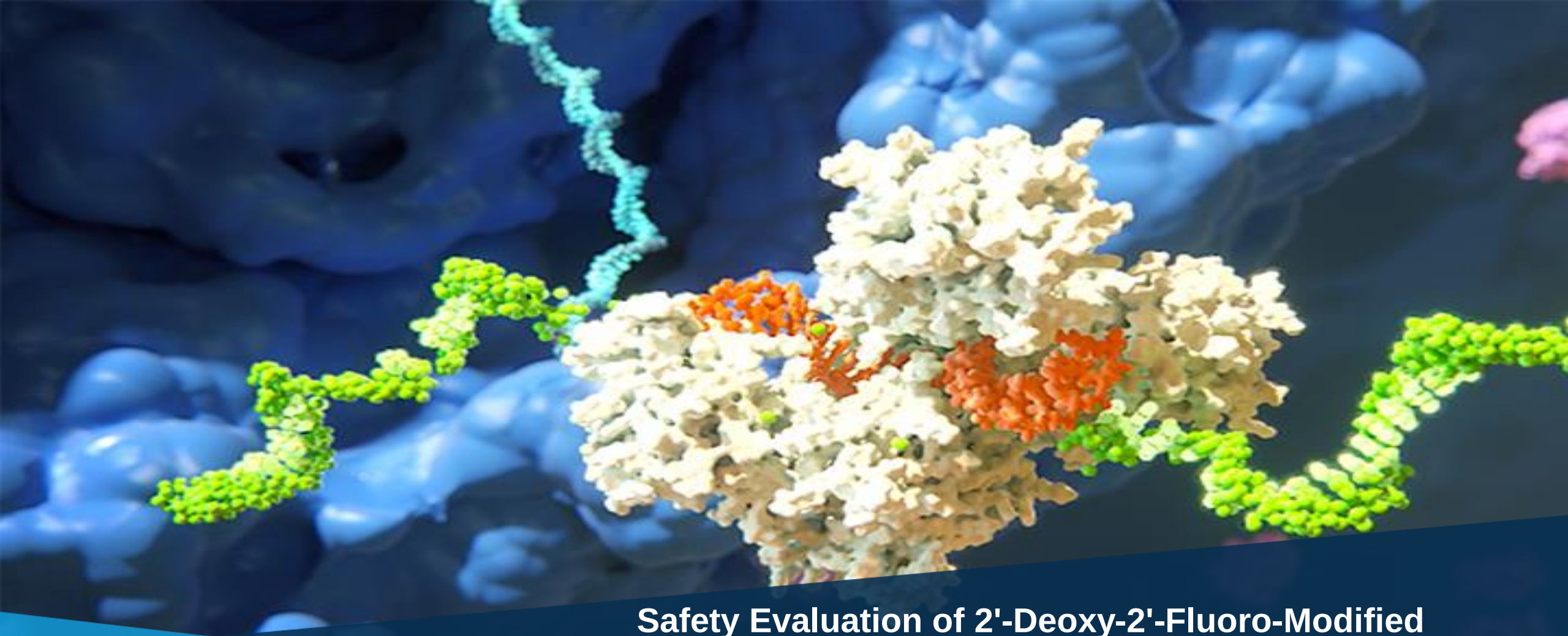

Safety Evaluation of 2'-Deoxy-2'-Fluoro-Modified Nucleotides in GalNAc-siRNA Conjugates

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Principal Scientist, Early Development

14th Annual Meeting of the OTS
October 3rd, 2018

Alnylam® PHARMACEUTICALS

Alnylam Forward Looking Statements

This presentation contains forward-looking statements, within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the Securities Exchange Act of 1934. There are a number of important factors that could cause actual results to differ materially from the results anticipated by these forward-looking statements. These important factors include our ability to discover and develop novel drug candidates and delivery approaches and successfully demonstrate the efficacy and safety of our product candidates; pre-clinical and clinical results for our product candidates; actions or advice of regulatory agencies; delays, interruptions or failures in the manufacture and supply of our product candidates; our ability to obtain, maintain and protect intellectual property, enforce our intellectual property rights and defend our patent portfolio; our ability to obtain and maintain regulatory approval, pricing and reimbursement for products; our progress in establishing a commercial and ex-United States infrastructure; our ability to successfully launch, market and sell our approved products globally; our ability to successfully expand the indication for ONPATTRO™ (patisiran) in the future; competition from others using similar technology and developing products for similar uses; our ability to manage our growth and operating expenses, obtain additional funding to support our business activities and establish and maintain business alliances; the outcome of litigation; and the risk of government investigations; as well as those risks more fully discussed in our most recent report on Form 10-Q under the caption “Risk Factors.” If one or more of these factors materialize, or if any underlying assumptions prove incorrect, our actual results, performance or achievements may vary materially from any future results, performance or achievements expressed or implied by these forward-looking statements. All forward-looking statements speak only as of the date of this presentation and, except as required by law, we undertake no obligation to update such statements.

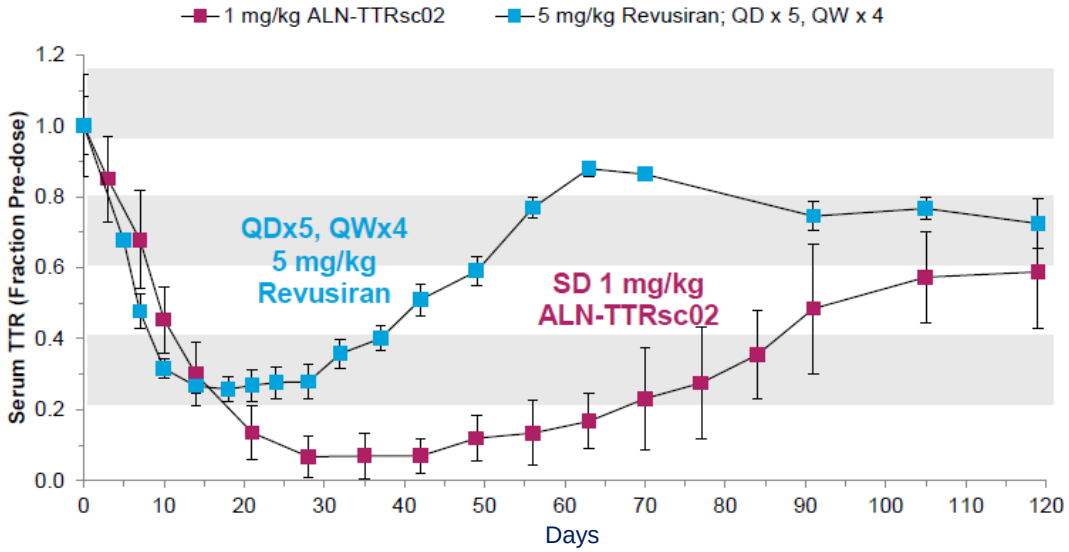
Conflicts of Interest

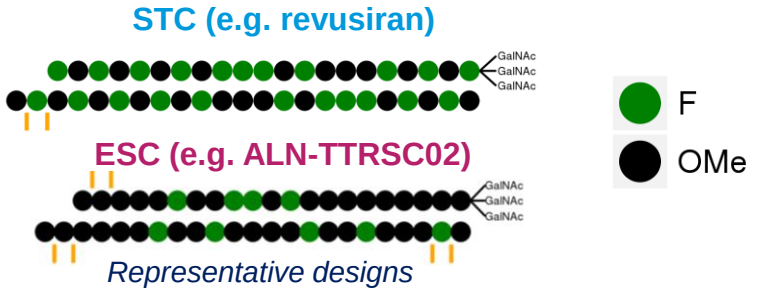
I am an employee of Alnylam Pharmaceuticals.

Evolution of GalNAc-siRNA Conjugate Design

From Standard Template Chemistry (STC) to Enhanced Stabilization Chemistry (ESC)

NHP Activity: ALN-TTRSC02 compared to Revusiran





Representative designs

	Total Dose	AUEC for % TTR lowering
Revusiran	45 mg/kg	4154
ALN-TTRsc02	1 mg/kg	8139

Adjusting for dose difference (45-fold) and AUEC (1.95-fold), ALN-TTRSC02 shows **~88-fold** *in vivo* potency improvement over Revusiran

AUEC= Area under effect curve

280-fold reduction in the annual projected human dose of ALN-TTRSC02 (25 mg Q3M) in Ph3 over Revusiran (500 mg QDx5, QW)

Nair et al. *NAR* **2017**
Foster et al. *Mol. Ther.* **2018**

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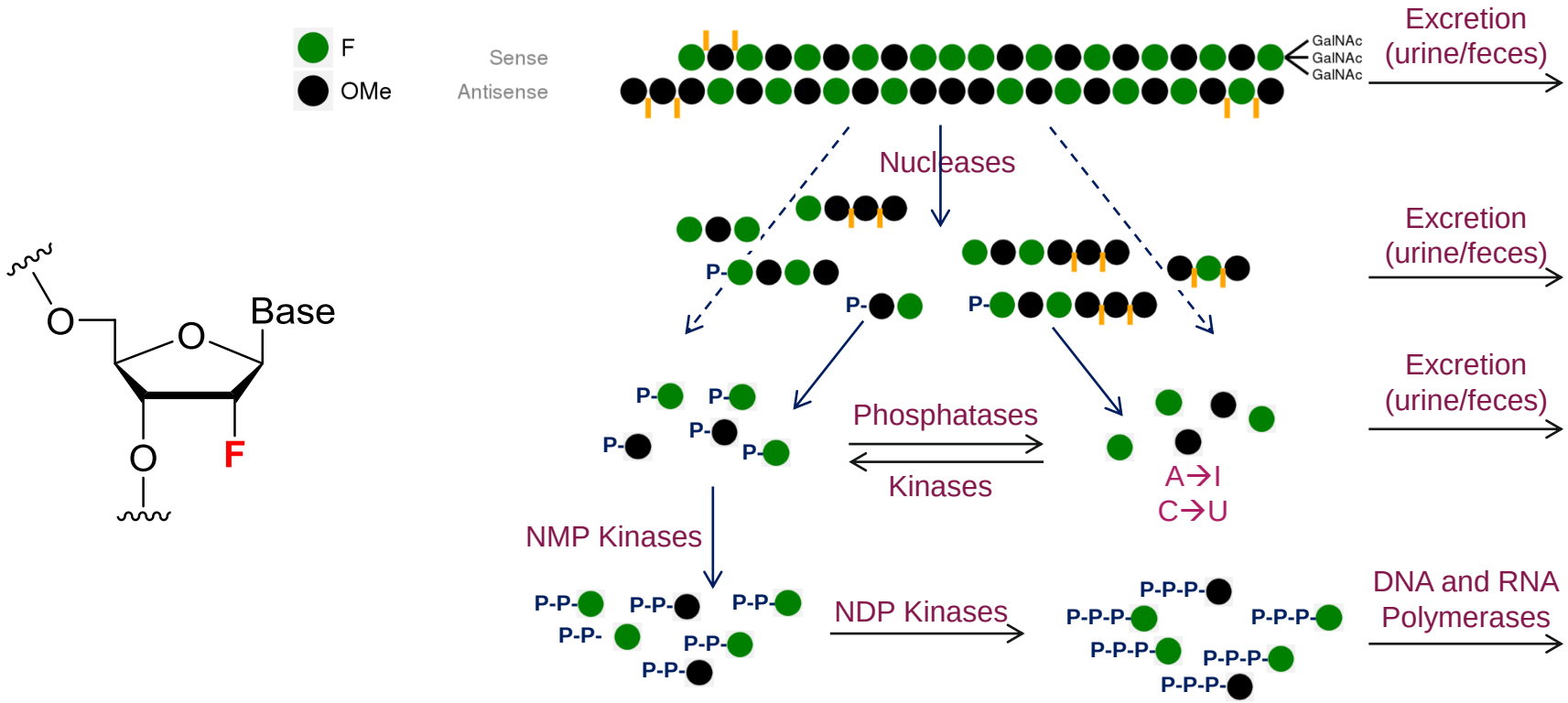
Fate of Modified Nucleosides/Nucleotides after Nucleolytic Degradation of GalNAc-siRNA



Mitochondrial polymerases (Pol-γ and POLRMT) are more sensitive to modified NTPs than nuclear polymerases because of:

(1) Poor selectivity and poor exonuclease activity

(2) Continuous replication of mitochondrial DNA, including in post-mitotic cells

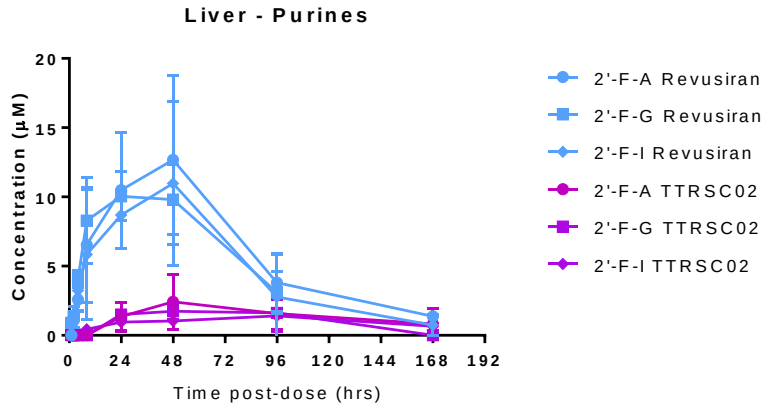
2'-F-Monomer Safety Evaluation Summary

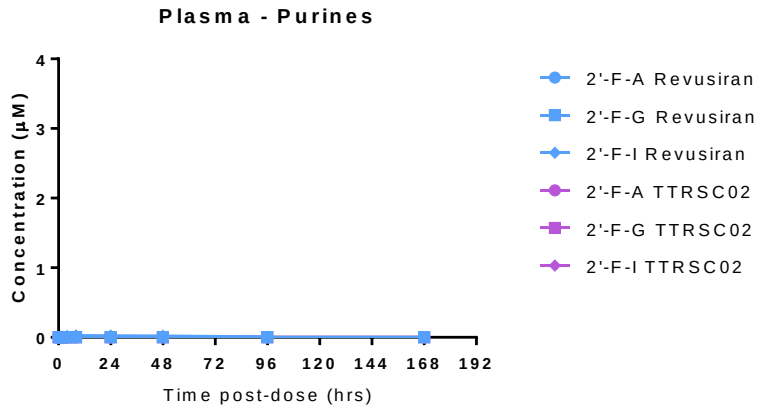
Monomer generation from STC siRNA in rat and human	**Low**
Monomer generation from ESC siRNA in rat and human	**Mostly undetectable**
Polymerase inhibition (Pol-γ, Pol-α, Pol-β, POLRMT)	**Not expected***
Polymerase substrate (Pol-γ, POLRMT)	**Poor**
Obligate chain termination	**No**
Non-obligate chain termination	**No**
Cytotoxicity and mtDNA effects *in vitro*	**In a subset of cell lines at concentrations > 16-fold higher than generated *in vivo* from STC siRNA**
2-year rat carcinogenicity study with STC siRNAs	**No effects on survival or tumor incidence; no apparent effects on mitochondrial function**

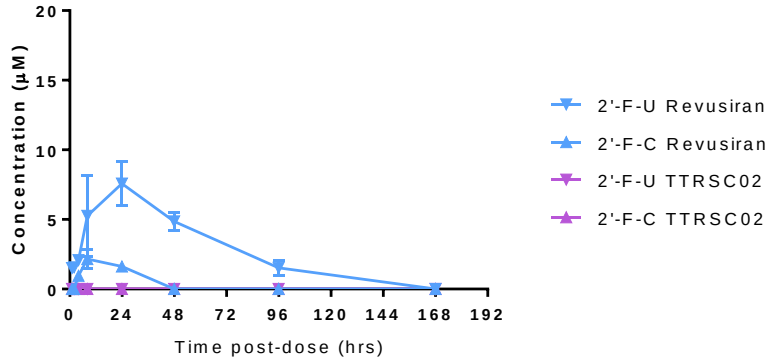
* 2'-F-ITP pending

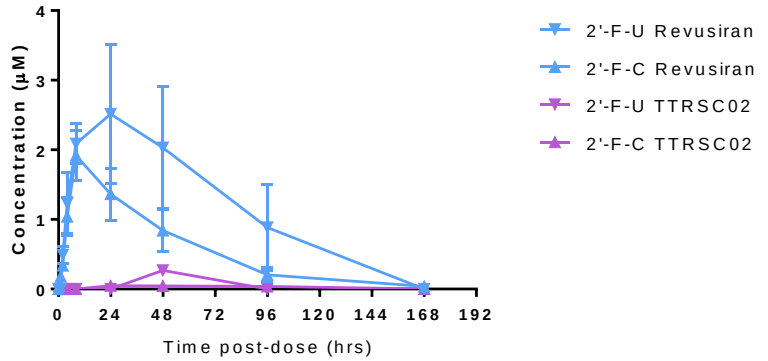
Monomer Generation Is Minimized with the ESC Design In Vivo

Rat, 30 mg/kg Single Subcutaneous Dose of Revusiran or ALN-TTRSC02





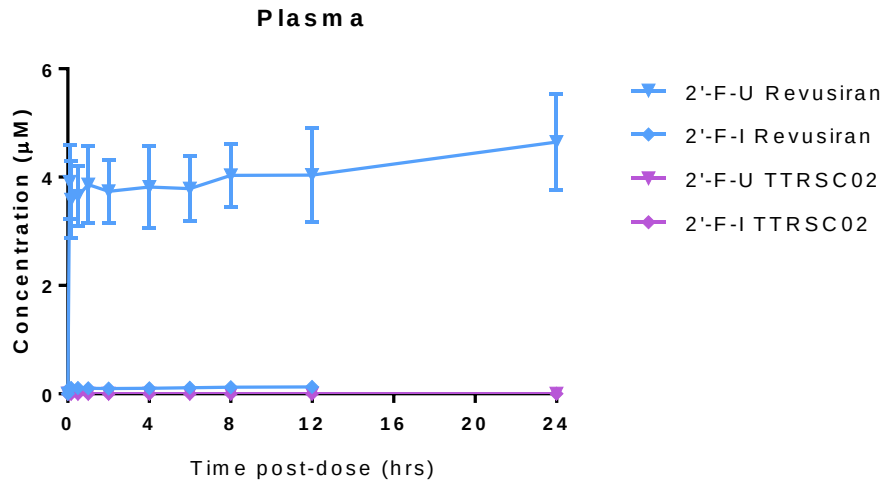


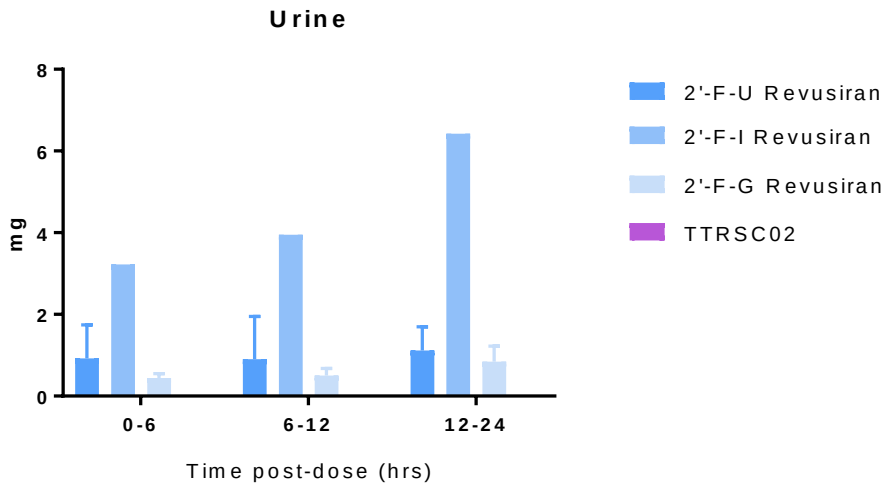


- 2'-F-monomer half-life of 1-2 days indicates that no accumulation is expected with weekly or less frequent dosing
- Only 2'-F-pyrimidines appear to re-distribute systemically after generation in the liver and kidney

2'-F-Monomers Are Not Detectable in Human Plasma or Urine at Therapeutically-Relevant Doses of ALN-TTRSC02

Revusiran: 7.5 mg/kg qd x 5 to healthy volunteers
ALN-TTRSC02: 50 mg (~0.83 mg/kg) single dose to healthy volunteers





- 2'-F-monomers reached steady state in plasma and urine by Day 4, indicating a half-life of ~ 1 day and therefore no accumulation with weekly or less frequent dosing
- Up to ~15 % of total monomer dose is excreted in urine, and therefore even revusiran is likely mainly excreted in oligomeric forms

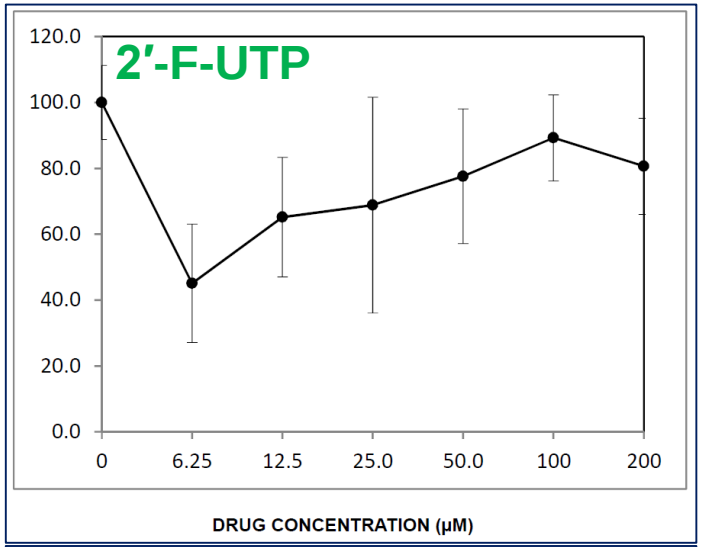
2′-F-NTPs Are Not Polymerase Inhibitors, in Contrast to FIAU-TP

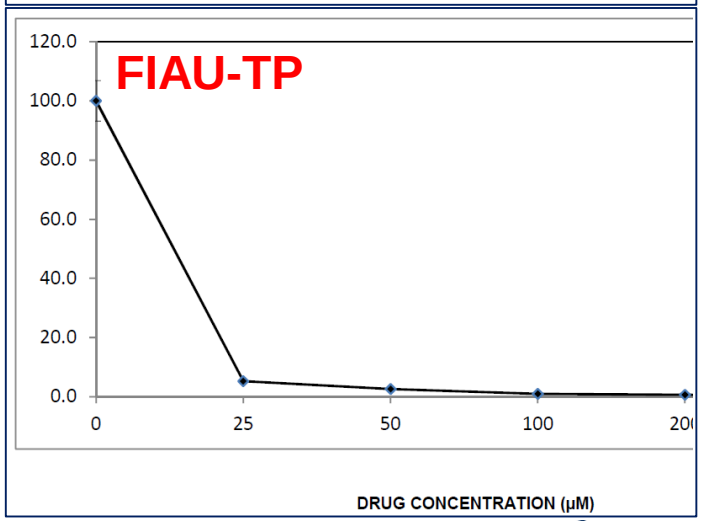
```
Template AGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGAC
                                 ||||||||||||||||||
Primer      <-------------------- ACCGCGGGCTTGTCCCTG
```

1. Polymerase of interest
   dATP, dCTP, dGTP, dTTP + ^{3}H-dTTP spike
   NTP of interest
2. Incubate @ 37 °C,1 hour
3. Precipitate DNA, wash, and count

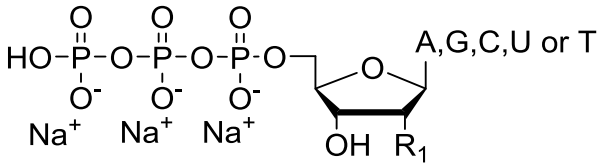
Test Article	Pol-α		Pol-β		Pol-γ	
	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)	IC_{50} (μM)	IC_{90} (μM)
2′-F-ATP	> 200	> 200	175	> 200	> 200	> 200
2′-F-CTP	> 200	> 200	> 200	> 200	> 200	> 200
2′-F-GTP	> 200	> 200	> 200	> 200	> 200	> 200
2′-F-UTP	> 200	> 200	91.6	> 200	> 200	> 200
2′-F-ITP	Pending		Pending		Pending	
FIAU-TP	0.27	13.7	0.37	5.56	1.04	13.4

Pol-γ

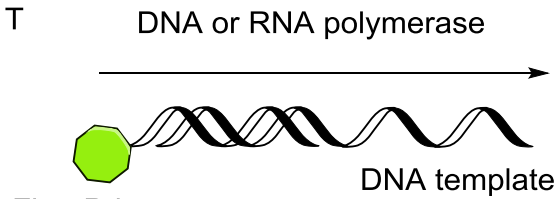




2′-F-NTPs Are Poor Substrates for Human Polymerases, in Contrast to FIAU-TP



natural or modified NTP



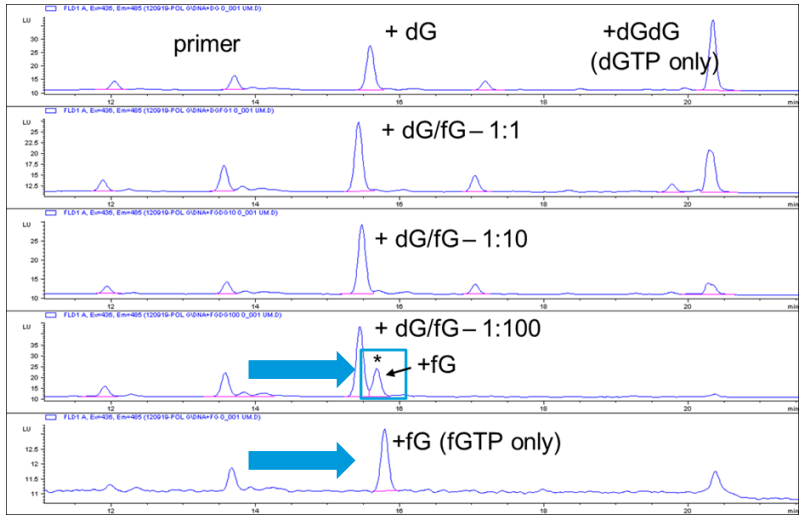
Fluo-Primer
DNA or RNA

N+1 Fluo-Primer
DNA or RNA

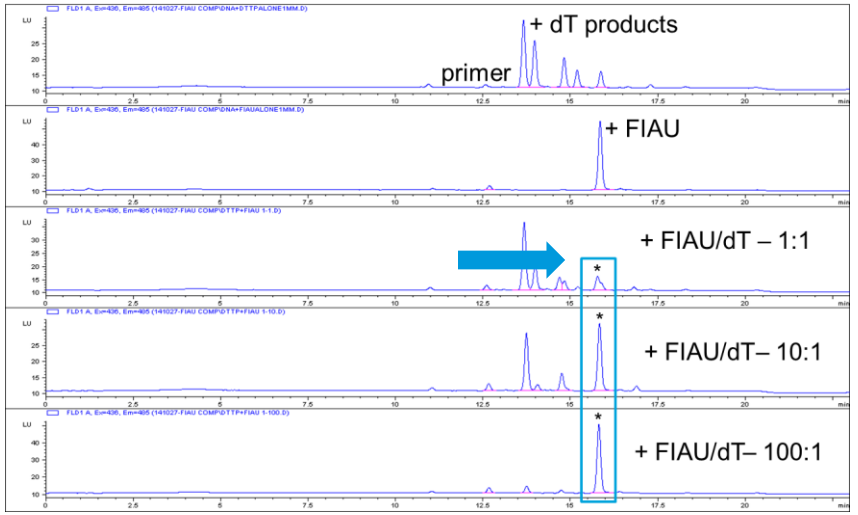
R_1 = H, OH, F

2′-F-NTPs: Incorporation at 10-100x excess to endogenous NTPs

Pol-γ

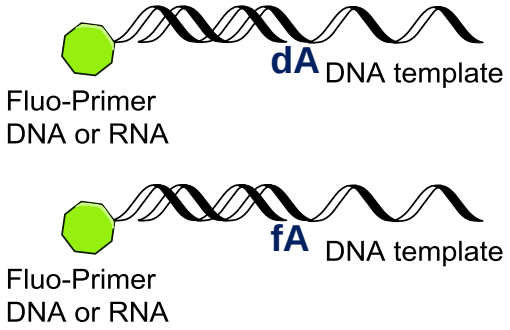


FIAU-TP: Incorporation at 1:1 ratio to endogenous NTPs

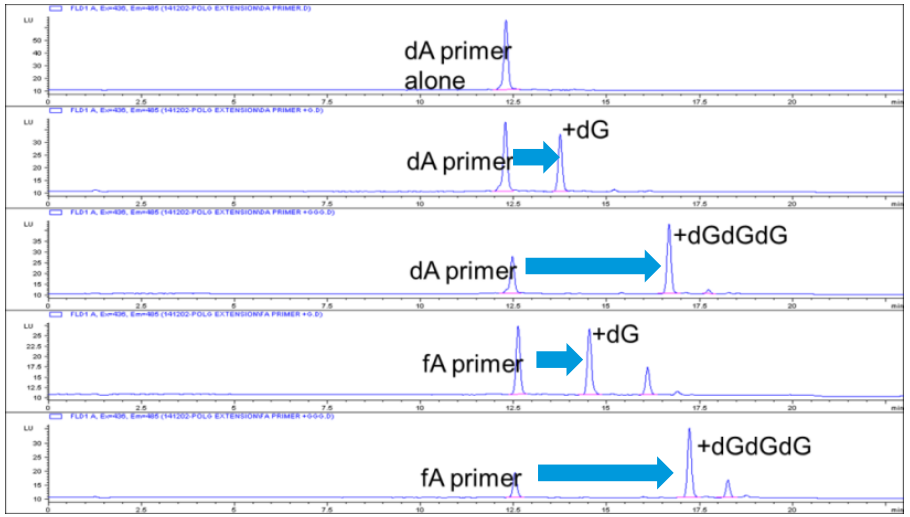


2′-F-Monomers Are Neither Chain Terminators Nor Non-Obligate Chain Terminators, in Contrast to FIAU-TP

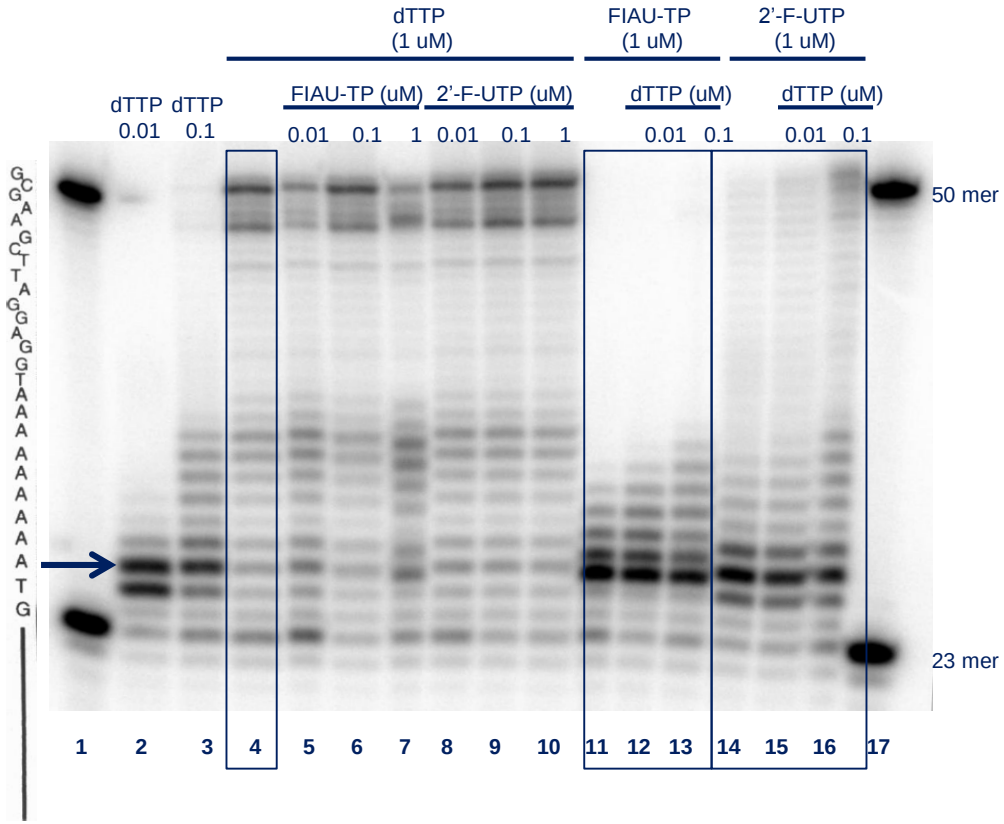
Pol-γ primer extension is unaffected by synthetic incorporation of 2′-F-monomers



Pol-γ



No evidence of Pol-γ non-obligate chain termination with 2′-F-UTP

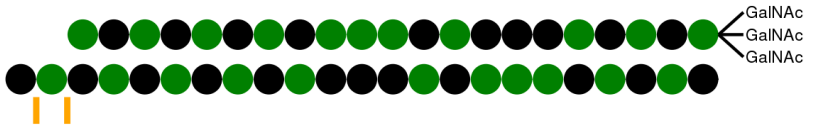


Lewis et al. Proc Natl Acad Sci U S A. 1996 Apr 16;93(8):3592

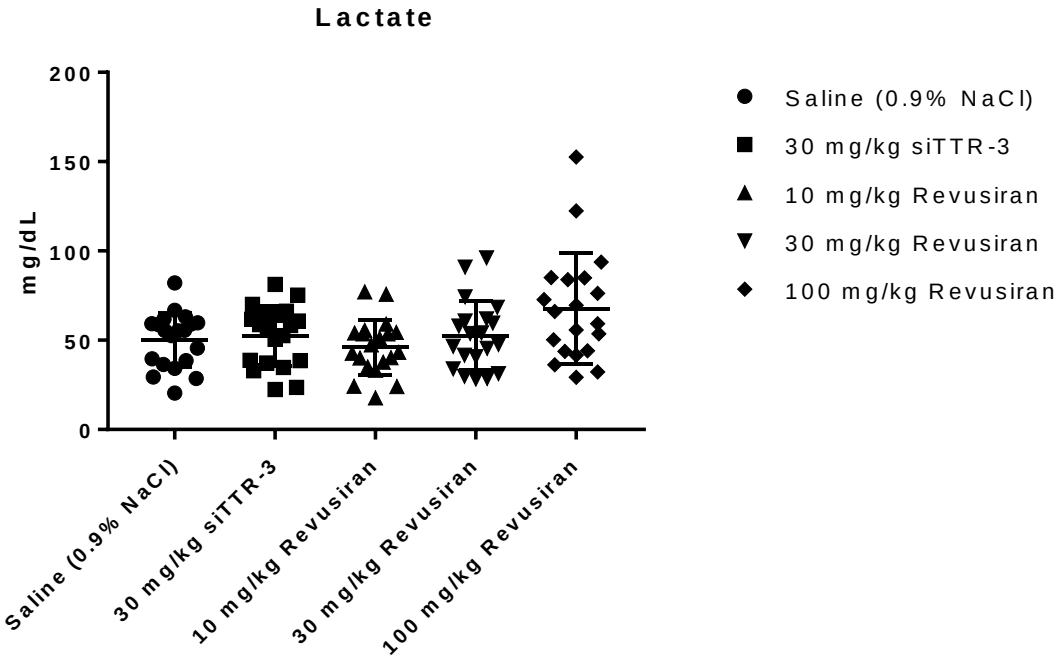
No Apparent Functional Impact on Mitochondria in a Two-Year Rat Carcinogenicity Study with Two STC GalNAc-siRNAs

10, 30, 100 mg/kg QW subcutaneous dose of Revusiran

30 mg/kg QW subcutaneous dose of rodent surrogate siTTR-3



- No effects on tumor incidence
- No effects on overall survival
- No statistically-significant plasma lactate elevations
- Increased incidence of elongated and ring-shaped/cup-shaped mitochondria in liver and skeletal muscle but not in heart or nerve, which was considered a non-adverse adaptive response to limited nutrient availability*
- No effects on mitochondrial cristae morphology, no mitophagy, and no mitochondrial degeneration/necrosis in all the tissues examined by TEM (liver, skeletal muscle, heart, sural nerve, dorsal root ganglia)

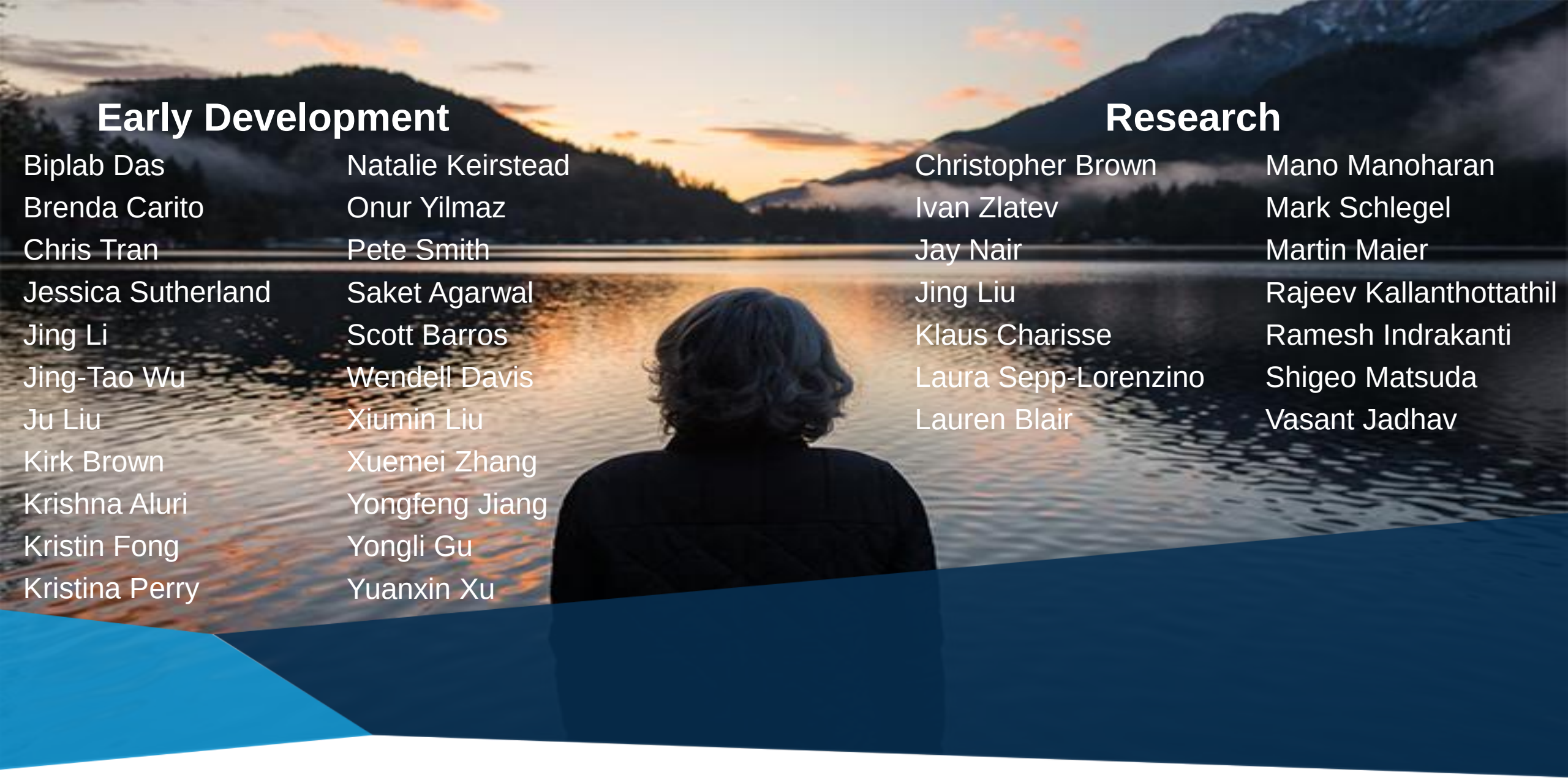


*Gomes et al. Biochim Biophys Acta 1833, 205-212 (2013)

Conclusions

The overall risk that 2′-F-monomer metabolites of GalNAc-siRNAs mediate mitochondrial toxicity or other toxic side effects is very low

- 2′-F-monomer generation is minimized with ESC designs with increased metabolic stability and substantially lower dose required for therapeutic activity
- Unlike known toxic nucleoside analogs (e.g. FIAU), 2′-F-NTPs of monomer metabolites of GalNAc-siRNAs are poor polymerase substrates and are unlikely to cause polymerase inhibition
  - In addition, no chain termination nor non-obligate chain termination was observed
- No apparent impact observed on mitochondrial function with chronic dosing of STC conjugates in a two-year rat study (up to 97 weekly doses of 100 mg/kg)



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