

Cattle genome editing For resistance to trypanosmiasis









A transgenic approach to trypanosome resistance

- Proof of concept
- Vehicle for stakeholder engagement
- Product with massive potential for impact





BILL & MELINDA GATES foundation









African trypanosomiasis

- Caused by extracellular protozoan parasites Trypanosoma
- Transmitted between mammals by Tsetse flies (*Glossina* sp.)
- Prevalent in 36 countries of sub-Sahara Africa.

In cattle

- A chronic debilitating and fatal disease.
- A major constraint on livestock and agricultural production in Africa.
- Costs US\$ 1 billion annually.

In human (Human Sleeping Sickness)

- Fatal
- 60,000 people die every year
- Both wild and domestic animals are the major reservoir of the parasites for human infection.





Trypanosomiasis in livestock costs billions of dollars and excludes livestock from much of Africa

	tion .	No. of Concession, Name	
	Sudan	Entropia Kenya	Somalia n Ocean
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Livestock are also reservoirs of human disease





T brucei rhodesiense T brucei gambiense

- T. congolense,
- T. vivax

Trypanosomes lyse when exposed to primate serum, which contains the pore-forming toxin APOLI

Mouse/Cow/Goat Serum (no APOLI) Primate serum (with APOLI)



The Mzima Cow– a transgenic approach







Targeting the Transgene to the ROSA26 Locus Using CRISPR/CAS



Are transfected mice protected from infection by African trypanosomes?

Mice expressing human APOLI are immune to infection by the cattle/goat infective *T. b. brucei*



But humans are susceptible to infection by *T. b. rhodesiense*





Mice expressing human APOLI are susceptible to infection by the human infective *T. b. rhodesiense*



Days Post Infection

But mice expressing baboon APOLI are immune to infection by the human infective *T. b. rhodesiense*



Experimental plan



The Mzima Cow

A transgenic approach to trypanosome resistance

Tumaini (*Hope*), the world's first cloned Boran at ILRI



Tumaini resulted from SCNT at ILRI. He is healthy and fertile.

The APOL1 resistance gene has now been inserted into the same source cell line

CRISPR/Cas9 mediated insertion of TLF protects mice



This construct is now in 'Tumaini's blastocysts' ready for implantation into recipient cows at ILRI



The Mzima Cow Project

A transgenic approach to trypanosome resistance

Currently, the Mzima cow methodology is based on CRSPR-Cas9 with Somatic Cell Nuclear Transfer SCNT using material derived from embryo skin cell lines.

• Produce bulls transgenic for your target gene



An interesting alternative approach is use of spermatogonial stem cell lines(SSC's)

- Produce *modified* males capable of hosting sperm carrying 'any' target gene
- Requires a host male with his own sperm production ablated

Nanos C2HC-Type Zinc Finger 2

Plays a key role in the sexual differentiation of germ cells by promoting the male fate but suppressing the female fate. Represses the female fate pathways by suppressing melosis, which in turn results in the promotion of the male fate. Maintains the suppression of meiosis by preventing STRA8 expression, which is required for premeiotic DNA replication, after CYP26B1 is decreased. Regulates the localization of the CCR4-NOT deadenylation complex to Pbodies and plays a role in recruiting the complex to trigger the degradation of mRNAs involved in meiosis. Required for the maintenance of the spermatogonial stem cell population. Not essential for the assembly of P-bodies but is required for the maintenance of their normal state (By similarity).





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The Mzima Cow Project

"Mzima Goat" by spermatogonial stem cell transplantation



The Mzima Cow Project

Disseminating preferred genetics

In vitro genomic selection requires *in vitro* gametogenesis

Sex selected sperm

Improved methods for sperm/embryo distribution

Translating Genetic Research to Adoption & Social Value

Preparing the regulatory, safety & public awareness environment



Kenyan and regional regulators and stakeholders meet in ILRI with their global counterparts to discuss the Mzima Cow project



- Ground-breaking Science
- The Challenge of Adoption
- Defining the Testing Routemap
 - "Systems Change at the Speed of Trust":

Establishing trust is key to regulatory progress and the eventual adoption and understanding of genome edited livestock









The Mzima Cow Regulatory and public awareness

Developing a Theory of Change

- To have a positive impact on human wellbeing we need a clear Theory of Change ie define the goals, actors and paths to success
- Our first workshop was held in January 2017 with stakeholders representing the following domains: agricultural economics, anthropology, veterinary science, public health, pastoralist, national parks, biosafety regulation, genetics, parasitology, science communication, land owners, agricultural marketing, small holders, community driven programmes
- The participants investigated the issues of the burden of trypanosomiasis, stakeholder and power mapping, impact analysis of Mzima Cattle and the challenges and the priorities for action

The Mzima Cow Regulatory and public awareness

USDA Workshop on *International Animal Biotechnology Regulation.* Charlottesville July 2017. Recommended:

- Seek specific new regulatory frameworks for animal biotechnology
- Engage with regulatory authorities early and openly
- Encourage cross-border regulatory harmonization or coordination
- Produce roadmaps for research progress
- Produce roadmaps for eventual approval and adoption of successful research products
- Produce realistic timetables for all of these

The Mzima Cow Regulatory and public awareness



Regulators, policy makers and stakeholders at the Mzima Cow Strategy & Theory of Change workshop 26-27th February at ILRI Nairobi, Kenya



The Mzima Cow Testing Roadmap

Systems Change at the Speed of Trust": Establishing trust is key to regulatory progress, adoption and understanding

- February 2018 workshop to gain multiple perspectives on the domains of *trypanosome resistance, animal welfare, human welfare & environmental welfare*
- Containment and alignment with (emerging) National Biosafety Guidelines
- Setting up a comprehensive and transparent testing schema, or route map, is the first step in building the knowledge to provide confidence in the viability and safety of these cattle

The Mzima Cow Testing Roadmap

Next steps

- Development and implementation of communications and monitoring & evaluation strategies
- Impact modelling
- Expanding to more breeds in more centres
- Development a rapid field-testing kit for the transgene and its product
- Workshops and planning for introduction of cattle to markets
- Expanding the programme to Mzima Goats (which due to cost, size and breeding speed could have benefits for both the research programme as well as consumers)

Mzima Cow Project A transgenics approach to the basic mechanisms underlying trypanosome resistance

The Mzima Project Experimental Objectives

The Challenge

We are developing transgenic cattle resistant to an important disease, trypanosomiasis, that could have a major impact across Africa. Our long-term aim is to generate genetically modified cattle which carry a gene that imparts resistance to African trynanosomes. The gene APOL1, encodes for the pore forming protein component applicoprotein I-Lof



Trypanosome Lytic Factor Trypanosome lytic factor is a highdensity lipoprotein (HDL) (the good cholesterol) that circulates in the blood A-I (APOA-I), haptoglobin related protein (HPR), and the pore forming apolipoprotein L-I (APOL1).



Mechanism of Action of Trypanosome Lytic Factor TLF binds in the flagellar pocket to a recentor (mediated by HPR) and is endocytosed by the parasite. TLF is activated in the acidic endosome and APOL1 is released from the particle and inserts into the membrane forming a



The activation can be blocked by the weak neutralizes the endolvsosomal system. The pore allows the equilibration of ions down their concentration gradients leading to and the influx of water such that the parasite swells and bursts

STIC:





Apolipoprotein L-I targeted transgenic murine matings: F1 heterozygote mice are Trypanolytic African Bos indicus brees High resistance to ticks and heat Can endure scarcity of water

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Generating APOL1 and HPR Knock-In Bovine Cells

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Select ideal CRISPR Guide RN

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Broader Impacts

Trypanosome resistant cattle will survive

in the tsetse belt - 10 million square mile The women who till the land by hand (90%)

of sub-Saharan Africa) can use cattle for:

- crop production could increase 10-fold

less vulnerable to critical harvest time

Haulage, traction and soil fertility

Have milk and meat products

Have a store of wealth for future

imposed by plants

USDA HUNTER Centre for Tropical Liverteck CGIAR

Mzima Cow Project

A Transgenics Approach to Introducing Resistance to Trypanosomiasis Translating Genetic Research to Adoption and Social Value

The Challenge

Bovine Trypanosomiasis (sleeping sickness) is a significant health and economic issue, especially in sub-Saharan Africa. In Africa, the disease vector is the Tsetse fly- The "Tsetse belt" of sub-Saharan Africa has effectively been closed to mixed agricultural development, because cattle do not thrive, and

fertilisation and food There is no innate resistance in cattle. One variety (N'dama) is trypano-tolerant, but this is a complex trait that conventional techniques successfully breed into other varieties

Drugs are available, but are toxic and expensive

Ground-breaking Science

There is now the potential to produce cattle with 100% resistance to trypanosomiasis (sleeping sickness)

· Some primate hosts (including humans) are have been identified by Prof Javne Raper, City University of New York (CUNY)

Introgression of a synthetic 'construct' of these genes has been demonstrated to confer resistance in mice

 Profs Raper and Kemp saw the potential of this approach for livestock early on and since then the partnership between CUNY, the International Livestock Research Institute (ILRI) and the Centre for Tropical Livestock Genetics & Health (CTLGH) has been evolving in order to take this further

 ERI has now developed the skills and infrastructure to undertake this work in Africa, and has successfully produced Turnair ('Hope'), the first cloned bull in Africa, as a first

The Challenge of Adoption

- The scientific research elements of this programm
 "" double and understood e increasingly well developed and under
- The programme team are aware of the complexities of introducing new technologies i general use
- Animal biotechnology is a novel area particularly for Africa regulators
- Starting the change programme early is laying the way for the eventual smooth uptake of

There has been a significant broadening in focus as the team grows to include broader programme management, social science and adoption of



Developing a Theory of Change

A first step in establishing a programme aiming to have a positive impact on animal and human wellbeing is the construction of a Theory of Change: defining the goals, actors and paths to success

representing the following domains, softwartual economics, anthropology, veterinary science, public health, pastaralist, national parks, biosafety regulation, genetics, parasitology, science communication, land owners, agricultural marketing, small holders, community driven

The participants investigated the issues of the burden of trypanosomiasis, stakeholder and power mapping, impact analysis of Azima Cattle and the challenges and the priorities for action

kers and stakeholders at the Maima Cow S













Our first workshop was held in January 2017 with stakeholders

Defining the Testing Routemap

· "Systems Change at the Speed of Trust": Establishing trust is key to regulatory progress and the eventual adoption and understanding of these Mzima cattle

 Setting up a comprehensive and transparent testing schema, or routemap, is the first step in building the knowledge to provide confidence in the viability and safety of these cattle

 A workshop was held in February 2018 to gain multiple perspectives on the domains of trypanas resistance, animal welfare, human welfare and environmental welfare

· In addition, we addressed issues of containment and alianment with (emerging) National Biosafety Guideline

· At this workshop were Kenyan and internatio experts on issues relating to biosafety and animal

The report from this workshop is available from the meeting organisers and at [sti4d.com/mzima]

Next steps

Field trials

 Consolidating the testing routemap: defining the plan · Producing the first transgenic Boran calves (Late

2018/Early 2019) · Securing funding for and implementing:

Further Theory of Change and regulatory workshops

communications and monitoring & evaluation strategies

Expanding to more breeds in more centres

Development a rapid field-testing kit for the

 Workshops and planning for introduction of cattle to markets

Expanding the programme to Mzima Goats (which due to cost, size and breeding speed could have benefits for both the research programme as well



innovation expertise

ILRI



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