

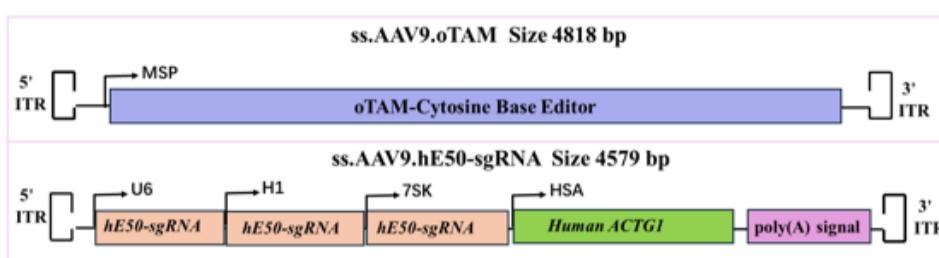
# Potency assessment for GEN6050X, a transformative DMD Cytosine Base editing Drug

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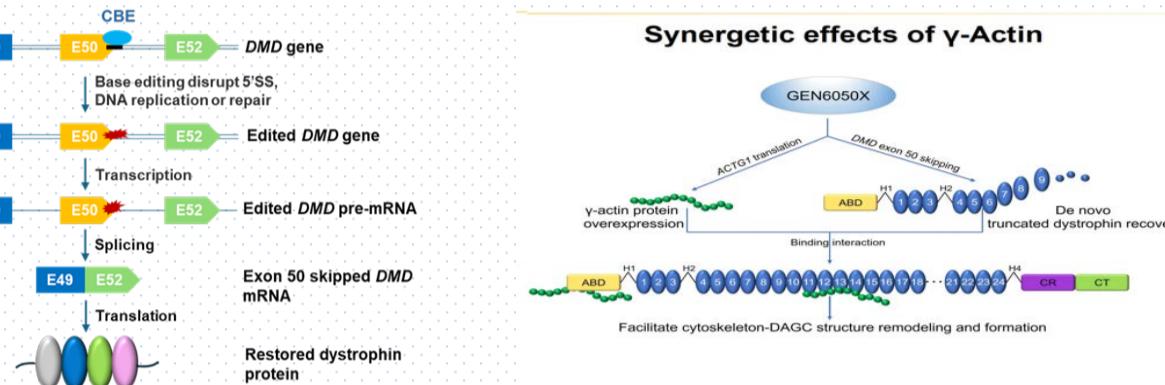
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**Background** GEN6050X is an intravenously administered drug containing two adeno-associated virus serotype 9 (AAV9) vectors: (1) ss-AAV9.oTAM encodes muscle-specific promoter-driven Targeted-AID mediated mutagenesis induced cytosine base editor (TAM CBE) and (2) ss-AAV9.hE50-sgRNA carries encodes 3 copies of hE50 sgRNA targeting 5'SS of DMD IVS50 to induce DMD exon 50 skipping. In addition, the ss-AAV9.hE50-sgRNA vector carries a HAS-driven human ACTG1 gene, which can bind to de novo restored dystrophin to rapidly facilitate costamere and Dystroglycan-associated complex.

## A.GEN6050X



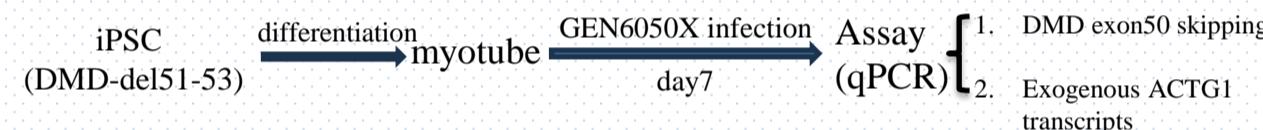
## B. Mechanism of Action (MOA) of GEN6050X



## Objective

This study was to develop a potency assay for the cGMP release of GEN6050X. The specificity, linearity, precision and accuracy of the method were assessed according the requirements of FDA guidelines<sup>1,2,3</sup>

## Workflow



## Results

Table1. Specificity of DMD Exon 50 Skipping

sample	MOI	DMD exon50 skipping ratio					mean	CV	Whether detected or not
		replicate1	replicate2	replicate3	replicate4	replicate5			
DMD-del51-53 myotube	NA	-	-	-	NA	NA	-	-	No
Formulation buffer	NA	-	-	-	NA	NA	-	-	No
GA501 reference standard	5E5	-	-	-	NA	NA	-	-	No
	1E6	-	-	-	NA	NA	-	-	No
GA502 reference standard	5E5	-	-	-	NA	NA	-	-	No
	1E6	-	-	-	NA	NA	-	-	No
GEN6050X	5E5	45.98%	48.23%	43.80%	47.56%	51.53%	47.42%	5.4%	Yes
	1E6	57.10%	60.92%	54.18%	63.02%	60.30%	59.10%	5.2%	Yes

Table2. Specificity of ACTG1 Transcripts

sample	MOI	ACTG1 Transcripts Copy Number (copy number/ng total RNA)					mean	CV	Whether detected or not
		replicate1	replicate2	replicate3	replicate4	replicate5			
DMD-del51-53 myotube	NA	-	-	-	NA	NA	-	-	No
Formulation buffer	NA	-	-	-	NA	NA	-	-	No
GA501 reference standard	5E5	-	-	-	NA	NA	-	-	No
	1E6	-	-	-	NA	NA	-	-	No
GA502 reference standard	5E5	1.9E+03	1.6E+03	1.5E+03	NA	NA	1.7E+03	8.2%	Yes
	1E6	2.3E+03	3.1E+03	1.9E+03	NA	NA	2.4E+03	21%	Yes
GEN6050X	5E5	2.3E+03	2.7E+03	2.4E+03	2.8E+03	2.6E+03	2.6E+03	6.7%	Yes
	1E6	1.9E+03	3.6E+03	3.7E+03	3.6E+03	4.0E+03	3.2E+03	25%	Yes

Table1. Mean and CV(Coefficient of Variation) of DMD Exon 50 Skipping Ratio for specificity of GEN6050X potency;Table2. Mean and CV(Coefficient of Variation) of ACTG1 Transcripts Copy Number for specificity of GEN6050X potency.

Figure1. Linear Regression of DMD Exon 50 Skipping Ratio and ACTG1 transcripts copy number

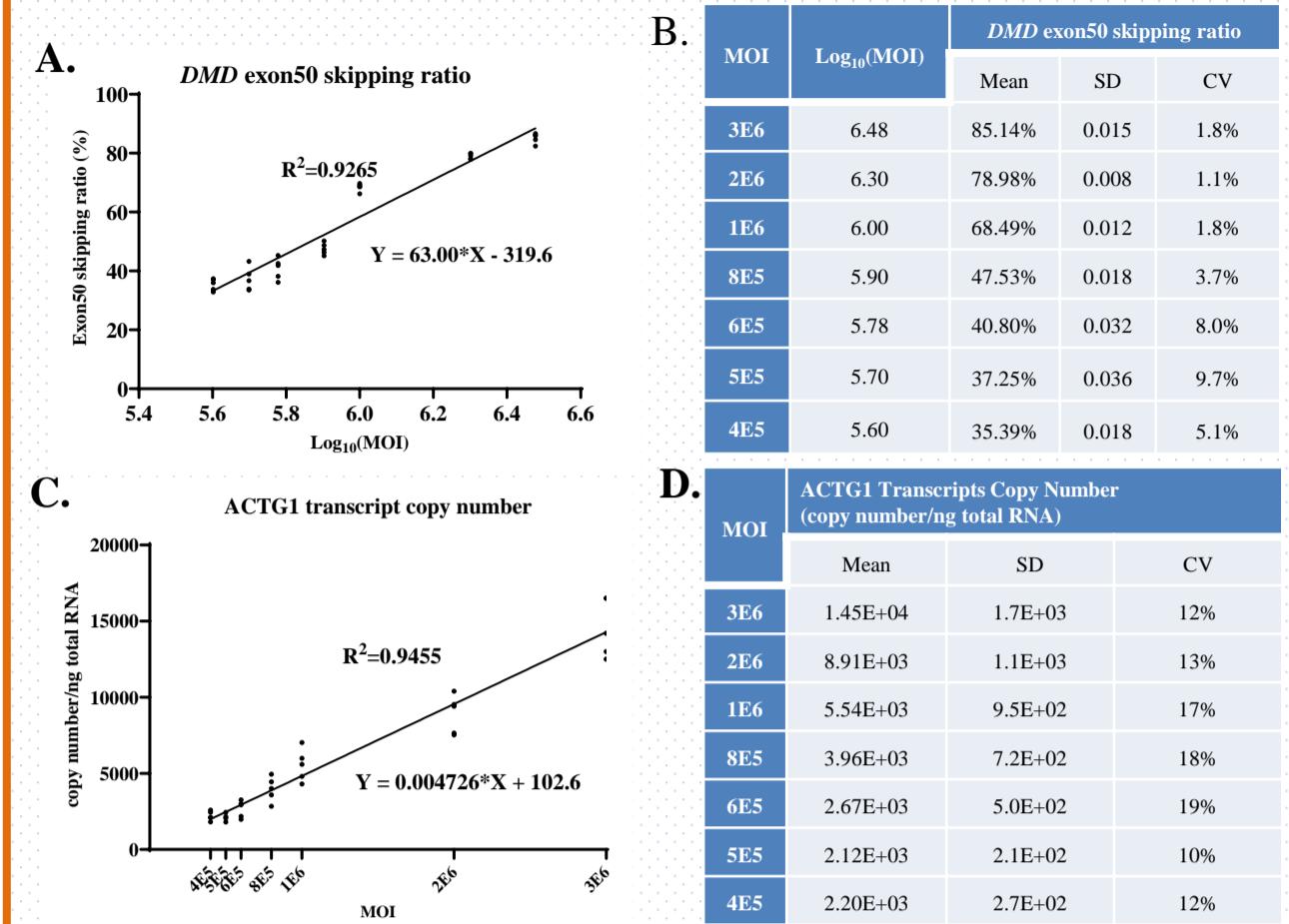


Figure1A. DMD Exon50 exon skipping ratio was linearly fitted against log10(MOI), with the R<sup>2</sup> fitting parameter;Figure1B. The mean and CV of Exon 50 skipping ratio when infecting with GEN6050X at different MOI. Figure1C.ACTG1 transcripts copy number was linearly fitted with log10(MOI), with the R<sup>2</sup> fitting parameter. Figure1D. The mean and CV of ACTG1 copy number infected with GEN6050X.

Table3. Inter-Group Precision and Accuracy Results of DMD Exon 50 Skipping

Replicate	DMD Exon 50 Skipping Ratio	Mean	SD	CV	Accuracy	Within 50-200%
1	47.15%	53.59%	0.082	15%	88%	Yes
2	51.21%				96%	Yes
3	43.24%				81%	Yes
4	52.34%				89%	Yes
5	68.49%				128%	Yes
6	59.10%				110%	Yes

Table4. Inter-Group Precision and Accuracy Results of ACTG1 Transcripts

Replicate	ACTG1 Transcripts Copy Number (copy number/ng total RNA)	Mean	SD	CV	Accuracy	Within 50-200%
1	4.60E+03	4.24E+03	9.7E+02	23%	108%	Yes
2	4.62E+03				109%	Yes
3	2.74E+03				65%	Yes
4	4.79E+03				113%	Yes
5	5.54E+03				131%	Yes
6	3.16E+03				75%	Yes

Table3 and 4.Six independent experiments were performed to detect mean for DMD exon50 skipping and ACTG1 transcripts copy number, then determine the inter-group precision and accuracy.

Table5. Performance criteria to potency assessment of GEN6050X by qPCR

Performance criteria	Qualified	Comment regarding each criterion
Specificity	Yes	Table1.and Table2. only GEN6050X can simultaneously induce DMD Exon50 skipping and ACTG1 transcription.
Linearity	Yes	Figure1A and 1C R2 of standard must be $\geq 0.9$ .
Precision(%CV, intra-assay)	Yes	Table3.and Table4. Conduct six independent experiments to determine precision for DMD exon50 skipping ratio and ACTG1 transcripts copy number. Intra-assay CV should be $\leq 30\%$ .
Accuracy	Yes	Table3. and Table4. Conduct six independent experiments. The accuracy must be within 50-200%.

## Conclusion

1. The potency assay of GEN6050X has been validated for specificity, linearity, precision, and accuracy, meeting the required criteria. This method can be effectively utilized to test the activity of cGMP-grade GEN6050X.
2. Limitation: This method was developed based a specific *DMD* mutation. The potency activity may vary in other mutations amenable for exon 50 skipping.

## References

1. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Potency Assurance for Cellular and Gene Therapy Products: Draft Guidance for Industry . December 2023.
2. Q2(R2): Validation of Analytical Procedures International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2023). Validation of Analytical Procedures: Q2(R2) Guideline . Final version. Adopted on 1 November 2023.
3. Q14: Analytical Procedure Development International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2023). Analytical Procedure Development: Q14 Guideline . Final version. Adopted on 1 November 2023.