Anticoagulant Therapy

Oral

1916
Vitamin K antagonists
Clinical use of warfarin

1936
1940s
1950s
1976
1980s
1990s
2000s
2009
2010s
Direct thrombin inhibitors
Direct factor Xa inhibitors

Parenteral

1916
Heparin Discovered

1936
Clinical use of heparin

1940s
LMWH Discovered

1950s
LMWH Clinical Trials

2000s
Direct thrombin inhibitors

2009
Indirect factor Xa inhibitors
Anticoagulants Commonly Used Today

- Unfractionated Heparin
- Low Molecular Weight Heparin
- Fondaparinux
- Warfarin

Diagram shows the interactions and pathways of different anticoagulants.
What are aptamers?

- Synthetic ssDNA or RNA molecules.
- They bind with high affinity and specificity to their target protein ($K_D$ in the nM to pM range).
- They are similar to monoclonal antibodies.
- They form an elaborate three dimensional structure.
Aptamers vs. Monoclonal Antibodies

- *In vitro selection*
- Target range (i.e. toxins and other molecules that do not elicit immune responses)
- Low molecular weight mass and structural flexibility
- Low immunogenic potential
- Produced by chemical or enzymatic reactions
Examples of RNA aptamers in clinical trials

- Macugen (age related macular degeneration/diabetic macular edema/proliferative diabetic retinopathy)
- E10030 and ARC1905 (Neovascular age related macular degeneration-awaiting Phase III)
- RB006 (Coronary artery disease-awaiting Phase II)
- ARC19499 (Hemophilia-Phase I/II)
- AS1411 (Renal cell carcinoma/non-small cell lung cancer – awaiting Phase III)
REG1 Anticoagulation System

![Diagram showing the interaction between RB006 and RB007 forming a complex]

**Active**: 5’P-L-G-C-idT 3’

**Inactive**: 3’c-G-L-P 5’

The diagram illustrates the formation of a complex between RB006 and RB007, leading to a change in conformation and function.
Develop RNA aptamers to Antithrombin III
SELEX (Systematic Evolution of Ligands by Exponential Enrichment)
AT specific RNA aptamer (AT 7-4.16)

Kd=250 nM
## Effect of RNA Aptamer 7-4.16 to Accelerate AT-Protease Inhibition

<table>
<thead>
<tr>
<th>Reaction Condition</th>
<th>Aptamer 7-4.16 or heparin (nM)</th>
<th>$K_2 \times 10^5$ (M$^{-1}$ s$^{-1}$)</th>
<th>Fold-acceleration</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT+ FXa + Aptamer 7-4.16</td>
<td>50</td>
<td>4.2</td>
<td>83</td>
</tr>
<tr>
<td>AT+ FXa + Aptamer 7-4.16</td>
<td>500</td>
<td>25</td>
<td>510</td>
</tr>
<tr>
<td>AT+ FXa + Heparin</td>
<td>40</td>
<td>20</td>
<td>400</td>
</tr>
<tr>
<td>AT+ thrombin + Aptamer 7-4.16</td>
<td>500</td>
<td>0.11</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Unfractionated heparin

1. AT
2. AT
3. AT
4. AT

Low-molecular-weight heparin

1. AT
2. AT
3. AT
4. AT

Fondaparinux

1. AT
2. AT
3. AT
4. AT

Source: Am J Health-Syst Pharm © 2002 American Society of Health-System Pharmacists
Competition Assay
FeCl₃-induced Saphenous Vein Thrombosis in Wild Type C57B6 Mice*

*Injury: 10% FeCl₃ on 2 x 5 mm filter paper for 2 min, mice treated with saline, unfractionated heparin (300 U/Kg) or RNA aptamer 7-4.16 (3 and 6 pmol/g)*
Oligonucleotides to AT Specific RNA Aptamer

Table 2: Synthesized Complimentary Oligonucleotides to Aptamer 7-4.16

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ap7-4.16:</td>
<td>AAGAAGGCCGAUAGAAGGCCGAUGCCUCUGCGAAUCGGGAGCGGC</td>
</tr>
<tr>
<td>ATanti-1:</td>
<td>CCGCTATCTTCCGC---------------------------------</td>
</tr>
<tr>
<td>ATanti-2:</td>
<td>CCGCTACGCGACGCTT-------------------------------</td>
</tr>
<tr>
<td>ATanti-3:</td>
<td>ACGCTTAGCCCTCGCCCG---------------------------</td>
</tr>
<tr>
<td>ATanti-4:</td>
<td>CTCGCCGCTAT-----------------------------------</td>
</tr>
</tbody>
</table>
## Effect of RNA Aptamer 7-4.16 to Accelerate AT-Protease Inhibition

<table>
<thead>
<tr>
<th>Reaction Condition</th>
<th>Aptamer 7-4.16 or heparin (nM)</th>
<th>$K_2 \times 10^5$ (M$^{-1}$ s$^{-1}$)</th>
<th>Fold-acceleration</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT+ FXa + Aptamer 7-4.16</td>
<td>50</td>
<td>4.2</td>
<td>83</td>
</tr>
<tr>
<td>AT+ FXa + Aptamer 7-4.16</td>
<td>500</td>
<td>25</td>
<td>510</td>
</tr>
<tr>
<td>AT+ FXa + Heparin</td>
<td>40</td>
<td>20</td>
<td>400</td>
</tr>
<tr>
<td>AT+ FXa + Aptamer 7-4.16 + Antidote oligo</td>
<td>500</td>
<td>8.0</td>
<td>300</td>
</tr>
</tbody>
</table>
FeCl₃-induced Saphenous Vein Thrombosis in Wild Type C57B6 Mice*

*This study was supported by the National Institutes of Health (NIH) grant number 1R01AR062585-01A1.
Conclusions

- AT RNA aptamer 7-4.16 accelerates the AT-factor Xa inhibition reaction in vitro and in preliminary studies, promotes an anticoagulant response in vivo using a vascular injury model.
- AT RNA aptamer 7-4.16 does mimic the action of heparin in terms of accelerating the AT-Xa inhibition reaction, but it has no effect to accelerate the AT-thrombin inhibition reaction.
- AT RNA aptamer 7-4.16 may not bind at the heparin-binding site, could it bind at the AT-Xa-specific exosite?
- AT RNA aptamer 7-4.16 binds relatively poorly to AT, and newer RNA aptamers based on this format are needed.
- Antidote oligonucleotide is able to partly reverse the effect of AT RNA aptamer 7-4.16
Acknowledgements

- Frank Church, PH.D. (UNC-Chapel Hill)
- Herbert Whinna, MD PH.D. (UNC-Chapel Hill)
- Katie Hsia BS
- Stephanie Brandal MS