May 16th, 9:45 AM

**Keynote Address: New Poverty-Related Neglected Diseases (‘The NTDs’)**

Peter Hotez  
*Baylor College of Medicine*

Let us know how access to this document benefits you.  
Follow this and additional works at: [https://escholarship.umassmed.edu/cts_retreat](https://escholarship.umassmed.edu/cts_retreat)

Part of the International Public Health Commons, Translational Medical Research Commons, and the Tropical Medicine Commons


Creative Commons License

This work is licensed under a [Creative Commons Attribution-Noncommercial-Share Alike 3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/). This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in UMass Center for Clinical and Translational Science Research Retreat by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
New Poverty-Related Neglected Diseases (‘The NTDs’)
From the MDGs to the SDGs

2000-15 MDGs
The Millennium Development Goals

1. Eradicate extreme poverty and hunger.
2. Achieve universal primary education.
3. Promote gender equality and empower women.
4. Reduce child mortality.
5. Improve maternal health.
7. Ensure environmental sustainability.
8. Develop a global partnership for development.
The Global Burden of Disease Study

Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010

Expanded use of vaccines

- 83% reduction in measles deaths
- 82% reduction in tetanus deaths
- 57% reduction in diphtheria/pertussis deaths
- 45% reduction in Hib deaths

Development new vaccines

- Pneumococcal disease (36% reduction in deaths)
- Rotavirus (63% reduction in deaths)

2.5 million childhood lives saved through these initiatives
2015 Measles Cases in the U.S.

January 1 to June 26, 2015

- **Cases***:
  - 0
  - 1-4
  - 5-9
  - 10-19
  - 20+

*Provisional data reported to CDC's National Center for Immunization and Respiratory Diseases*
The spread of anti-vax sentiment in California

Share of public school kindergartners with personal belief exemptions to vaccination requirements

2000

2007

2013

WASHINGTONPOST.COM/WONKBLOG

Source: California Department of Public Health
• Texas ranks at the bottom of fully immunized children
• 45,000 Personal Belief Exemptions in Texas
Patches of Disorganization in the Neocortex of Children with Autism

Rich Stoner, Ph.D., Maggie L. Chow, Ph.D., Maureen P. Boyle, Ph.D., Susan M. Sunkin, Ph.D., Peter R. Mouton, Ph.D., Subhojit Roy, M.D., Ph.D., Anthony Wynshaw-Boris, M.D., Ph.D., Sophia A. Colamarino, Ph.D., Ed S. Lein, Ph.D., and Eric Courchesne, Ph.D.

ABSTRACT

BACKGROUND
Autism involves early brain overgrowth and dysfunction, which is most strongly evident in the prefrontal cortex. As assessed on pathological analysis, an excess of neurons in the prefrontal cortex among children with autism signals a disturbance in prenatal development and may be concomitant with abnormal cell type and
The Millennium Development Goals

1. Eradicate extreme poverty and hunger.
2. Achieve universal primary education.
3. Promote gender equality and empower women.
4. Reduce child mortality.
5. Improve maternal health.
7. Ensure environmental sustainability.
8. Develop a global partnership for development.
Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013

“Other Diseases”
The Neglected Tropical Diseases

- 13-14 tropical infections:
  - Highly prevalent among the poor
  - Endemic in rural areas of low-income countries
  - Ancient afflictions
  - Chronic
  - Disabling (growth delays, blindness or disfigurement)
  - Stigmatizing
  - Poverty promoting
## NEGLECTED TROPICAL DISEASES:
NTDs infect more than 1 BILLION of the world’s poorest people

<table>
<thead>
<tr>
<th>Disease</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascariasis</td>
<td>761.9 million</td>
</tr>
<tr>
<td>Trichuriasis</td>
<td>463.7 million</td>
</tr>
<tr>
<td>Hookworm Disease</td>
<td>428.8 million</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>252.2 million</td>
</tr>
<tr>
<td>Dengue and other arboviruses</td>
<td>79.6 million</td>
</tr>
<tr>
<td>Food-borne trematodiases</td>
<td>71.1 million</td>
</tr>
<tr>
<td>Lymphatic Filariasis</td>
<td>38.5 million</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>15.5 million</td>
</tr>
<tr>
<td>Chagas disease</td>
<td>6.7 million</td>
</tr>
<tr>
<td>Leishmaniasis</td>
<td>3.9 million</td>
</tr>
<tr>
<td>Trachoma</td>
<td>3.6 million</td>
</tr>
<tr>
<td>Cysticercosis</td>
<td>1.9 million</td>
</tr>
<tr>
<td>Echinococcosis</td>
<td>1.4 million</td>
</tr>
<tr>
<td>Hansen’s Disease</td>
<td>514,200</td>
</tr>
<tr>
<td>Rabies</td>
<td>17,400</td>
</tr>
<tr>
<td>African Trypanosomiasis</td>
<td>10,700</td>
</tr>
<tr>
<td>Guinea worm</td>
<td>&lt;1,000</td>
</tr>
<tr>
<td>Yaws</td>
<td>Not determined</td>
</tr>
<tr>
<td>Buruli ulcer</td>
<td>Not determined</td>
</tr>
<tr>
<td>Mycetoma</td>
<td>Not determined</td>
</tr>
<tr>
<td>Zika</td>
<td>4 million</td>
</tr>
<tr>
<td>Ebola</td>
<td>2,800</td>
</tr>
</tbody>
</table>
“It’s quite a problem for me when I have to stand at work for long periods.”

Lymphatic Filariasis (“Elephantiasis”)
NTDs and Girls & Women
Female Genital Schistosomiasis

Zimbabwe
OR = 3 increase in HIV/AIDS
Kjetland et al. AIDS 2006

Tanzania
OR = 4 increase in HIV/AIDS
Downs et al. AJTMH 2011

100 million girls & women
Africa’s most common gynecologic condition?
The Bottom Billion Suffers from Multiple NTDs!

Ascariasis, Trichuriasis, Hookworm, Schistosomiasis, LF, Onchocerciasis, Trachoma

NTD Scale up with the U.S. Government + Drug Donations from

George W. Bush 2001-2009
USAID NTD Program

Funding in Millions USD

>450 million People Rx: Elimination of some NTDs
10 SIGNIFICANT GAINS

Mass Drug Administration (MDA)

Lymphatic filariasis
-52% (2005-15)

Onchocerciasis
-52% (1990-2013)

Trachoma
-65% (1990-2013)

Ascariasis
-20% (2005-2015)

Yaws
Not determined

Case detection + Rx + Vector control

African trypanosomiasis
-78%
(2005-2015)

Dracunculiasis
-99%
(1990-2013)

Rabies (Canine)
-53%
(2005-2015)

Cysticercosis
-21%
(2005-2015)

WASH

Leprosy

Other approaches

Elimination targets:
LF
Trachoma
Yaws
African trypanosomiasis
Dracunculiasis
Leprosy (Hansen’s Disease)

Source: GBD 2015 and GBD 2013
9 MAJOR SETBACKS

Mass Drug Administration (MDA)

Schistosomiasis +30% (1990-2013)

Hookworm -5% (1990-2013)

Trichuriasis -12% (1990-2013)

Case detection + Rx + Vector control

Leishmaniasis +174% (1990-2013)

Chagas disease +22% (1990-2013)

Dengue & Other Arbovirus Infections +610% (1990-2013)

WASH

Ebola +28,000% (2005-2015)

Coronaviruses

Other approaches

Food-borne Trematodiases +51% (1990-2013)

Losing the Battle:
Vector-borne Neglected Diseases
Arthropods
Snails
Zoonotic Neglected Diseases
Viral Diseases

Source: GBD 2015 and GBD 2013
Are we playing “global health whack-a-mole”? 

MDGs
- AIDS
- Malaria
- Some NTDs
- Childhood dz

SDGs
- NTDs V.2.0
- Vector-borne NTDs
- Zoonotic NTDs
Explosive Outbreaks in the Americas

Emergence of Dengue in the New World in 1980s, 1990s

Emergence of Chikungunya in New World in 2013 (Saint Martin)

Zika Path: Explosive Pacific Outbreaks
Emerging Vector-borne Disease in Southern Europe

EMERGING VECTOR BORNE NEGLLECTED DISEASE IN SOUTHERN EUROPE
The **Anthropocene** is a proposed epoch that begins when human activities started to have a significant global impact on Earth's geology and ecosystems.
Anthropocene forces promoting NTDs

Poverty

Deforestation

Conflict and Political Destabilization

Urbanization and Human Migrations

Climate Change
POVERTY: “Blue Marble Health”

- Neglected diseases of the poor living amidst wealth
- A new framework for global science policy and the poverty-related diseases
Blue Marble Health:
The poor living among the wealthy (G20 + Nigeria)

WHO + GBD 2013
- 73-78% Leprosy
- 61-78% Chagas
- 60-61% Dengue
- 57-60% TB
- 45-67% VL
- 50-52% Helminths

G20 + Nigeria = 54% Population and 86% Global Economy

http://www.plosntd.org/article/info:doi/10.1371/journal.pntd.0002570
Chagas disease in Argentina, Brazil, Mexico

• Ranking By GDP
  - 1. Brazil
  - 2. Mexico
  - 3. Argentina

• Ranking By Chagas
  - 1. Argentina 1.5 million
  - 2. Brazil 1.2 million
  - 3. Mexico 0.9 million
# Brazil and Blue Marble Health

## Introduction to Brazil

<table>
<thead>
<tr>
<th>Rank</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LARGEST</td>
<td>Economy in Latin America</td>
</tr>
<tr>
<td>5th</td>
<td>Largest country by land mass + population</td>
</tr>
<tr>
<td>7th</td>
<td>Largest economy by nominal GDP</td>
</tr>
</tbody>
</table>

- Member of MERCOSUL
- Member of BRICS

## Poverty & Disease NE Brazil:

- Schistosomiasis
- Leishmaniasis
- Chagas
- Dengue
Zika Microcephaly cases in NE Brazil

Updated as of Epidemiological Week 48 (Nov 29 - Dec 5, 2015)

Microcephaly rates by state in Brazil, 2015 (cases per 100,000 live births)
- 1
- 10
- 100

Microcephaly rates by state in Brazil, 2010-2014 (cases per 100,000 live births)
- 1
- 5
- 10

Countries with Zika confirmed cases
- in 2015
- in 2014
- Country limits
- Brazil state boundaries

One case of autochthonous transmission of Zika virus infection in Easter Island, Chile, 2014. The presence of the virus was reported until June of the same year and was not detected later.

Data Source:
Reported from the IHR National Focal Points and through the Ministry of Health websites.

Map Production:
PAHO-WHO/ICH/IR/180
Poverty in Northeastern Brazil

Recife

Salvador de Bahia
Spread of Zika in the Americas

- Countries where the virus is normally found
- Countries where the virus has spread

- Thailand
- Indonesia
- Philippines
- Mexico
- Colombia
- Texas
- Venezuela
- Brazil

2014
2015
2016
POVERTY IN TEXAS


Fifth Ward, Houston Texas
Anna Grove

South Texas “Colonias” Shaghayegh Tajvidi.
WAR & POLITICAL DESTABILIZATION: Ebola

Data are based on reported cases up to the end of 13 September 2014 for Guinea and Sierra Leone. Data for Liberia are based on reported cases up to the end of 9 September 2014. The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.
ISIS-Occupied Syria, Iraq, Libya
Yemen

Measles/Polio
Leishmaniasis
Schistosomiasis
Brucellosis
MERS CoV
Dengue
Malaria/TB
Rift Valley Fever
Vaccinating Against The Anthropocene’s NTDs
Pepsi has a new Doritos-flavored Mountain Dew. No, we don't have an Ebola vaccine, but we do have the Doritos-flavored Mountain Dew.

—David Letterman—
Coalition for Epidemic Preparedness Innovations (CEPI)

Building on the WHO R&D blueprint
• Need for improved R&D preparedness for diseases of epidemic potential
• Prioritization of pathogens
• Identification of R&D priorities
• Exploration of funding models for R&D preparedness and response
  Nipah
  Lassa
  MERS CoV

Vaccines
Putting shots in the locker

How to anticipate epidemics
Sep 3rd 2016 1 From the print edition

FOREWARNED, the proverb has it, is forearmed. But what happens when there is no warning? That was the case in December 2013, when an outbreak of Ebola haemorrhagic fever began in Guinea. It spread rapidly to Liberia and Sierra Leone and raged on for over a year. Around 29,000 people were infected. More than 11,000 of them died.

The world responded to this crisis, shipping in doctors, nurses and medical equipment. But what it could not ship in, for none existed, was the thing that would most quickly have stopped the epidemic: a vaccine. Such a vaccine was created eventually, but by the time it was ready, the outbreak was all but over. Had it been available from the beginning, things could have been different.

Next time, though, they might be, for on August 31st a new organisation came into being. CEPI, the Coalition for Epidemic Preparedness Innovations, was founded this week in London, at the headquarters of the Wellcome Trust, a medical charity. It is the joint brainchild of the Wellcome, the Bill and Melinda Gates Foundation, the World Economic Forum and the government of Norway, and its purpose is precisely to forewarn the world against future outbreaks of disease, without foreknowledge of what those outbreaks will be.

Paradoxically, part of the inspiration for CEPI’s creation was not the failure to deliver an Ebola vaccine in time for it to be useful, but how close that project came to success. Creating a new vaccine from scratch is a long-winded undertaking, but in the case of Ebola several candidate vaccines were already on the shelf thanks to earlier, but stalled, work by America’s army and that country’s National Institutes of Health. There were also three pharma companies, GlaxoSmithKline, Johnson & Johnson and Merck, willing, pro bono publico, to take these candidates and try to turn them into the real thing as quickly as possible. That they succeeded in doing so by the summer of 2015 was, by most standards, extraordinary.
Sabin PDP Pipeline and Disease Portfolio

2000 to 2004
• Built structure
• Launched Hookworm Program

2004 to 2011
• Expanded Hookworm Program
• Schisto Program
• Relocated to TMC

2011 to 2015
• Added 7 additional programs
• Expansion of capabilities

PROGRAM R&D PRE-CLINICAL PHASE 1 PHASE 2 AND 3
HOEKWORM

SCHISTOSOMIASIS

LEISHMANIASIS

CHAGAS DISEASE

TRICHURIASIS

ASCARISIS

WEST NILE

SARS

ONCHOCERCIASIS

A Handful Of ‘Antipoverty’ Vaccines Exist For Neglected Diseases, But The World’s Poorest Billion People Need More

ABSTRACT: Severe neglected tropical diseases are the most common infections of the world’s poor, affecting 1.4 billion people who live below the poverty line defined by the World Bank—nearly twice the number of people infected with HIV/AIDS. An estimated 125,000 to 400,000 people die each year from these diseases, which are often invisible, silent, and easily preventable. Although millions of dollars have been spent on vaccine development, vaccines are not available to the poorest in the world. The Neglected Tropical Disease Initiative for Children is working to change this by developing, testing, and scaling up vaccines for some of the most neglected diseases. This initiative highlights the potential of vaccines to combat tropical diseases, and the critical need for global partnerships to accelerate vaccine development and delivery.
Rino Rappuoli: Reverse Vaccinology

In silico prediction of vaccine candidate antigens

Genome sequence of *Neisseria meningitidis* serogroup B

600 potential vaccine candidates identified

350 proteins successfully expressed in *E. coli*

91 novel surface-exposed proteins identified

28 novel proteins have bactericidal activity

Vaccine candidates selected for development

Protein purification

Mouse immunization

Testing mouse sera
Reverse vaccinology as a ‘holy grail’ for complex eukaryotic organisms

- Large genomes of similar complexity to human genome
- Inadequacy of bacterial expression systems for eukaryotic antigens
- Low throughput not high throughput
- Deficiencies in animal models
Vaccine Targeting
Hookworm and Schistosomiasis Co-Infections

A MULTIVALENT VACCINE TARGETING HOOKWORM + SCHISTO
Goals of Anti-schistosome Vaccine

- Worm burden reduction
- Egg reduction
- Reduced end-organ pathology
- Reduction in inflammation
- Reduction in anemia and malnutrition
Immunomics Approach:
Schistosome protein microarrays

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Expression Pattern</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SjDiag</td>
<td>High expression</td>
<td>Hypothetical protein</td>
</tr>
<tr>
<td>Sj23</td>
<td>Moderate expression</td>
<td>23 kDa integral membrane protein</td>
</tr>
<tr>
<td>Sj22.6</td>
<td>Low expression</td>
<td>Tegmental antigen</td>
</tr>
<tr>
<td>Filamin</td>
<td>Very low expression</td>
<td>Similar to acetylcholinesterase precursor</td>
</tr>
<tr>
<td>Calponin homolog</td>
<td>Low expression</td>
<td>Sim. XP 217452 prot.</td>
</tr>
<tr>
<td>Sim. myosin heavy chain</td>
<td>Low expression</td>
<td>Hypothetical protein</td>
</tr>
<tr>
<td>SjCUT</td>
<td>Low expression</td>
<td>SNAK1</td>
</tr>
<tr>
<td>Sim. tetraspanin TE736</td>
<td>Low expression</td>
<td>Sm29</td>
</tr>
<tr>
<td>Sim. Sm</td>
<td>Low expression</td>
<td>Putative uncharacterised prot.</td>
</tr>
<tr>
<td>Hypothetical protein</td>
<td>Low expression</td>
<td>Hypo. prot. /DNA-binding SAP domain</td>
</tr>
<tr>
<td>Smp96760(S1CF)</td>
<td>Low expression</td>
<td>Phosphoglycerate mutase</td>
</tr>
<tr>
<td>Sm ACTIN2</td>
<td>Low expression</td>
<td>Sim. alkaline phosphatase</td>
</tr>
<tr>
<td>Sm facilitated glucose transporter 8</td>
<td>Low expression</td>
<td></td>
</tr>
</tbody>
</table>
Immune localization of $Sm$-TSP-2
Suppression of tsp-2 mRNA expression results in impaired tegument turnover in vitro

Tran et al. PLoS Path 2010
**Intestinal Schistosomiasis Vaccine**

**Introduction**

Schistosomiasis is a parasitic infection caused by blood flukes of the genus *Schistosoma*. Today, human schistosomiasis is considered one of the most important human helminthic infections in terms of morbidity and mortality, especially in Africa, where more than 50% of the cases occur.\(^1\) Recent studies indicate that approximately 200 million people are infected worldwide, with 800 million people at risk.\(^2\) However, additional studies indicate that this number may be at a higher level and as many as 600-800 million people may be infected with schistosomes.

Although schistosomiasis is a treatable infection, the current treatment of choice does not provide an optimal strategy for controlling the disease.\(^3\) Since 1990, praziquantel (PZQ) based mass chemotherapy has been the major approach in controlling schistosomiasis, by primarily targeting school-aged children with annual administration.\(^4\) However, the sustainability of PZQ treatment for the long-term control and elimination of schistosomiasis remains a concern and has limitations. For instance, PZQ does not cure infestations in resistant patients. Although there is no clear evidence for the existence of PZQ-resistant schistosome strains, decreased susceptibility to the drug has been observed in several studies.\(^5\) To overcome these challenges, a prophylactic vaccine or a vaccine-induced chemotherapy would be ideal to complement the existing treatment options.\(^6\) Evidence for the feasibility of developing a schistosome vaccine includes studies showing that immunization with irradiated schistosome cercariae results in high levels of protection in experimental animal models, with boosting further increasing the level of protection.\(^7\) In addition, a subset of human populations living in endemic areas has been shown to develop various degrees of natural resistance,\(^8\) while veterinary schistosome vaccines against other schistosome parasites have been developed successfully and applied in practice.\(^9\)

Expanded information on the mechanisms of immunity to schistosomiasis and the recent availability of the schistosome genome for both *S. mansoni* and *S. bovis\(^10\) have resulted in the discovery of several schistosome antigens, while additional candidates are now being found through proteomic approaches.\(^11,12\) Within the last 5 years results of a Phase 1 trial for a parasite (2nd generation) vaccine against *S. mansoni* have been obtained and are encouraging.

**Expression at a 20L scale and purification of the extracellular domain of the Schistosoma mansoni TSP-2 recombinant protein**

A vaccine candidate for human intestinal schistosomiasis

Elena Curtil\(^1\), Clifford Kweise\(^2\), Fara Dhan\(^2\), Portia Gillepple\(^1\), Jill Bradfield\(^1\), Yohali Dieup\(^1\), Jordan Plaskett\(^1\), WandaPow\(^2\), Sami Reza\(^2\), Eric Tsao\(^1\), Bose Kalabun\(^1\), Robert Houser\(^1\), and Maria Elena Bettazz\(^\text{a}^1,\text{b}\)

Department of Pediatrics and Microbiology and Immunology; National School of Tropical Medicine; Baylor College of Medicine; Houston, TX; SAGE Vaccine Institute and Texas Children’s Hospital Center for Vaccine Development; Houston, TX; SAGE Department of Microbiology, Immunology and Tropical Medicine, the George Washington University; Washington, DC; Eton BioViva, Medford, MA; James A. Baker III Institute for Public Policy, Rice University; Houston, TX; USA

*Correspondence: Elena Curtil, ecurtil@bcm.edu; Maria Elena Bettazz, mel@bcm.edu

Keywords: Schistosomiasis, Schistosoma mansoni, neglected tropical diseases, vaccine, Sch-TSP-2, Public Partners, recombinant protein

A novel recombinant protein vaccine for human schistosomiasis caused by *Schistosoma mansoni* is under development. The Sch-TSP-2 recombinant protein vaccine is comprised of a 94 kDa recombinant protein corresponding to the extracellular domain of *S. mansoni* TSP-2. Here, we describe the cloning and expression of the extracellular domain of *S. mansoni* TSP-2 recombinant protein protein by PhosFlo\textsuperscript{TM} the process development at 20L scale fermentation, and the titering purification, which resulted in a protein recovery yield of 2.1% and a protein purity of 97%. The developed processes are suitable for the production of purified protein for subsequent formulation and Phase 1 clinical studies.

**The Schistosome Tetraspanins**

Entering phase 1 trials – Baylor VTEU
The Rise of Emerging + Neglected Diseases in the “New Texas”

• Leading TX NTDs
  - Toxocariasis 700,000
  - Trichomoniasis 450,000
  - Chagas disease 37,000
  - WNV 183-1,900
  - Intestinal protozoan 1,000
  - Cysticercosis 195-754
  - Murine Typhus >100
  - Dengue, Zika, Chik
The role of trypanocidal therapy in patients with established Chagas’ cardiomyopathy is unproven.

Trypanocidal therapy with benznidazole in patients with established Chagas’ cardiomyopathy significantly reduced serum parasite detection but did not significantly reduce cardiac clinical deterioration through 5 years of follow-up.
Tc24 protein combined with E6020 in a Stable Squalene Emulsion as a lead candidate vaccine

**Candidate Antigen**

Tc24- 24kDa *Trypanosoma Cruzi* Flagellar Calcium Binding Protein

**Candidate Adjuvant**

TLR4 agonist:

E6020

Additional antigens also under evaluation and development
ADVANCES IN A THERAPEUTIC CHAGAS VACCINE INITIATIVE

• Preclinical Efficacy:
  - Pilot studies were performed to evaluate efficacy of recombinant Tc24 combined with imiquimod or MPLA when used as a preventative vaccine

• Preliminary Results
  • Reduce parasitemia
  • Increase survival during acute phase
  • Antigen specific IgG2a
  • Antigen specific IFNγ
  • Reduced cardiac parasite burden
Western Blot comparison of Tc24-WT (A), Tc24-C2 (B), and Tc24-C4 (C) purified proteins. Lanes 1-3: Non-Reduced. Lane 4: SeeBlue Plus Molecular Weight Marker. Lanes 5-7 Reduced. Lanes 1,5: Sample before size-exclusion chromatography (SEC) 8 µg load. Lanes 2,6: Post SEC low load (3 µg). Lanes 3,7: Post SEC high load (8 µg). Detection was performed using mouse polyclonal antibody against Tc24 expressed in *Pichia pastoris* as primary antibody diluted 1:2,500 in PBST and an alkaline phosphatase conjugated goat anti-mouse secondary antibody diluted 1:7,500 in PBST.
Structural comparison of Tc24 constructs. a) Circular Dichroism (CD). Far UV CD spectrum of different constructs of Tc24 were taken on a Jasco J-1500. All tested Tc24 protein have a virtual identical CD profile with overlapping spectra. Negative peaks at 222 nm and 208 nm and a positive peak at 193 nm indicate that Tc24 is an \( \alpha \)-helical protein. b) Thermal melting profile of Tc24-WT and Tc24-C4 measured using Protein Thermal Shift™ kit (Life Technologies).
Therapeutic Chagas Disease Vaccine

Experimental Chagas disease vaccine improves Cardiac Echocardiography function