Connecting Changes in AAV Capsid Protein Charge Species to In Vitro Protein Expression

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INTRODUCTION

Thermal stress has been shown to have a negative impact on the *in vitro* potency of adeno-associated virus (AAV) vectors. A key driver is the increase of capsid protein modifications, which can impair the ability of the vector to enter the cell. Techniques such as capillary isoelectric focusing (cIEF) provide an aggregate measurement of capsid modifications; however, to better understand how capsids respond to thermal stress, a deeper dive is necessary. Analysis of post-translational modifications (PTM) by liquid chromatography – mass spectrometry (LC-MS) can provide residue-level resolution of changes to capsid proteins.

These studies explored the changes induced by thermal stress on the capsid proteins of POLARIS-101™ (formerly known as AAV-SLB101) and wildtype AAV8 (wtAAV8) including assessments of *in vitro* protein expression. POLARIS-101™ is the north star for a new generation of capsids designed by Solid Biosciences to enhance AAV transgene delivery in skeletal and cardiac muscle tissues.

These findings can have implications on manufacturing processes (e.g., hold steps) and clinical protocols (e.g., drug product thaw procedures) to ensure optimal product quality.

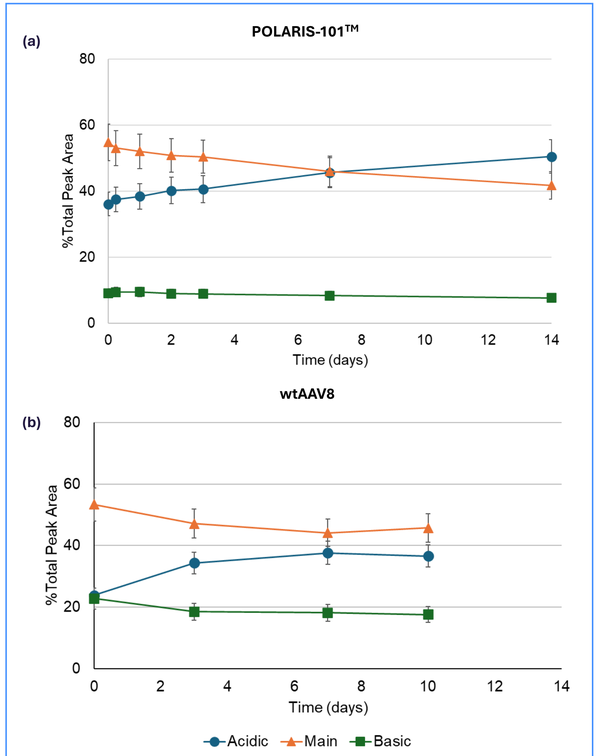
MATERIALS & METHODS

- POLARIS-101™ and wtAAV8 products were aliquoted in scaled-down DP container-closures.
- AAV products were incubated at 25°C for up to 14 days.
- An aggregate measure of the effect of thermal stress on capsid protein charge distribution was generated with cIEF.
- PTM analysis was performed by LC-MS following tryptic digestion.
- *In vitro* protein expression was evaluated by product-specific cell-based assays with enzyme-linked immunosorbent assay (ELISA) or Meso Scale Discovery (MSD) readouts and reported relative to a product-specific reference standard.
- Additional attributes were assessed including capsid protein purity by capillary electrophoresis sodium dodecyl sulfate (CE-SDS), capsid aggregation by size exclusion high performance liquid chromatography (SE-HPLC), genome concentration by singleplex droplet digital polymerase chain reaction (ddPCR), and genome integrity by multiplex ddPCR.

RESULTS

AGGREGATE MEASURE OF VECTOR PROTEIN CHARGE DISTRIBUTION

Figure 1. Vector Protein Charge Distribution by cIEF after incubation at 25°C



a) Trending plot of charge distribution for POLARIS-101™ over 14 days of incubation
b) Trending plot of charge distribution for wtAAV8 over 10 days of incubation

While unstressed POLARIS-101™ (Figure 1a) and wtAAV8 (Figure 1b) contained different distributions of acidic, main, and basic species of the vector proteins, incubation at 25°C resulted in an increase in acidic species with a corresponding decrease in the main species. Basic species also decreased but to a lesser extent than the main species. Notably, for POLARIS-101™, the acidic species continued to rise and was the predominant form after 14 days. For wtAAV8, the main species remained the dominant form and the distribution of species stabilized after 3 days.

VECTOR PROTEIN CHARGE DISTRIBUTION BY PEPTIDE MAPPING ANALYSIS

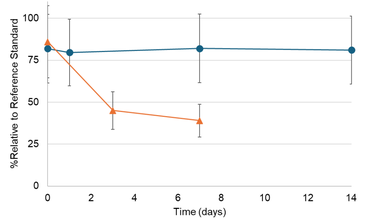
Table 1. PTM Profile by LC-MS after incubation at 25°C

Modification	POLARIS-101 Site	POLARIS-101 %Abundance T0	1d	7d	14d	wtAAV8 Site	wtAAV8 %Abundance T0	3d	7d
Acetylation	N-term	67	100	100	100	not detected			
Deamidation	not detected					N34	5	10	10
	N57	30	25	32	25	N56	30	34	31
	not detected					N261	41	24	17
	N287	8	11	12	11	not detected			
	N329	18	13	21	17	not detected			
	not detected					N382	44	22	19
	not detected					N407	11	3	3
	N452	35	40	44	43	not detected			
	N512	11	11	10	14	N511	23	24	20
	not detected					N537	23	19	18
	not detected					N667	17	3	3
	N698	11	10	18	12	not detected			
Oxidation	M203	63	60	61	67	M203	21	19	16
	not detected					M210	28	23	30
	M373	78	66	71	74	M372	53	55	54
	M404	81	81	77	78	M402	72	66	73
	M436	65	62	61	62	M434	62	57	62
	M471	76	74	72	72	M470	75	67	74
	M518	39	29	31	30	not detected			
	M524	24	28	25	30	M523	63	57	64
	M559	69	55	57	64	M558	48	47	53
	not detected					M604	56	43	49
	M612	69	66	69	68	not detected			
	not detected					M634	62	58	74
	M642	60	39	43	52	not detected			
	M647	49	52	48	50	not detected			
Phosphorylation	S149	11	10	9	11	S148	33	30	29
	S205	13	20	14	13	not detected			
Mapped by residue:	Least abundant → Most abundant								

The most abundant PTMs observed in POLARIS-101™ (Table 1) are N-terminal acetylation and methionine oxidation; however, none of the modifications differed significantly from unstressed material. Similarly for wtAAV8, oxidation was the most abundant PTM and the oxidation levels in incubated samples were comparable to unstressed sample. In contrast, unstressed wtAAV8 deamidated residues N261, N382, N407, and N667 showed a time-dependent decrease in abundance, suggesting a more nuanced degradation pathway. PTM trends did not correlate with the observed increase in acidic species observed by cIEF analysis, however, differences in the abundance of PTMs at residues in similar positions for both serotypes were observed.

IN VITRO POTENCY

Figure 3. Protein Expression after Incubation at 25°C



Trending plots of protein expression relative to product-specific reference standards for POLARIS-101™ and wtAAV8 over incubation times of 14 days and 7 days, respectively.

No loss of relative *in vitro* protein expression was observed for POLARIS-101™ (Figure 3) despite the changes observed in vector charge distribution measured by cIEF where the acidic species ultimately became the predominant form. The changes observed in wtAAV8, however, did result in an appreciable drop in protein expression after 3 days. PTM analysis did not identify a specific modification or set of modifications that may correspond to the observed loss of potency.

SUPPORTING METHODS

Table 2. Summary of Effects of 25°C Incubation on Reported Attributes

Attribute	Assay	Reportable	POLARIS-101™	wtAAV8
Vector Protein Charge Distribution	cIEF	%Acidic	Increase	Increase
		%Main	Decrease	Decrease
		%Basic	No change	Decrease
PTMs	LC-MS	Acetylation	Increase	Not detected
		Deamidation	No change	No change or decrease
		Oxidation	No change	No change
		Phosphorylation	No change	No change
In Vitro Potency	Product-specific cell-based assay	%Relative Expression	No change	Decrease
Capsid Protein Purity	CE-SDS	%Purity	No change	No change
Capsid Aggregation	SE-HPLC	%Monomer	No change	No change
Vector Genome Titer	Singleplex ddPCR (GOI)	vg/mL	No change	No change
Vector Genome Integrity	Multiplex ddPCR	%Double Positive Droplets	No change	No change

Summarized in Table 2 are high level results of the featured assays and additional supportive testing.

CONCLUSIONS

- The stress conditions utilized in this study selectively induce changes in the charge variant profiles, as measured by cIEF, for both the novel capsid POLARIS-101™ and wtAAV8 while other attributes remained unchanged.
- No PTMs were identified that correlated with the increase in acidic species observed by cIEF and additional testing and analytical approaches will be explored to further investigate the connection between PTMs and aggregate measures of vector protein charge.
- Despite the changes in charge profile, the *in vitro* potency of POLARIS-101™ was unaffected whereas wtAAV8 showed a significant loss of potency.

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