【S-14】

Pharmacokinetics and Toxicity of Oligonucleotide Therapeutics: Similarities and Differences with Other Biotherapeutic Agents

Arthur A. Levin, Tae-won Kim, Richard Geary, Rosie Yu, and Scott Henry

Isis Pharmaceuticals, Carlsbad, CA 92008 USA

Antisense oligonucleotide drugs (ASO) are being tested in clinical studies for a wide variety of diseases like inflammation, cancer, infections, diabetes and cardiovascular disease. The theory behind antisense activity is remarkably simple: create a short oligonucleotide that is complementary to the mRNA of a target gene, so that it can bind to a target mRNA and prevent the translation of that message in to protein, reducing the expression of that protein. Converting the simple theory into drugs is the topic of this presentation. ASOs are generally 20 nucleotides in length, polyanionic, and water soluble. These properties are similar from sequence to sequence so that many of the pharmacokinetic and toxicologic properties are similar from sequence to sequence independent of the target mRNA or intended medical application. Recent advances in the pharmacology, pharmacokinetics, and toxicology demonstrate the potential promise of this technology.

Pharmacokinetics and Toxicology of Oligonucleotide Therapeutics: Similarities and Differences with Other Biotherapeutic Agents

Arthur A. Levin, Tae-won Kim, Richard Geary, Rosie Yu, and Scott Henry Isis Pharmaceuticals,

Carlsbad, CA 92008 USA

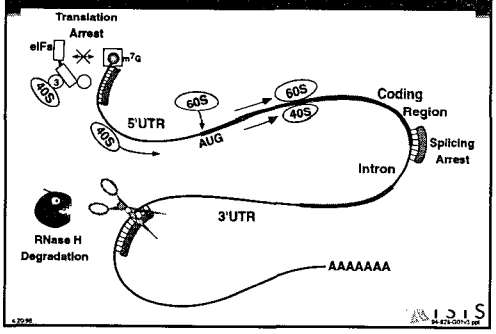
alevin@isisph.com

Antisense Pharmacology

Receptor	mRNA
Drugs	Oligonucleotides
Receptor Binding Motif	Watson Crick
Post Receptor Binding Events	Degradation

ISIS

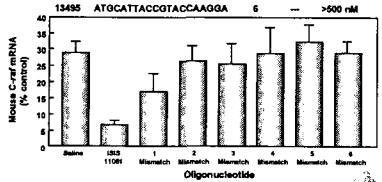
Antisense Mechanism of Action



ISIS

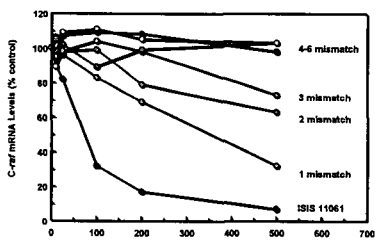
Mismatches in C-*Raf* Kinase Antisense Oligonucleotides Reduce Potency

ISIS #	Sequence	Mismatches	T_m	mRNA (IC_{50})
11061	ATGCATTCTGCCCCCAAGGA	0	60.5	75 nM
13490	ATGCATTCTCCCCCCAAGGA	1	45.8	300 nM
13491	ATGCATTCTCGCCCCCAAGGA	2	42.2	>500 nM
13492	ATGCATTCCCGCCCCCAAGGA	3	33.8	>500 nM
13493	ATGCATTCCCGTCCCCAAGGA	4	25.0	>500 nM
13494	ATGCATTACCGTCCCAAGGA	5	—	>500 nM
13495	ATGCATTACCGTACCAAGGA	6	—	>500 nM

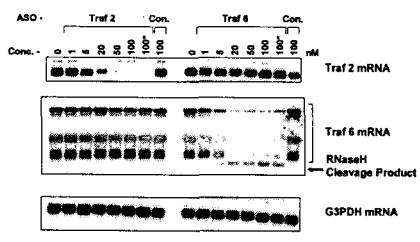


ISIS

Antisense Inhibition of C-*raf* mRNA Levels in Mouse bEND Cells - *Mismatch Series* -

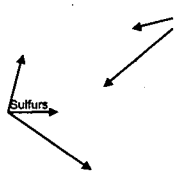


ISIS



ISIS

Modifications are Made to a Non-Bridging Oxygen and at the 2' Site of the Ribose



- Greater potency due to enhanced affinity for target RNA.
- Dramatic increase in stability permits relaxed dosing regimens (once a week or less frequent).
- Decreased potential for acute toxicities.
- Does Not Support Enzymatic Degradation of mRNA

ISIS

Structure of Typical ASO

ISIS 301012

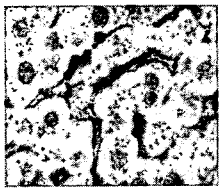
5'-GCCTC agtctgcttc GCACC-3'

ISIS

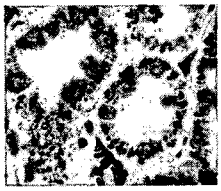
Do Oligos Get into Cells?
Immunostaining

(20 mg/kg at 2 hr)

Liver

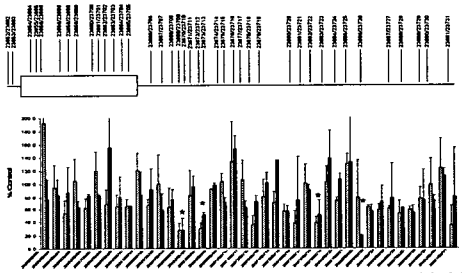


Kidney



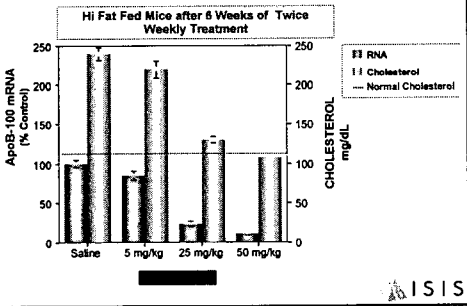
ISIS

Not Every Antisense Oligonucleotide is Active

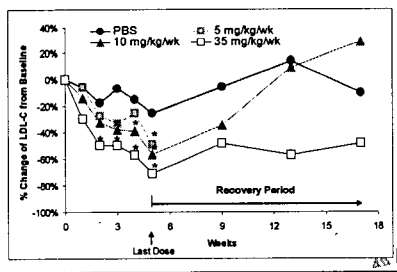


ISIS

Dose-Dependent Reduction in Total Cholesterol to Normal levels

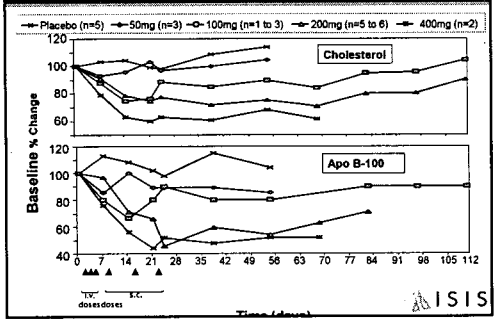


ISIS



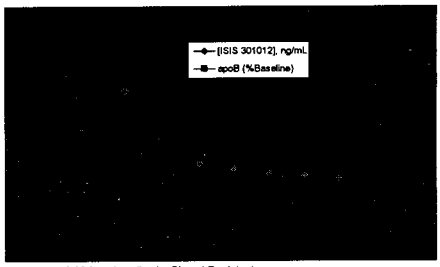
ISIS

ISIS 301012 Effect on Cholesterol Levels and ApoB-100 in Normal Volunteers



ISIS

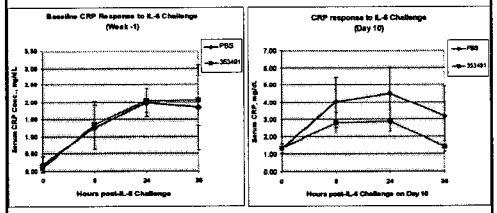
Trough Plasma Conc Correlates with Reductions in apoB-100 in Human Subjects (200 mg, n= 8)



* p< 0.02 from baseline by Signed Rank test.
Error bar represents standard error.

ISIS

Antisense to C-reactive protein Reduces the Response to IL-6 Challenge in Monkeys (Data from KIT Study # IN05019.



Prior to dosing with ASO

After 4 doses of ISIS 353491

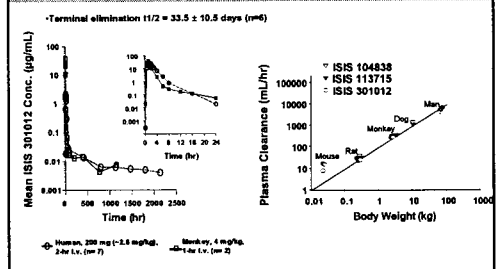
Key Points in the Pharmacokinetics of Oligonucleotides

- Oligonucleotides are polyanionic with a molecular wt of ~6kDa
- Rapid polyphasic elimination from plasma distribution half-life approximately 1 hr
  - distribution complete by 24 hr
- Bound to plasma proteins (>95% in man)
  - binds to hydrophilic sites on albumin
- Little urinary or fecal excretion of intact drug
- Plasma cleared by uptake into tissues
- Tissue cleared by nuclease-mediated metabolism
  - Not P450 mediated
  - Tissue elimination half-life ranges from 10-30 days
  - Urinary excretion of shortened metabolites
- Complete SC bioavailability by 48 hr

Confidential

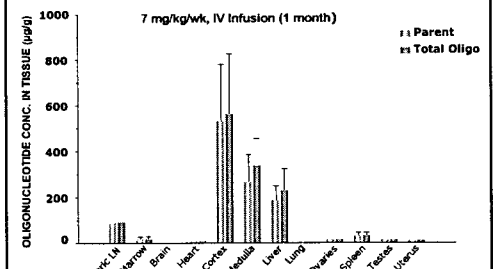
ISIS

Similarities in Pharmacokinetics Across Species



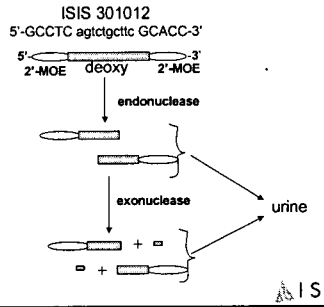
ISIS

Tissue Distribution in Monkeys- 48 hr after last dose



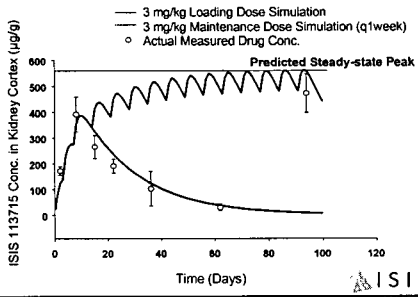
ISIS

Gapmers are Metabolized by Endonucleases and then Exonucleases



ISIS

Repeated Dose Accumulation Is Predictive and Not Progressive



ISIS

Characterization of Toxicity

Toxicity of ASOs

- Class- and chemistry-related toxicity predominate: tox profile often predictable from sequence to sequence
- Species specific toxicities identified
- Target organs for toxicity have been identified
- Numerous potential toxicities – not observed
- Experience across oligonucleotides in animals and man gives indication of clinical relevance of tox
  - 4,000 patients dosed:
- Similarities among compounds combined with clinical experience with other ASOs provides a basis for relating Tox and Clinical data

ISIS

Scope of Toxicity Studies with ASOs

- IND Toxicology Studies for 8 PS ODN and 6 MOE ASO
  - Approximately 70 GLP Studies in monkeys
  - Toxicity testing typically done in mice and monkeys
  - Doses up to 100 mg/kg/wk in mice and 80 mg/kg/wk in monkeys acutely tolerated
    - High dose in subchronic studies are typically 80 mg/kg/wk in mice and 35 mg/kg/wk and monkeys
  - Every 4 to 7 days dosing by IV infusion or SC injection
  - Duration of 6 and 9 months in mice and monkeys
  - Single Carcinogenicity study with a 1st generation drug
  - Reprotox and Gentox batteries negative
  - Both human and species-specific oligos used in Tox Studies when scientifically justified

ISIS

Class-Related Effects

- Mice
  - Pro-inflammatory effects-lymphoid proliferation
  - Lymphohistiocytic infiltrates in various tissues
  - Slight increase in AST and ALT at ≥25 mg/kg
  - Endosomes accumulate oligo in basophilic granules in Kupffer and proximal convoluted tubules at ≥12.5 mg/kg
- Monkey
  - Pro-inflammatory effects not prominent
  - Complement activation at doses > 20 mg/kg
  - Transient ↑ in APTT at high doses during the first 4hrs
  - Minimal degeneration in proximal tubular cells after 3-month treatment in monkeys treated with doses~10 mg/kg/wk
  - Basophilic granules in Kupffer cells and in proximal convoluted tubules
  - Minimal SC injection site reaction

ISIS

Safety Issues Excluded

- Antibody formulation
- Bone marrow
- Skeletal muscle
- CNS
- Mitochondrial
- Cardiac
- Gastrointestinal
- Cytochrome P450 interactions
- Respiratory
- Hepatic
- Genotoxicity
- Teratogenicity

ISIS

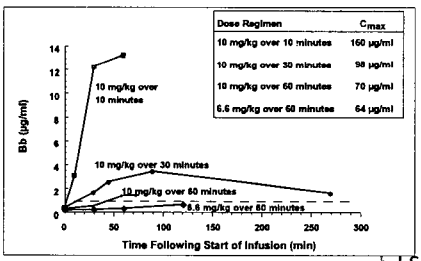
Complement Activation - Preclinical (Monkey)

- Activation of alternative pathway of complement
  - ◆Increases in complement split products C3a, C5a, and Bb observed following rapid intravenous dosing of monkeys
  - ◆Severe hypotension & cardiovascular collapse in some monkeys
- Threshold effect related to peak plasma concentration
- Activation independent of ASO sequence
- Mechanism is related to inhibition of negative regulators of alternative pathway for complement cascade
- Monkey more sensitive than man

Ref: Henry et al. (1997). JPET 281: 810
Henry et al. (2002). Int J Immunopharm 2: 1657

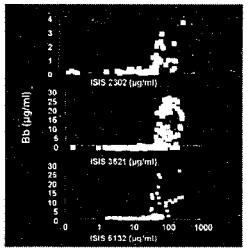
ISIS

In Monkey: Complement Pathway Activation Related to C_{max} and Modulated by Rate of Infusion



ISIS

Complement Activation in Monkey is a Threshold Effect Independent of ASO Sequence



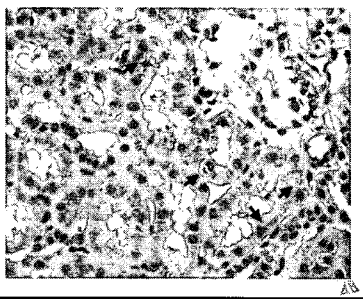
ISIS

Sequence-Independent Effects: Renal Granules

- Oligos taken up by proximal tubular epithelium at the brush border
  - ◆ Processed into phagolysomes: normal phagocytic function
  - ◆ Stain with hematoxylin, allowing phagolysosomes to be visualized
  - ◆ Generally independent of sequence
  - ◆ Independent of species
  - ◆ Dependent on concentration
  - ◆ Reversible
- Not considered toxicologically significant
  - ◆ Evidence of oligonucleotide uptake
  - ◆ Also present in Kupffer cells and other tissue macrophages

ISIS

Oligonucleotides Are Taken up by Lysosomes and Appear as Basophilic Granules in Proximal Tubules



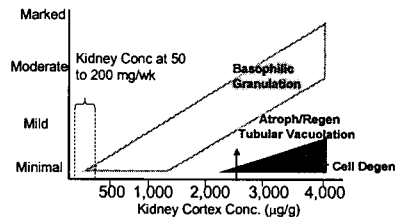
ISIS

Granules

- Granules are phagolysosomes filled with oligo that stain with hematoxylin
- Oligos are small, water-soluble molecules that may not be well fixed by formalin
- During fixation, osmotic imbalances result in extraction of oligo and appearance of vacuoles
- Uptake of oligo and therefore appearance of granules and vacuoles is dose-related
- May be artifact of fixation or exacerbated by fixation process

ISIS

Monkey Kidney Cortex Concentration & Histologic Changes in Proximal Tubules



doses of 20 mg/kg/wk produce up to 3,500 to 4,500 µg/g cortex conc.

ISIS

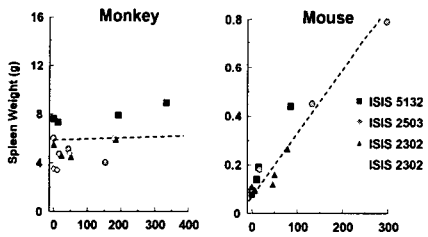
Effects on Renal Functional Assessments

- Monkeys loaded and then treated with 40 mg/kg/wk x 4
  - Normal BUN, creatinine
  - Normal urine protein, glucose, and electrolytes
  - Normal GFR and renal blood flow
  - Normal glucose reabsorption after glucose challenge
  - No increases in urinary NAG and α-GST
  - Concentrating ability maintained after water deprivation

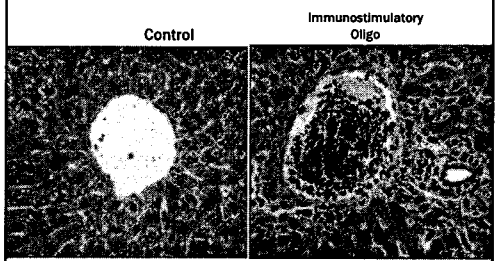
ISIS

Relevance of Animal Models

Correlation Between Oligonucleotide Exposure and Spleen Weight Effects in Mice and Monkeys

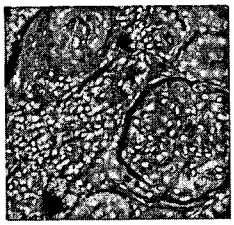


ISIS

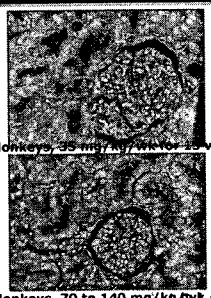


ISIS

No Infiltrates in Monkey Tissues



Rats, 30 mg/kg/wk for 2-3 wk
Kidney Conc. = 1800µg/g



Monkeys, 70 to 140 mg/kg/wk for 5 wk

ISIS

Secondary Effects of Pro-inflammatory Changes in Rodents

- Thrombocytopenia in rodents- correlates with splenomegaly
- ALT/AST increases- exacerbated by pro-inflammatory effects
- Premature delivery/abortion
- Chronic proliferative pressure and potential for tumor promotion

Conclusion: Because of their sensitivity to proinflammatory effects, rodents may display nonrelevant responses to ASOs and thus over predict toxicities.

ISIS

ASO Class Toxicities

Toxicity	Mechanism	Dose in Man
Increased APTT	Inhibition of tenase complex	Peak plasma concentration related – self-limited
Pro-inflammatory effects	Release of cytokines via activation of toll-like and other receptors	>18mg/kg with first generation ASOs - Not observed with second generation ASOs
Complement activation (in monkeys)	Inhibition (or activation) of complement inhibitors	Not observed in man (monkey only)
Renal tubular degeneration	Concentration in kidneys – mechanisms not known	No renal effects observed in man to date
Injection site reactions to SQ administration	Local point inflammatory effects	Dose dependent, but less with second generation ASOs

ISIS

Antisense Oligonucleotides versus Monoclonal Abs

Antisense	Monoclonals
Can target both intra- and extra cellular proteins	Can only target extracellular proteins
Long half-life in tissues	Long half-life in plasma
Synthesized chemically	Fermented
Non-antigenic	Antigenic
Injection, infusion and oral	Injection or infusion only
Tox studies predictable from sequence to sequence	Tox studies predictable from mAb to mAb
Species specific drugs used in tox studies (surrogates)	Occasionally surrogate mAbs are used in tox studies

ISIS

Conclusions

- Ground work has been performed to make antisense compounds drugs
- Pharmacology is sequence, dose and time dependent
- PK and Tox have been well characterized
- There are species-specific toxicities and most toxicities are related to the chemical class not the sequence

ISIS