Aptamers and SELEX: The Past, Present and Future

Paloma H Giangrande, PhD
University of Iowa
Aptamers

• Short, single-stranded DNA or RNA molecules

• Discovered in 1990
  • Tuerk and Gold – SELEX*
  • Robertson and Joyce – SELEX
  • Ellington and Szostak – coined the term aptamer

• Aptamer: *aptus* = to fit (*Latin*) + *meros* = part (*Greek*)
Advantages of aptamers as therapeutics

‘Chemical antibodies’
‘Nucleic acid antibodies’

• High affinity & specificity
• Very small – good tissue penetration
• Easily chemically modifiable for:
  increased stability
  reduced toxicity
  combination therapy
• Easily manufactured (<60 nt)
• Easy scale-up
• Rapid in vitro discovery— SELEX

PSMA
SELEX – systematic evolution of ligands by exponential enrichment

Initial dsDNA library → evaluation → ssRNA library (2’F-NTPs) → binding → target

- Purified protein
- Whole cell
- Live animal

Regeneration

amplification

7-15 cycles

wah

elution

Unmodified 2’-Fluoro
Recent advances in SELEX technology

• Efficient partitioning and recovery
  • Negative selection
  • Specialized partitioning technologies – CE, AFM, flow cytometry, microfluidics, Biacore SPR

• Accurate amplification
  • Emulsion PCR (ePCR)
  • Droplet digital PCR (ddPCR)

• Global analysis of sequencing data
  • High throughput sequencing (HTS) technology
  • HT-SELEX

Tuesday, Session VII: Aptamers
Tom Soh (Stanford)
Rapid Identification of Cell-Specific, Internalizing RNA Aptamers with Bioinformatics Analyses of a Cell-Based Aptamer Selection

William H. Thiel¹, Thomas Bair¹, Andrew S. Peek², Xiuying Liu¹, Justin Dassie¹, Katie R. Stockdale¹, Mark A. Behlke³, Francis J. Miller, Jr.¹, Paloma H. Giangrande¹,⁴

¹ Department of Internal Medicine, University of Iowa, Iowa City, Iowa, United States of America, ² Roche Molecular Systems, San Francisco, California, United States of America, ³ Integrated DNA Technologies, Coralville, Iowa, United States of America, ⁴ Department of Radiation Oncology, University of Iowa, Iowa City, Iowa, United States of America

SELEX

- DNA Library
  - RNA Library
    - RNA
      - DNA
    - RNA
      - DNA

Illumina (HTS)

- DNA Library
  - RNA Library
    - RNA
      - DNA
    - RNA
      - DNA

Bioinformatics

- Tree Distance (Structure)
  - Edit Distance (Sequence)

Thiel WH, PLoS ONE 2012
Recent progress in aptamer-based therapeutics

• Aptamer as ANTAGONISTS Inhibitors
Targeted Inhibition of Prostate Cancer Metastases with an RNA Aptamer to Prostate-specific Membrane Antigen


![H&E images](10x, 40x)

**% mice with mets**

<table>
<thead>
<tr>
<th></th>
<th>DPBS</th>
<th>PSMA</th>
<th>Control</th>
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<tbody>
<tr>
<td>0</td>
<td>20</td>
<td>0</td>
<td>80</td>
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</table>

<table>
<thead>
<tr>
<th>mets/mouse</th>
<th>DPBS</th>
<th>PSMA</th>
<th>Control</th>
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<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>6</td>
<td>10</td>
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</tbody>
</table>

* *p<0.05*  
* *p<0.01*  
NS
Recent progress in aptamer-based therapeutics

- Aptamer as **ANTAGONISTS**
  - Inhibitors

- Aptamer as **AGONISTS**
  - Activators
Aptamer as agonists (activators)

- **T cell costimulatory receptors**
  - **4-1BB**: McNamara et al. JCI, 2008
  - **CD28**: Pastor et al. MTNA 2013
  - Cancer immunotherapy; immune-response modulators

- **CD40**
  - Antigen presenting cells (APCs); B- cell lymphoma
  - Soldevilla et al. Biomaterials, 2015
  - Monovalent aptamer = antagonist
  - Divalent aptamer = agonist
  - Aptamer-shRNA conjugate
  - Cancer immunotherapy; Bone marrow aplasia
Recent progress in aptamer-based therapeutics

- Aptamer as **ANTAGONISTS** Inhibitors
- Aptamer as **AGONISTS** Activators
- Aptamer as **DELIVERY AGENTS**
Aptamer as delivery agents

• Therapeutic oligonucleotides
  • RNAi
    • siRNAs (Giangrande, Rossi, Gilboa, Lieberman, Ellington)
    • shRNAs (Lupold, Pastor)
    • miRNAs (Giangrande, de Franciscis)
  • Aptamers (Gilboa, Pastor)
  • Antisense (Sullenger)

• Drug conjugates
  • Small molecule drugs (Farokhzad and Langer; Leong)
  • Protein drugs (Giangrande, Sullenger)
**In vivo** Tumor Targeting Specificity

NIR-PSMA (Targeting)

NIR-Control (Non-Targeting)

Tumor targeting: not due to enhanced permeability and retention (EPR) effect

*Dassie, Molecular Therapy 2014*
<table>
<thead>
<tr>
<th>Company Name</th>
<th>Focus/ Exclusive Technology</th>
<th>Featured Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfizer/Eyetech</td>
<td>Modified RNA aptamer for eye diseases</td>
<td>Approved: Macugen® (pegaptanib sodium) VEGF&lt;sub&gt;165&lt;/sub&gt;</td>
</tr>
<tr>
<td>NOXXON Pharma AG</td>
<td>Mirror-image chemistry Spiegelmer® (RNA or DNA-L-stereoisomer)-based therapeutics for cancer, inflammation, obesity or other diseases</td>
<td>Clinical pipeline: NOX-A12 (CXCL12/SDF-1); NOX-36 (CCL2); NOX-H94 (Hepcidin)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre-clinical pipeline: NOX-S93 (SIP); NOX-D21 (C&lt;sub&gt;5&lt;/sub&gt;a); NOX-G16 (Glucagon); NOX-L41 (CGRP)</td>
</tr>
<tr>
<td>Ophthotech Corp.</td>
<td>Modified RNA or DNA aptamers for eye diseases</td>
<td>Clinical pipeline: Zimura® (C&lt;sub&gt;5&lt;/sub&gt;); Fovista® (PDGF)</td>
</tr>
<tr>
<td>Archemix (acquired by Baxter in 2010)</td>
<td>Modified RNA or DNA aptamers for cardiovascular, hematology, and oncology diseases</td>
<td>Clinical pipeline: ARC1779 (von Willebrand factor); ARC19799 (TFPI)</td>
</tr>
<tr>
<td>NeXstar (merged with Gilead)</td>
<td>SELEX license/Modified RNA or DNA aptamers for transplant rejection and other immunological responses</td>
<td>Pre-clinical pipeline: G-quadruplex DNA aptamer (thrombin); HDD22 (thrombin exosite II)</td>
</tr>
</tbody>
</table>
Challenges in the development of aptamer-based therapeutics

- Nuclease degradation
  - Chemical modifications (fluoro, amino, O-methyl)
  - Modification strategies: In-SELEX (2’-position) or post-SELEX (base, 2’-position, sugar ring, phosphate group)

- Renal filtration
  - Renal glomerulus cut-off = 30 – 50 kDa
  - Aptamers diameter (6 – 30 kDa) is < 5 nm
  - Bulky groups: HMW PEG, cholesterol, proteins, liposomes, organic or inorganic nanomaterials

- Toxicity
  - Negatively charged molecules display nonspecific binding to proteins in serum
  - Chemical modifications can be a double-edged sword
Letter to the editor

Pre-existing anti–polyethylene glycol antibody linked to first-exposure allergic reactions to pegnivacogin, a PEGylated RNA aptamer

Journal of Allergy and Clinical Immunology
137: 1610–1613, 2016

Tuesday, Session VII: Aptamers
Bruce Sullenger (Duke)
Formulation as a multimer /combination
An aptamer-antibody complex (oligobody) as a novel delivery platform for targeted cancer therapies

Kyun Heo a, Sung-Won Min a, Ho Jin Sung a, Han Gyul Kim a, Hyun Jung Kim a, Yun Hee Kim a,b, Beom Kyu Choi a, Sewoon Han c, Seok Chung c, Eun Sook Lee a,b, Junho Chung d,e, In-Hoo Kim a,b,c,e

Inert antibody
# Pharmacokinetics of a Cholesterol-conjugated Aptamer Against the Hepatitis C Virus (HCV) NS5B Protein

Chang Ho Lee¹, Soo-Han Lee², Ji Hyun Kim¹, Yook-Hwan Noh³, Gyu-Jeong Noh¹,⁴ and Seong-Wook Lee¹

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## Table 1 Noncompartmental pharmacokinetic parameters of cholesterol and non-cholesterol aptamer following intravenous (IV) or intraperitoneal (IP) administration in mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1 mg·kg⁻¹ (IV)</th>
<th>10 mg·kg⁻¹ (IV)</th>
<th>100 mg·kg⁻¹ (IV)</th>
<th>100 mg·kg⁻¹ (IP)</th>
<th>100 mg·kg⁻¹ (IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₀ (hours)</td>
<td>10 ± 5.8</td>
<td>14 ± 10</td>
<td>11 ± 12</td>
<td>2.4 ± 0.62</td>
<td>5.8 ± 2.1</td>
</tr>
<tr>
<td>tₘ₅ (hours)</td>
<td>0.25 ± 0.00</td>
<td>0.25 ± 0.00</td>
<td>0.25 ± 0.00</td>
<td>0.67 ± 0.29</td>
<td>0.25 ± 0.00</td>
</tr>
<tr>
<td>Cₘ₅ (µg·ml⁻¹)</td>
<td>2.7 ± 0.60</td>
<td>46 ± 12</td>
<td>728 ± 47</td>
<td>109 ± 45</td>
<td>97 ± 8.7</td>
</tr>
<tr>
<td>AUCₘ₅ (µg·hour·ml⁻¹)</td>
<td>2.0 ± 1.1</td>
<td>13 ± 3.9</td>
<td>382 ± 40</td>
<td>160 ± 103</td>
<td>46 ± 17</td>
</tr>
<tr>
<td>AUCₘ₅ (µg·hour·ml⁻¹)</td>
<td>2.6 ± 1.8</td>
<td>13 ± 3.9</td>
<td>385 ± 39</td>
<td>161 ± 103</td>
<td>46 ± 17</td>
</tr>
<tr>
<td>AUC₂ₜ (µg·hour·ml⁻¹)</td>
<td>21 ± 8.0</td>
<td>0.57 ± 0.62</td>
<td>0.58 ± 0.72</td>
<td>0.10 ± 0.16</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>V₂ (l·kg⁻¹)</td>
<td>5.9 ± 0.89</td>
<td>17 ± 15</td>
<td>4.4 ± 4.5</td>
<td>2.9 ± 1.8</td>
<td>21 ± 14</td>
</tr>
<tr>
<td>Cl (l·hour⁻¹·kg⁻¹)</td>
<td>0.50 ± 0.27</td>
<td>0.81 ± 0.26</td>
<td>0.26 ± 0.025</td>
<td>0.78 ± 0.37</td>
<td>2.4 ± 0.86</td>
</tr>
<tr>
<td>MRTₘ₅ (hours)</td>
<td>6.2 ± 1.0</td>
<td>0.38 ± 0.05</td>
<td>0.64 ± 0.07</td>
<td>1.3 ± 0.14</td>
<td>0.53 ± 0.21</td>
</tr>
<tr>
<td>Vₛ (l·kg⁻¹)</td>
<td>5.7 ± 1.4</td>
<td>0.61 ± 0.53</td>
<td>0.25 ± 0.12</td>
<td>—</td>
<td>1.2 ± 0.01</td>
</tr>
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</table>

Data are expressed as mean ± SD (n = 3 for each administrations).
AUCₘ₅, area under the curve from administration to the last measured concentration; AUC₂ₜ, area under the curve from administration to infinity; AUC₂ₜ, area under the curve at the total area under the curve; Cₘ₅, maximal concentration; Cl, clearance volume of the plasma cleared of the aptamer per unit time; MRTₘ₅, Mean residence time to the last measured concentration; t₀, terminal half-life; tₘ₅, time at maximal concentration; V₂, volume of distribution; Vₛ, volume of distribution at steady state.

*Volume and clearance for IP administration are actually volume/F or clearance/F where F is the fraction of dose absorbed.*
The isolation of an RNA aptamer targeting to p53 protein with single amino acid mutation


School of Life Sciences and Chinese Academy of Sciences Key Laboratory of Brain Function and Disease, University of Science and Technology of China, Hefei, Anhui Province 230027, China

Edited by Jack W. Szostak, Massachusetts General Hospital, Boston, MA, and approved July 6, 2015 (received for review February 2, 2015)

**Nanoparticles**

**Intratumoral**

<table>
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<th>Tumor volume (cm³)</th>
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<td>Days post tumor inoculation</td>
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- scramble
- p53R175H-APT

**Intravenous**

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<th>Tumor volume (cm³)</th>
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- scramble
- p53R175H-APT
Pluronic gel

Smooth Muscle Cell–targeted RNA Aptamer Inhibits Neointimal Formation

William H Thiel, Carla L Esposito, David D Dickey, Justin P Dassie, Matthew E Long, Joshua Adam, Jennifer Streeter, Brandon Schickling, Maysam Takapoo, Katie S Flenker, Julia Klesney-Tait, Vittorio de Franciscis, Francis J Miller, Jr, and Paloma H Giangrande

1Department of Internal Medicine, University of Iowa, Iowa City, Iowa, USA; 2Istituto di Endocrinologia ed Oncologia Sperimentale, CNR, Naples, Italy; 3Department of Microbiology, University of Washington, Seattle, Washington, USA; 4The Veterans Affairs Medical Center, Iowa City, Iowa, USA

Carotid Injury Ligation Model

Intimal hyperplasia

Pluronic Gel + Aptamer
Diagnostics

• SOMAmer (Slow Off-rate Modified Aptamer)

• Products and Services
  • SOMAmer reagents (therapeutics, quantitative analysis, affinity purification, flow cytometry, etc.)
  • SOMApanel (smaller groups of SOMAmers for qualitative or quantitative analysis)
  • SOMASuite (professional software tool for proteomic data analysis)
  • SOMAmer discovery service (proteomics service)

http://www.somalogic.com/About-Us.aspx

Tuesday, Session VII: Aptamers

Larry Gold

bimarker analysis,
qualitative or quantitative analysis, proteomic data

http://www.somalogic.com/About-Us.aspx
Future...

- End of exclusive intellectual property for SELEX technology - limited initial distribution
- Lessons learned: Outcomes of clinical trials/technological advances
- Better understanding of best medical formulation, PK/PD properties, and toxicity
- Unique advantages of aptamers could fill a niche market
  Ex. Viral treatments: fast-track vaccines to overcome viral emergence and mutation