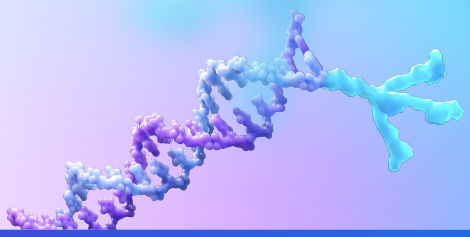
WuXi TIDES



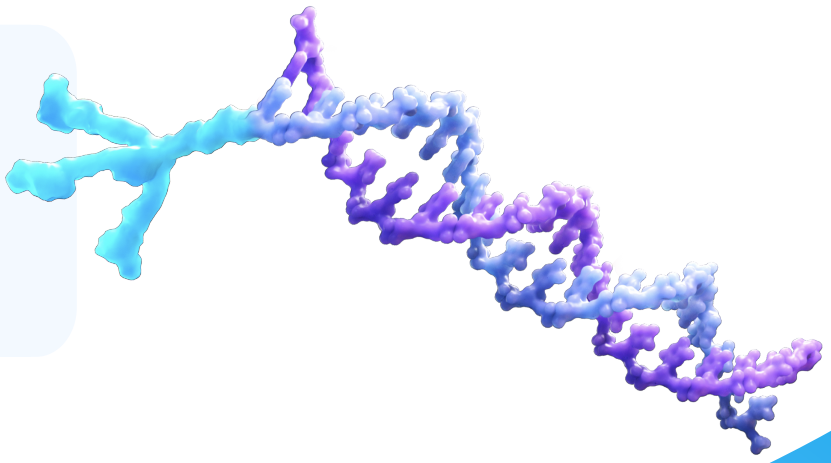
Case Study

Fast-Track to Phase I: Two siRNA IND CMC Packages Completed in 14 Months

A biotech company engaged WuXi TIDES to develop a siRNA-GalNAc drug candidate for cardiovascular disease. The original synthesis process for this 23-mer siRNA resulted in a yield of 13% and a crude purity of 18%. In addition, the GalNAc moiety was not commercially available. Our team's objective was to accelerate this challenging molecule to IND filing as fast as possible. Utilizing innovative conjugation chemistry and tailored analytical studies, we significantly improved the crude purity and yield to 75% and 62%, respectively, delivering on this project in a timely and efficient manner.

Process Optimization Results

crude purity	final yield
75%	62%
18%	13%



Challenges

01 The project faced a supply chain challenge due to the need for a non-standard GalNAc moiety and a specific monomer, which was costly.

02 Process development challenges included improving the yield and purity of the sense strand synthesis. The instability of the intermediate oligonucleotides, due to the phosphodiester bond, required innovative stabilization strategies.

18%
crude purity

13%
Initial yield

Solution Highlights

Our integrated Chemistry, Manufacturing, and Controls (CMC) services effectively managed the complexities of oligonucleotide synthesis, GalNAc supply, formulation development and manufacturing, as well as analytical support for both drug substance and drug product. This collaborative effort, coupled with a tailored and optimized process workflow, resulted in the delivery of two siRNA-GalNAc conjugate drug candidates ready for IND submission within 14 months.

- **GalNAc Process Development and Manufacturing**

Our Drug Substance (DS) team initiated the project by synthesizing and optimizing the required GalNAc fragment. Leveraging advanced process chemistry techniques, we developed an improved synthetic route that enhanced the yield of the GalNAc moiety. Specifically, we achieved a high recrystallization yield of 94.8% by employing flow chemistry and selecting the optimal solvent, which minimized impurities and maximized crystal purity. Establishing an internal supply of GalNAc ensured a reliable and consistent source, facilitating the conjugation process. The team successfully delivered 4.5 kg of high-purity GalNAc product in-house within 4 months, significantly accelerating the project timeline.

- **GalNAc-siRNA Conjugate Process Development and Manufacturing**

Recognizing the potential of click chemistry for its high specificity and yield, our scientists explored its application in the synthesis of GalNAc-siRNA conjugates to address the major challenges of yield and purity post-synthesis. Click chemistry reactions are highly selective, producing minimal byproducts, which is crucial for therapeutic efficacy. Our team developed specific on-resin click reaction conditions to simplify subsequent downstream process steps, thereby recovering excess azides before oligonucleotide deprotection and reducing cost. This method allowed conjugates to be cleaved from the solid support in a single step, resulting in higher purity products with enhanced crude stability.

By streamlining the synthesis and purification processes, we accelerated the development timeline for the siRNA-GalNAc conjugate drug candidate. This approach improved the efficiency and yield of the conjugates, ensuring high purity and stability suitable for clinical trials. Our optimized process design enabled us to control impurity degradation through on-resin click chemistry, reducing purification steps from three rounds to one, and achieving an endotoxin level below 0.1 EU/mg, along with a crude purity of 75% (vs. originally 18%) and a final yield of 62% (vs. originally 13%).

- **Formulation Development and Manufacturing**

Meanwhile, our Drug Product (DP) team initiated formulation development alongside drug candidate synthesis. A 25 g demo batch was delivered for formulation development at month 4, followed by a 500 g GLP batch delivered within 6 months of development. Concurrently, process optimization continued, culminating in the delivery of a 500 g GMP batch used to manufacture the clinical trial material (CTM) batch ready for IND submission at month 10. This synchronized effort across multiple teams enabled the rapid advancement of the siRNA-GalNAc conjugate formulation, ultimately accelerating the IND submission process.

During GMP production, we achieved a line loss of less than 1%, significantly minimizing the loss of costly API. Additionally, the optimal study design during the development phase allowed us to streamline the scale-up process, resulting in fewer trials and more efficient production runs. We minimized the API required for formulation research and development and analytical testing to less than 20 g.

3 Months
Formulation Development and Optimization

6 Weeks
CTM Production and Shipment

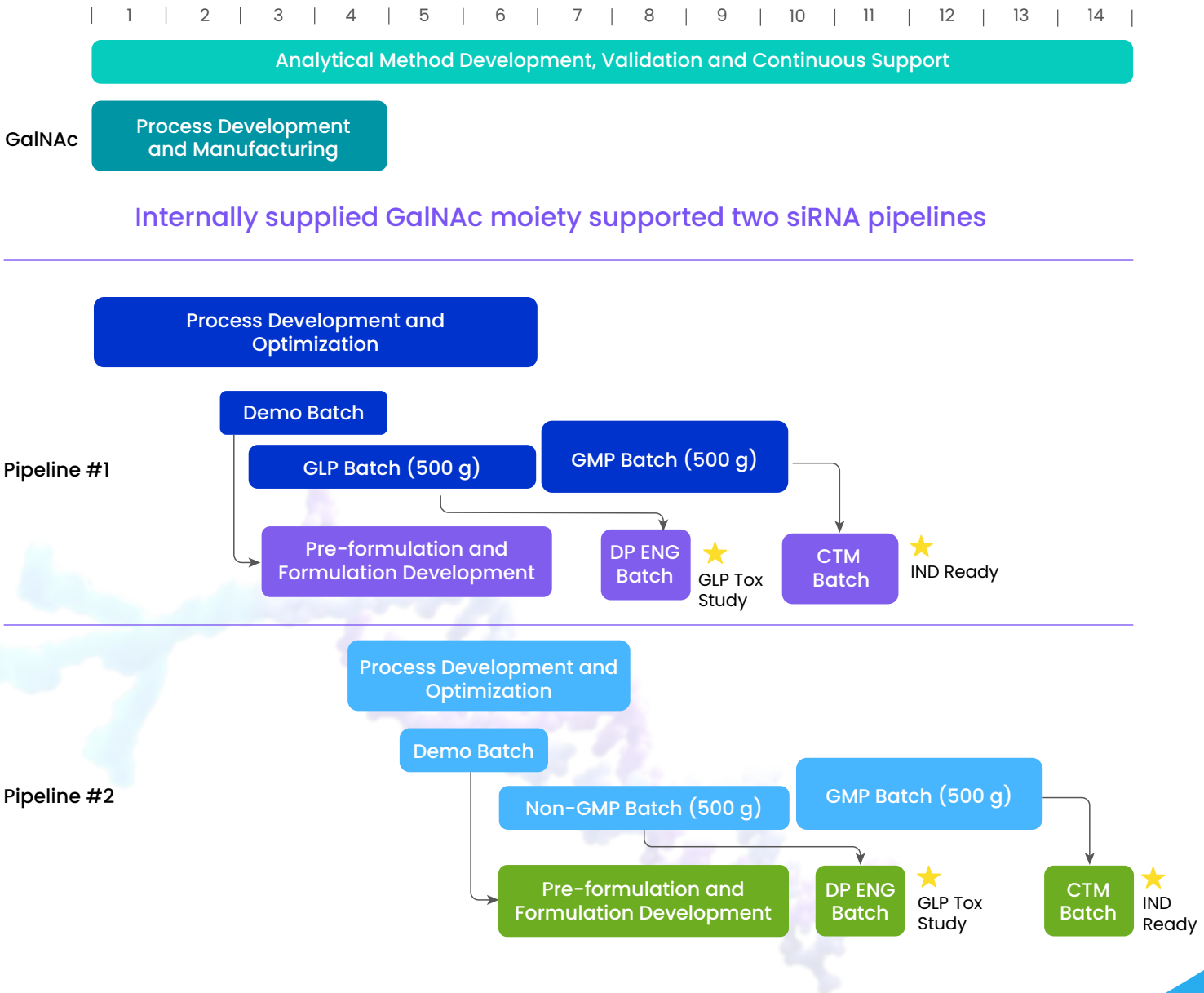
<1% Line Loss
in GMP Production

- **Analytical Development**

Our integrated quality system enabled seamless analytical method transfer from API to Drug Product, leveraging the shared analytical methods between the DS and DP teams. Our multidisciplinary approach, combining expertise in oligonucleotide synthesis, GalNAc chemistry, formulation development, and analytical validation, allowed us to overcome the inherent challenges of this complex modality.

By streamlining processes, optimizing yields, and ensuring reliable supply chains, we successfully delivered two high-quality siRNA-GalNAc conjugate drug candidates, ready for clinical evaluation within an accelerated timeline.

Two siRNA IND CMC Packages Completed in 14 Months



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