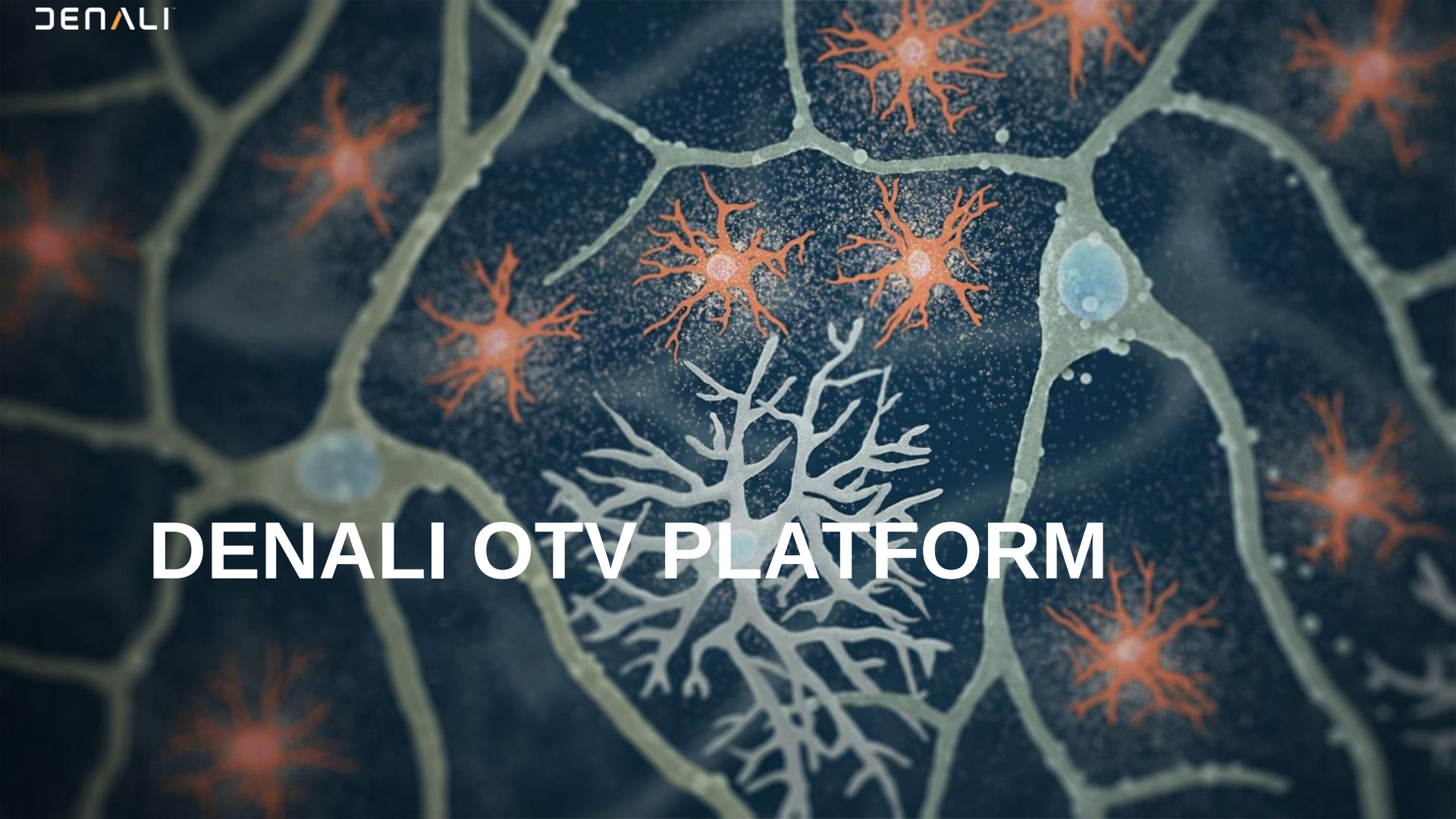
Considerations for Developing Oligonucleotide Transport Vehicle Conjugates

Amelia Adams
CASSS CMC Strategy Forum
July 16, 2024

DISCLAIMER

I am a full-time employee at Denali Therapeutics and own Denali shares.

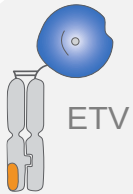

DENALI
DENALI OTV PLATFORM

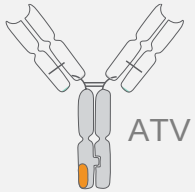
TV ENABLES MULTIPLE MODALITIES FOR BRAIN DELIVERY

Denali is focused on tackling neurodegenerative diseases leveraging the transport vehicle platform to deliver therapies across the blood-brain barrier.

Enzyme Transport Vehicle

Deliver **enzymes** to the brain to replace deficient or missing enzyme activity



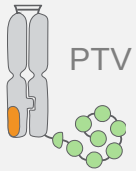


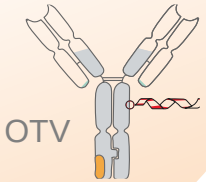
Antibody Transport Vehicle

Deliver **antibodies** in bivalent or bispecific format to the brain

Protein Transport Vehicle

Deliver **proteins** to the brain to replace deficient or missing protein





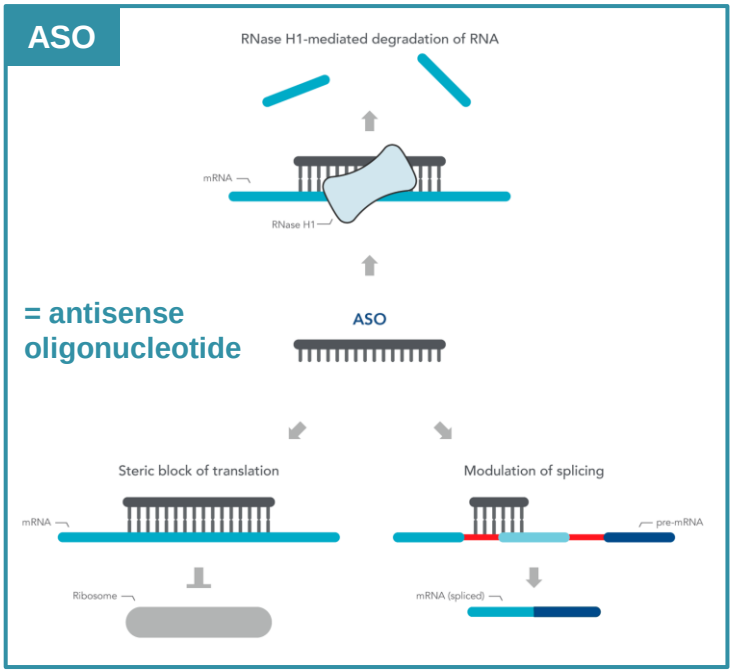
Oligonucleotide Transport Vehicle

Deliver **oligonucleotides** to the brain and modify gene expression

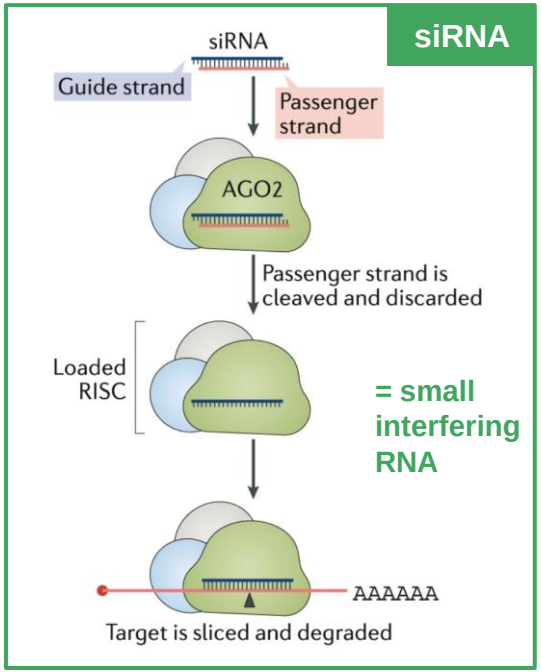
OLIGONUCLEOTIDES AS THERAPEUTICS

Oligonucleotide therapeutics can specifically modify gene expression through gene knockdown, gene regulation, or modulating splicing.

ASO



siRNA



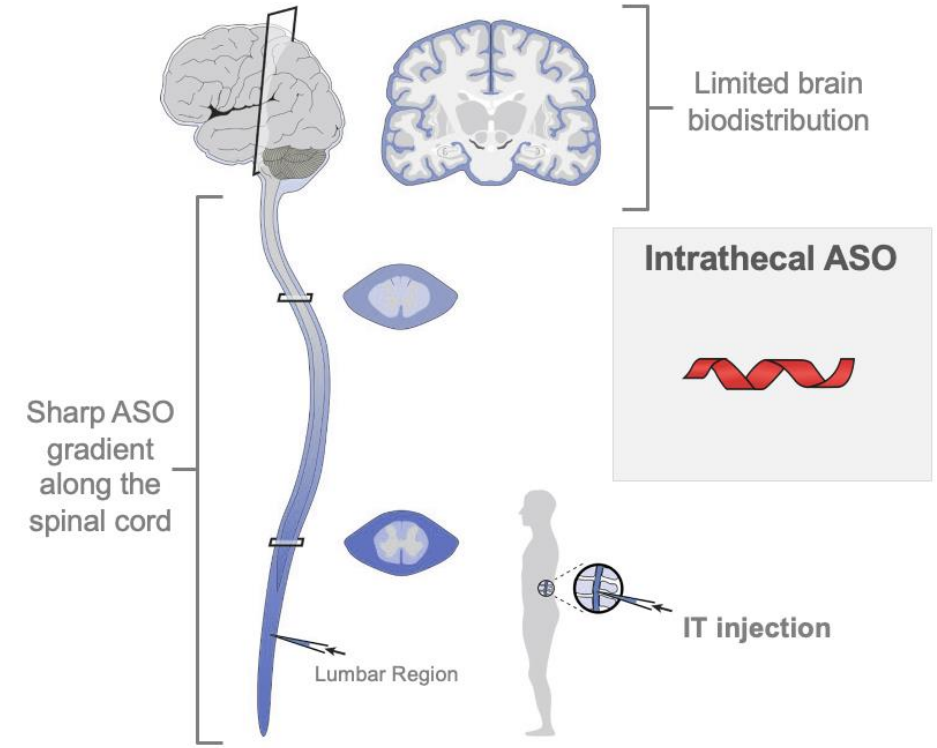
Other modalities

- microRNA
- tRNA
- aptamers
- sgRNA (CRISPR-Cas9 systems)
- etc.

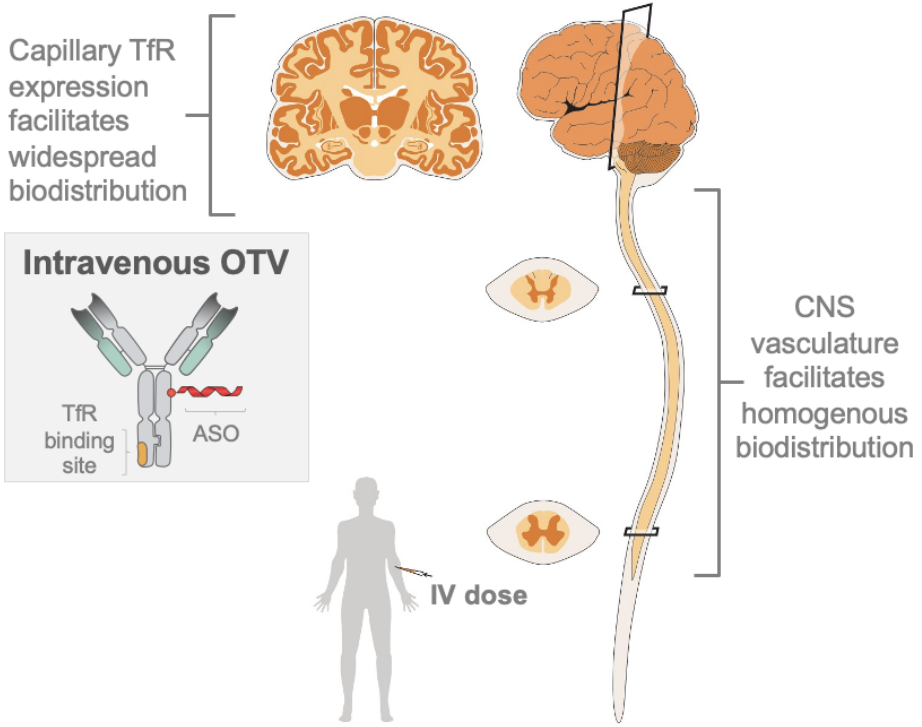
https://www.idtdna.com/pages/products/functional-genomics/antisense-oligos; Roberts, T.C., et al. Nature Reviews Drug Discovery (2020)

SOLVING THE BBB CHALLENGE FOR BRAIN DELIVERY OF BIOTHERAPEUTICS

LIMITED BIODISTRIBUTION WITH INTRATHECAL DOSING



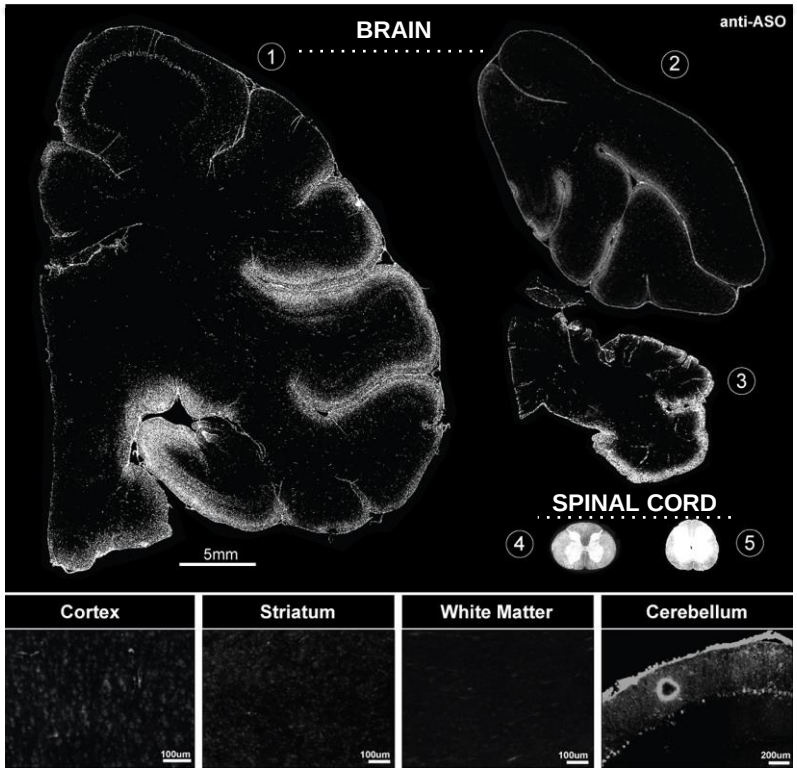
WIDESPREAD BIODISTRIBUTION WITH INTRAVENOUS, BBB-CROSSING ASO



OTV PROVIDES UNIFORM ASO DEPOSITION ACROSS THE CNS WITH IV DELIVERY

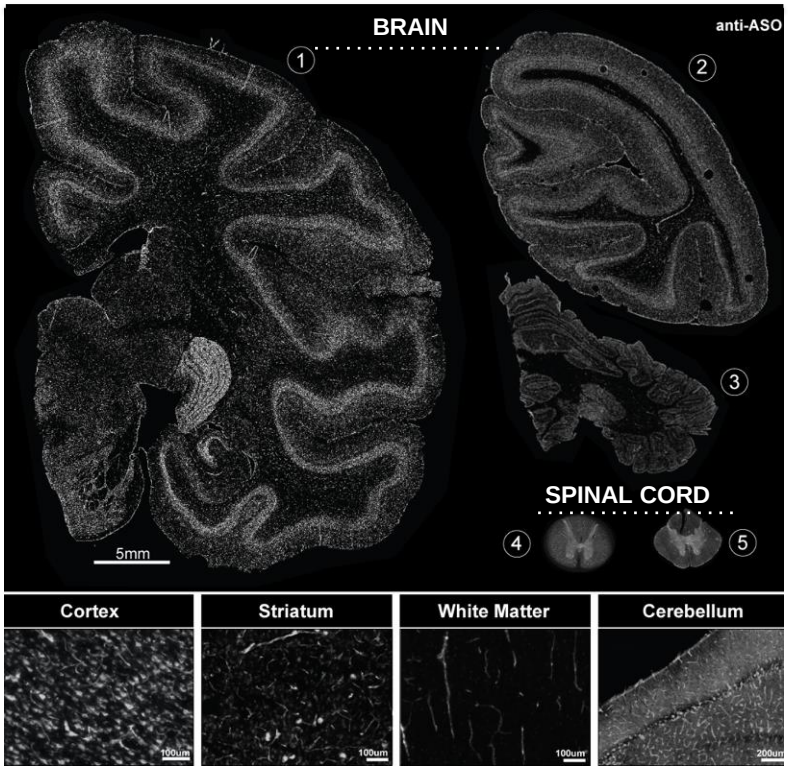
NAKED ASO INTRATHECAL (IT) DELIVERY

Limited ASO Biodistribution



OTV INTRAVENOUS (IV) DELIVERY

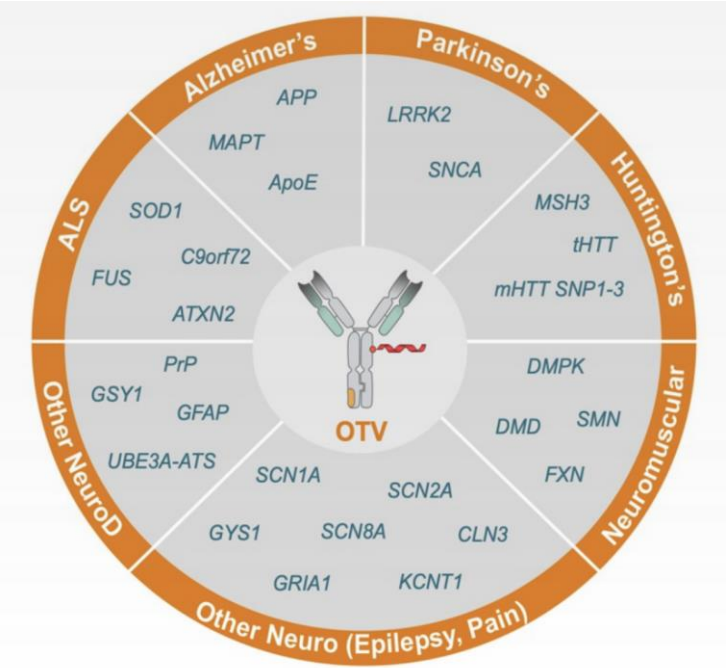
Widespread ASO Biodistribution



(1) Full Hemibrain Section (2) Posterior Cortex overlaying Cerebellum (3) Cerebellum (4) Cervical Spinal Cord (5) Lumbar Spinal Cord

Barker et al. 2023 *BioRxiv*

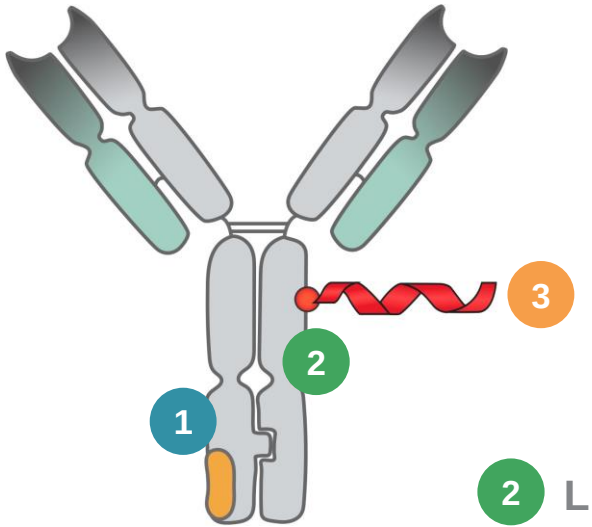
OTV PLATFORM HAS LARGE POTENTIAL TARGET SPACE



- Therapeutic oligonucleotides have the potential to address challenging targets
- OTV is designed to
  - Achieve **superior biodistribution** of ASOs across brain regions
  - Provide knockdown of target gene expression **across all cell types**
  - Enable **less invasive** dosing methods (e.g. intravenous)
- OTV opens a **large potential indication space** in neurodegeneration


DENALI
OTV CMC

COMPONENTS OF AN OTV



1 Transport Vehicle

Delivery vehicle containing a binding site targeting hTfR or other receptor of interest, enabling transport of the OTV across the blood brain barrier.

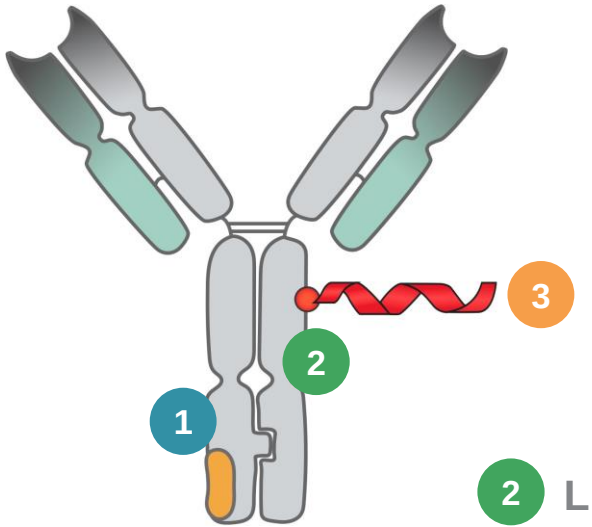
2 Linker

Chemical moiety used to conjugate the oligonucleotide to the transport vehicle.

3 Oligonucleotide

Therapeutic molecule targeting gene or sequence of interest.

COMPONENTS OF AN OTV – DESIGN VARIABLES



1 Transport Vehicle

- Structure
- BBB target
- Non-binding Fabs
- Conjugation site(s)

2 Linker

- Linker-oligo attachment
- Linker-transport vehicle attachment

3 Oligonucleotide

- Oligo modality
- Secondary structure
- Sequence
- Modifications

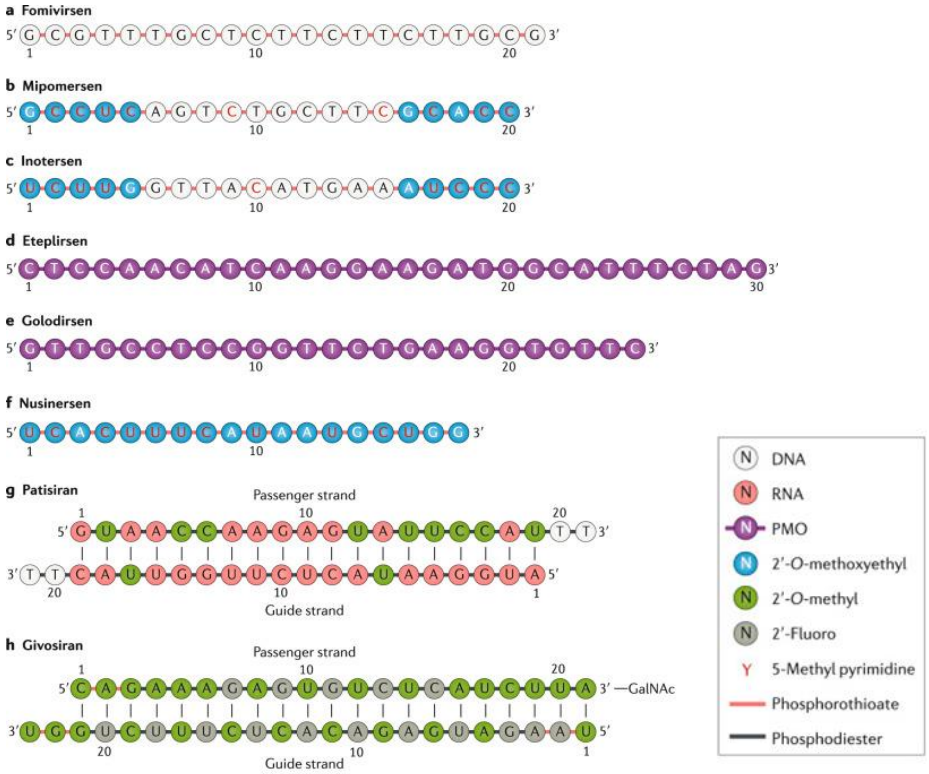
THERAPEUTIC OLIGONUCLEOTIDES CAN BE WIDELY VARIABLE

Design is informed by **mechanism of action** and **pharmacology**.

Typical variables include:

1) Modality
(ASO, siRNA, PMO, etc.)
2) Nucleotide chemical modifications
3) Number of nucleotides
(per strand)
4) Secondary structure

Variables can significantly impact **synthesis**, **conjugation**, and **analytics**.



Roberts, T.C., et al. Nature Reviews Drug Discovery (2020)

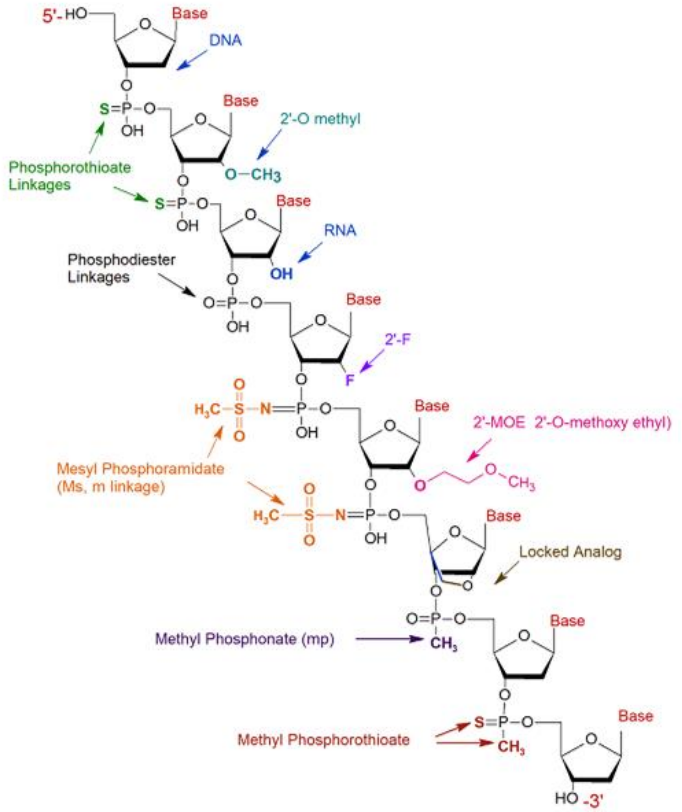
CHEMICAL MODIFICATIONS ADD FURTHER CMC COMPLEXITY

Chemical modifications typically have two purposes:

1) increasing *in vivo* **stability**
   (for example, protecting against exo- or endo-nuclease activity)

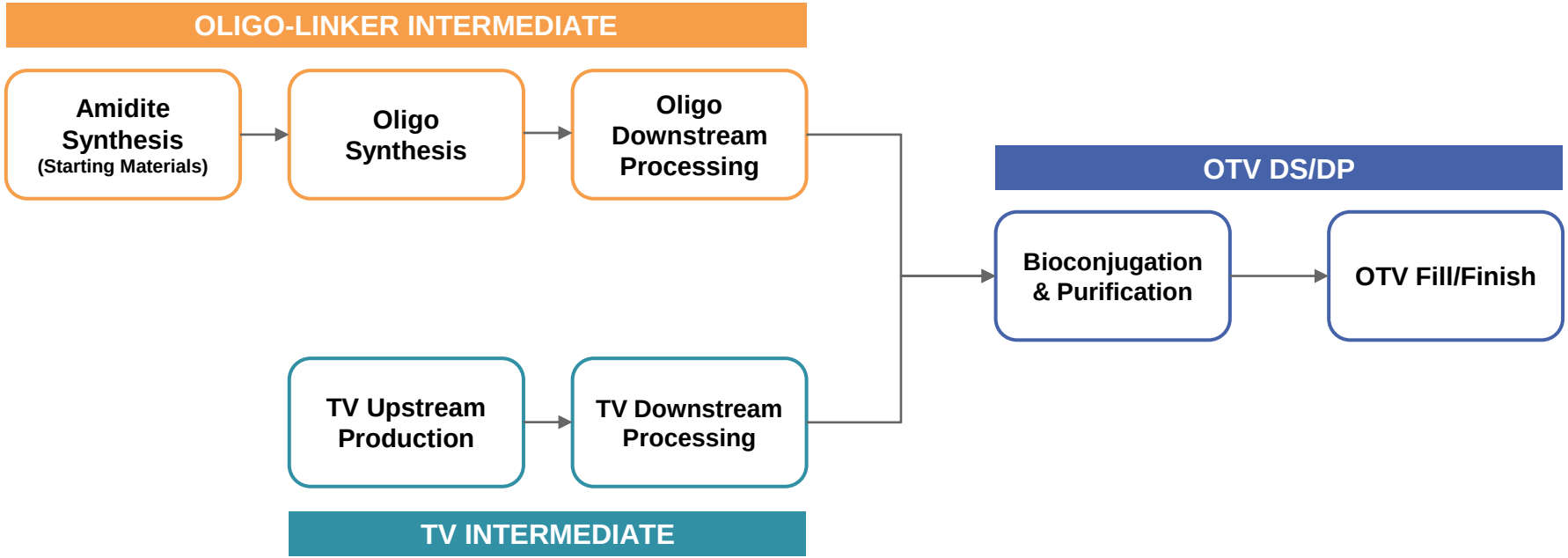
1) increasing **potency**

Modifications are typically introduced during oligo synthesis or as part of amidite (starting material) synthesis.



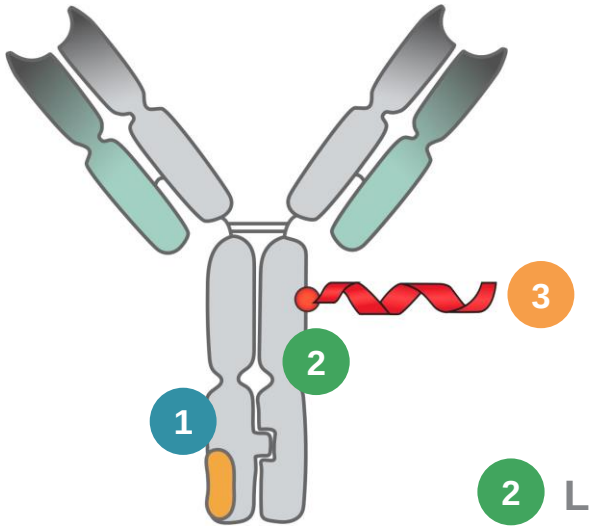
https://www.genelink.com/oligo_modifications_reference/

OTV PLATFORM PRODUCTION PROCESS



PLATFORM APPROACH TO OTV DESIGN

In an optimal “platform” approach, all variables are held constant with the exception of oligonucleotide sequence.



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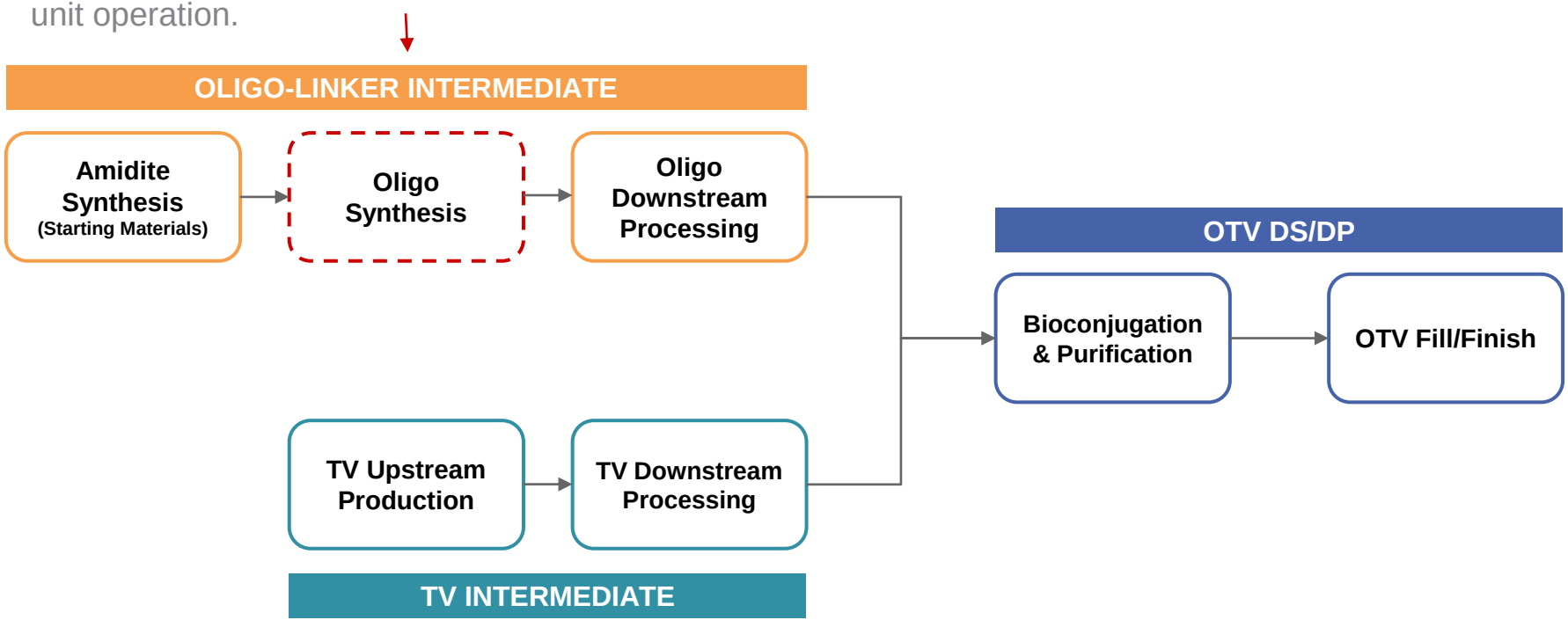
- Linker-transport vehicle attachment

3 Oligonucleotide

- Oligo modality
- Secondary structure
- **Sequence**
- Modifications

OTV PLATFORM PRODUCTION PROCESS

In an optimal “platform” approach, the oligo synthesis step may be the only significantly changed unit operation.



BENEFITS OF PLATFORM APPROACH (CMC)

1 Transport Vehicle

- Same TV drug substance intermediate can be leveraged across multiple programs, reducing process & analytical development work per program
  - ★ *Opposite of ADCs, where drug-linker is typically conserved and antibody is variable*
- Multiple programs can leverage large batches of transport vehicle

2 Linker

- Conjugation process parameters can be largely maintained across conjugate programs, reducing process & analytical development time

3 Oligonucleotide

- Solid phase synthesis process can be optimized for a given oligo type and modification profile, reducing process & analytical development time

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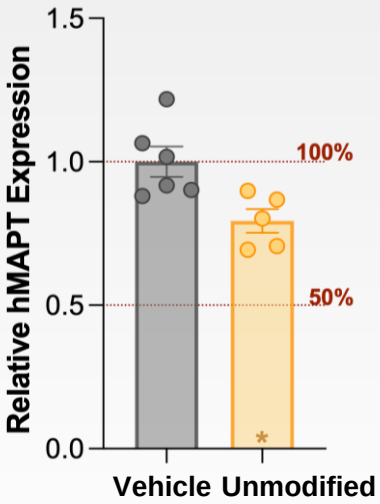
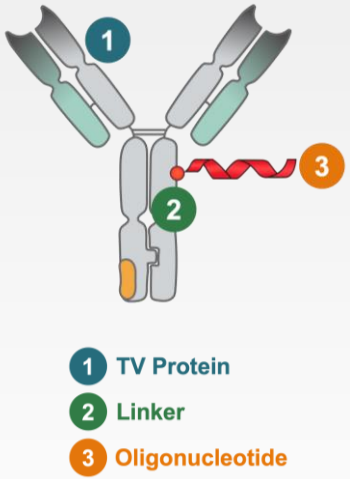
OPTIMIZING GENE KNOCKDOWN CAN LEAD TO VARYING FROM PLATFORM

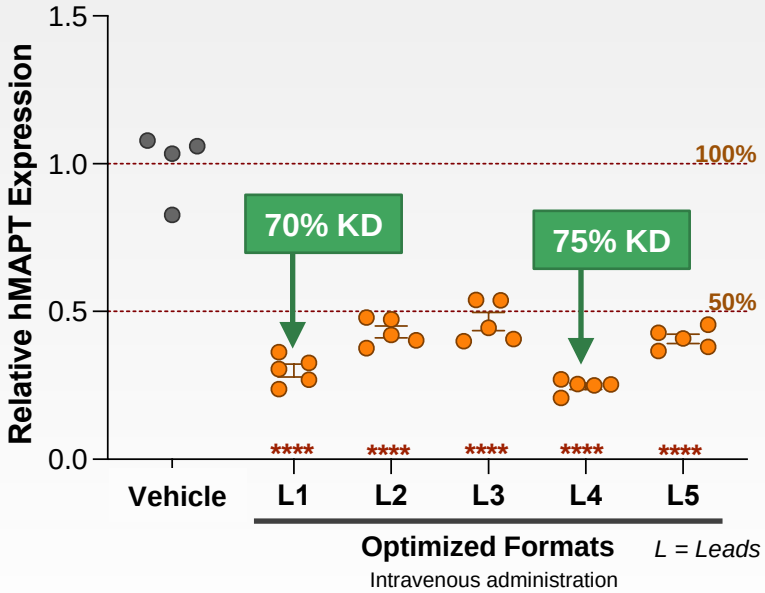
Unmodified OTV Format



Optimization of OTV Format



OTV Optimization Enables Enhanced Brain MAPT Knockdown



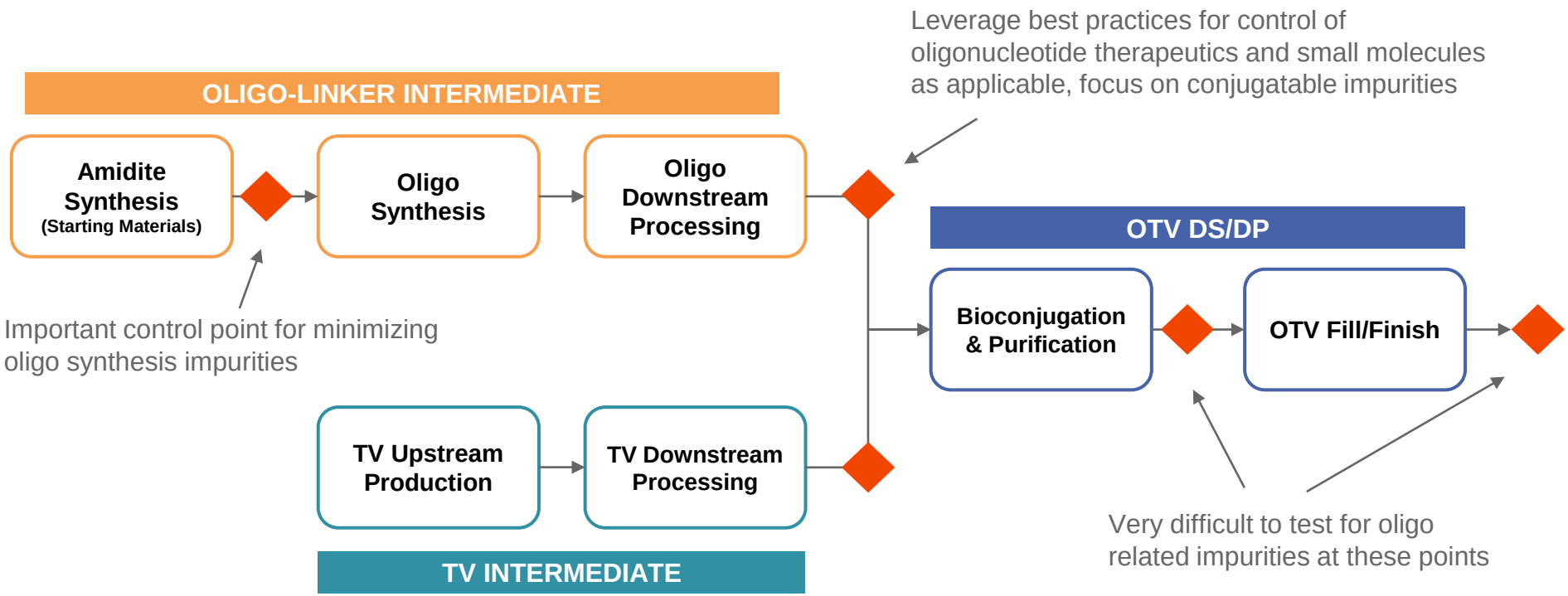
hTau x TfR$^{mu/hu}$ KI mice dosed IV at 1mg/kg ASO eq. on d1, d8, d15, d22; Collect d29. Data shown as Mean +/- SEM; Student t-test (left), One-way ANOVA w/ Dunnett's multiple comparisons test (right)

Relative gene expression normalized to Gapdh (housekeeping gene); expression relative to Vehicle Control
KD – knockdown

IMPACT OF CHANGES FROM PLATFORM ON CMC

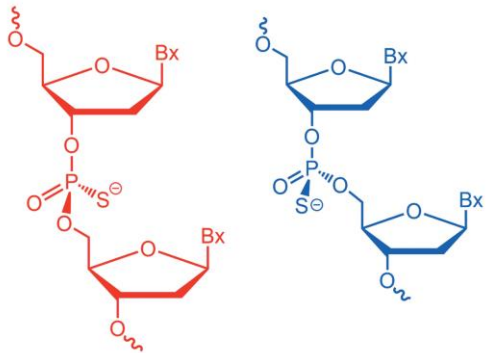
	Variable	Potential therapeutic benefit	CMC Impact
Transport Vehicle	**BBB target**	Alter biodistribution by targeting a new receptor	**High** New cell line, upstream and downstream processes, formulation, and analytics
	Conjugation site(s)	Increase or decrease payload delivery	
Linker	**Linker moiety**	Alter release of payload	**Medium/Low** New conjugation process and formulation, potential impact to analytics Potential impact to oligo synthesis and purification
Oligo-nucleotide	**Oligo type & structure (ASO, siRNA, etc)**	Different mechanisms of action	**High** New amidites, synthesis and purification process New bioconjugation and purification processes, formulation, and analytics
	Modifications	Increase potency and/or stability	**Medium** Potential impact to synthesis, purification, analytics, and stability
	Sequence	Target new therapeutic indication	**Low** Limited anticipated impact to oligo synthesis, conjugation process & analytics

OTV PRODUCTION CONTROL & RELEASE POINTS



UNIQUE CONSIDERATIONS FOR CONTROL OF OTVs

- No official guidance yet from FDA or EMA on control of oligonucleotide therapeutics - leverage industry white papers/best practices and relevant small molecule guidance
  - While ASO's are out of scope, general principles of ICH guidance are good starting points for development of control strategies
- Identification testing for ASO requires confirmation of full sequence by LC-MS/MS
- Precise measurements of relative amounts of specific diastereomers may be impossible due to large number of potential chiral P
  - # diastereomers = $2^{(\#\ \text{chiral P})}$
  - Compared to small molecules, more complex analytical methods and statistical analysis are required to mitigate potential impacts to PK/PD



Capaldi, D., et al. Nucleic Acid Therapeutics (2017)
Arrico, L., et al. Nucleic Acid Therapeutics (2022)

UNIQUE CONSIDERATIONS FOR CONTROL OF OTVs

- Conjugatable impurities include “full length product”-related impurities, which may still have biological activity and low toxicity concern
  - Impurities are typically grouped into families (i.e., n+1, n-1, P=O)
  - Limits for impurities can be less tight than those generally acceptable for small molecules
- OTV DS has a complex mechanism of action, requiring careful design of potency method(s)
- Conjugation of large transport vehicle to the oligonucleotide makes assessing certain oligo quality attributes difficult at the DS and DP step
  - Perform risk assessments and/or extended characterization to assess the impact of the conjugation process on oligo impurity levels and quality attributes
  - Perform forced degradation studies on model conjugates (peptide+ASO) to learn what to look for in real samples

Capaldi, D., et al. Nucleic Acid Therapeutics (2017)

EXAMPLE OLIGO CONTROL STRATEGY

Test		Oligo-Linker Intermediate
General	Appearance	✓
	Counterion Identity	✓
	Counterion Content by IC	✓
	Water Content	✓
	Assay	✓
Identity	Identification by LC/MS	✓
	Sequence Verification by MS^n	✓
	Identification of Duplex by UV Tm - if applicable	✓*
Purity	Chemical Purity	✓
	Related Substances (i.e., n+1, n-1, P=O)	✓
	Chiral purity - if applicable	✓*

ID & purity testing after conjugation is extremely difficult, but of interest during process development

**need for testing determined by oligo design (siRNA v. ASO, modifications, etc)*

EXAMPLE OLIGO CONTROL STRATEGY

Test		Oligo-Linker Intermediate	OTV DS	OTV DP
General	Appearance	✓	No longer relevant after conjugation.	
	Counterion Identity	✓		
	Counterion Content by IC	✓		
	Water Content	✓		
	Assay	✓		
Identity	Identification by LC/MS	✓	Leverage post-conjugation potency assays.	
	Sequence Verification by MS^n	✓		
	Identification of Duplex by UV Tm - if applicable	✓*		
Purity	Chemical Purity	✓	Characterize impact of conjugation steps on impurity levels and determine need for additional testing.	
	Related Substances (i.e., n+1, n-1, P=O)	✓		
	Chiral purity - if applicable	✓*		

**need for testing determined by oligo design (siRNA v. ASO, modifications, etc)*

EXAMPLE OLIGO CONTROL STRATEGY

Test		Oligo-Linker Intermediate
Residual Impurities	Inorganics	✓
	Residual Linker + Related Products	✓
	Residual Solvents (OVI)	✓
	PMI (ie; acrylomide, azide)	✓
Safety	Microbial Testing	✓
	Endotoxin	✓
	Sterility	

← Test limits will be less tight than for oligos intended for intrathecal injection

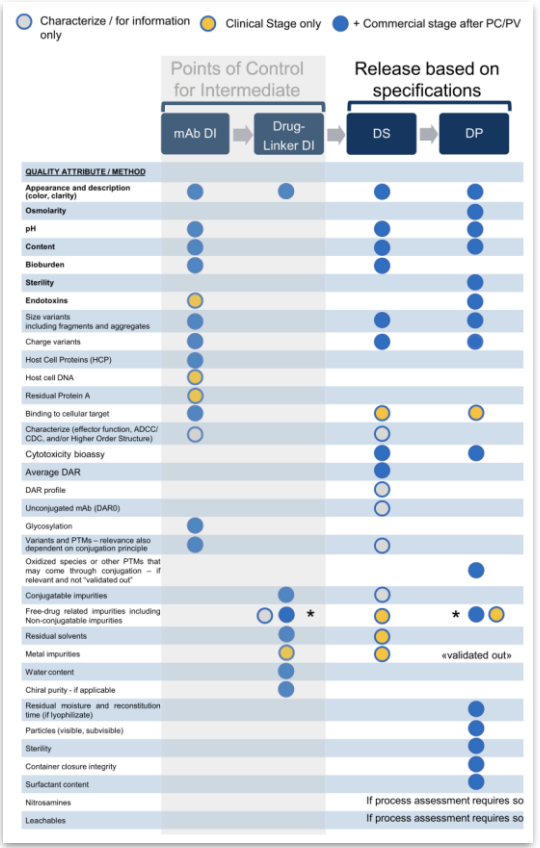
EXAMPLE OLIGO CONTROL STRATEGY

Test		Oligo-Linker Intermediate	OTV DS	OTV DP
Residual Impurities	Inorganics	✓	Purge factors anticipated to be large. Characterize during clinical development.	
	Residual Linker + Related Products	✓		
	Residual Solvents (OVI)	✓		
	PMI (ie; acrylomide, azide)	✓		
Safety	Microbial Testing	✓	✓	
	Endotoxin	✓	✓	✓
	Sterility			✓

After conjugation, follow safety testing practices for biologics

CERTAIN ADC STRATEGIES CAN BE APPLIED TO OTVs

- **DAR → Oligonucleotide Transport Vehicle Ratio**
  - Characterizing OTR profile and quantifying amount of target OTR material remains critical for OTVs
- **mAb DI → TV Intermediate**
  - Tests for TV-specific impurities may only be required at TV intermediate release step (i.e., rHCP, rDNA, rProA)
  - Addition of negatively charged oligo, complexity of oligo diastereomers, and/or oligo FLP impurities may make testing certain TV-specific quality attributes difficult after conjugation (i.e., charge variants)
- **Free drug → Residual ASO**
  - Free oligo/oligo-linker may be less of a concern than free cytotoxic drug due to high systemic clearance and low toxicity
  - May not need a DS release test for residual ASO if it's possible to demonstrate sufficient clearance over OTV purification steps



Bechtold-Peters, K., et al. Journal of Pharmaceutical Sciences (2023)

WRAP UP

OTV therapeutics combine the biodistribution benefit of the transport vehicle with the specific gene expression modifying capabilities of oligonucleotides

OTVs are an opportunity for a platform CMC approach, with "mix-and-match" modifications to the TV, linker, and oligo based on desired therapeutic properties

- To maintain rapid timelines, it is critical to understand the potential CMC impact of new variables ***early***

ADC control strategy considerations can be applied to OTVs with some caveats

- Oligonucleotides have unique analytical testing considerations and potential conjugatable impurities
- Some transport vehicle-specific and oligo-specific quality attributes may not be easily evaluable after oligo conjugation, requiring mechanisms of control other than DS & DP release tests


THANK YOU

REFERENCES

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