A novel vaccine technology platform

Plasmid Launched Live Attenuated Virus (PLLAV) vaccines

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www.antivirals.be
www.facebook.com/NeytsLab
The Neyts-lab of Virology, Antiviral Drug & Vaccine Research

Our Mission: The development of (i) antiviral strategies against a range of (RNA) viruses and (ii) a game-changing novel vaccine technology platform.

- Flaviviridae
- Picornaviridae
- Norovirus
- Respiratory syncytial virus
- Chikungunya virus
- Coronavirus
- Hepatitis E virus
- Bunyaviridae
- Hepatitis B virus
Yellow fever

~200,000 cases/year
~30,000 deaths/year

Staples et al. MMWR 2010; www.who.int
Fears rise over yellow fever’s next move

Scientists warn vaccine stocks would be overwhelmed in the event of large urban outbreaks.

WHERE MIGHT YELLOW FEVER GO NEXT?

An ongoing outbreak of yellow fever in Angola has scientists worried that the virus might spread to cities that harbour its urban carrier, the Aedes aegypti mosquito.

People in cities on Brazil’s eastern seaboard are not routinely vaccinated.

Yellow fever has never taken hold in Asia, although conditions seem ripe for its spread.

Estimated distribution of Aedes aegypti

High

Low

2015–16 Angola outbreak.

Areas with risk of yellow fever virus transmission

- Endemic (vaccination recommended)
- Intermediate/low risk

Darfour (2012/13): 2M
Angola (2015/16): 6M
Congo (2016): 11M
Brazil (2016/17): 3.5M
USA July 2017: breakdown supply
The Live-attenuated YFV vaccine (YFV-17D)

**PROs**
- Safe
  - Massively used since 1938
  - Rare complications
- Efficient
  - Fast neutralizing immune response
  - One dose; long-lasting protection

**CONS**
- Production
  - Need for embryonated egg
  - Q/C & batch release time
- Storage & transport
  - Need for strict cold-chain

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*Max Theiler*


[https://smart.servier.com/smart_image/injectable/](https://smart.servier.com/smart_image/injectable/)
PLLAV: Plasmid-launched live-attenuated viral vaccine

Proprietary BAC shuttle vector:

- Stable maintenance in *E. coli*
- Inducible high yield production
- Modularity and high vector capacity
- No cold chain required
- Needle-free administration

ADVANTAGES OF DNA VACCINE

Live-attenuated YFV vaccine strain (cDNA):

- Replicates in mammalian cells
- Induction of strong immune response
- Insertion of foreign antigens possible

ADVANTAGES OF LAV

Dallmeier and Neyts, WO/2014/174078
Identical virus replication of Stamaril® and PLLAV-YF17D in tissue culture
PLLAV-YF17D is highly stable in *E. coli*

- **High copy n° plasmid**
  - 92%
  - large deletions (> 1000 bp)
  - restriction pattern changed

- **Passage P0**
- **10x overnight 1:100**
- **Passage P10**
- **PLLAV-YF17D**
  - 4%
  - non synonymous
  - ORF remains open
Immunogenicity and protection from challenge viremia

Dr. J. Julander, Utah State University, Logan under NIH/NIAID Service Contract
Protection from disease and mortality

**LIVER**

**WEIGHT**

**SURVIVAL**

Dr. J. Julander, Utah State University, Logan under NIH/NIAID Service Contract
Rapid antibody response in hamsters

**Stamaril®**

<table>
<thead>
<tr>
<th></th>
<th>Log_{10} IFA end titer</th>
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<tbody>
<tr>
<td>Day 0</td>
<td></td>
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<tr>
<td>Day 7</td>
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<td>Day 14</td>
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<td>Day 21</td>
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<td>Day 28</td>
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**PLLAV-YF17D**

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**anti-YFV IgG**

**nAb**
Towards the best route of administration

**A**

<table>
<thead>
<tr>
<th>YF17D</th>
<th>1 days</th>
<th>3 days</th>
<th>5 days</th>
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<tbody>
<tr>
<td>PLLAV : 2,5µg</td>
<td>Stamaril® 10⁴ PFU</td>
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<tr>
<td>PEI : Poly-ethylenimine condenses DNA into positively charged particles facilitating endocytosis</td>
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**B**

PLLAV

**C**

PLLAV ± PEI

**D**

Total flux (photons/sec), log10

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<thead>
<tr>
<th>Method</th>
<th>Subc.</th>
<th>Subc. + PEI</th>
<th>Jet</th>
<th>Jet + PEI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of positive spot delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>67% (31/46)</td>
<td>20% (2/10)</td>
<td>73% (19/26)</td>
<td>100% (8/8)</td>
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</tbody>
</table>

PEI : Poly-ethylenimine condenses DNA into positively charged particles facilitating endocytosis
PLLAV-YF17D can be administered to pigs
Proof-of-concept: Immunogenicity of PLLAV-YF17D in rhesus macaques

<table>
<thead>
<tr>
<th>Seroconversion</th>
<th>Stamaril</th>
<th>PLLAV-YF17D</th>
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<tbody>
<tr>
<td>Anti-YFV IgM/IgG (IIFA)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Neutralizing titer (CPNT)</td>
<td>1 : 500</td>
<td>1 : 1500</td>
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<tr>
<td></td>
<td>1 : 800</td>
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CoP by serum transfer to AG129 mice

Save, no adverse effects
PLLAV as a platform technology

Chimeric flavivirus vaccines

Japanese encephalitis virus
Zika virus

Transgenic vaccines

Ebola
Rabies
Influenza
Leishmania
HBV
...
PLLAV as platform for chimeric vaccines
Japanese encephalitis

A very safe and highly efficient ZIKV vaccine

VIREMIA

Log_{10} RNA copies/ml

Non-Vaccinated | Vaccinated

1x vax 10^2 PFU

AG129

10^5× LD_{50}

ZIKV MR766

SURVIVAL

Mean weight change (%)

Non-Vaccinated | Vaccinated

PLLAV as platform for transgenic vaccines (HBV)

Induction of T-cell response to HBV core
PLLAV as platform for transgenic vaccines (rabies)
PLLAV as platform for transgenic vaccines (rabies)

RabG insertion between E and NS1 (PLLAV-YF/RabG)

Day 14
YFV seroconversion 5/5
RABV seroconversion 5/5
RABV nAb: 1.5 [0.25-3.3]

Day 28
YFV seroconversion 5/5
RABV seroconversion 5/5
RABV nAb: 5.3 [0.54-11.5]
PLLAV as platform for transgenic vaccines (Lassa)

This year, the rats that carry Lassa fever may be more numerous, or more likely to harbor the virus. 

**Health workers scramble to contain deadly rat-borne fever in Nigeria**

By Leslie Roberts | Mar. 12, 2018, 4:29 PM

Science, March 2018

Towards region specific vaccines
To summarize...

Commercial YFV-17D vaccine  OR  PLLAV-17D
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Acknowledgements

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