Development of new tools to control infections due to parasites of the Trypanosomatidae family (NANOTRYP)

Prof. Dr. ir. Stefan Magez

Lab. Cellular and Molecular Immunology
Vrije Universiteit Brussel
Dept. Molecular and Cellular Interactions
Flanders Institute for Biotechnology

stemagez@vub.ac.be
Sleeping Sickness and camel antibodies: what keeps us awake?
African Trypanosomiasis: the disease
African Trypanosomiasis: the life cycle

- **Metacyclic**
  - **« Slender » bloodstream**
    - T. brucei
    - T. b. gambiense
    - T. b. rhodesiense
    - T. congolesne
    - T. evansi
    - T. vivax

- **Mammal**

- **Procyclic**
  - **« Stumpy » bloodstream**

- **Glossina** (by P.B. Pippa and E.M. Vivares)
Trypanosome defense = antigenic variation

The coat changes at a frequency of $10^{-2}$ to $10^{-6}$ / cell / generation.
Only the hypervariable VSG loops are accessible on the intact parasites
Trypanosome defense = antigenic variation
How do we diagnose ‘sleeping sickness’?

In the “old” days ....... and today...
How do we diagnose ‘sleeping sickness’?

More recently: CATT
How do we diagnose ‘sleeping sickness’?
How do we diagnose ‘sleeping sickness’?
Why are camels a man’s best friend?
Serum immunoglobulins

Serum from camelids → IgG purification on Protein A / Protein G

H2L2 = conv. Ab
H2 = HCAb

IgG1  IgG2  IgG3

H

L

67

43

30
Camel & Llama/alpaca antibodies

Classical antibody (IgG1)

Camel Heavy-Chain antibody (IgG2 & IgG3)

Single domain antigen binding fragment (15 kDa)
NANOBODY
Monomeric
Prolate particle:
Diameter 2.4 nm
Height 4 nm
Isolation of specific VHHs/Nbs

- Immunization
- Collect blood
- PBL isolation
- Purify RNA
- Amplify VHH gene fragments
- Transform in E.coli
- Ligation in phagemid vector
- Rescue library with helper phages
- Panning
- Select
- Production
- Specific VHH or Nb
Trypanosomes and VSG-specific Nanobodies®

Only Nanobodies® has access to hidden VSG epitopes

Here the academic funding stops!
FP7 - NANOTRYP

FIND – Foundation for Innovative and New Diagnostics
NANOTRYP

- Flanders Institute for Biotechnology VIB Belgium
- Fondation para la Investigacion Biosanitaria de Andalucia Oriental FIBAO Spain
- Institute of Primate Research IPR Kenya
- University Eduardo Mondlane UEM Mozambique
- Foundation for New and Innovative Diagnostics FIND Switzerland
- ARTTIC in Brussels Belgium
NANOTRYP

- WP1: generation of libraries for T. brucei, T. congolense, T. vivax and T. evansi

- WP2: developing a Nb-based dip-stick
- WP3: developing a Nb-based Ag capturing tool

- WP4: producing a commercial dip-stick format and testing this tool in a field setting

- WP5: using Nbs as specific drug delivery tools

- WP6: testing Nbs in green vervet monkey model, both as diagnostic and drug delivery tool

- WP7: testing Nbs in cattle/goat models and field infections, both as diagnostic and drug delivery tool

- WP8: training, joined PhD and awareness campaigning

- WP9 project management
Detecting parasites with fluorescent Nbs

Trypanosoma congolense

- FIXED PARASITES:
- LIVING PARASITES:
Detecting parasites with fluorescent Nbs
NANOTRYP

-WP1: generation of libraries for T. brucei, T. congolense, T. vivax and T. evansi

-WP2: developing a Nb-based dip-stick
-WP3: developing a Nb-based Ag capturing tool

-WP4: producing a commercial dip-stick format and testing this tool in a field setting

-WP5: using Nbs as specific drug delivery tools

-WP6: testing Nbs in green vervet monkey model, both as diagnostic and drug delivery tool

-WP7: testing Nbs in cattle/goat models and field infections, both as diagnostic and drug delivery tool

-WP8: training, joined PhD and awareness campaigning
EU-FP7 NANOTRYP project

Assay building blocks:

1) Infected blood sample containing
   - Parasites
   - Released antigen
   - Infection-induced conventional host antibodies against VSGs, ISGs etc.

2) Biotin coupled anti-*T. brucei* specific pan-reactive capturing Nanobody® (Nb)

3) Detecting anti-*T. brucei* specific pan-reactive Nb or Nb-collection

4) Streptavidine coated carrier (beads / membranes)
Dipstick assay = capturing of released antigen:

1) Collect infected blood sample and induce antigen release
   *This sample contains host antibodies...so immune complexes are likely to be formed.*

2) Mix sample with Nbs
   *Capturing Nb is coupled to biotin*

3) Allow the sample to migrate to streptavidine band
EU-FP7 proposal - Dipstick
EU-FP7 proposal - Dipstick
EU-FP7 proposal - Dipstick

10^4 par/ml
EU-FP7 proposal - PickPen®

Nb-PickPen® Trypanosome purification/isolation

www.bio-mobile.com
Capturing of assay-induced released VSG:

1) Collect infected blood sample and induce antigen
   (This sample contains host antibodies)

2) Add Strep-paramagnetic beads + biotin anti-VSG Nb
EU-FP7 proposal - PickPen®

Capturing of assay-induced released VSG:

1) Collect infected blood sample and induce antigen (This sample contains host antibodies)

2) Add Strep-paramagnetic beads + biotin anti-VSG Nb
Capturing of assay-induced released VSG:

1) Collect infected blood sample and induce antigen (This sample contains host antibodies)

2) Add Strep-paramagnetic beads + biotinilated anti-VSG Nb + paired specific detecting Nb(s)

3) Fish out beads with Pickpen®

wash, detect
Detection of AnTat 1.1 VSG

Equivalent of 100 parasites/sample (equivalent to Tryp/ml)
NANOTRYP

- WP1: generation of libraries for *T. brucei*, *T. congolense*, *T. vivax* and *T. evansi*
- WP2: developing a Nb-based dip-stick
- WP3: developing a Nb-based Ag capturing tool

- WP4: *producing a commercial dip-stick format and testing this tool in a field setting*

- WP5: *using Nbs as specific drug delivery tools*

- WP6: testing Nbs in green vervet monkey model, both as diagnostic and drug delivery tool

- WP7: testing Nbs in cattle/goat models and field infections, both as diagnostic and drug delivery tool

- WP8: training, joined PhD and awareness campaigning

- WP9 project management
How do we treat ‘Sleeping Sickness’ today?

Berenil

Mel B

Pentamidine

Melarsen oxide

Suramin

Tryparsamide

DFMO

p-Aminophenylarsenoxide
How to ‘use’ Nanobodies®
Drug targeting with Nanobodies®

Nanotrip

Para site $(10^6/\text{ml})$

$\begin{array}{c}
\text{Days after infection} \\
0 & 2 & 4 & 6 & 8 & 10 & 20 & 40 & 60
\end{array}$

$\begin{array}{c}
\text{Percent survival} \\
0 & 25 & 50 & 75 & 100
\end{array}$

- Targeted (20µg)
- Targeted (10µg)
- Non-targeted (20µg)
- Control

*Nat. Med.*, but: Here the academic funding stops!
1. Couple existing cyclodextrin encapsulated anti-trypanosome drugs to Nbs

First experiment: fluorophore
Berenil 15 μM Nb-CD pH 7.5
Next: Nifurtimox
**WP6:**
- Immunogenicity testing of Nbs in monkey model: so far no indication of any immunogenicity
- Establishing Green Vervet monkey models for *T. rhodensiense* and *T. gambiense*, in order to allow later for Nb testing in BBB-crossing context

**WP7:**
- Testing of Nb diagnostics for *T. congolense* and *T. vivax* under field conditions
- A large batch of field samples has been prepared and 3 anti-*T. congolense* + 11 anti-*T. vivax* Nb-fluo are ready to be shipped to Mozambique

**WP8:**
- PhD training
- Research exchange and training + workshops in African partner countries
- Awareness campaigning in areas where diagnostic testing will be performed

**WP9:** Management
Why are camels a man’s best friend

THANK YOU
Why are camels a man’s best friend

Can we use Nanobodies in the fight against trypanosomiasis?
How do we treat ‘Sleeping Sickness’ today?
2. Genetic construction of Nb-peptide(lytic) constructs
   - construct has been made, chimeric protein has been produced, checked, purified
   - New construct in preparation: 4 peptides/5 cleaving sites
Nb properties versus scFv and Fab

- Efficient identification of Ag binders
- Good expression yields
- Good stability
- Good solubility
- Antigen specific
- High affinity for the Ag
- Easy tailoring

• Nb > scFv = Fab
• Nb > Fab > scFv
• Nb = Fab = scFv
• Nb > scFv = Fab