Aptamers: Cutting Edge Technology to Combat HIV/AIDS

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South African Burden of Diseases

HIV/AIDS (38% YLLs)

Diseases of poverty, e.g. TB (25% YLLs)

Chronic diseases, e.g. Heart diseases (21% YLLs)

Injuries (16% YLLs)

Bradshaw et al. 2003. Initial Burden of disease estimates for South Africa. SAMJ. 76(9): 682-8
Common Denominator in the African burden of disease

- HIV/AIDS is a common denominator in at least three of the South African quadruple burden of diseases
  - HIV/AIDS fuels the TB epidemic (disease of poverty)
  - HIV/AIDS one of the underlying cause of some chronic diseases (e.g. cardiomyopathy)

- HIV/AIDS is the defining public health problem of our generation.

- Greatest challenge facing South Africa and the entire African continent today.

- The epidemic has attained a scale at which the impact on the economy and, even more broadly, on our society, is both evident and very serious
Now that we know the defining public health problem of our generation

What do we do?
Do We Deny It?

AIDS DENIALISM!
Or...

“Do we accept the diagnosis but defy the verdict”

Norman Cousins, 1989: The Biology of Hope

But How?
Aptamers: Part of the feasible solution
Aptamers: An innovative technology platform

- Artificial nucleic acid ligands (ssRNA) selected \textit{in vitro} for specific binding to a target.
- Form well-defined 3-D shapes, allowing them to bind target molecules in a manner conceptually similar to antibodies (Abs).
- Have molecular recognition properties of Abs.
- Small (6 kDa- 40 kDa) to probe protein structure and can penetrate viral defence mechanisms.
- Combine optimal characteristics of small molecules and Abs: High affinity & specificity.
- Functional products in their own right.
- Low immunogenicity.
- Resistant to nucleases.
- A new approach to drug discovery.
Structural lessons from aptamer-protein complexes

- Aptamers are prone to bind to functional domains of the target protein.
  - E.g. substrate binding pockets or allosteric sites
  - Modulate the biological function of the target molecule
- Aptamers are pre-existing molecules that have not been exploited during evolution

Aptamers: a Paradigm of Darwinian Evolution in a Test Tube

Made to fit?

Off the peg?

X

✓
Aptamers as therapeutics & multifunctional tools

Target validation
e.g. Marro et al., 2005

Aptamers as diagnostics
e.g. Fredrikson, 2002

High-throughput screening
e.g. Green et al., 2001

Aptamers as therapeutics
e.g. Rusconi et al., 2004
Our Application of the Aptamer Technology to Combat HIV/AIDS
Problem Identified!

- Almost all of the ARV drugs currently in clinical use only act on the virus after it has infected target cells.

- Treatment of infected individuals is costly to our health service.

- Drug resistance compounds problem.
HIV cunningly shield itself from the immune system attack:

- Occlusion of receptor binding sites
- Hypervariable loops
- Conformational shifts
- Extensive glycosylation
- Su gp120 is both a multiple lock system and the master key
- Lynchpin and Achilles' heel
- Strong and weak point
- Desirable target for therapeutic intervention

BIASElection: Isolation of anti-gp120 aptamers

DNA Library (10^{15})

SELEX protocol

RT-PCR

Elute bound RNA with 7M urea

Wash off weak binders

discard unbound

Allow dissociation of weak binders

gp120 (HIV-1_{Ba-L})

Transcribe 2′F RNA

Neutralization of HIV-1\textsubscript{Ba-L} by aptamers

Virus input (log\textsubscript{10} dilution)

Control aptamer (SA19)  Aptamer B4

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<thead>
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LTR-PCR

Beta-globin PCR

Limiting-dilution infectivity assay in human PBMC

Utility of aptamers against clinically relevant HIV PIs

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<tr>
<th>HIV-1 Isolate</th>
<th>Subtype</th>
<th>Characteristic</th>
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<tr>
<td>93BR029</td>
<td>F</td>
<td>Isolated from seropositive individual in Brazil</td>
</tr>
<tr>
<td>92TH021</td>
<td>E</td>
<td>Derived from asymptomatic individual in Thailand</td>
</tr>
<tr>
<td>92UG035</td>
<td>D</td>
<td>Isolated from seropositive individual in Uganda</td>
</tr>
<tr>
<td>97ZA003</td>
<td>C</td>
<td>Isolated from seropositive individual in South Africa</td>
</tr>
<tr>
<td>93MW960</td>
<td>C</td>
<td>Isolated from seropositive individual in Malawi</td>
</tr>
<tr>
<td>97USSN54</td>
<td>A</td>
<td>Isolated from a Senegalese woman living in the USA with full blown AIDS</td>
</tr>
<tr>
<td>BCF07</td>
<td>Group O</td>
<td>Isolated from a 29 year old woman from Cameroon</td>
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Pan-clade neutralization of HIV-1 clinical isolates by aptamers

Mechanism of neutralization: Competition of aptamers with NAbs for binding to gp120

Aptamers interfere with gp120 binding to its natural receptors

Dey, Khati et al. (Nov 2005) J. Virol. 79 (21)
Use aptamers for analysis and neutralization of endemic, South African strains of HIV-1 from adult and pediatric patients at various stages of disease.

Exploit aptamers to provide structural leads for the development of potent and even smaller molecules that can mimic their HIV neutralizing properties.

Determine if the structural mimetic would provide hope for salvage therapy for patients failing current ARV’s including HAART, as well as alternatives for initial therapy for newly infected individuals.
Exploit aptamers to elucidate and treat HIV associated cardiomyopathy

Isolate aptamers against early markers of active TB and develop rapid and reliable diagnostics
In the face of adversity
It is not all gloom!