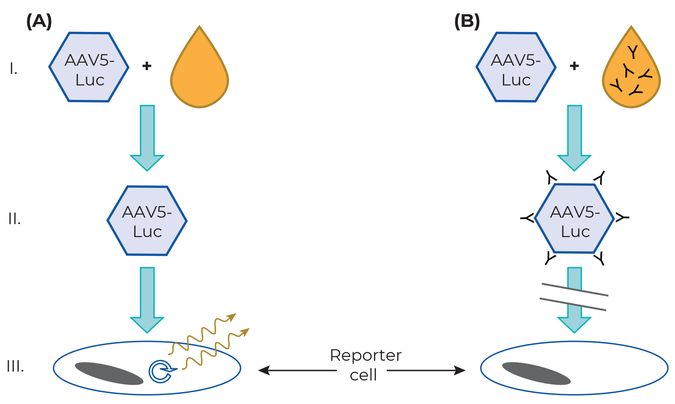
Background

- AAV NAbs may be present in the population following infection with naturally occurring AAVs[1]
- NAbs are measured using an *in vitro* cell-based transduction inhibition assay[2,3] with a similar design principle to others[4]
  - The assay measures viral transduction in a cell line by the expression of a luminescent-based reporter gene engineered into the viral vector. Patient serum can inhibit (or neutralize) viral vector transduction based on the functional activity of NAbs in patient serum (**Figure 1**)[3]
- In the pivotal Phase 3 trial HOPE-B, patients treated with etranacogene dezaparvovec who had a preexisting AAV serotype 5 (AAV5) NAb titer of up to 678 at baseline responded safely to treatment[5]
  - The original AAV5 NAb clinical trial assay was modified to extend the reportable measuring range

Figure 1: A cell-based transduction inhibition assay to measure NAbs to etranacogene dezaparvovec



(**A**) In the absence of AAV5 NAb, the unaffected reporter vector (I) can transduce the reporter cells (II), thereby establishing luciferase expression, which can be measured by luminescence (III). (**B**) AAV5 NAb present in the test serum will bind to the reporter vector particles, impairing their ability to transduce the reporter cells and resulting in lack of luciferase expression.
AAV5, adeno-associated virus serotype 5; Luc, Luciferase; NAb, neutralizing antibody.
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Objective

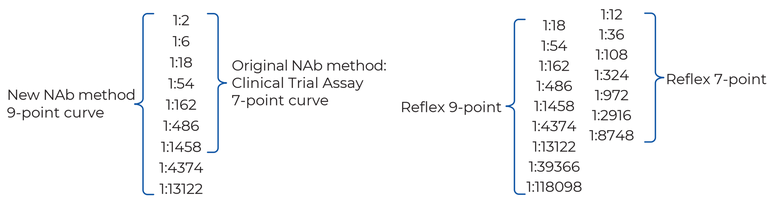
- **To analytically validate a modified NAb assay with an extended reportable measuring range to more precisely measure preexisting AAV5 NAb titers of patients eligible for etranacogene dezaparvovec and compare titers to the original clinical trial assay**

Methods

ASSAY DESCRIPTION

- Validation studies were conducted on a modified NAb assay that uses the same principle as the phase 3 clinical trial assay moving from 7 serum dilution steps to 9 (**Figure 2**)[3]
  - Samples with titers greater than the linear range of the standard assay starting at 1:2 dilution (titers >1400) are retested starting with a higher starting dilution (1:18) in the reflex test

Figure 2: Comparison of serial dilution schemes



NAb, neutralizing antibody.
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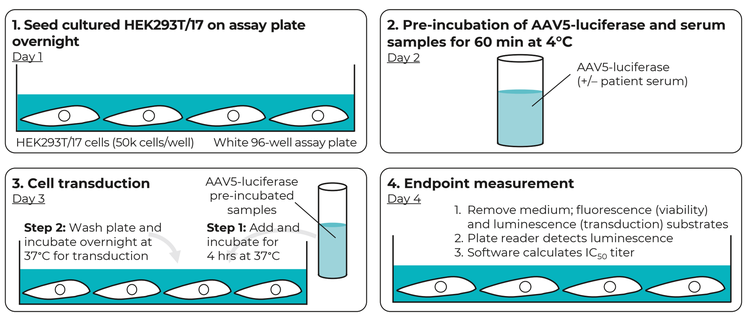
- The NAb assay was performed in microtiter plates coated with HEK293T/17 cells over the course of 3 days (**Figure 3**)
  - The percent neutralization of a sample dilution is calculated from its luminescence signal relative to the average signal of the negative transduction control (0% transduction) and the average signal of the positive transduction control (100% transduction)
  - The AAV5 NAb titer of a test sample is defined as the "IC_{50}" (midpoint of the titration curve) and is determined by performing 4-parameter regression to percent neutralization as a function of sample dilution

Figure 3: NAb assay performed in HEK293T/17 cells



AAV5, adeno-associated virus serotype 5; IC_{50}, half maximal inhibitory concentration; NAb, neutralizing antibody; RT, room temperature.

Methods (cont.)

We conducted precision, linearity, method comparison, reference range, sample stability, cross-contamination, cross-reactivity with humanized antibodies against hepatitis C virus (HCV) and HIV, and endogenous interferent validation studies. The results of the first four studies are presented here. All validation studies met acceptance criteria (data not shown)[3]

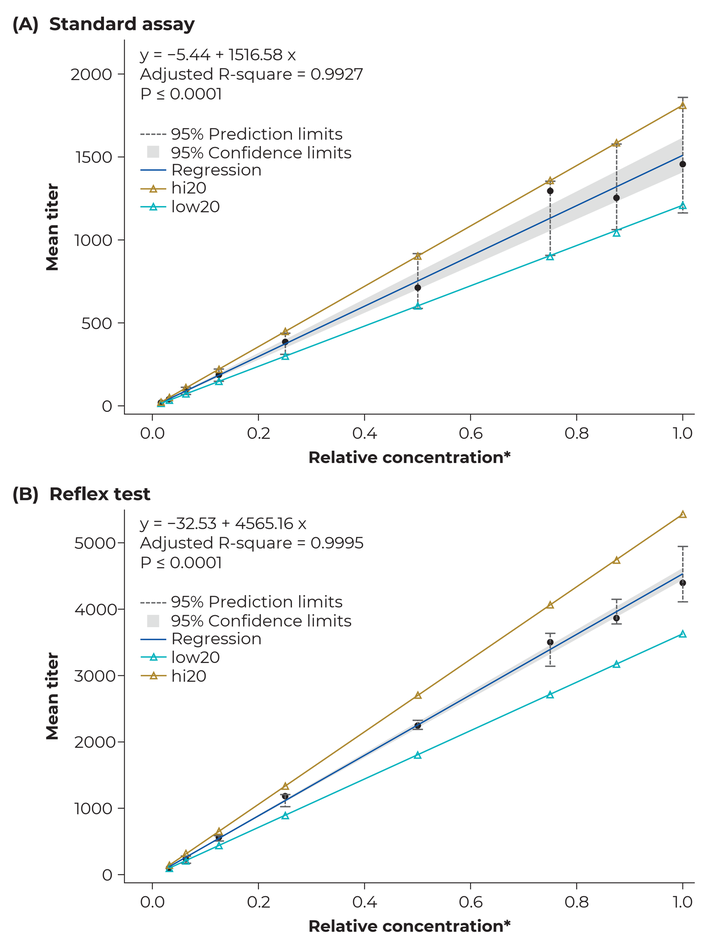
- **Linearity:** one high titer AAV5 NAb serum sample was mixed at different volume ratios with negative serum to produce 9 and 8 samples for standard and reflex linearity, respectively
  - Each sample was tested in four replicates, and a replicate is defined as a 9-point titration with triplicate wells at each point
  - High titer samples were analyzed with the reflex test. Acceptable deviation from linearity was ≤20%
- **Precision:** intra-assay precision, operator-to-operator, between instrument, and lot-to-lot variability were assessed
  - Each study used four AAV5 NAb standard serum samples with titers of 110, 300, 600, and 900
  - Intra-assay precision was evaluated with 20 replicates per standard in a single day
  - Operator precision was tested by three operators with 15 replicates per standard per operator over 3 days and two instruments
  - For lot-to-lot precision, five replicates per standard per day over 3 days tested two lots of critical reagents
  - For the reflex test starting at 1:18 dilution, an operator-to-operator precision study was conducted utilizing three operators testing two standards (titers of 1507, 3701) with five replicates per standard over 3 days
  - Acceptance criteria was ≤ 25% coefficient of variation (CV) for intra-assay and ≤ 35% CV for total within laboratory precision
- **Reference range:** sera were tested from 60 healthy adult males residing in the northeast United States
- **Method comparison:** samples from 30 donors with detectable and two donors with undetectable NAb were tested in three replicates in the 7-point and 9-point assays
  - For both reflex assays, 12 contrived test samples were prepared by mixing high and negative serum. Results were log-transformed prior to linear regression

Results

LINEARITY

- Linearity was supported from 18.5 (the lower limit of quantification) to 1100 in the standard assay as the third sample of the linearity series (1:18 dilution) had a higher mean titer than the second (**Figure 4A**)[3]
- The upper limit of quantification of the assay is extended with reflex testing to 4400 (**Figure 4B**)[3]

Figure 4: Linearity of the modified 9-point NAb assay



*Relative concentration: concentration of AAV5 NAb relative to the highest titer sample tested.
AAV, adeno-associated virus; NAb, neutralizing antibody.
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PRECISION

- Precision of the standard assay is summarized in **Table 1**
- The reflex assay had ≤ 16% CV within run precision and ≤ 15% CV for operator-to-operator precision

Table 1: Precision of the modified 9-point NAb assay

Standard	Intra-assay precision		Total precision*	
	Replicates	CV%	Replicates	CV%
110	20	10	130	25
200	20	11	110	18
600	20	13	114	21
900	20	13	115	19

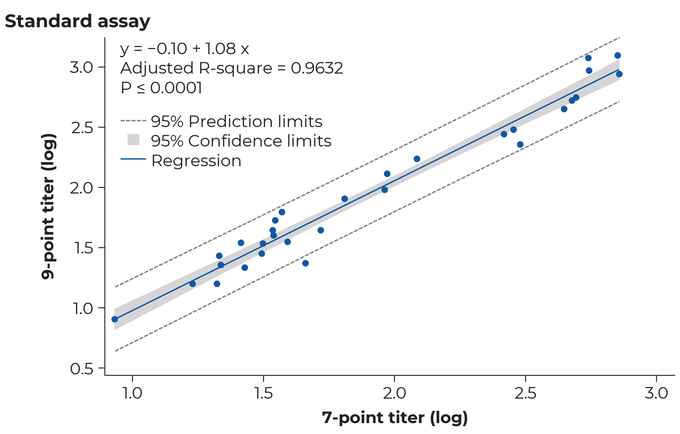
*Total precision includes variances for intra-assay, inter-operator, lot-lot, between instrument precision.
CV, coefficient of variation; NAb, neutralizing antibody.

Results (cont.)

COMPARISON OF THE 7-POINT AND 9-POINT NAB ASSAY

- The modified 9-point assay has a close linear relationship with the original 7-point clinical trial assay with a R^2 of 0.96 by regression analysis (**Figure 5**)[3]
  - The association between the two reflex assays had an R^2 of 0.99 and equation, y = –0.1181 + 1.0732x (data not shown)

Figure 5: Linear comparison of the 7-point and 9-point NAb assay



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- **Table 2** shows simulated values based on these equations to relate the equivalent titers between the NAb methods[3]

Table 2: Relationship of equivalent titer values determined by 7-point and 9-point NAb assays

7-Point	10	50	100	300	678	1500*	3212*
9-Point	9	54	114	372	898	1952*	4417*

*Point estimates calculated from the 7- vs 9-point reflex assay comparison.
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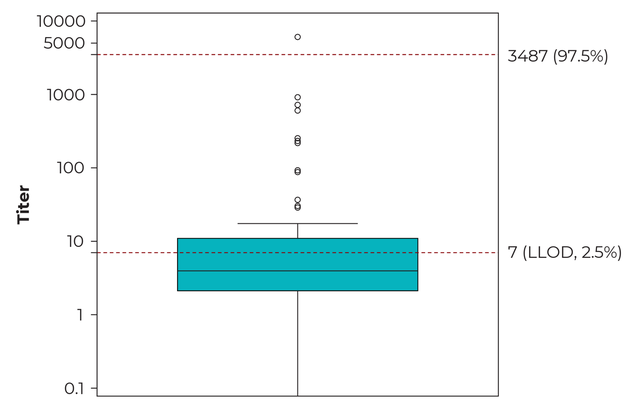
REFERENCE RANGE

- The reference range based on AAV5-NAb results of 60 healthy adult males was <18.5 to 3487 (the lower limit of quantification and the 97.5th percentile including reflex test), with 75% titers below the lower limit of detection (LLOD) (**Figure 6**)[3]

Figure 6: Reference range of AAV5 NAb titers from the 9-point assay



AAV5, adeno-associated virus, serotype 5; LLOD, lower limit of detection; NAb, neutralizing antibody.
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Conclusions

- **A modified etranacogene dezaparvovec NAb assay is based on the same principle as the original clinical trial assay, sharing the same seven dilution measurements with two additional dilutions at the high end**
- **The two additional dilutions extended the reporting range of the assay from 18.5 to 1100 in the standard (or initial test) and up to 4400 with additional reflex testing**
- **A method comparison demonstrated a close relationship between the original and modified assay with improved linearity and accuracy at NAb titers of approximately 300 or more, resulting in higher reported titers**
  - **This does not represent a change in the amount of the AAV5 NAb, rather the improved assay response curve of the new method yields a comparatively higher titer than the original assay**
- **Improved detection of AAV5 NAb titers may provide valuable insights for clinicians who wish to prescribe etranacogene dezaparvovec for patients with NAbs**

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Disclosures

J.T. and S.B.E.F. is an employee of CSL Behring and stockholder of CSL Behring. **H.L., D. B-K., and D. Z-C** are employees of CSL Behring. **A.F.** is an employee of CSL Innovation. **J.M.** is a co-owner and partner in Wild Type Ventures, LLC (dba Wild Type Advisors) and is a paid consultant to CSL Behring through this business. **T.H., C.S., V.G., D.P.** are employees of Precision for Medicine.

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