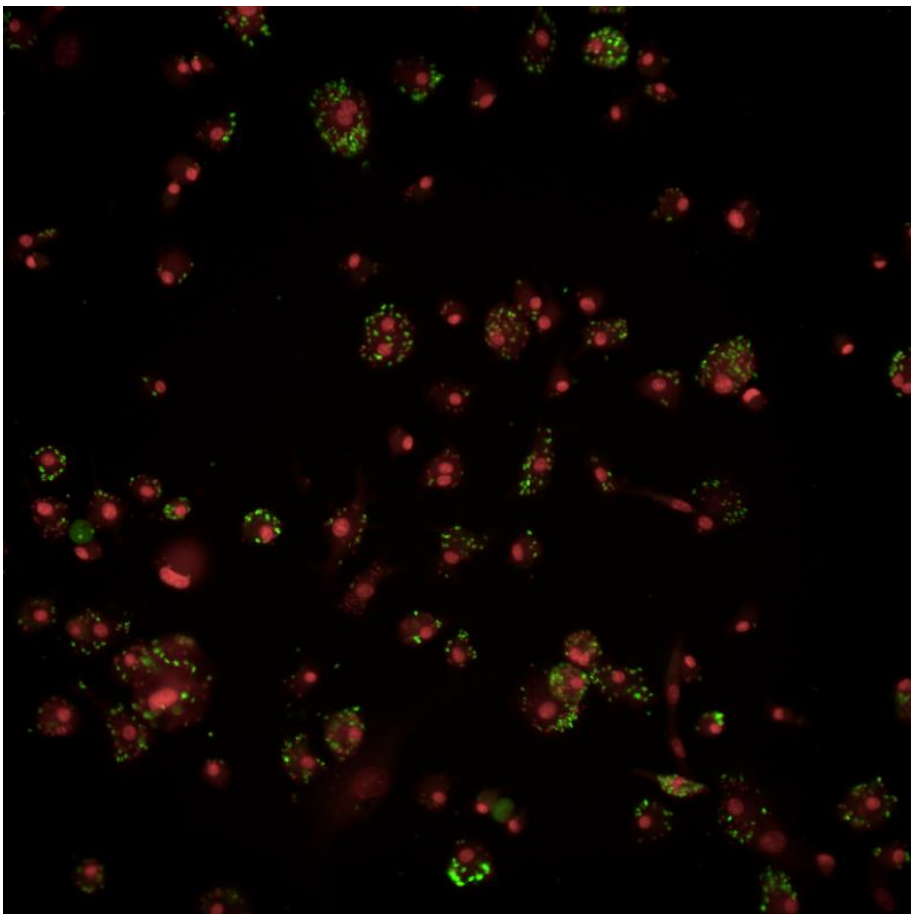
DRUG SCREENING IS A NUMBERS GAME

The Usage of Gene Technology in Drug Screening for Chagas Disease



Term Paper February 2023

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1. Preface

For our term paper we chose to take a closer look into how gene technology can be used to find and improve new drugs. We liked the idea of working on a neglected tropical disease because they aren't talked about enough in our society as they mostly affect rural areas and less wealthy regions. In this paper we will use "Chagas disease" as an example because there is a lab in Basel working on this topic and the disease is transmitted by an interesting parasite.

Our questions regarding the topic are:

- What techniques are used in the drug screening?
- What is the role of gene technology in drug screening?
- What are the characteristics of Chagas disease?
- Why are there only drugs with low efficiency to treat Chagas disease?

2. Introduction

Chagas disease is a potentially life-threatening illness, of which an estimated 6-7 million people are infected worldwide. The disease was discovered by and named after Carlos Ribeiro Justiniano Chagas, a Brazilian physician and researcher, in 1909. It is caused by the protozoan parasite *Trypanosoma cruzi,* which was transmitted to humans and other mammals through contact with faeces or urine of triatomine bugs (vector). The triatomine bugs are endemic in Latin America, but due to migration and traveling the Chagas disease has spread worldwide. The triatomine bugs do not have to be present for the T. cruzi to spread, since it can be transmitted through passage from mother to her new-born during pregnancy or childbirth, through contaminated blood- and organ donations, consumption of food or beverages contaminated with T. cruzi and laboratory accidents.

Chagas disease presents in two phases, the acute and the chronic phase. During the acute phase, which lasts for about two months after infection, a high number of parasites circulate in the blood, but in most cases, symptoms are absent or mild and unspecific. About one in three people infected will develop a chronic pathology. The consequences are enlargement of the heart, megaoesophagus or megacolon up to organ failure.[1]

There is no vaccine against the Chagas disease. It can be treated with benznidazole or nifurtimox, both of which have numerous side effects and are not approved of during pregnancy. Therefore, there is an urgent need for better drugs with less side effects.

In order to test the efficacy of possible new drugs, scientists have recently developed a new technique in which the gene coding of a luminescent protein is introduced into the parasite's DNA and the samples are being analysed with high content microscopy.[2]

3. The Parasite *Trypanosoma cruzi* and Chagas Disease

Parasite

To analyse the screening methods, we first need to understand the parasite *Trypanosoma cruzi* itself. Its life cycle is particularly interesting, because the parasite undergoes "changes in morphology, metabolism and gene expression". *Trypanosoma cruzi* is a heteroxenic protozoan. Therefore, in distinct hosts we can find the parasite in different stages of its life cycle. The parasite uses these different stages to adapt to the different environments. Every stage looks different and has its own characteristics and functions.[3][4]

1 'Chagas Disease'.
2 Fesser, Kaiser, and Mäser, 'Neue Parameter Für Die Wirkstofftestung Gegen Trypanosoma Cruzi'.
3 Gonçalves et al., 'Revisiting the Trypanosoma Cruzi Metacyclogenesis'.
4 Moody et al., Atlas of Medical Helminthology and Protozoology.

Life Stages

The most important life stages of *Trypanosoma cruzi* are the trypomastigotes, amastigotes and epimastigotes. Trypomastigotes have a characteristic C or S shape. This stage is found in the blood of humans and in the guts of insects. Trypomastigotes are non-proliferative and infective. Amastigotes are found in human cells and are able to replicate in host cells. The epimastigotes mostly exist in the insect vectors gut. They are responsible for the replicative stage in the vector cycle. Both of amastigotes and epimastigotes are believed to be non-infective.[5][6]

Insect Vector

The most common vector transmitting *Trypanosoma cruzi* is the triatomine bug. A vector is a living organism which can transmit infectious pathogens. Often those vectors are blood sucking insects. The pathogen replicates in the vector and is transmitted to a new host, often through biting the new host.[7]

Life Cycle of *Trypanosoma cruzi*

The triatomine bug is infected by feeding on an infected individual. The ingested trypomastigotes travel to the midgut and undergo metamorphosis, becoming epimastigotes. The epimastigotes then multiply and differentiate into small metacyclic trypomastigotes in the hindgut. These infectious metacyclic trypomastigotes are passed on to the host.[8][9]

The vector cycle where bloodstream trypomastigotes become epimastigotes, multiply and later differentiate into metacyclic trypomastigotes and are passed on to a new host is called metacyclogenesis.[10] This cycle can be triggered in in vitro assays in the lab to cultivate a parasite through temperature changes.[11]

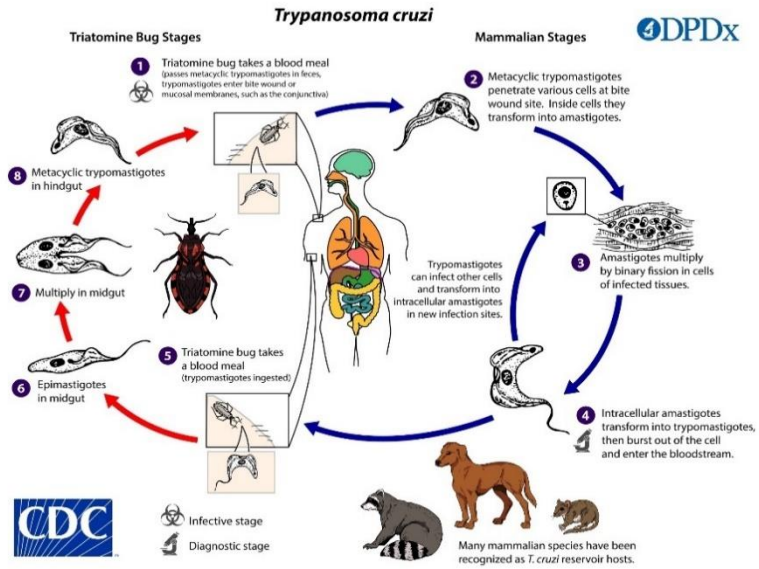

Figure 1 Life Cycle of Trypanosoma cruzi

When an infected vector bites a person, it releases its faeces containing the parasites near the wound. By rubbing them into the wound metacyclic trypomastigotes can enter the body. Trypomastigotes can also be transmitted over intact mucosal membranes by eating something containing parasites or over the conjunctiva.[12]

In the host, trypomastigotes invade the cells and there differentiate into amastigotes. There they multiply by binary fission. When there are so many amastigotes that the cell ruptures, they metamorphose back to bloodstream trypomastigotes and invade the blood and lymphs. Replication in the host only happens in the amastigote stage in host cells. The trypomastigotes travel through blood streams and can infect more and more tissues of the body by entering new cells and there replicating as amastigotes.[13][14]

5 Gonçalves et al., 'Revisiting the Trypanosoma Cruzi Metacyclogenesis'.
6 Moody et al., Atlas of Medical Helminthology and Protozoology.
7 'Vector-Borne Diseases'.
8 Moody et al., Atlas of Medical Helminthology and Protozoology.
9 Prevention, 'CDC - Chagas Disease'.
10 Gonçalves et al., 'Revisiting the Trypanosoma Cruzi Metacyclogenesis'.
11 Fesser, Interview with Biologist Anna Fesser.
12 Prevention, 'CDC - Chagas Disease'.
13 Moody et al., Atlas of Medical Helminthology and Protozoology.
14 Prevention, 'CDC - Chagas Disease'.

Disease Progression

Some of the amastigote parasites, instead of multiplying, stop even without drug treatment. Those non-multiplying amastigotes go quiet and can survive the drug treatment. Active amastigotes are killed by the aggressive medication. The surviving parasites continue multiplication months or years after, causing a relapse in the patient. Those “quiet” forms are so-called dormant amastigotes. In many patients, the chemotherapy using benznidazole or nifurtimox cannot eliminate the illness completely. The lack of drug efficacy is most likely linked to the dormant state. Finding new, more effective drugs is the goal of drug screening.[15]

4. Procedure and Techniques

Cultivation of Parasites

Mouse embryonic fibroblasts (MEF) are cultivated and infected with metacyclic trypomastigotes of T. cruzi STIB980 clone 1 (DTU TcI) which was obtained by A. Osuna in 1983. The metacyclogenesis is simulated in order to cultivate the parasites.

Determination of Basic Efficacy

The active ingredient of the possible new drug that needs to be tested is added to the infected cells. The used strain of *T. cruzi* is transgenic and expresses β-galactosidase. A chromogenic substrate is added, which reacts with the β-galactosidase and causes the parasites to turn pink. Using high content microscopy, the number of infected host cells and the number of parasites per infected cell are counted, and the efficacy of the substance can be determined. This gives a first impression on the efficacy of the tested substance. If the substance is promising, further information needs to be collected.

Introduction of Enhanced Green Fluorescent Protein

In order to determine the effects of the tested active ingredient on the replication rate of the parasites, the gene coding for an enhanced green fluorescent protein (eGFP) is introduced into the parasite’s DNA. The gene coding for the eGFP lies on a Plasmid which also contains a gene coding for an antibiotic resistance, which prevents the parasite from cutting out the plasmid DNA and functions as a marker. It also contains a ribosomal promoter and an untranslated constitutional active 3‘ area to make sure that the plasmid DNA is being transcribed and translated. The Plasmid is introduced into the parasite by electroporation.

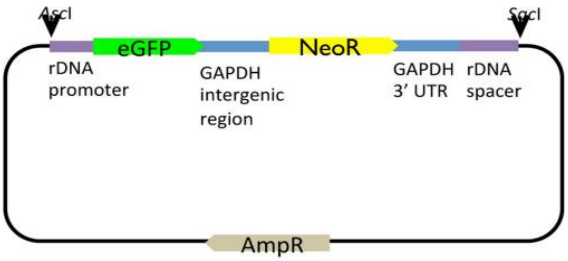

Figure 2 Composition of Plasmid

15 Sánchez-Valdéz et al., ‘Spontaneous Dormancy Protects Trypanosoma Cruzi during Extended Drug Exposure’, n.d.

Evaluation with High Content Screening

If the plasmid was successfully introduced into the parasites DNA and this parasite replicates, the eGFP should be expressed. Therefore, the replicating stages become green fluorescent. Fluorescence and light microscopic images are taken every four hours in a high content microscope. From these images, the number of parasites is determined. The DNA of host cells and parasites gets stained with Hoechst 33342. This allows us to determine whether the number of green-fluorescent parasites per image corresponds to the absolute number of parasites. In the treated wells, the replication rate decreases as a function of concentration and duration of treatment. A graph of the calculated rate of change of concentration and duration of treatment shows an individual profile for each individual tested substance. This way an impression of whether the substance belongs to the class of concentration-dependent or time-dependent substances and how well it works is gained.[16][17]

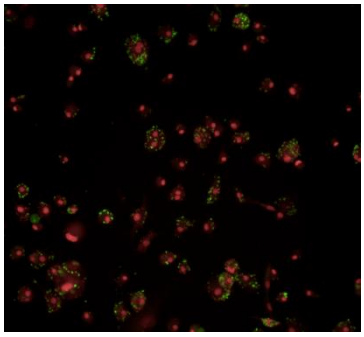

Figure 3 High content microscopy image of eGFP expressing T. cruzi and with Hoechst 33342 stained host cells and parasites

High Content Screening

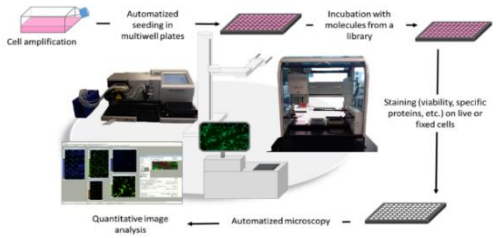

Figure 4 five steps of high content screening

Hence high content screening is a type of phenotypic screen conducted in cells involving the analysis of whole cells or components of cells with simultaneous readout of several parameters. It is a combination of modern cell biology, with all its molecular tools, with automated high-resolution microscopy and robotic handling. High content imaging is based on 5 steps: First, the wells are seeded in multiwell plates. Then, they are incubated with molecules from a library. After incubation, cells can be labelled to study some live cell parameters, as the viability or cell proliferation for example. Then, cells are fixed and stained by immunocytochemistry or like in our case by fluorescent probes in order to study expression and localization of proteins of interest, of subcellular components etc. The results are acquired by fluorescence microscopy, and quantitatively analysed. The development of high content screening in the 90s was a major breakthrough in drug discovery as it makes it possible to analyse more substances in a short amount of time.[18][19]

5. Interview and Lab Visit

For our term paper we consulted with biologist and epidemiologist Anna Fesser. We were able to contact her through family friend Kirsten Gillingwater. Anna Fesser studied at the University of Basel and worked for the Swiss Tropical & Public Health Institute, where she did her PhD. Now she works as an epidemiologist for the BAG. She is also one of the authors from our main source, the research paper "*Neue Parameter für die Wirkstofftestung gegen Trypanosoma cruzi*".[20] Therefore, she was the perfect researcher for our interview. The following is a short section from our Interview with her. The full interview can be found in the appendix.

Figure 5 Biologist Anna Fesser

[16] Fesser, Kaiser, and Mäser, 'Neue Parameter Für Die Wirkstofftestung Gegen Trypanosoma Cruzi'.
17 Fesser et al., 'Non-Invasive Monitoring of Drug Action'.
18 Conto, 'What Is High Content Screening (HCS)?'
19 'High-Content Screening'.
20 Fesser, Kaiser, and Mäser, 'Neue Parameter Für Die Wirkstofftestung Gegen Trypanosoma Cruzi'.

Interview with Anna Fesser, Basel, 29.01.2023

Q: What is your personal motivation to work or have worked in this field/ on this project?

A: My personal motivation is to work on a disease that, on a global scale, impacts people who are on the margins of society. Chagas disease basically only affects Latin American people but also mostly people who are from rural areas or have a migration background. Even in Switzerland, where Chagas disease is present, this often affects "sans-papiers". […]

Q: What methods did you use to introduce the gene coding for the fluorescent protein into the parasites DNA?

A: So, when I was doing my PhD, I used the traditional method which is homologues recombination. This works not for all organism but t. cruzi has - like mammals - this ability to use some pieces of similar DNA and then repairs breaks in their genome with that. So, whenever there are breaks in their genome, they use pieces of the same or at least similar regions to repair their genome. And this is being used traditionally in genetics to do the repair. […]

Q: So, you need to rely on the cells breaking by themselves?

A: Yes, exactly. You can break the genome and get the plasmid in with electric shocks but what I really was dreaming of was the use of Crispr cas9, which was at the time popular in other organisms but not yet established in t. cruzi. You probably have heard of that. That would solve the problem of relying on an accidental break of the DNA. The you can go and say okay, I give you the postcode and you go there, and you cut that area. Then about 50% of the cells get the break exactly on the point where you want it to be. It's much easier to select and much easier to work with.

Currently, the woman who is doing her PhD after me, she's using that and I'm really curious whether that will work better or not.

Q: Was there a reason why you were not able to use the Crispr cas9 method?

A: Yes because you have to have an organism which already has Crispr cas9. So, you have to introduce Crispr cas9 by this way. And I was the first one in our lab to even do transfections or to do genetic manipulation on t. cruzi. So, I got material from other labs who've already done the methodology and were just about to develop the Crispr cas9 technology. […]

Q: So Crispr cas9 will be an improvement for future steps?

A: Yes, right now there are a number of cas proteins which I honestly don't really know. One I have heard of is cas13 where you're cutting RNA and you can regulate translation. In 2018 this method won the "breakthrough of the year" of the science magazine, which I think was fully granted. I mean when you think of genetic manipulation you mostly think of "designer babies" in China but most of the genetical manipulation happens like in my case just for being able to detect a parasite, for a drug screening assay or to study certain genes. […]

Q: What do you think are the advantages of this fluorescent protein?

A: The advantages of it were that it was available to me, I mean it is a very bright protein and it worked to use it. Should I already start with the disadvantages? [laughs] It has a major one that is fluorescent itself has a lot of electrons moving, so where you have a fluorescent protein, you always have oxidative stress which is like electrons moving around which might hit another protein and they shouldn't go there but then they do. There are now more modern versions of fluorescent proteins which reduce that stress. […]

Q: Is there a risk of misuse or abuse of this technology?

A: I'd say no. I mean there's always this lab leakage question but then if my parasites would get out of my lab, the risk is more that they are parasites and not that they carry around this green fluorescent protein. This might even help us detect them and they are a little hindered by that. The big risk is really that they are parasites. […]

Q: What are your plans for the future?

A: I will start doing a statistical class to get more into clinical statistics or statistical epidemiology which is where I am at the BAG right now. In my PhD I realised how much I like statistics but for a long time I really wanted to become a professor, I wanted to work in neglected tropical disease and Chagas disease and maybe also leishmaniosis (this is like a cousin's disease of Chagas disease, very similar organisms but a quite different disease). […]

Q: If you had unlimited research funds, what would you do with them?

A: I think one step would be – I even applied for this position – in a lab, they use single cell sequencing, they separate every cell in a flow cytometry and then look inside the cell which RNAs are inside that cell or which genes are being produced in that specific cell. […] *…do a lot of drug screening if you have unlimited funds because drug discovery is always a "numbers game". It has two aspects – you have to make sure you're looking for the right thing for Chagas disease it's not only which concentrations numbers decrease the parasite, but you also want to look at "do I really kill all of the parasites?". That's the question you want to ask in drugs screening for Chagas disease which might be different in another disease.*

Visiting the Lab

We also contacted Monica Cal to organize our lab visit. She works at the Swiss Tropical & Public Health Institute (Swiss TPH) and helped us with our research. With Monica Cal we were able to visit the Swiss TPH and its labs which are usually not open for visits. She gave us a tour of the institute, showed us the high content screener, and explained many interesting details about the parasite and the testing in the lab. Unfortunately, we couldn't look at *Trypanosoma cruzi* in the lab because it requires bio safety level 3, for which you need training beforehand. But we were able to look at a very similar parasite, *Trypanosoma brucei*. It was in the trypomastigote-state, where it looks almost identical to the *Trypanosoma cruzi* trypomastigotes.

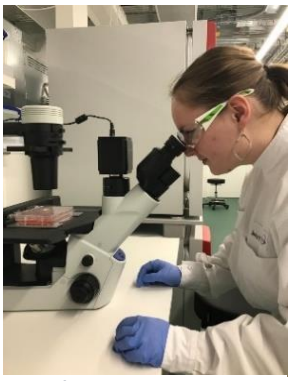

Figure 6 Trypanosoma brucei under the microscope

Impressions

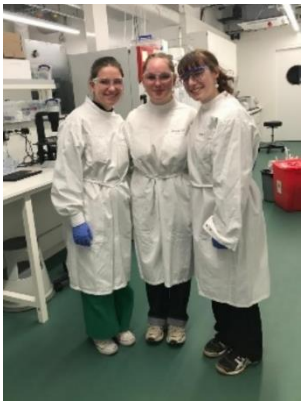

Figure 7 Us in the laboratory

We learned a lot from our interview and visit to the lab. It was interesting to see the behind the scenes of a bio laboratory. Monica Cal gave us an insight in a potential job for the future. A job in which you have many opportunities and definitely a diverse everyday life. It was also very nice to apply the theory from school lessons in practice and to actively use our knowledge and skills. Dealing with and learning about a topic that is not so well known was very enriching for us. Chaga's disease is relatively unknown and, as Anna Fesser said, there are only about two hundred people researching about this parasite and the disease. A disease which is still unccureable and in most cases deadly. We hope that the tropical infection gets a little bit more attention through our term paper and we raised awarness and inspired people to dedicate their time and effort into research about the disease and the parasite *Trypanosoma cruzi*.

6. Discussion

Progress with the Application of High Content Screening

Introducing fluorescence into *T. cruzi* enables the application of high content screening of active ingredients for possible new drugs against Chagas disease. This enables scientists to distinguish dead or non-active parasites from the reproducing ones. The application makes the drug screening much more efficient because a huge amount of data can be gathered in little time and scientists don't have to spend hours in the lab counting parasites as it was the case before. Using electroporation to introduce the Plasmid has a much higher success rate ccompared to the traditional method of homologues recombination.

Ethical Aspects

Chagas disease mostly occurs in poorer regions in the world because healthcare in these areas is less developed and the triatomine bug that transmits the parasite lives in such tropical and often third world countries. It also occurs in Europe, but generally "*impacts people who are on the margins of society*".[21] Chagas disease is a neglected tropical disease. Those are almost completely ignored by the global health agenda. No profit is in sight when studying *Trypanosoma cruzi.* This is why research funds are often hard to come by.

Although medication for the disease exists, we must ask ourselves important ethical questions, as the side effects are drastic. They include gastro-internal effects, chest pain, headache, dizziness etc. It's also important to mention that the existing medication is almost only effective in the active state of the disease. The medications (benznidazole and nifurtimox) get less effective the more time has passed since the infection. So, there is a need for new medication with less side effects. Additionally licensing requirements for new medication are much stricter today, so the approval for a new drug is even harder to get. The requirements for infectious diseases are the same as for other drugs. A possibly carcinogenic molecule may not be given to a patient, even if the patient will most possibly die of Chagas in the timespan of a few months. This makes the possibility of discovering a new active substance which is not too harmful even smaller.[22][23][24]

Using gene technology to improve drug screening and understanding the parasite better leads to a higher chance of finding new drugs for Chagas disease. This would be a huge opportunity for all the people suffering and possibly dying from Chagas. The possibility of curing all these people or at least finding a drug with less side effects is the ultimate goal.

Pros and cons

Pros:

- The induced protein made the parasite visible under a luminescent microscope.
- Using luminescence makes the high content screening possible as it makes the infected spots visible.
- The luminescence shows in reproducing parasites only. You can distinguish dead/non active parasites from the reproducing ones.
- The luminescent protein was available at the time and compared to other methods not very expensive.
- A huge amount of data can be gathered in little time by using high content screening. Instead of counting parasites by hand for hours in the lab, scientists can let the HCS system do the laborious work for them.
- More active ingredients can be tested, because of less work time used for one screening process.
- There is little risk of misuse of this technique.

Cons:

- When using electroporation to introduce the eGFP into the parasites DNA, only one in 10^7 cells successfully include the plasmid into their DNA.
- The parasites lose their fluorescence in the generations because the eGFP causes oxidative stress. They can only be used for a maximum of 4 weeks.
- High content screening is very expensive. An HCS system costs 500'000 Fr.
- The high content screening creates huge amounts of data which are hard to handle.

21 Fesser, Interview with Biologist Anna Fesser.
22 'Chagas Disease'.
23 'Nifurtimox Side Effects'.
24 'Benznidazole Side Effects'.

Future Research Steps

One major future goal would be to study the parasite and finally figure out how exactly it works. The ability of the amastigotes to go into a dormant stage must be studied. The question why certain amastigotes suddenly stop multiplying and start again up to years later and what could trigger this phenomenon remains a mystery. Until we figure out this quiet stage of the illness, it will be nearly impossible to find a drug that doesn't have a high chance of relapse. According to Anna Fesser, using single cell RNA sequencing to compare the "active" and "dormant" amastigotes could be a topic for future research.[25] When looking at mRNA it gives us a lot of information about cellular traits and changes in cellular state because we look at what is expressed.[26] Getting more knowledge might improve drugs for the chronic state of the disease and will help to kill off all the parasites in the body.[27][28]

Another question is, if it would be possible to create a vaccine as a precaution which could stop the parasite to be infectious.[29]

To be able to manipulate the parasite better Crispr cas was a huge breakthrough too. Currently scientists at Swiss TPH are working on using this technique to induce other helpful genes such as a fluorescence.[30]

Another branch which needs future steps is making the in-vitro assays and the high content screening better in order to make the drug screening easier and as accurate as possible. Also, a lot of scientists, including the ones we talked to, are convinced that Chagas research needs to be done on in-vivo assays to better understand replication, differentiation, and the effect of drugs.[31] In the Siss TPH lab scientists are going to start working on rats in the next few years.[32]

In the end, as Anna Fesser said, drug discovery remains a "*numbers game*"[33]. Even with improved techniques, more knowledge and unlimited research funds the only way to find a new drug is testing one molecule after the other until one has an effect on the parasite. Sot, the more tests we run, the more likely it is that we will find a cure to Chagas disease.

7. Summary

It is a difficult task to find a good drug for curing the Chagas disease as it is transmitted by a vector, it presents asymptomatic for a long time after the infection and the parasites can enter so called dormant stages in which they are inactive and therefore very hard to detect and kill. Since Chagas is a neglected disease research funds are very limited. Despite all these facts, the development of high content screening with the help of a fluorescent protein being introduced into the parasites DNA is a big first step in finding better drugs to cure the Chagas disease. Scientists are already taking further steps by introducing Crisper cas into the parasites. We are certain that if scientists keep playing the numbers game of drug screening, a new and better drug against the Chagas disease will be found soon.

25 Fesser, Interview with Biologist Anna Fesser.
26 Haque et al., 'A Practical Guide to Single-Cell RNA-Sequencing for Biomedical Research and Clinical Applications'.
27 Sánchez-Valdéz et al., 'Spontaneous Dormancy Protects Trypanosoma Cruzi during Extended Drug Exposure', n.d.
28 'Improving antichagasic drug discovery through stage-specific monitoring of Trypanosoma cruzi'.
29 Fesser, Interview with Biologist Anna Fesser.
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Images

9. Appendix

Interview

The full Interview with our **questions** and *answers* by Anna Fesser, 29.01.2023, Basel

Q: What is your personal motivation to work or have worked in this field/ on this project?

A: My personal motivation is to work on a disease that, on a global scale, impacts people who are on the margins of society. Chagas disease basically only affects Latin American people but also mostly people who are from rural areas or have a migration background. Even in Switzerland, where Chagas disease is present, this often affects "sans-papiers". So, for me, when I started working in biology, I always liked the interaction of things like within molecules for example, but then you also have interaction between cells and organisms up to now where your kind of working with the interaction between humans which can make a huge difference. All of these interactions are interesting to study but, whenever you're working in science, you have to give a lot into it. So now, if I have to give a lot of myself, I want to do something that is important and has an impact. But now at the BAG, it's also really nice to just do something you're interested in, even if the impact might not be as good. I had to leave the field because I wasn't seen as "good enough" and there for my research didn't get enough funding. So, then I also couldn't do an impact. And now it's like whenever I do something that's good and not bad, I make an impact. And now people see me and my work and that's the way I want to go. People also think that the most problematic tropical diseases are located in Africa but that's not true. There are many diseases which are not located in Africa but still affect mostly poor people.

Q: What methods did you use to introduce the gene coding for the fluorescent protein into the parasites DNA?

A: So, when I was doing my PhD, I used the traditional method which is homologues recombination. This works not for all organism but t. cruzi has - like mammals - this ability to use some pieces of similar DNA and then repairs breaks in their genome with that. So, whenever there are breaks in their genome, they use pieces of the same or at least similar regions to repair their genome. And this is being used traditionally in genetics to do the repair. So, what we are doing is that we have the gene of interest which in my sense is the enhanced green fluorescent protein to put around some areas which make sure that the gene will be first translated and then later transcribed. You have regions where the polymerase transcribes and then you also have some untranslated regions to make sure that the gene really is translated into the protein at the right time. I used the non-coding region, which is supposed to be translated always what later turned out to not always be translated but mostly the replicating areas. Then you have the antibiotic resistance which makes sure that the gene is maintained there and not the parasite sorting it out because it thinks it's not using that for its personal benefits. This also happened in my study. And then you also have another part outside where it's flanking the region where it's targeted in the genome. There you have a break in the genome. Only about 1 in 10^7 of the parasites have a break in this area and then also repair it and then you select them.

Q: So, you need to rely on the cells breaking by themselves?

A: Yes, exactly. You can break the genome and get the plasmid in with electric shocks but what I really was dreaming of was the use of Crispr cas9, which was at the time popular in other organisms but not yet established in t. cruzi. You probably have heard of that. That would solve the problem of relying on an accidental break of the DNA. The you can go and say okay, I give you the postcode and you go there, and you cut that area. Then about 50% of the cells get the break exactly on the point where you want it to be. It's much easier to select and much easier to work with.

Currently, the woman who is doing her PhD after me, she's using that and I'm really curious whether that will work better or not.

Q: Was there a reason why you were not able to use the Crispr cas9 method?

A: Yes, because you have to have an organism which already has Crispr cas9. So, you have to introduce Crispr cas9 by this way. And I was the first one in our lab to even do transfections or to do

genetic manipulation on t. cruzi. So, I got material from other labs who've already done the methodology and were just about to develop the Crispr cas9 technology. So actually, later on I went to lab which used that and brought the plasmids with me back, so the woman who's doing her PhD now can use that. But it was already clear it was too late for me to use it. And also, at the end what I did was to just get some fluorescence in the parasite. The area I was targeting was the ribosomal area, there are at least 10'000 copies of the genome, so it enhances the chances to not have one right spot but maybe ten or even a hundred spots where you could get it in. So, for my work it wasn't as necessary to use Crispr. It gets more complicated if you want to use one specific gene and you need to make sure you only hit that one specific area.

Q: So Crispr cas9 will be an improvement for future steps?

A: Yes, right now there are a number of cas proteins which I honestly don't really know. One I have heard of is cas13 where you're cutting RNA and you can regulate translation. In 2018 this method won the "breakthrough of the year" of the science magazine, which I think was fully granted. I mean when you think of genetic manipulation you mostly think of "designer babies" in China but most of the genetical manipulation happens like in my case just for being able to detect a parasite, for a drug screening assay or to study certain genes. Most of the genetic manipulation stays on a laboratory level and never leaves that. But it enhances research for basically all organisms because the chances are so much higher to introduce the genetic material you want to work with. I'm a big fan of that but it has to be used wisely for sure. You want to think about what you're doing and want you want to do with it. But it's a big breakthrough.

Q: What do you think are the advantages of this fluorescent protein?

A: The advantages of it were that it was available to me, I mean it is a very bright protein and it worked to use it.

Should I already start with the disadvantages? [laughs] It has a major one that is fluorescent itself has a lot of electrons moving, so where you have a fluorescent protein, you always have oxidative stress which is like electrons moving around which might hit another protein and they shouldn't go there but then they do. There are now more modern versions of fluorescent proteins which reduce that stress. This was one of the reasons my parasites lost their fluorescents in the generations. After three rounds, half of the population didn't express dfp [Diisopropyl fluorophosphate] *anymore. I believe they were already under a major stress because they are inside the cell and then they also had the stress of electrons moving around inside them which shouldn't be moving around. But I mean yes, whenever you do drug screening there is always the question "how can I find out how many parasites are in there?" and therefor fluorescent is a big thing because then you can see them (of course also with fluorescent microscopy) but then you are able to count them. That's what really matters. And honestly speaking I was also quite fascinated when I was in the lab, and I had the first culture transvector and the first look myself at the fluorescent protein. It's very dark and then you see those bright green spots and then your like "yeah" [laughs]. And you can make pretty pictures with them.*

Q: Is there a risk of misuse or abuse of this technology?

A: I'd say no. I mean there's always this lab leakage question but then if my parasites would get out of my lab, the risk is more that they are parasites and not that they carry around this green fluorescent protein. This might even help us detect them and they are a little hindered by that. The big risk is really that they are parasites. We work in a level 3 facility, where you use glasses, two pairs of gloves and you have an overall as well and some people even use more than that. We don't really use masks, but we use glasses to at least not infect our eyes. Because the parasites are pretty versatile wherever they get. The skin is a good barrier but if you have the slightest lesion or touch your mouth, that would be really bad. But the genetic manipulation is not the dangerous part in this case.

Q: What are your plans for the future?

A: I will start doing a statistical class to get more into clinical statistics or statistical epidemiology which is where I am at the BAG right now. In my PhD I realised how much I like statistics but for a long time I really wanted to become a professor, I wanted to work in neglected tropical disease and Chagas disease and maybe also leishmaniosis (this is like a cousin's disease of Chagas disease, very similar

organisms but a quite different disease). I really wanted to know – I don't know how much you know about Chagas disease – the parasite stays inside the body for years and infects many organs and the major problem is the heart. It might not be always infected but every now and then it is infected and the immune system fights against this, but it also simultaneously destroys heart cells which leave scars at the heart, and this leads to heart problems. This is the major cause of death. The big question in the beginning was where these parasites actually reside, and this question was solved right when I started my PhD – they can reside everywhere but often they stay in the gap tissue. There was also a big trial to find new drugs. There was this one new drug where parasite numbers would go down rapidly but as soon as you release the drug, the numbers would go high up again. So, they realised they didn't kill all the parasites, they got them into a resting stage, and this might be very similar to the stage they are already in when they staid over 30 years in your body. The big question is how do they stay so long in your body? How active are they? Are they metabolically active? Are they reproducing? Are they increasing their numbers? Are they down regulating the immune system? So I actually wanted to study how the regulation of this parasite works. What makes the cell decide if it divides and becomes more cells or stays there quietly until maybe one reproduces again. I wanted to study what are these decisions and there is a lot of research being done in a lot of different organisms which actually have a little bit of a similar problem. You always have some cells which are not really active but when "the rock hits" they survive and come up again. So I wanted to do some more approaches they had done in cancer research or look at some proteins which seemed important in cancer regulation and then use genetic manipulation to knock them out and get rid of them or down regulate them (at a certain moment in their life) and look what happens. That was my major interest.

Q: So, you could use genetic manipulation for finding new drugs and also finding new treatment?

A: Yes, I really believe especially in Chagas disease we don't know how the parasite works (when does it decide to go quite, when does it decide to get active, when is it a good target for drugs when is it not and also what kind of drug might work) So I really wanted to know how the parasite works first and then decide which kind of drugs could you use to work against them and also when you're doing drug screening, what do you want to look for as a sign the parasite is really gone.

Q: If you had unlimited research funds, what would you do with them?

A: I think one step would be – I even applied for this position – in a lab, they use single cell sequencing, they separate every cell in a flow cytometry and then look inside the cell which RNAs are inside that cell or which genes are being produced in that specific cell. So, with single cell sequencing you can look at the whole population of parasitic cells and see where are the quite ones and where are the active ones and how do the differentiate between each other. And then with this look which genes are the ones which thrive the quiescence and then you would experiment with that, you knock out the gene and maybe introduce different copies of that gene to look at certain areas of the protein which might change its regulation or just put more copies of it in and see which replicates more often or less often. I think that's the way to go with that disease. And I mean Kirsten [Gillingwater] *would probably say do a lot of drug screening if you have unlimited funds because drug discovery is always a "numbers game". It has two aspects – you have to make sure you're looking for the right thing for Chagas disease it's not only which concentrations numbers decrease the parasite, but you also want to look at "do I really kill all of the parasites?". That's the question you want to ask in drugs screening for Chagas disease which might be different in another disease. So, make sure you ask the right question and then test all the molecules available to make sure whether one of them works because you can always have a rational for why a certain drug might work or not but most often you just stumble across them.*

Q: You kind of already touched on that but what are the steps that need to be taken in order to find treatment?

A: I think there are really different tracks to go on. One definitely being go study the parasite and really understand – most of the researches for Chagas disease, which are about a hundred to two hundred people, are pretty certain that this quiet stage is the stage we have to tackle and we have to understand and I'd completely agree with that. Maybe there's even a way to kill them that stage or a way to wake them up to make sure they are all woken up. On idea is also that the immune system isn't able to find them it that quiet stage as well and is there a way to help the immune system find them better.

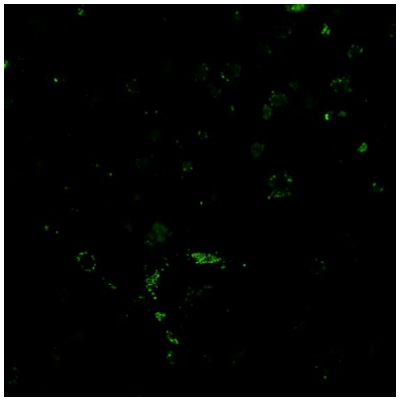
So far, we don't have a vaccine, so that would also be a question is there a certain thing you could vaccinate. Either that the parasite can't infect people – that would be great – or the people who are infected could be treated with the vaccine. So that is one branch. The other branch is to make the in-vitro assays better. That's what I kind of tried in my PhD, didn't really work out that well. But find a way to really see if there's no parasite left in your treatment. And then just do the assays. The next step is not very popular but once you have discovered in a petri dish or a well-plate a drug, you'll have to go on to the animal testing. There you have to make sure to find the parasite which is in an animal very different to the lab. That's also a bit of a tough job to work on the animals.

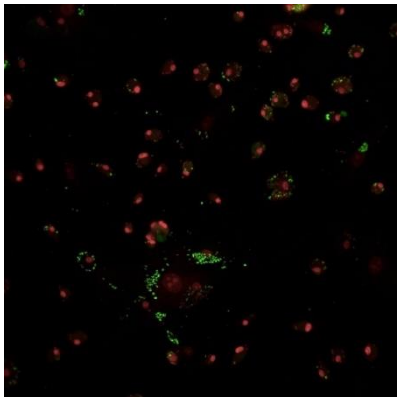
Q: I was wondering, in the next few years, which techniques apart from Crispr will make a difference?

A: I mean we touched on single cell RNA sequencing, but I have done parasitology which is always a little bit behind than f. e. cancer biology which is way ahead (because they have more money and more people working on it). I don't really know what's the "hot shit" in cancer biology but as far as I see, we're increasing recognising whatever organism we have or organ or even when we look at cancer or when we look at parasite infection, we don't have one homogenous ball of cells. Cells are different, cells have different functions so there might be a cell or let's say the community decided they go quiet, and the rest is more like the warriors – in cancer this would be the metastases – to go settle somewhere else. So, in biology we have to make sure to capture those differences and really know if there's a real difference or not the just the difference in one pipet or in another. So, I'd say the single RNA sequencing to really make sure we know the differences. And also, the whole regulation of genes and epigenetics. I think even when I studied biology from 2008 to 2012, we were kind of taught there's like 1 to 5 % in the human genome that is coding material and the rest is junk, but they already told us that that's wrong. But it still was kind of like a thing and we were more and more recognising how it's not junk but it's regulating and fine-tuning things. So, all these things [...] (single cell RNA sequencing, high content microscopy (where you improve the technique by using more wells and sides, doing more pictures)) are ways to improve research in the future.

Extra Pictures

High content microscopy images:





BSL 3 laboratory:

High content screener in the Swiss TPH: