# Designing and Selecting Antibody-Oligonucleotide Conjugates for Enhanced Therapeutic Activity

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## Introduction

Oligonucleotides are an outstanding class of therapeutics. Their exquisite ability to modulate the expression of a target gene provides unique opportunities to develop therapies for the treatment of otherwise undruggable diseases.

Although the unique properties of oligonucleotides have been successfully harnessed with the regulatory approval of over 20 ASOs and siRNAs, oligonucleotides present several features limiting their clinical efficacy. They are rapidly cleared from systemic circulation, and their large size and negative charge affect their ability to permeate the cell membrane, limiting their therapeutic activity.

Antibody-Oligonucleotide Conjugates (AOCs) have emerged as a promising modality to achieve more efficient delivery to the target tissue and uptake into the target cells. AOCs are complex molecules though, requiring appropriate design and optimization of each of their individual components, oligonucleotide, antibody and linker, to deliver their full potential.

## Designing Antibodies For Targeted Oligonucleotide Delivery

A large variety of antibody formats with differentiated pharmacokinetics and tissue penetration profiles can be explored for targeted oligonucleotide delivery, from full IgG to VHHs. Different clones with different target epitopes and binding affinities as well as various monovalent and bivalent formats are typically included in the panel of constructs investigated to select an optimal format for targeted antibody delivery.

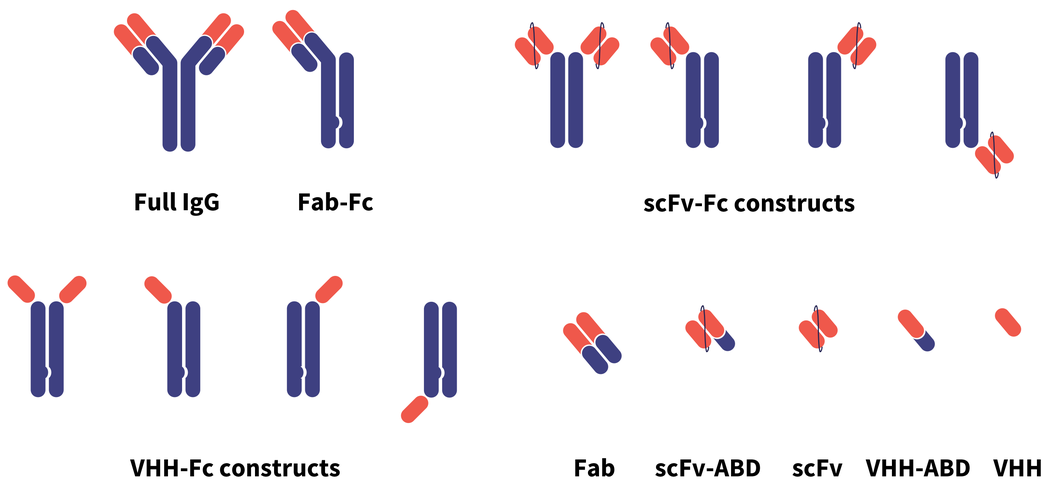
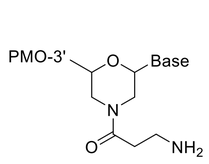


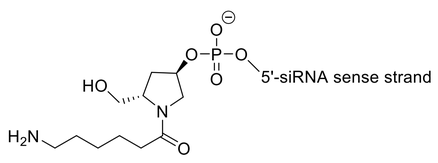

**Figure 1. Representative panel of antibody designs for AOC development.** Larger antibody formats and antibody formats incorporating an Fc domain or an Albumin Binding Domain (ABD) benefit from a longer circulatory half-life compared to smaller antibody formats but have reduced ability to penetrate tissues. Antibody formats developed for AOCs typically lack Fc domains or include silencing mutations in the Fc domain to prevent undesirable interactions of AOCs with Fc receptors on immune cells. Monovalent antibody formats frequently outperform bivalent ones for the delivery of AOCs, especially to the CNS.

## Modifying Oligonucleotides For Conjugation Linker Coupling

For coupling to conjugation linkers, oligonucleotides need to be modified with suitable moieties such as amino-hexyl, orthopyridyl disulfide or azido-hexyl during their synthesis.
Oligonucleotides largely tolerate modifications at their 5' or 3' end, which usually do not alter their activity. For siRNAs, the sense strand is usually modified for coupling to conjugation linkers.




**β-alanine 3' end modified PMO**



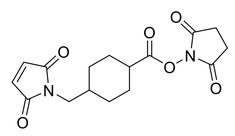

**amino 5' end modified siRNA sense strand**

**Figure 2. Example of common oligonucleotide modifications for coupling to conjugation linkers.** Amino modifications allow straightforward attachment of oligonucleotides to a broad range of non-cleavable and enzyme cleavable conjugation linkers.

## Designing Linkers For Antibody-Oligonucleotide Conjugates

Linkers used for AOCs often have simple structures, reuse design elements originally developed for ADC linkers, and place emphasis on high conjugation reaction efficiency with frequent use of thiol-maleimide or 'click' chemistry.

**delpacibart braxlosiran**



**SMCC linker**

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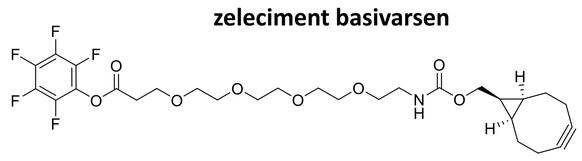
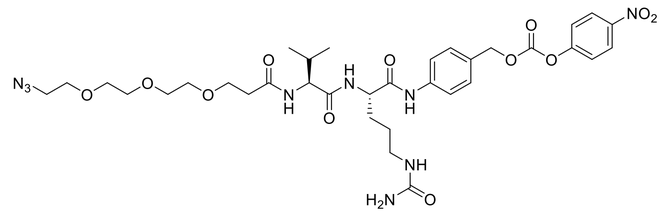


**Endo BCN-PEG4-PFP ester linker – For antibody pre-conjugation**



**Azido-PEG3-Val-Cit-PAB-PNP linker – For oligonucleotide coupling**

**Figure 3. Example of linkers present in AOCs in late-stage clinical trials.**

## Comparing Bioconjugation Strategies To Generate OAR 1 AOCs

The oligonucleotide-to-antibody ratio (OAR) is a parameter typically investigated during the lead optimization stage of a bioconjugate. While PMO AOCs tend to be developed at an OAR of 3 or 4, siRNA and ASO AOCs tend to be developed with an OAR 1. Different bioconjugation strategies can be employed to generate OAR 1 AOCs, each with advantages and limitations.

| Strategy | Advantages | Drawbacks |
|---|---|---|
| **Stochastic Conjugation on Native mAb** | ✓ Utilizes native mAb (no engineering)<br>✓ Minimal input requirements | × Generates mixture of OAR species<br>× Low yield for isolated OAR 1 species<br>× Requires capping of residual Cysteines |
| **Stochastic Conjugation on Engineered Cys mAb** | ✓ Utilizes standard engineered Cysteine mAb<br>✓ Minimal input requirements<br>✓ Higher conversion to OAR 1 than for purely stochastic conjugation | × Requires mAb engineering<br>× Moderate yield for isolated OAR 1 species<br>× May result in disulfide scrambling<br>× Requires capping of residual Cysteines |
| **Site-specific Conjugation on Single Engineered Cys Bispecific mAb** | ✓ High conversion to target OAR 1<br>✓ Choice of conjugation location | × Requires expression of a bispecific mAb with an engineered Cysteine<br>× May result in disulfide scrambling |
| **Site-specific Enzymatic Conjugation on Single Tag Bispecific mAb** | ✓ High conversion to target OAR 1<br>✓ Choice of conjugation location<br>✓ No engineered Cysteine | × Requires expression of a bispecific mAb<br>× Requires an enzyme supply |
| **ThioBridge® Conjugation to Fab** | ✓ High conversion to target OAR 1<br>✓ Utilizes native Fab<br>✓ Stable Conjugation<br>✓ High overall yield | × Strategy more challenging to apply to native mAbs for generation of OAR 1 conjugates |

**Figure 4. Comparison of different bioconjugation strategies to generate OAR 1 AOCs**

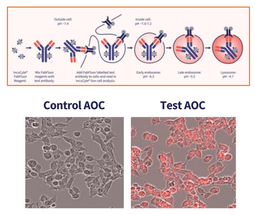
## Selecting a Lead Antibody-Oligonucleotide Conjugate

To identify the most promising candidates for further evaluation and development, AOCs are screened using a series of assays that assess their internalization into target cells, their ability to knock down target gene expression, and their resistance to thermal stress and stability in serum.

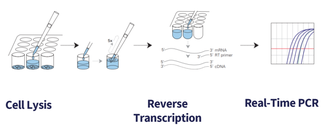
**Sartorius IncuCyte S3 Imaging System**

**QuantStudio™ 3 Real-Time PCR System**

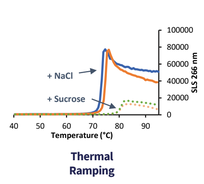
**Prometheus Panta Biostability Platform**



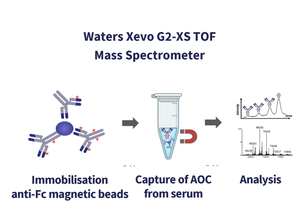

**Internalisation Assay**




**Gene Knock-Down Assay**




**Biophysical Stability Assay**




**Ex vivo Serum Stability Assay**

**Figure 5. Panel of in vitro assays applied for the screening of AOCs and selection of lead candidates.** AOCs are evaluated for internalisation, gene knock-down activity, biophysical stability and serum stability to select the best leads for further evaluation and development.

## Summary

Antibody-Oligonucleotide Conjugates are a promising modality to expand the therapeutic use of oligonucleotides beyond their traditional indications.

As highly complex molecules, AOCs require appropriate design and optimization of each of their components, oligonucleotide, antibody and linker, to deliver their full potential.

Leveraging our bioconjugation toolbox and multi-disciplinary expertise, Abzena rapidly assemble panels of AOC constructs, screen their properties across a range of biophysical and biological assays to accelerate the selection the best designs for clinical development.