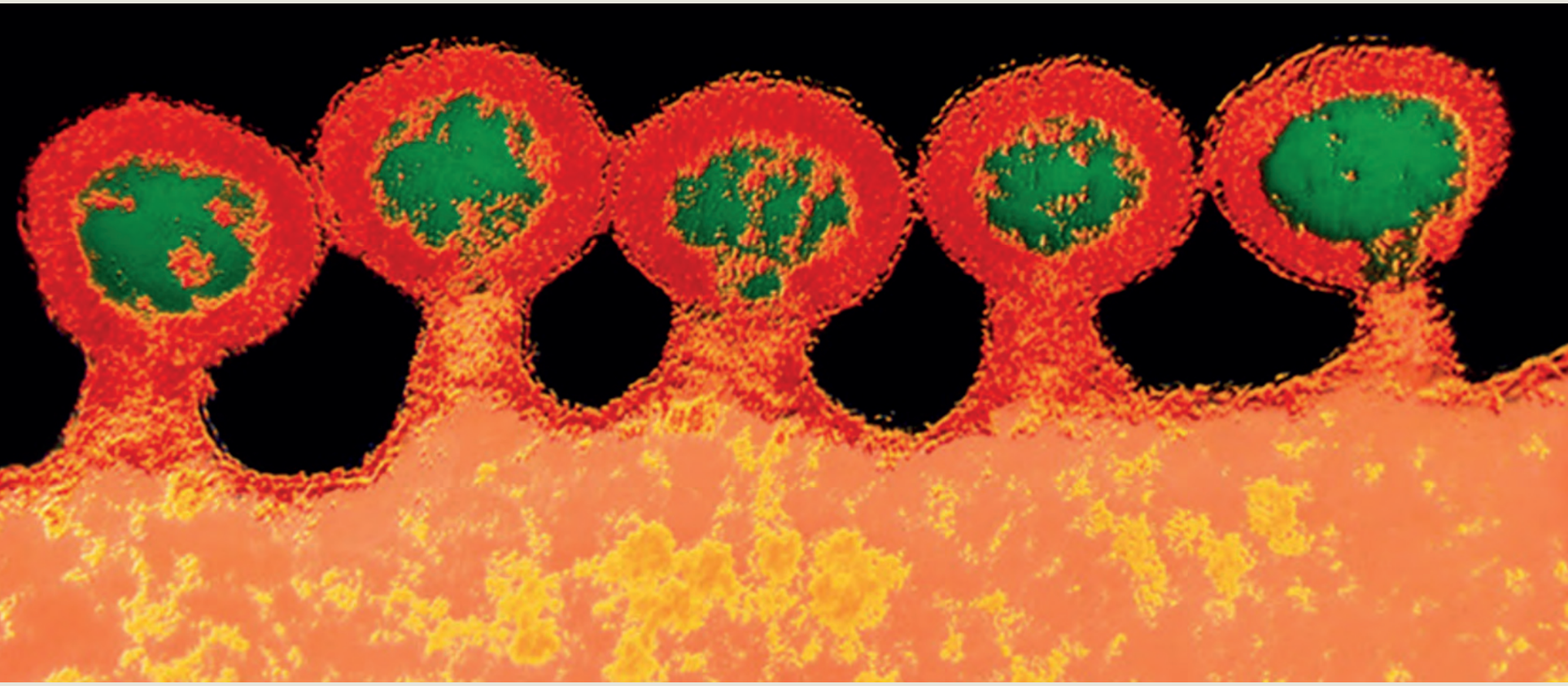
2

JOINT RESEARCH PROJECTS

COMMUNICABLE DISEASES



HI Virus

Sanitation, food control and antibiotics have reduced the toll of communicable diseases, saving millions of lives. Smallpox was eradicated in 1977, and poliomyelitis eradication is close. Measles mortality has reduced drastically yet outbreaks occur where immunisation lags.

But for every step forward, a substantial obstacle has appeared. For example, micro-organisms have developed resistance to some antibiotic drugs and they continue to mutate in ways that make their eradication ever more difficult. The human immunodeficiency virus (HIV) emerged in the 1980s, grew into a global HIV/AIDS pandemic costing millions of lives and, despite progress, remains a major global health issue. Highly active antiretroviral therapy (HAART) has transformed HIV into a manageable chronic disease; however, significant HAART-induced and/or HIV-related morbidity continues to exist.

Malaria and tuberculosis (TB) still cause millions of deaths. The worldwide prevalence of malaria is estimated to be in the order of 250 million clinical cases annually, of which one million people die.

TB is a disease that is thousands of years old and, while other diseases that have subsequently emerged have been effectively controlled, TB still stands out today as one of the worst public health threats with which modern medicine is battling. More people are dying of TB than ever before and Mycobacterium tuberculosis (MTB), the causative agent of TB,

is now responsible for the largest numbers of human deaths due to a single bacterial pathogen.

The lethal combination of HIV and TB, coupled with the evolution and spread of multi- and extensively-drug-resistant MTB strains, has magnified the burden of disease, particularly in developing countries.

Influenza pandemics with new, deadly versions continue to appear. Neglected tropical diseases are responding to global donor efforts, but newly emerging diseases move to new regions and become endemic, while deadly localised haemorrhagic fevers threaten to transmit more widely. Rapid mass travel allows infectious diseases in isolated villages to quickly become global threats and has brought the opportunity for nearly anyone anywhere to become infected with what were formerly thought to be "exotic" diseases.

About one new infectious disease organism has been discovered each year for the past 50 years. "The 21st Century is likely to be marked by a proliferation of infectious viral illnesses," says Antonio Hernandez Conte in *Infectious Diseases*. "There are few new antibiotics under development to combat gram-negative organisms." (Conte, 2018).

New strains of viruses, antibiotic resistance and micro-organisms causing chronic diseases are challenges for infectious disease control requiring continuing political, financial and scientific support and much tenacity (Tulchinsky and Varavikova, 2015).

South Africa is still battling the burden of infectious diseases, but according to health findings published in a dedicated issue of *The Lancet* as part of the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), South Africans are living longer lives than they were 10 years ago. In South Africa, HIV/AIDS was the leading killer, resulting in 112 243 deaths in 2015. The second and third top causes of death were ischemic heart disease and tuberculosis related to HIV/AIDS, killing 45 119 and 42 943 people respectively (Centre for Disease Control and Prevention).

The CDC in South Africa has implemented a quality improvement system within 1 159 facilities providing HIV-rapid testing within the 27 focus districts in the country. The first cycle of proficiency testing has seen a pass rate of 97% for all facilities enrolled.

TB still remains a crisis in South Africa. At the time of the launch of the latest National Strategic Plan in 2017, the South African Government said: "We have made major gains in terms of treating millions of people living with HIV and TB, slashing the death toll due to these infections, and reducing the number of new infections. However, there is still a great deal to be done." The National Strategic Plan aims to intensify efforts in the geographic areas that are most affected. In addition, the highest impact interventions are to be used in these areas.

Switzerland has an internationally recognised history in supporting research and implementation of pilot approaches to control communicable diseases, mainly in the field of malaria and neglected diseases, such as Trypanosomiasis (Health Network of the Swiss Agency for Development and Cooperation – SDC).

Global, national and local capacities are strengthened to reduce the morbidity and mortality related to communicable diseases such as HIV and AIDS, malaria and TB as well as to achieve and/or sustain high levels of immunisation.

Other communicable diseases – such as diarrhoea or acute respiratory infections (pneumonia) – are also prioritised, as they are major causes of mortality among children under the age of five in low-income countries. Neglected tropical diseases affecting the poorest quintiles of society remain a priority for the SDC. Throughout its programming, SDC promotes a multisectoral and systemic approach, including the mainstreaming of HIV and AIDS. It also promotes the integration of HIV and AIDS and sexual and reproductive health services. To mitigate the impact of the epidemic in countries with a high HIV prevalence, SDC prioritises prevention activities, psychosocial support and social protection mechanisms.

OUTCOME OF THE COMMUNICABLE DISEASES DOMAIN: ECONOMIC VALUE

Projects in this domain featured strong industry support, with the resultant social impact. Of particular importance is the innovation impact of the projects and their potential going forward.

INNOVATIONS	TOTALS	%
Project achieved innovation	8	40
Impact innovation achieved	5	25
Projects have innovation potential	11	55
Innovation potential beyond project	11	55

INDUSTRY LINKAGES	TOTALS	%
Research support from industry	7	20
Industry funding	3	15
Industry partner SA	7	20
Industry partner CH	2	10
Industry interested	2	10
SA Industry funds received	0	0
Swiss Industry funds received	2	10

INTELLECTUAL PROPERTY	TOTALS	%
Joint IP	1	5
Swiss IP	2	10
SA IP	3	15
Swiss IP protected	2	10
SA IP protected	3	15
Joint IP projected	1	5
Open innovation	1	5

Joint Research Projects

Outcomes of the Communicable Diseases Domain (20 projects)

RESEARCH DOMAIN: COMMUNICABLE DISEASES

TOTAL FUNDS INCLUDING THIRD-PARTY FUNDING:
CHF 9 362 172 ZAR 144 525 148

UNIVERSITY PARTNERS

University of Basel
University of Bern
Esperanza Medicines Foundation
Swiss Tropical and Public Health Institute
Swiss Federal Institute of Technology in Lausanne
Swiss Federal Laboratories for Materials Science and Technology
University of Lausanne
University of Geneva
Swiss Institute of Bioinformatics
Swiss Federal Institute of Technology Zurich
University of Zurich
University of KwaZulu-Natal
University of Cape Town
University of the Witwatersrand
University of Pretoria
University of Western Cape
Nelson Mandela University
North-West University
National Institute for Communicable Diseases
National Health Laboratory Service

SEED FUNDING

PHASE I — 2008 – 2012 — 6 projects
PHASE II — 2012 – 2013 — 5 projects
PHASE II — 2013 – 2016 — 6 projects
PHASE III — 2017 – 2020 — 3 projects

Phase III projects have not as yet reached full scale scientific outputs.

BENEFICIATION

ECONOMIC
- **35%** Technology development
- **45%** Economic platform
- **35%** Industry development
- **15%** Africa economy
- **15%** Knowledge economy

GLOBAL CHALLENGES
- **90%** Global disease
- **85%** Africa challenge
- **75%** Neglected tropical diseases

NATIONAL OBJECTIVES
- **25%** Policy beneficiation
- **70%** National strategies in South Africa
- **45%** HCD of historically disadvantaged
- **5%** Gender balance redress in SER

PUBLICATIONS

12 48 25

85 TOTAL

POSTGRADUATES

11	MSc	52
8	PhD	37
7	Postdoc	14

129 TOTAL

CONFERENCES & PRESENTATIONS

26 55 52 133

EXCHANGES

31 TO SWITZERLAND
61
30 TO SOUTH AFRICA

BOOK CONTRIBUTIONS

2 2 1

5 TOTAL

HDI

24	MSc	7
14	PhD	5
5	Postdoc	1

56 TOTAL

WORKSHOPS

7 **12**

APPRECIATION

- **COLLABORATION** — **65%** Alignment of PIs objectives **70%** Joint knowledge **5%** Joint publications **45%** Joint exchanges **25%** workshops
- **HUMAN CAPITAL DEVELOPMENT** — **65%** Appreciate Swiss contribution **80%** HCD in general **50%** Should demonstrate South Africa research excellence
- **RESEARCH FACILITIES** — **50%** Access to unique research environment in South Africa **30%** Opportunity for applied research
- **GENERAL APPRECIATION** — **35%** New research opportunities **40%** Leverage funds from other grants **5%** adequate project funds

RESEARCH LINKAGES AND BENEFICIATION

INTERNATIONAL
- 5% BRICS COUNTRIES
- 35% EU COUNTRIES
- 15% AFRICAN COUNTRIES
- 5% NORTH AMERICA

UNIVERSITIES AND NETWORKS
- 45% SOUTH AFRICAN UNIVERSITIES
- 20% SWISS UNIVERSITIES
- 30% CONTACTS AND NETWORKS ESTABLISHED
- 15% EXTEND COLLABORATION TO INTERNATIONAL NETWORKS

BENEFITS OF LINKAGES
- 20% NEW RESEARCH TOPIC
- 70% EXTENT COLLABORATION WITH SWISS AND SOUTH AFRICAN PARTNERS
- 20% GOVERNMENT INTEREST IN PROJECT

CHALLENGES

IN THE FIELD
- **25%** Exchange and transfer of research material to and from CH
- **15%** Inadequate equipment and funding thereof
- **10%** Challenge reaching research environment for fieldwork
- **5%** Experiment challenges
- **30%** Inadequate support for HCD (e.g. scholarships)

GENERAL
- **5%** Decrease in ZAR value - decrease in project funds
- **5%** Lack of follow-up funding
- **5%** High flight costs to EU

SWITZERLAND GLOBAL JOINT
SOUTH AFRICA TOTALS

Novartis – a Swiss Private Sector Company in Public Health Research and Innovation

As a one of the global leaders in Research & Development (R&D) and employing 23 000 scientists worldwide, Novartis invests in scientific capability development as part of an integrated strategy to strengthen healthcare systems in middle/lower income countries. The Swiss pharmaceutical giant invests US$9 billion in R&D every year to ensure patients around the world can have access to transformative medicines.

Through its science and healthcare development partnership with South Africa, Novartis' business activities contributed US$250 million towards the GDP of South Africa in 2018. Furthermore, Novartis' total employment impact exceeds 5 000 jobs in South Africa with direct jobs totalling more than 700 and an indirect employment impact of just over 1 000 jobs. The indirect impact is as a result of Novartis buying goods and services from local vendors.

Novartis also delivers a significant positive human capital impact. These include various initiatives each year to enhance the skills and knowledge of healthcare professionals, as well as multiple programmes to build medical research capacity in South Africa.

Novartis drives the Chronic Disease Foundation initiative to sustainably improve healthcare delivery for chronic diseases and focuses on the localisation of products in pursuit of locally relevant and globally competitive research and innovation (Novartis, 2018).

Novartis, the DST and SAMRC signed an MoU on 25 May 2017 in Cape Town. The agreement not only brought opportunities for additional collaboration in education, research and innovation but builds on the longstanding support that Novartis provides to South Africa in research capabilities, human capacity development and innovation.

Novartis Global CEO Vasant Narasimhan said during his first visit to Ghana, Kenya and South Africa on 7 June 2018, that after just one year of signing the MoU the collaboration showed promise for successful partnerships in communicable and non-communicable diseases. For Novartis, increasing clinical research skills had the potential for multiple positive knock-on effects to strengthen local healthcare systems, while innovation would attract further investment with positive outcomes for the economy and job creation. (Professional Healthcare Press Room, 2018).

"Previous efforts to build capacity have tended to focus on well-recognised academic facilities. In partnership with the DST and the MRC, we have now been able to identify candidates and programmes in under-resourced facilities, where excellent work deserving of our support is being carried out. It has been encouraging to see the scope of research in under-resourced facilities, and it has been gratifying to contribute to building capability and supporting these facilities," said Narasimhan.

A key contribution of Novartis to South Africa specifically and to Africa in general is the support for drug discovery, initially for malaria and TB. The H3-D Director, Prof Kelly Chibale, and his research team are benefiting from the collaboration with the Novartis Institute for BioMedical Research (NIBR), ensuring that they take basic science and clinical research to that of innovation. Novartis will also provide H3-D with new chemical starting points for drug discovery against tuberculosis.

Key elements of the Novartis collaboration with H3-D include: human capacity development in pre-clinical and clinical research areas, including FDA-level clinical study sites for the testing of new molecular entities; exchange programmes through internships, postdoctoral fellowships and sabbaticals; and financial support from the Novartis Research Foundation for training and infrastructure.

INITIATIVES

Novartis South Africa initiatives include: • The young physicians' skills development programme, funding postgraduate studies in Clinical Epidemiology at Stellenbosch University • The Next Generation Scientist programme at the University of Kwa-Zulu-Natal • Partnering on the MSc degree course in Regulatory Sciences at UWC and Hibernia College • Clinical trials capability building with UCT • Young Scientist training in Genetic Research in collaboration with Wits University and drug discovery and clinical trials (28 trials involving 1 602 patients across 163 trial sites in the private and public sector).

Novartis South Africa is also a corporate citizen that is relevant and sensitive towards the needs of the communities it operates in. The company has partnered with the Clicks Foundation to address a challenge faced by adolescent girls in the country.

According to UNICEF, one in 10 girls in Africa miss four days of school per month during their menstrual cycle due to a lack of sanitary towels. This means in a year, 10% of girls that have reached puberty are absent from school for a total of 48 days (nearly two months) for this reason. This has an impact on their ability to perform in class at the same level as their male counterparts.

To address this challenge, the Novartis and Clicks Foundation partnership saw 1 000 girls in two schools in Diepsloot, Johannesburg receive reusable sanitary towels with a life span of 3 – 5 years. This is a huge saving for families in the low income community.

Discovery and development of novel natural plant products as leads against neglected tropical diseases

UNIVERSITY PARTNERS

University of Basel
Professor Matthias Hamburger
Swiss Tropical and Public Health Institute
Professor Dr Reto Brun
University of Pretoria
Professor Vinesh Maharaj

Data generated by the WHO indicates that approximately 800 million individuals in several countries worldwide have succumbed to neglected infectious diseases, with protozoan diseases such as Leishmaniasis, African Trypanosomiasis and Chagas disease high on this list.

Unfortunately, there has been drug resistance by the protozoan, an expression of undesired effects, as well as reduced drug availability and access. Due to the high cost of research and development in potential drugs and the lack of return on their investment because of the economically disadvantaged position of the patients, this group of diseases has become unpopular to players in the pharmaceutical industry.

This project aimed to investigate novel natural plant molecules against malaria, Leishmaniasis and Trypanosomiasis parasites and develop these as far as the preclinical candidate stage. This would subsequently lead to reduced cases of disease-induced mortality and morbidity as well as poverty among the affected populations in the disease-endemic countries.

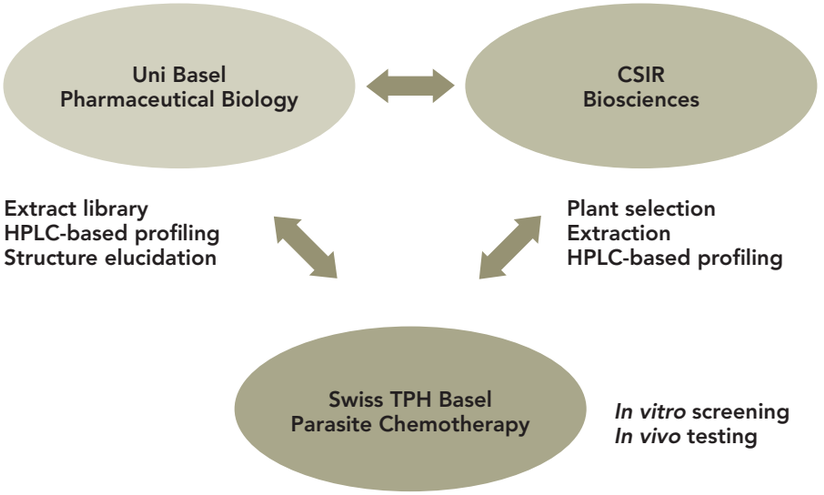
The researchers could not identify lead compounds that would be of direct interest to industry. The compounds identified from *Abrus precatorius* could have potential as starting point for a medicinal chemistry effort, with the aim of providing material for *in vivo* testing, and possible structural optimisation.

A collaborative network was established between the two countries, which exposed South African plant biodiversity to biological assays that are not readily accessible to South African scientists. Modern chromatographic technologies to rapidly identify active compounds from complex plant extracts have been transferred to South Africa. This technique does not rely on the classical bioassay-guided fractionation, which is time-consuming and results in wasted resources, but rather on a miniaturised, resource-sparing and rapid approach.

The collaboration has provided the Swiss scientist with new, unique and unexplored biological materials. South Africa's largely untapped biodiversity was accessed to identify new chemotypes for natural product-based lead

Photograph courtesy of Reto Brun

From left: Dr Heindrich Hoppe, Dr Joe Molete, Dr Paolo Meoni, Dr Nivan Moodley, Professor Vinesh Maharaj and Dr Dashnie Naidoo.



discovery in the area of neglected diseases. Knowledge generated from the project provides new entries for medicinal chemistry and potential activities in the areas of drug development.

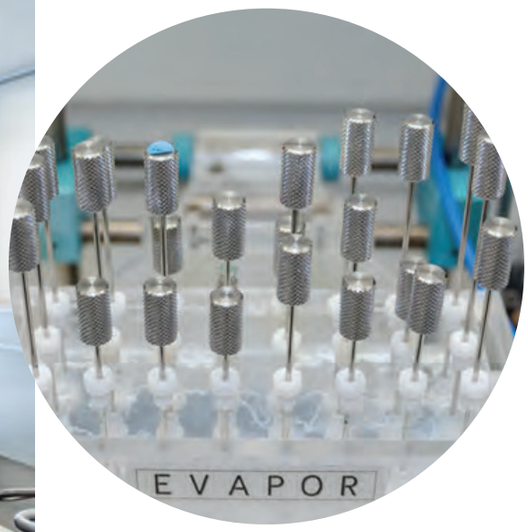
Of the 300 plant extracts prepared, *in vitro* biological evaluation against the protozoan parasites resulted in 102 (34%) being identified as "hits". The hits were selected for further research after review of the selectivity of their biological efficacy across the various parasites, analysing parameters such as ethnobotany strength, plant part used and probable compound type present. This resulted in two lists totalling 30 selected hits. Twenty of the more favoured candidates underwent immediate fractionation with the remaining hits reserved as back-up in the event of unacceptable results from the favoured candidate group.

At CSIR and University of Basel, the selected hits were submitted to a so-called HPLC-based activity, profiling a miniaturised approach to localise the active compounds in the extracts. Testing of the minute fractions obtained in 96-well format was performed at Swiss TPH. At the University of Basel, this approach was combined with spectroscopic detectors enabling, to some extent, a structural characterisation. The follow-up on the most promising extracts via a targeted preparative purification led to a range of compounds with *in vitro* activity. They represented different structural classes of secondary metabolites. Unfortunately, the most promising compounds, with high potency and selectivity *in vitro*, and favourable physico-chemical properties (a measure for "drug-likeness"), could not be progressed to *in vivo* testing due to the minute amounts of compounds obtained.

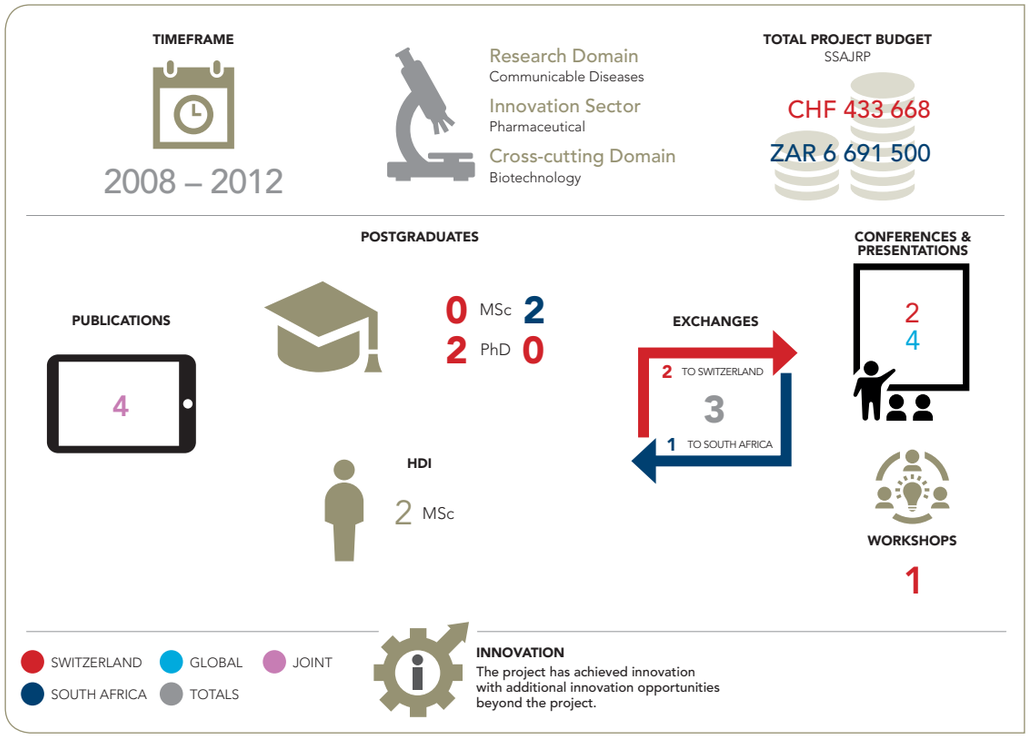
The research data has provided scientific data that substantiates the traditional use of medicinal plants, which have been used to treat neglected diseases. The data ultimately will be incorporated into research reports for communities, an initiative driven by the DST.

The collaboration included the University of Basel (with a focus on Pharmaceutical Biology); the CSIR (with a focus on Biosciences), and the Swiss Tropical and Public Health Institute (with a focus on Parasite Chemotherapy).

Microprobe NMR instrument for structure analysis.



Small scale evaporation of active extracts.



Impact of disease burden on schoolchildren's physical fitness and psychosocial health

UNIVERSITY PARTNERS
University of Basel
Professor Dr Uwe Pühse
Nelson Mandela University
Professor Cheryl Walter

Schoolchildren participating in the standardised 20m shuttle run test, which measures cardiovascular endurance.

The goal of the project was to assess the burden and distribution of communicable diseases and non-communicable chronic conditions among children in selected schools near Port Elizabeth, South Africa, and to assess their impact on physical fitness, cognitive performance and psychosocial health.

The objective was to undertake the scientific component with the SSAJRP grant and follow up with a school-based health promotion intervention for which the participants still had to source funding at the time.

The scientific component included an assessment of the extent of non-communicable chronic conditions (diabetes and obesity) and communicable diseases (helminth infections) and how they affect children in disadvantaged South African schools. Further aspects included assessment of the anthropometric indicators of the children where the results were correlated with the communicable and non-communicable diseases in the study population; a randomised pilot study to assess the effect of specific interventions, (lifestyle interventions and deworming); and an assessment of common allergens in children reporting allergies. The project

Deputy Vice-Chancellor for Research and Engagement at the Nelson Mandela University, Professor Dr Andrew Leitch, and the South African principal investigator, Professor Cheryl Walter, presents the university's Engagement Excellence Award 2017.

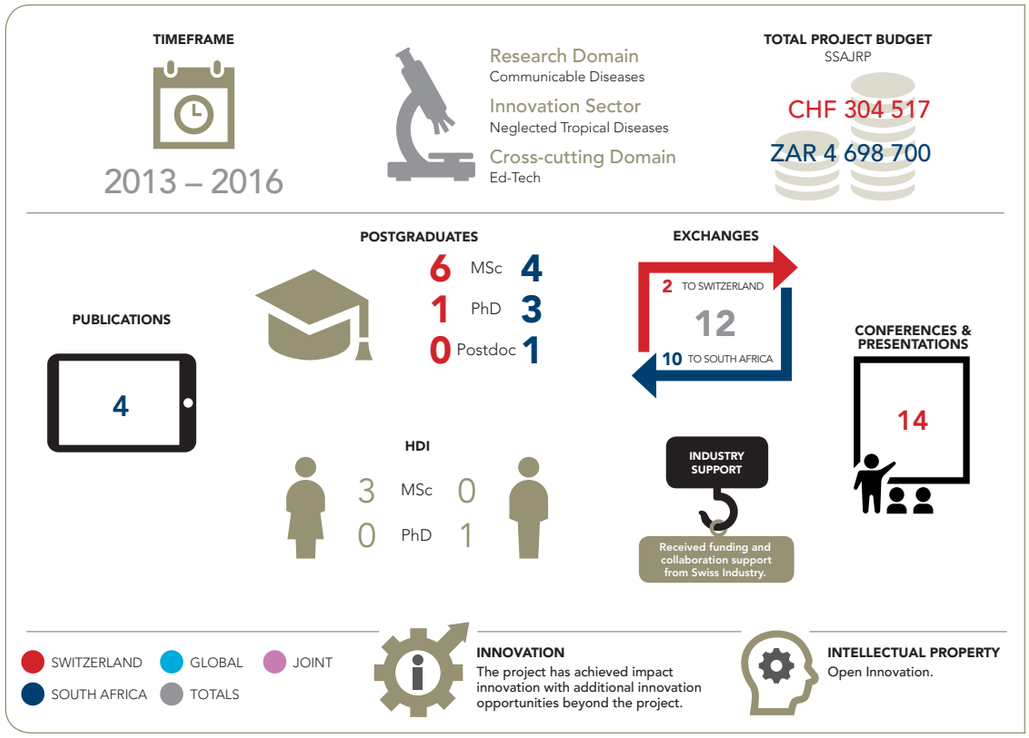
Photographs both courtesy of Nelson Mandela University

Qualified Medical Laboratory Sciences students conduct diagnostic tests on collected urine and stool samples.

assessed 1 000 children from eight project schools.

From the baseline results, schools were match-paired and divided into the intervention and control groups. The intervention Phase I included three main components: physical activity, health education and a nutritional intervention. Post-intervention testing took place when the entire set of tests was conducted again. The research team followed this up with the treatment of disease-positive schoolchildren, a second intervention phase and a last round of testing in 2016.

An allergy-testing component was added to assess sensitisation to common allergens, disease-burden among children with allergic symptoms and the correlation of allergy with socio-demographic and clinical characteristics.



HIV drug discovery from medicinal plants

UNIVERSITY PARTNERS

Esperanza Medicines Foundation
Professor Alex Matter
Professor Thomas Klimkait
University of Pretoria
Professor Vinesh Maharaj

The rapidly increasing number of HIV infection cases, especially in sub-Saharan Africa, has led to the increased use of traditional medicines in an effort to combat the disease. These traditional medicines have been noted to boost immune systems and control HIV in infected individuals. Although it is claimed that the general quality of life of the patients who take these traditional medicines is better than that of those not on the medication, there has not been scientific evidence to substantiate this claim to date.

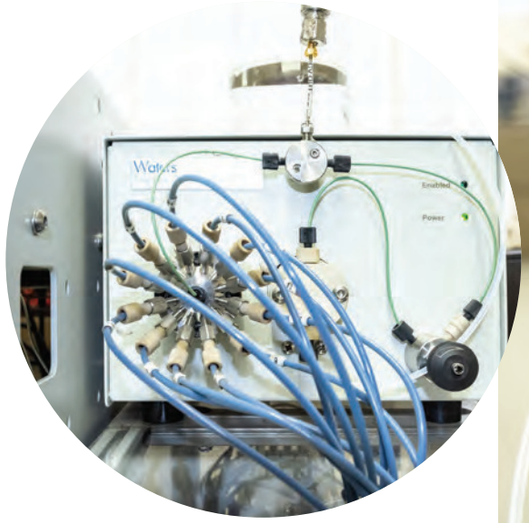
The successful isolation of the active ingredients from the traditional medicines should provide the necessary evidence to support these claims and will, more importantly, have a global impact on the availability of novel and less expensive natural-based HIV treatments. This project has the potential to increase global acceptance and legitimacy of traditional medicines.

This scientific endeavour was complemented by a combination of indigenous knowledge and the extensive biodiversity found in Africa.

The project team aimed to fill a compound pipeline with substances isolated from plants and lower organisms. They initially selected indigenous plants based on their traditional use related to HIV, and followed this up with the plant extract preparation. The extracts and pure compounds (obtained from the CSIR compound library) were divided into three batches and biologically assayed for their anti-HIV activity using a Swiss state-of-the-art cellular-based screening technology called the cellular infection anti-HIV system (deCIPh). Of the 88 plant extracts, six displayed potential for further development and were classified as "hits".

They evaluated a selection of 27 South African plants for anti-HIV activity, based on a desktop chemotaxonomic study of indigenous plants containing chemo-types with the backbone similar to those compounds in HIV clinical trials. Three plant extracts showed potent activity with no signs of toxicity while 24 extracts showed high potency but high levels of cytotoxicity. They also screened a traditional mixture of five plants traditionally used for the treatment of HIV, of which two exhibited good anti-HIV activity. The collaborative work they did on four Tanzanian medicinal plants and previous

PhD student from the University of Pretoria, Babalwa Tembeni.



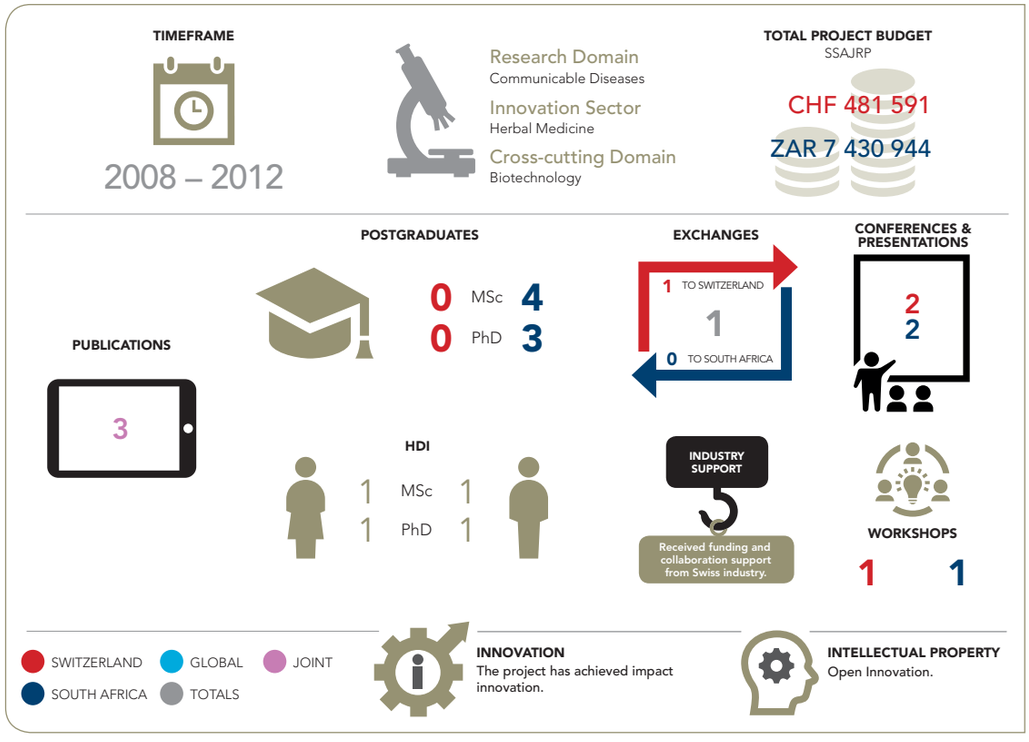
Maharaj laboratory

screening results of the extracts, showed that two plant extracts exhibited potent anti-HIV activity and these will be investigated further.

The team also screened crude organic and aqueous extracts as well as partially purified fractions of the South African *Helichrysum psilolepis* (Asteraceae family) for their anti-HIV-1 activity and found them to be very active at micro-Molar concentrations with two strains of HIV-1.

This project established the groundwork for collaboration between leading organisations in South Africa and Switzerland. It allowed South African scientists to further develop their skills in drug development based on the leads identified. A new technology has been introduced at the CSIR Biosciences: HPLC-based activity profiling of plant extracts into 96 well plates were implemented. In addition, the implementation of an accelerated approach to identifying the active compounds in complex plant extracts using semi-preparatory HPLC MS/MS technology has shown to be a powerful tool in drug discovery and a valuable asset to the CSIR.

Ultimately, plants identified with active ingredients will serve as the starting point to produce affordable and easy-to-produce anti-HIV/AIDS and anti-parasitic medicines. It has further led to traditional healers having their medicines exposed to modern biotechnology-based HIV and neglected diseases assays.



Evolution and epidemiology of rifampicin-resistant tuberculosis in Khayelitsha, Cape Town: implications for biology and disease control

UNIVERSITY PARTNERS

University of Basel
Professor Sebastien Gagneux
University of Cape Town
Associate Professor Helen Cox

Associate Professor Helen Cox from the University of Cape Town, Dr Lizma Streicher and Professor Robin Warren both from Stellenbosch University.

Professor Sebastien Gagneux from the University of Basel.

Bacteria resistant to multiple antibiotics are a growing threat for global public health and the economy. Multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant TB (XDR-TB) are particular problems in many parts of the world, including South Africa. Yet, little is known of the bacterial factors driving the global epidemics of MDR/XDR-TB.

In the past, the common view was that the *de novo* acquisition of drug-resistance determinants during patient treatment was the main driver of drug resistance in TB. It was thought that, due to the fitness costs associated with resistance, drug-resistant strains were less likely to transmit to other patients. However, recent experimental and epidemiological data show that the fitness of drug-resistant *Mycobacterium tuberculosis* (MTB) is heterogeneous and that most MDR-TB is in fact transmitted. Preventing ongoing transmission of drug-resistant TB (DR-TB) is fundamental to controlling the epidemic. Understanding the drivers behind the *de novo* emergence and subsequent transmission of DR-TB, and the impact of treatment with currently available treatment regimens and those that include newly available drugs, is essential to improving control strategies.

The research group is aiming to use next-generation whole genome sequencing (WGS) to describe and understand the evolution and epidemiology of DR-TB in Khayelitsha, a high HIV, TB and DR-TB burden setting in South Africa. As part of a community-based DR-TB treatment programme, initiated in Khayelitsha in 2008, a detailed patient-level clinical database is being maintained. Project members proposed to link this data to a biobank of stored DR-TB strains held at Stellenbosch University to address their objectives.

The first objective was to assess the between-host evolution and extent of transmission of DR-TB through analysis of WGS, among drug-resistance categories, over time (data was available for a 10-year period from 2008-2017), between HIV-negative and HIV-infected patients, and across geographical areas within Khayelitsha. Another objective was to assess within-patient strain evolution among initially DR-TB strains during treatment for individual

Processing TB isolates in a biosafety Level 3 laboratory.

Stored frozen TB isolates.

patients, focussing on resistance acquisition for key existing and new TB drugs and contrasting HIV-negative and infected individuals.

The study, which is currently under way, is both retrospective – using data and stored isolates from 2008-2015 – and prospective, utilising data from the Khayelitsha programme from 2016-2017. MTB isolates are sub-cultured and bacterial DNA extracted in South Africa and then sent to Switzerland for WGS. Data analyses will be performed in close collaboration between the teams.

Through describing the role of strain diversity and resistance acquisition in transmission of DR-TB in a high DR-TB setting, this study has the potential to dramatically improve understanding of DR-TB transmission and the impact of current control strategies. In addition, as Khayelitsha is one of the pilot sites for expansion of access to new TB drugs, this study provides opportunity to study the impact of expanded use of new drugs on resistance evolution. The data generated through this study also provides an opportunity to assess the potential feasibility and impact of using WGS for routine, rapid determination of TB drug resistance, in order to optimise and individualise second-line treatment.

TIMEFRAME

2017 – 2020

Research Domain
Communicable Diseases

Cross-cutting Domain
Bio-Infomatics

TOTAL PROJECT BUDGET
SSAJRP

CHF 512 985

ZAR 7 915 363

POSTGRADUATES

0 PhD 1

1 Postdoc 1

HDI

1 Postdoc

SWITZERLAND GLOBAL JOINT

SOUTH AFRICA TOTALS

Development and characterisation of Shigella glycoconjugate vaccines

UNIVERSITY PARTNERS

Swiss Federal Laboratories for Materials Science and Technology
Professor Dr Linda Thöny-Meyer
Dr Michael Kowarik
University of Cape Town
Professor Neil Ravenscroft
3rd Party
GlycoVaxyn AG, now LimmaTech Biologics AG

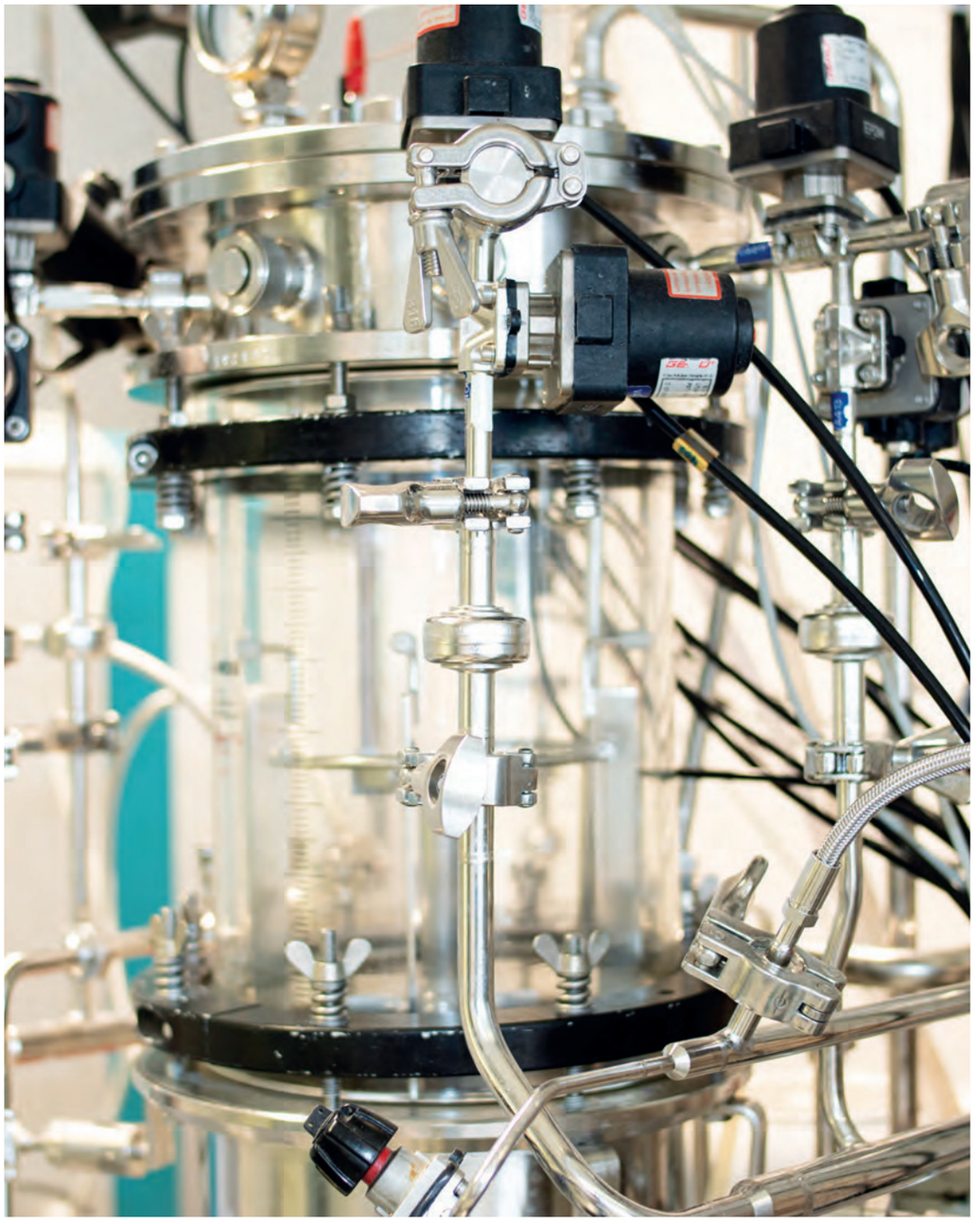
The participants in this project employed an interdisciplinary approach to the research and development of affordable vaccines to address health needs, such as Shigellosis, in the developing world. Shigellosis is an infectious disease caused by a group of bacteria called Shigella. Shigellosis results in diarrhoea, fever and stomach cramps one or two days after being exposed to the bacteria.

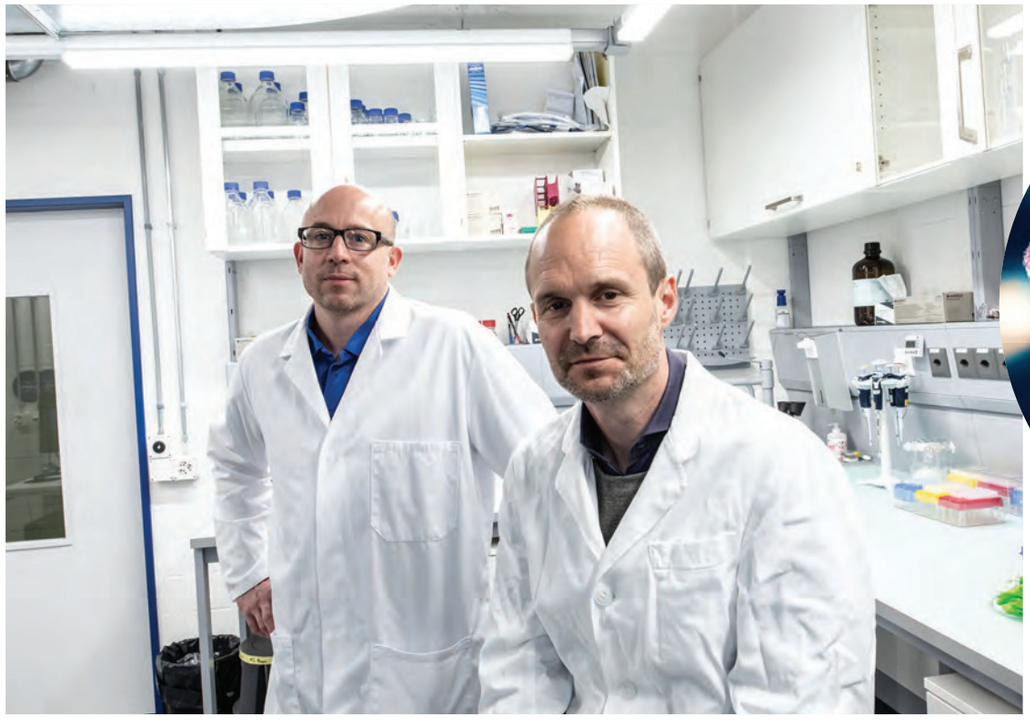
Shigellosis is a major public health concern with approximately 165 million cases annually, resulting in about five million hospitalisations and over 1,1 million deaths, mainly in the developing world. No vaccine is available.

The collaboration between the University of Cape Town, Empa St Gallen and GlycoVaxyn was aimed at *Shigella flexneri 2a* and *Shigella sonnei* glycoconjugate production, chemical synthesis of the *S. sonnei* O-antigen repeating unit and the physicochemical characterisation of the glycoconjugate vaccines. Empa St Gallen focused on developing a downscale model for upstream process development and GlycoVaxyn provided *S. flexneri 2a* glycoconjugate, auxiliary materials as well as analytical and process know-how. This collaboration resulted in successful *S. flexneri 2a* glycoconjugate production, detailed analytical characterisation and establishment of a downscale model, including the identification of critical process parameters for fermentation.

Researchers at UCT established protocols for the structural elucidation of the carbohydrate antigens and applied them to the bioconjugates produced by GlycoVaxyn and Empa St Gallen. They achieved even more detailed nuclear magnetic resonance (NMR) spectroscopy characterisation of the carbohydrate and associated amino acids on conjugates following proteolytic treatment and purification. By preparing a well-characterised glycoconjugate vaccine candidate against *S. flexneri 2a*, the partners have established a suitable platform for the production and analysis of other glycoconjugate vaccines.

Despite the many challenges presented by the zwitterionic *S. sonnei* antigen, the researchers





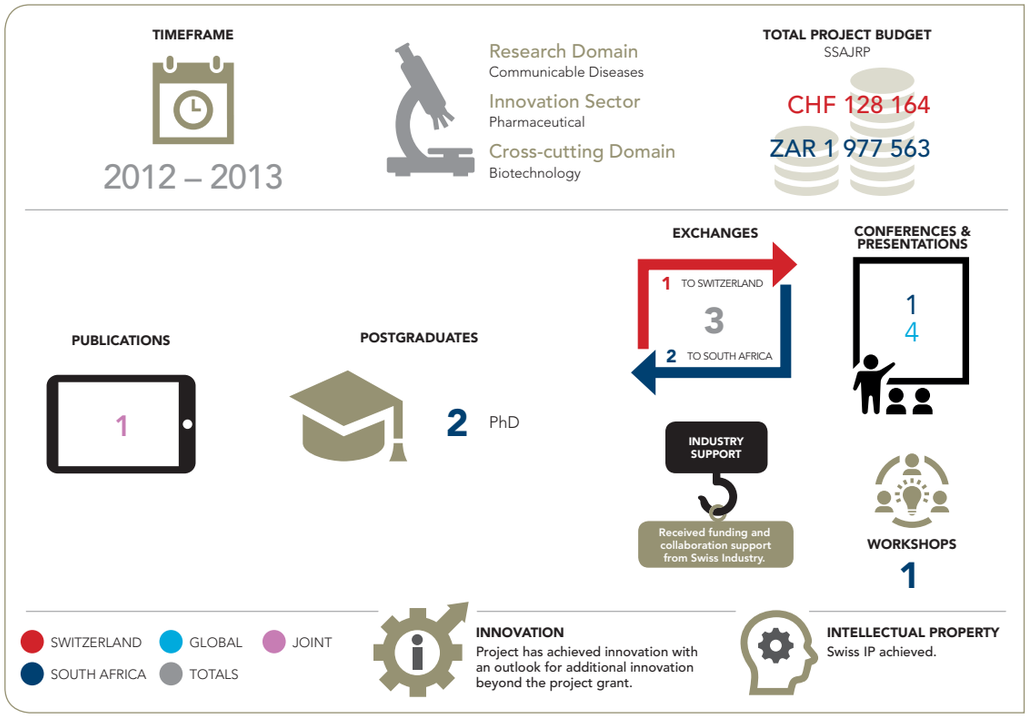
Michael Steffen and Dr Michael Kowarik.

Shigella bacteria.

made some progress with regards to the chemical synthesis and characterisation of the *S. sonnei* O-antigen, and production of the recombinant *S. sonnei* polysaccharide in *Escherichia coli* cells. However, the strain construction, manufacturing process and associated yield of the *S. sonnei* glycoconjugate require further attention.

Vaccine development and licensure take 8-10 years and even longer if a multivalent vaccine is required. The Global Enteric Multicenter Study showed that broad-spectrum vaccine protection against Shigellosis can be achieved with a tetravalent vaccine comprising O antigens from *S. sonnei, S. flexneri 2a, S. flexneri 3a,* and *S. flexneri 6.* No such vaccine is currently available.

GlycoVaxyn, now GSK (using its subcontractor LimmaTech Biologics AG), is continuing to develop a multivalent Shigella conjugate vaccine and has just completed a Phase 2b clinical study with the well-characterised bioconjugates vaccine against *S. flexneri 2a.* All the vaccines in development and clinical trials are based on the bioconjugates production and characterisation methodology investigated during this project.



Nano-vesicle and micro-sponge Pheroid® drug delivery system for antimalarial drugs with probable reversal of drug resistance for chloroquine

UNIVERSITY PARTNERS

Swiss Tropical and Public Health Institute
Professor Dr Reto Brun
North-West University
Professor Anne Grobler

Photograph courtesy of Anne Grobler

Research conducted in Professor Grobler's laboratory.

The worldwide prevalence of malaria is estimated to be in the order of 250 million clinical cases annually, of which half a million people die. This can mainly be ascribed to the rapidly escalating acquisition of drug-resistance by *Plasmodium falciparum* strains, which subsequently renders the administered treatments ineffective. In addition to drug resistance acquisition, other contributing factors that promote the global increase in malarial cases are the elevated cost and limited availability of conventional anti-malarial drugs.

The main aim of the project was to develop Pheroid®-entrapped antimalarial drugs and investigate the potential increases in efficacy and uptake or absorption of these antimalarial drugs in Pheroid®-drug micro-sponge and nano-vesicle carrier combinations. This project hinged on the nature of the Pheroid® technology and the versatile project task force with their particular facilities. Pheroid® is a stable lipid-based submicron emulsion delivery system. It is unique in that its morphology, structure, size and function can be manipulated as required. The non-toxic nature of this delivery system is attributed to its constituent natural and/or essential fatty acid compounds that have been formulated with various drugs for novel and innovative dosage forms.

The North-West University (NWU) successfully characterised the chemical and physical nature of the formulations and entrapment of antimalarials in Pheroid® micro-sponges and nano-vesicles. Both the NWU and the Swiss Tropical and Public Health Institute (Swiss TPH) performed *in vitro* and *in vivo* studies of the efficacy of the entrapped antimalarials on resistant malaria parasites. The entrapment of the antimalarials amodiaquine (AQ) and artemisone in Pheroid® vesicles resulted in enhanced uptake in mice. No toxicity or discomfort was exhibited by any of the animals in these studies.

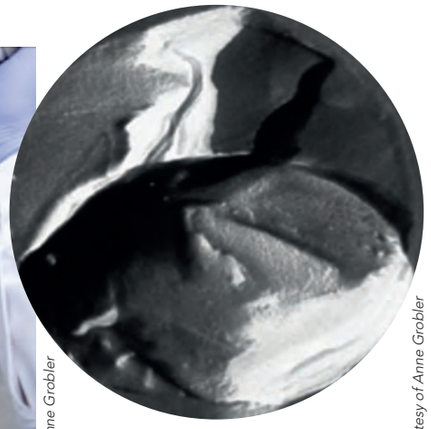
The re-use of cheaper raw materials in the low start-up and manufacturing cost requiring Pheroid® manufacturing process will significantly reduce the costs for pharmaceutical companies. There is still the potential to treat latent disease and multidrug-resistant malaria parasites. Pro-Pheroid® formulations did increase the stability of previously unstable active pharmaceutical ingredients (APIs). Novel adult and paediatric dosages can and have been formulated and the Pheroid®-based concept may be applied to the treatment of other parasitic diseases.

Photograph courtesy of Reto Brun

Initiation meeting of project at the NWU with representatives from STPHI, NWU, CSIP and UP.

Photograph courtesy of Anne Grobler

In vitro *evaluation of Pheroid®-antimalarial formulations at the Swiss Tropical and Public Health Institute.*



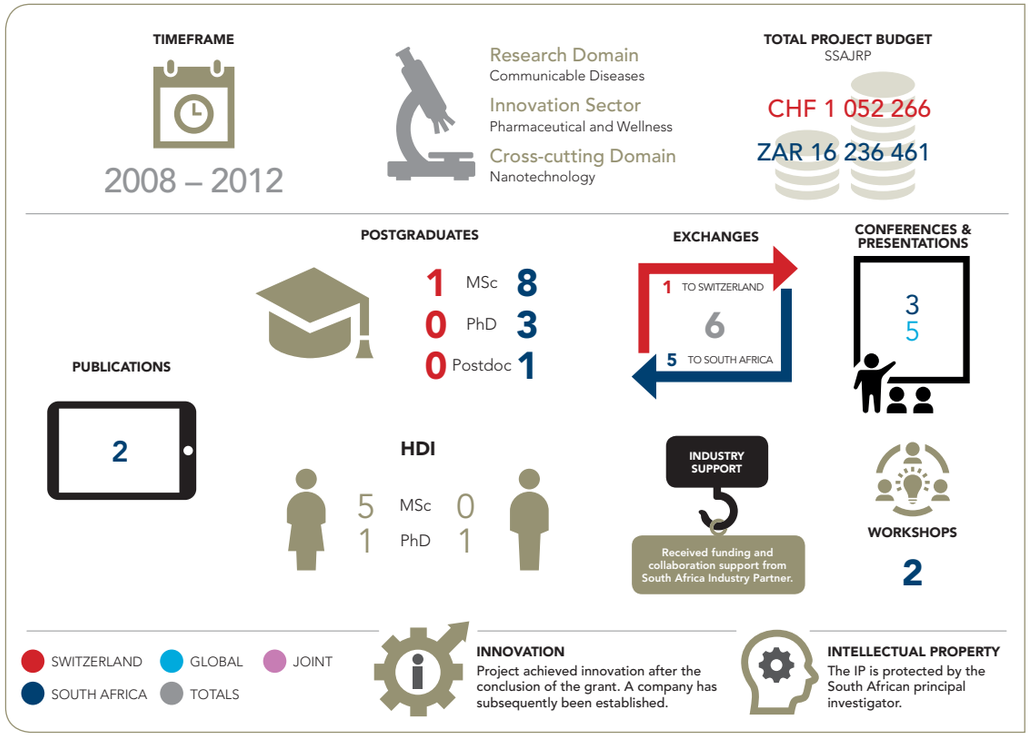
Photograph courtesy of Anne Grobler

Pheroid® with antimalarial compound entrapped below.

While the original aim was not reached, the contribution that the project made to the future commercialisation of home-grown South African technologies cannot be underestimated. The industrialisation phase of this research project is ongoing and is already making a contribution to job and wealth creation in the North-West Province.

To date, five Pheroid® antimalarial vesicle and micro-sponge complexes have been manufactured with upscaling to pilot batch size. Development and validation of Pheroid® size and morphology measurement methods were undertaken and entrapped drug verification is now feasible. Accelerated stability studies were performed for two of the antimalarial drugs entrapped in Pheroid®. The uptake and pharmacokinetics of the Pheroid® -based malarial formulations have since been determined in non-human primates. These studies showed clinically relevant improvement of the absorption of Pheroid® CQ (chloroquine) but not of Pheroid® AO (artemisone). Furthermore, in the case of Pheroid® CQ, preferential targeting to the erythrocytes were observed, which should result in improved efficacy. The use of a carrier system, such as the Pheroid®, is still under investigation at the NWU.

The project enabled the scientific exchange of scientists and technical personnel of the Swiss TPH and counterparts of the NWU in Potchefstroom and other institutions in South Africa. The project was an early enabler in the development of a Pheroid® manufacturing facility with the resultant creation of new jobs and capacity building. The cost-effective African-focussed new formulations studied during the course of the project are helping to improve the health and well-being of individuals outside the scope of these projects.



Protein bioinformatics resource development for important health-related pathogens

UNIVERSITY PARTNERS

Two parallel efforts to unravel the causes of drug resistance of M. tuberculosis and HIV:

Swiss Institute of Bioinformatics
Professor Ioannis Xenarios
Updated and extended the annotation of targeted sequence
Contrasted predictions with experimental literature
Updated and structured virus annotation around HIV-1 and host interactions

University of KwaZulu-Natal
Dr Tulio de Oliveira
Identified a subset of HIV viruses for selecting representative sequences, Predicted HIV resistance and used Viralzone knowledge representation

University of Cape Town
Professor Nicola Mulder
Provided a list of proteins for annotations
Provided network-based priority targets

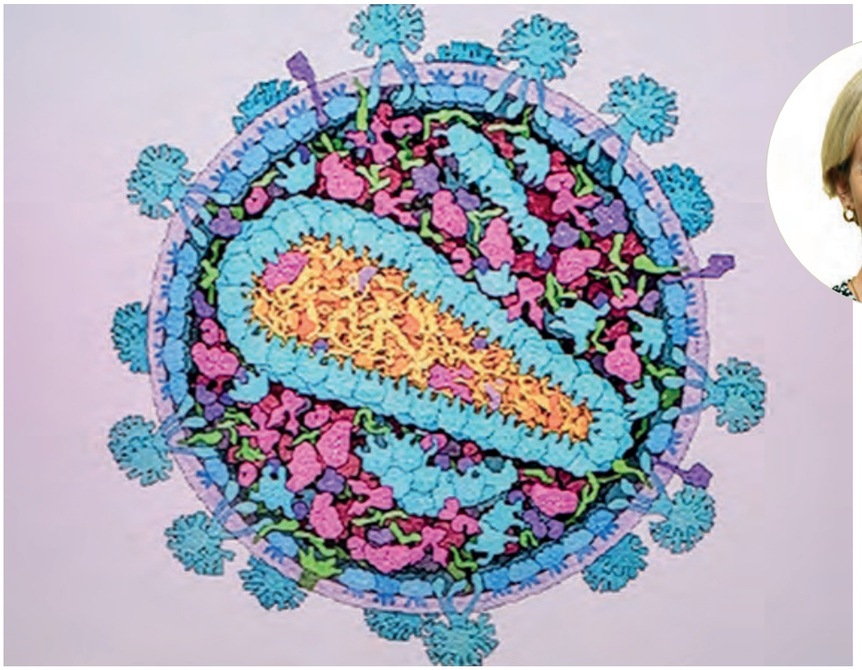
The **first phase** of this project aimed to create the first African protein annotation group to unravel the causes of drug resistance acquisition by some southern African HIV-1 and tuberculosis (TB) strains, and also to strengthen viral protein annotation and proteomics resources.

Human Immunodeficiency Virus (HIV) and Mycobacterium tuberculosis (MTB) are two of the most important health-related pathogens in Africa, causing Acquired Immune Deficiency Syndrome (AIDS) and tuberculosis (TB) respectively. It is, therefore, paramount for an inaugural African protein annotation group to be created to generate scientific research data that shed light on the drug resistance of southern African HIV-1 and TB strains, specifically in multiple and extremely resistant strains, and the characterisation of the MTB metabolome. Knowledge of this resistance and the ability to create drugs that fight against it can result in significant advancements in the fight against these diseases, especially in developing countries.

The researchers selected a set of MTB proteins that is adequate for both training and annotation purposes. They annotated a total of 102 MTB proteins from this set and 10 others have had their annotations updated. The MTB proteins cited in recent literature as being involved in virulence were also annotated. They assigned Enzyme Commission (EC) numbers to up to 60 uncharacterised proteins.

In the HIV research sector, team members carried out entries to update the 353 HIV-1 UniProtKB/SwissProt while they conducted the curation of approximately 2 500 genotypes with clinical data from antiretroviral (ARV) treatment cohorts in the South African region. The HIV proteomics resource and the RNA virus database were updated with information from Swiss-Prot. In addition, two drug-resistance databases, the mirrors of the Stanford HIV drug resistance and RegaDB database, were published.

Strengthening viral protein annotation and proteomics resource is important to the international medical research community. One of its many applications is as a tool that is used globally to feed the drug development pipeline. Capacity of South African researchers was built in the field of protein bioinformatics and resource development. This project thus addressed the national goal of human capacity development in a scarce skills area. Moreover, it allowed the expansion of resources in South Africa to fight the pandemic of HIV and AIDS via the publication of the "Public database for HIV drug resistance in Southern Africa" database in *Nature* (2010).



Professor Nicola Mulder

Characterisation of multiple and extremely drug-resistant HIV and MTB strains in Africa for potential treatment of HIV/AIDS and tuberculosis.

An integrated Mycobacterium tuberculosis resource for drug discovery

The **second phase** of the project aimed to provide the TB research community with a portal that integrates reference information on the MTB genome and proteins and research information derived from Mulder's laboratory in South Africa and Cole's laboratory in Switzerland. Reference information is based on TubercuList, the major knowledgebase for H37Rv, the reference MTB strain. The UniProt knowledgebase provides high-quality functional annotation and gene ontologies of thousands of proteins, including MTB proteins, for manual biocuration and annotation.

The Swiss group performed a comparison of the TubercuList and UniProtKB databases, determining which proteins in each database to focus annotation efforts on in order to provide a synchronised and more complete dataset to the scientific community. The Mulder laboratory developed and implemented the function prediction algorithms and used a functional interaction network between proteins in MTB to predict gene ontology (GO) biological process terms and TubercuList functional classes for unknown proteins. This increased the number of GO annotated proteins in this organism

substantially and predictions have been linked to relevant protein entries in TubercuList.

The group also used network properties to predict likely essential proteins and thus potential drug targets, and has compiled a list of known or predicted targets from a variety of sources to provide to UniProt and TubercuList. Host-pathogen interactions were predicted by the Mulder group using interologs based on intra- and inter-species interactions. These were filtered to ensure the interacting partners are expressed at the appropriate time and in the appropriate subcellular location. The two networks can be visualised using a new interactive network visualisation tool developed by the group.

The project has improved public resources and the data therein for TB, an important disease for Africa and other parts of the world. The resource is open to researchers world-wide and thus has the potential to impact global research on TB. Most scientists use the public databases such as UniProt and TubercuList in their research on all aspects of TB, including new drug design and vaccine development, therefore the added value provided in this project will improve the ability of scientists using the resources to achieve their research goals.

The **third phase** of the project aimed to represent fundamental knowledge on HIV/AIDS and MTB in an innovative and interactive way, with links to relevant databases.

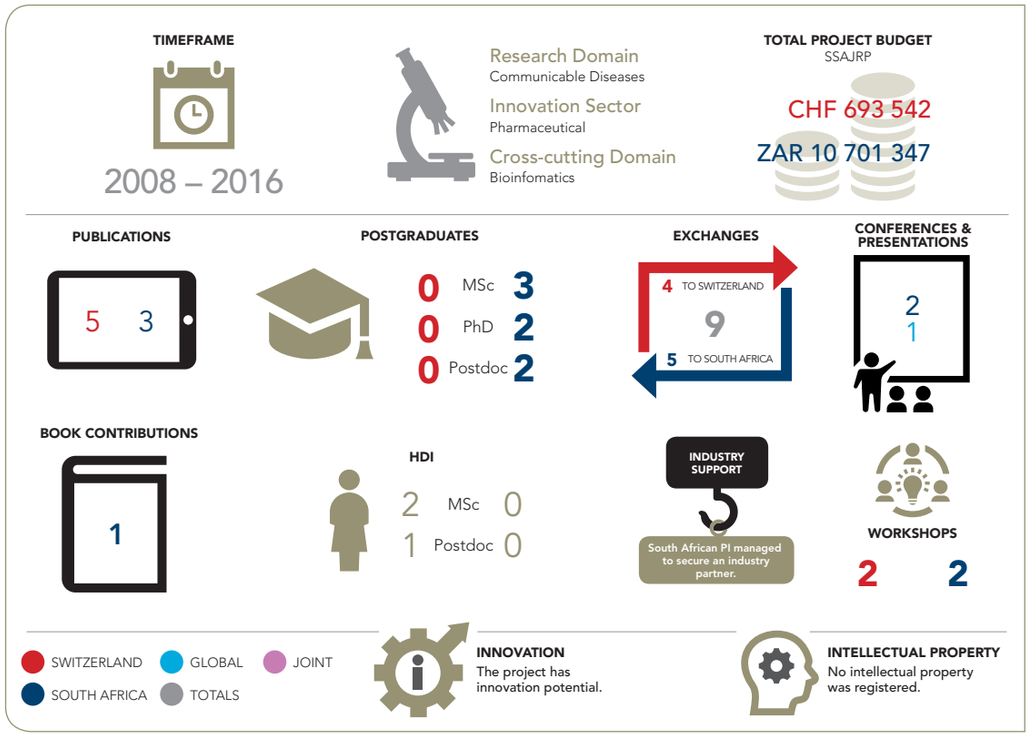
The Southern African Treatment Resistance Network (SATuRN), the Swiss Institute of Bioinformatics (SIB) and the University of Cape Town (UCT), constructed an online resource that provides specialised proteomic, host-pathogen interaction and bioinformatics information on HIV and TB and drug activity/resistance mechanisms. This unique web resource was constructed using one of the most advanced systems for protein data curation available in the world, in order to provide clinicians and researchers with new cutting-edge knowledge that can be used to combat drug resistance in Africa.

The UCT partner aimed to predict host-pathogen (MTB) interactions, do RNA-Seq analysis of TB strains, and develop an interaction visualisation tool. The host-pathogen predictions were complete, and around 200 interactions were predicted with validation from public expression data. Expression and mass spectrometry data from local strains were analysed. The proteomics data enabled proteogenomic analysis of the strains, and results are being fed to the Swiss partner to improve MTB annotations. A protein interaction network visualiser (PINV: http://biosual.cbio.uct.ac.za/pinv.html), which enables visualisation of intra- and interspecies interactions, was developed and published.

The project has produced an HIV proteome resource on the Swiss and South African websites. This resource has received over 50 000 visits since inception and has been published at *Database* (Oxford), the official journal of the biocuration community. The TB host-pathogen interaction and expression analysis has identified a number of key potential drug targets and proteins involved in virulence. The annotation has improved the quality of TB data in Swiss-Prot, which will benefit the entire TB community. The focus on drug targets for annotation will benefit industry partners.

A female South African student completed her MSc (cum laude) as part of this project, and a female coloured student registered for an MSc. Two previously disadvantaged individuals from De Oliveira's group attended Next Generation Sequencing training in Cape Town in 2016. Dr Mazandu, a black African, visited Geneva and Lausanne as part of this project.

Professor Ioannis Xenarios



Single cell analysis of peptidoglycan remodelling and resuscitation in Mycobacteria: implications for TB disease

UNIVERSITY PARTNERS

Swiss Federal Institute of Technology in Lausanne
Dr Neeraj Dhar
University of the Witwaterstrand
Dr Bavesh Kana

Measuring cylinders in the South African partnering laboratory of Dr Bavesh Kana, used to prepare of reagents that are required to grow Mycobacterium tuberculosis under laboratory containment.

Considering the importance of successful bacterial proliferation to TB disease and the predicted effect that efficient interruption of these processes will have on treatment outcome, the research team of the SSAJRP-funded collaborative research programme between Wits University and EPFL aimed their work at identifying new drug targets in bacterial cell division. They directed their research specifically towards understanding how bacteria remodel their cell walls during division and how the enzymes involved in this process ultimately regulate bacterial growth and cell division.

The underlying premise of their work was that this remodeling process is critical for pathogenesis and represents a vulnerability within the mycobacterial cell, which can be exploited for killing tubercle bacteria in the lung.

The project united the complementary skills from the South African team in gene manipulation and mycobacterial physiology with that of the Swiss partner, who is a global leader in the field of mycobacterial single-cell time-lapse microscopy. The result has been a vibrant collaboration, with

rich knowledge and student exchange, which has resulted in high-impact outcomes and substantive capacity development.

Since the discovery of penicillin in 1928, disruption of cell wall biosynthesis with antibiotics has proven to be a clinically successful strategy with bacterial infections for close to a century. However, these benefits have not accrued to TB due to the refractory nature of MTB to treatment with conventional penicillin-type antibiotics. The research conducted in this project has allowed for an extraordinary view of cell division in tubercle bacteria and the resulting findings have extended the scientific understanding in this field. The researchers identified enzymes that could possibly be used as targets for the development of new TB drugs.

Numerous collaborative discussions have allowed the South African partner to further develop its TB research portfolio, which has facilitated the development of verification reagents for novel diagnostics, for TB detection. These tools have been provided to the National Department of Health to facilitate rollout of new molecular diagnostics and have also been approved for use by the World Health Organization. As a result, Wits University has spun out a company to market these reagents in over 20 countries. These efforts have also led to the filing of a patent and the licensing of intellectual property.

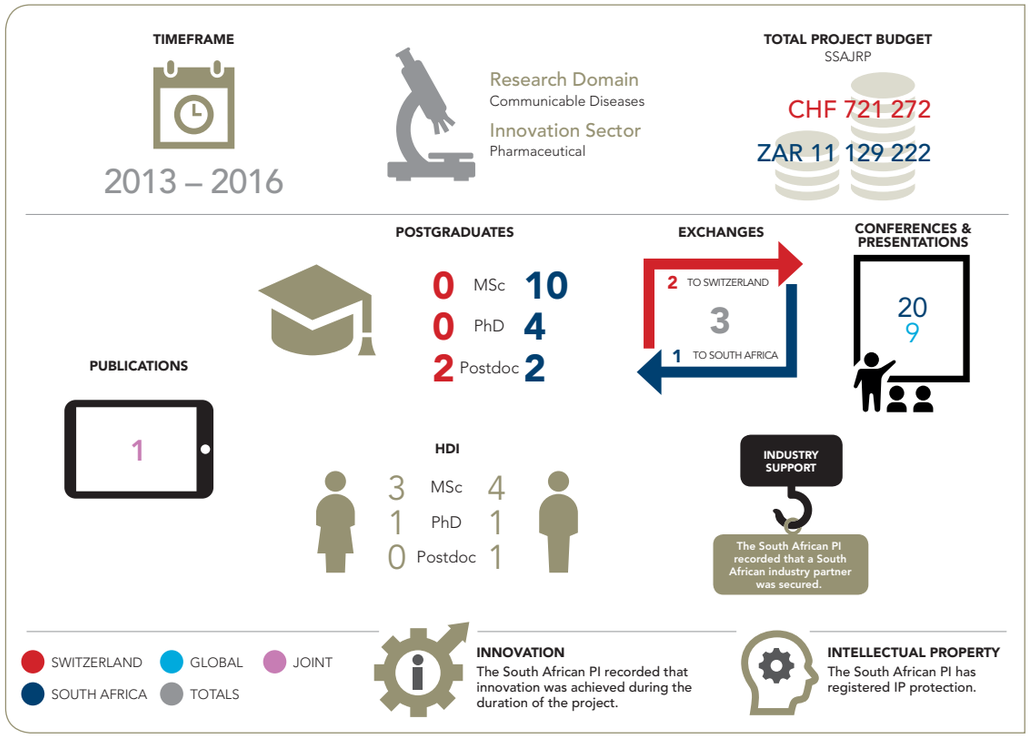
Students engaged in this programme have conducted numerous public awareness campaigns for TB and drug-resistant TB. These included participation in World TB Day activities and the hosting of stalls at local research days and symposia. The individuals within the research team that led this programme are community activists against TB. As an example, several students from the laboratory participated in the "Unmask Stigma" campaign, a social initiative targeted at reducing the stigma associated with TB and TB-HIV. Due to their unwavering commitment and innovative ideas, these students won a cash prize for their efforts.

Photograph courtesy of Neeraj Dhar

Dr Neeraj Dhar

Photograph courtesy of Bavesh Kana

Dr Bavesh Kana



Remodelling of Mycobacterial peptidoglycan during cell division in tuberculosis disease

UNIVERSITY PARTNERS

Swiss Federal Institute of Technology in Lausanne
Dr Neeraj Dhar
National Health Laboratory Service
Dr Bavesh Kana

The development of novel therapeutic options for the treatment of tuberculosis (TB) has become a deep-rooted urgency in modern medicine, driven by the rapid emergence of drug-resistant TB and the substantive loss of human life associated with this disease. The emergence of complex forms of drug-resistant TB, such as extensively drug-resistant (XDR) and totally drug-resistant (TDR) TB, has heightened the global crisis associated with TB. Furthermore, treatment of TB is complicated by the prolonged period of time required to eradicate persisting bacteria, which is necessary to achieve non-relapsing cure.

This project is aimed at identifying new drug targets for TB, through an analysis of the peptidoglycan component of the mycobacterial cell wall; and at assessing how remodelling of the cell wall contributes to bacterial persistence during treatment.

The main focus was directed at mycobacterial cell division as substantive remodelling of the bacterial surface is required for this process and disruption thereof will unmask new vulnerabilities in the mycobacterial cell that could be exploited for killing the organism. Remodelling of the cell surface is driven by cell wall hydrolases and recent studies reveal that these enzymes play important and divergent roles in mycobacterial cell division. The research group is assessing the role of distinct classes of peptidoglycan hydrolases in cell division and bacterial survival through a multi-pronged approach involving bacterial physiology, chemical biology and immunology.

Furthermore, it has been demonstrated that sputum samples from patients with active TB disease, before the initiation of treatment are characterised by a large proportion of organisms that are unable to grow under standard conditions. These organisms, termed differentially culturable tubercle bacilli (DCTB), selectively require cell wall remodelling enzymes for recovery and also display drug tolerance, suggesting that they comprise persisting organisms that are able to withstand treatment. This observation confirms an important role for cell wall remodelling processes in modulating bacterial growth dynamics in pulmonary TB in humans.

Using single-cell time-lapse microscopy, these processes will be studied to further understand how cell wall remodelling enzymes affect bacterial recovery from TB-diseased individuals.

Time-lapse microscopy serves as one of the principal tools to address mycobacterial phenotypic heterogeneity in Dr Dhar's laboratory.

The peptidoglycan component of the mycobacterial cell wall remains an underexplored area in TB drug development and in this context, the work proposed for this project has great promise to deliver new TB treatments.

In addition, the study of persisting bacterial organisms that display drug tolerance is essential to develop new treatment modalities that have a shorter duration and a reduced daily pill burden.

Considering this, the study of DCTB in patient sputum will provide insight into the environmental cues that tubercle bacilli use to modulate their growth and metabolism. The researchers expect that this enhanced understanding of bacterial growth will allow for the development of more accurate culture-based diagnostics and new measures of drug efficacy.

TIMEFRAME
2017 – 2020

Research Domain
Communicable Diseases
Innovation Sector
Pharmaceutical
Cross-cutting Domain
Bioinfomatics

TOTAL PROJECT BUDGET
SSAJRP
CHF 518 087
ZAR 7 994 089

PUBLICATIONS
1

POSTGRADUATES

		SWITZERLAND	SOUTH AFRICA
MSc		0	5
PhD		1	4

HDI

		SOUTH AFRICA	SWITZERLAND
MSc		3	0
PhD		2	0

SWITZERLAND SOUTH AFRICA GLOBAL JOINT TOTALS

Molecular mechanisms of propionate catabolism in Mycobacterium tuberculosis

UNIVERSITY PARTNERS

Swiss Federal Institute of Technology Lausanne
Professor Dr John McKinney
University of Cape Town
Professor Valerie Mizrahi

Tuberculosis (TB) is an infectious bacterial disease caused by Mycobacterium tuberculosis (MTB). Major advances in mycobacterial research have been achieved in recent years, but research to date has not fully elucidated the metabolism of MTB during infection. The overall aim of this study was to develop better insight into the metabolism of MTB.

As an obligate pathogen, MTB must survive within disparate host environments during successive cycles of infection, replication, persistence, and transmission. In turn, this suggests that the organism must possess the metabolic flexibility to adapt to variable nutrient availability, in particular a deficiency in glucose as a carbon source and an abundance of fatty acids. The consumption of alternative carbon sources – including odd- and branched-chain fatty acids, branched-chain amino acids, and cholesterol – generates the compound, propionyl coenzyme A (propionyl-CoA) as a three-carbon (C3) terminal product. Propionate is a high-energy metabolite, but is toxic to MTB if accumulated in high concentrations. This dual nature implicates propionate metabolism in the growth and persistence of MTB during host infection.

The methylmalonyl pathway, one of the potential pathways of propionate metabolism in MTB, uses the enzyme methylmalonyl CoA mutase, which is dependent on a vitamin B12-derived cofactor for activity. As such, it constitutes a natural fulcrum for this collaborative project, uniting two primary research interests of the applicant laboratories: the regulation and function of carbon metabolic pathways (Swiss) and the contributions of vitamin B12 biosynthesis and B12-dependent enzymes (South Africa) to MTB pathogenesis.

The researchers assessed the effect of combined methylcitrate cycle and methylmalonyl pathway loss on virulence by undertaking processes including MTB mutant strain construction. Central in addressing the main aim of the project are *in vitro* characterisation of growth on alternative carbon sources; investigation of the capacity of MTB to transport and utilise selected vitamin B12 and pseudovitamin B12 precursors to support the function of B12-dependent pathways; and the identification of proteins involved in the transport of vitamin B12, vitamin precursors and cobalt in MTB are central in addressing the main aim of the project.

Professor Dr John McKinney

Exposure to state-of-the-art technologies that have been established in the Swiss laboratory has benefited the South African collaboration team on a technological level. These include single-cell microbiology based on time-lapse video microscopy and microfluidics, as well as metabolomics (large-scale metabolite analysis). The application of these techniques is changing the face of research in the field of mycobacterial metabolism.

TIMEFRAME
2008 – 2012

Research Domain
Communicable Diseases

TOTAL PROJECT BUDGET
SSAJRP
CHF 398 166
ZAR 6 143 695

POSTGRADUATES

	MSc	
0		1
1	Postdoc	0

EXCHANGES
1 TO SWITZERLAND
2
1 TO SOUTH AFRICA

SWITZERLAND SOUTH AFRICA GLOBAL JOINT TOTALS

Novel host-protective functions against Leishmaniasis using transcriptomics and cell-specific gene-deficient mice

UNIVERSITY PARTNERS

University of Lausanne
Professor Fabienne Tacchini-Cottier
University of Cape Town
Associate Professor Reto Guler

Leishmaniasis, one of the neglected infectious diseases, is a significant health problem in Africa with a rising concern of Leishmania-HIV co-infection. There is an urgent need to develop more effective drugs and vaccines in the fight against Leishmanisasis. In sub-Saharan Africa, Leishmaniasis is responsible for high morbidity and mortality. There is no efficient vaccine and an increase in resistance against the commonly used drugs is observed. It is therefore important to dissect the immunological pathways involved in the development of a protective immune response to *L. major* infection.

This collaborative project brought new insights regarding the mechanism required to develop a protective immune response against the parasite. Better knowledge of the immunopathology contributed to improving the design of an efficient vaccine against Leishmaniasis.

The data provided important insights into understanding the immune response taking place following infection with *L. major*, contributing to the knowledge needed to design an effective vaccine.

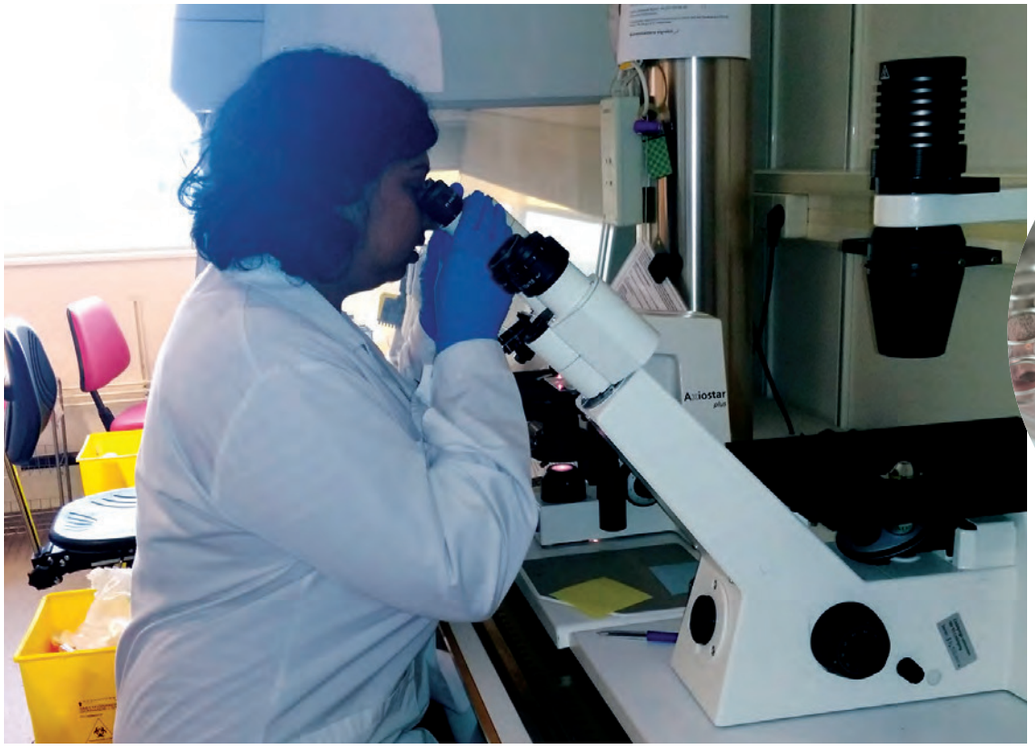
The short-term goal of the project was to better understand the cellular interactions between keratinocytes and other cells present in the microenvironment at the site of infection. They showed that IL-4 (interleukin-4, a small protein that plays an important part in certain immune reactions) interaction with keratinocytes during the first hours of infection is not critical in launching the adaptive immune response needed to cure the diseases. These results contributed to the team's long-term goal, which was to identify important therapeutics and drug targets for Leishmaniasis. The uncovered mechanisms may be relevant to immunopathogenesis of many other infectious diseases.

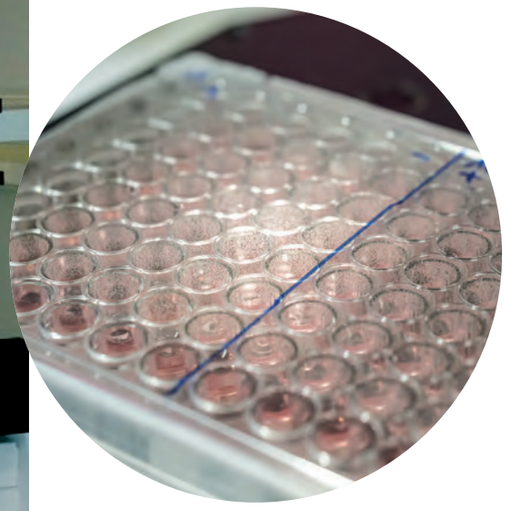
The collaboration of the Swiss and South African teams with complementary competencies in immunology, infectious disease and biotechnology enhanced capacity building of both partners' host institutes, and contributed in the long term to the much-needed international effort to fight neglected diseases such as Leishmaniasis.

Some of the laboratory members are (from left): Bere Salazar, PhD, Professor Fabienne Tacchini-Cottier and Katiuska Passelli, PhD student.

Research group of Associate Professor Reto Guler (UCT) with Dr Ramona Hurdayal, Shandra Pillay and Lorna Gcanga.

Photographs courtesy of Reto Guler



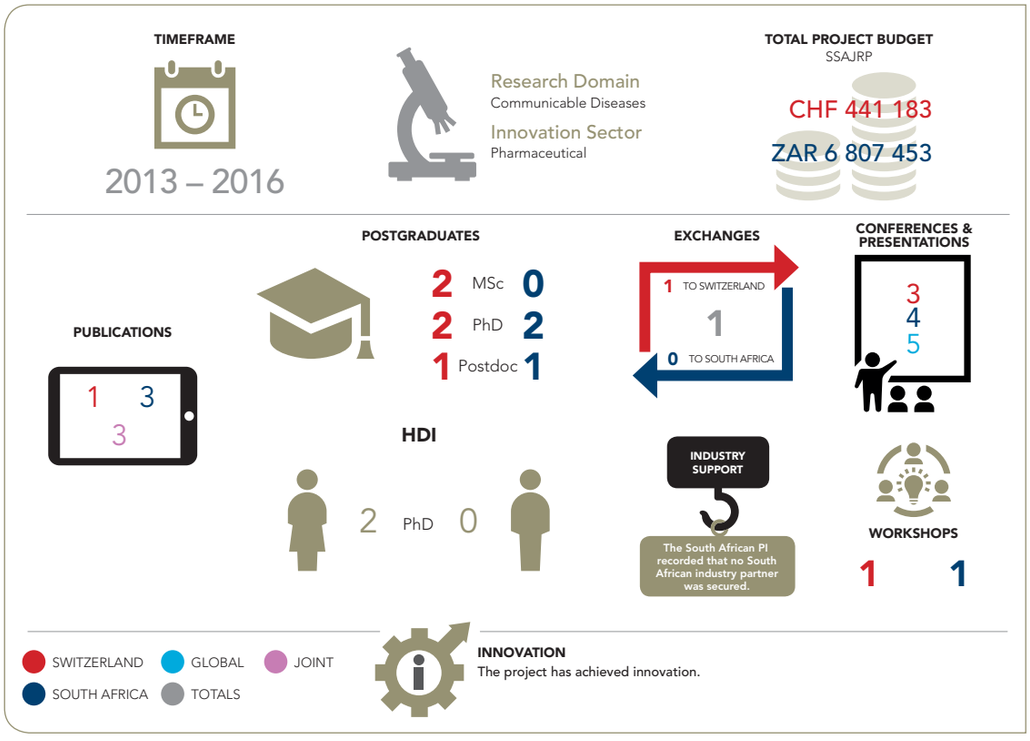


Photograph courtesy of Reto Guler

The lead author Dr Melissa Govender (UCT) evaluates parasites by microscopy.

The community at large will benefit from this project since this research work investigated the early protective immune response to *L. major* with a constructive impact for future development of potential novel host-directed therapy against Leishmaniasis. This research is of particular importance to developing countries where Leishmaniasis is highly prevalent with an estimated 1,3 million new cases occurring annually. It also raises the awareness for alternative host-directed drug treatments against Leishmaniasis with the benefits of avoiding drug resistance against Leishmania parasites.

The exposure of postgraduate students to international experts, along with technology transfer, provided scarce skill development and much-needed training for the South African biotechnology and industry sectors.



Virulence of pneumococcal serotypes in human meningitis

UNIVERSITY PARTNERS

University of Bern
Dr Lucy Hathaway
National Institute for Communicable Diseases
Professor Dr Anne von Gottberg

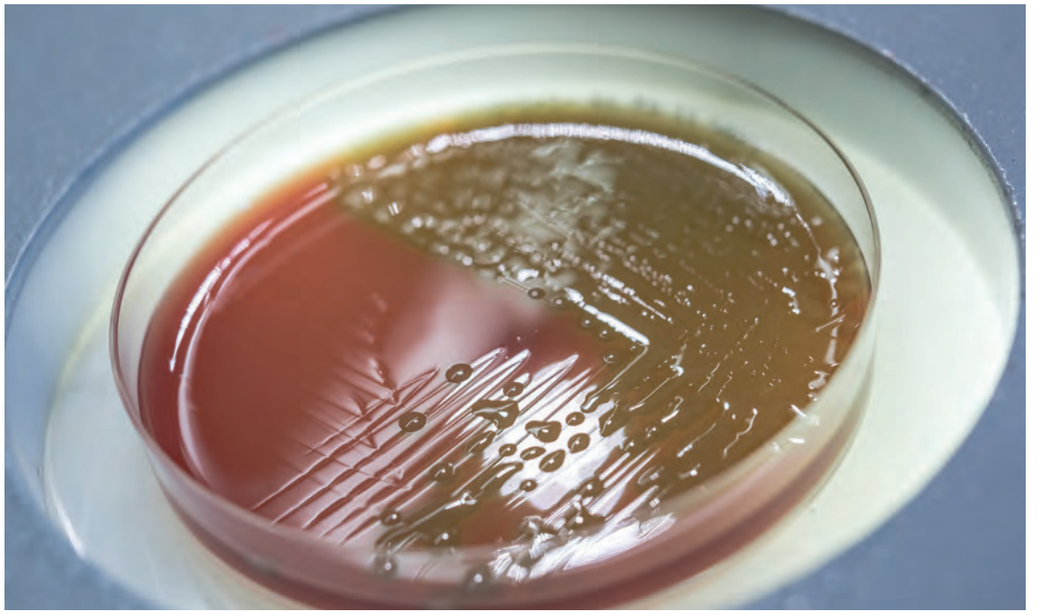
This project is aiming, firstly, to determine the relationship between pneumococcal serotype and the severity of infection and, secondly, to establish an *in vitro* model for the prediction of serotype severity in humans to inform future vaccine design. *Streptococcus pneumoniae* (or pneumococci) are Gram-positive bacteria known to cause infections such as pneumonia, meningitis, bacteremia, sepsis and otitis. The most severe form of disease is invasive pneumococcal disease (IPD) and includes bacteremia and meningitis.

In 2009, a published paper calculated that *S. pneumoniae* causes about 11% of all deaths in children aged 1 – 59 months, with Africa having the highest incidence rate and accounting for 66% of pneumococcal cases worldwide. In South Africa, the mortality rate from pneumococcal meningitis is particularly high.

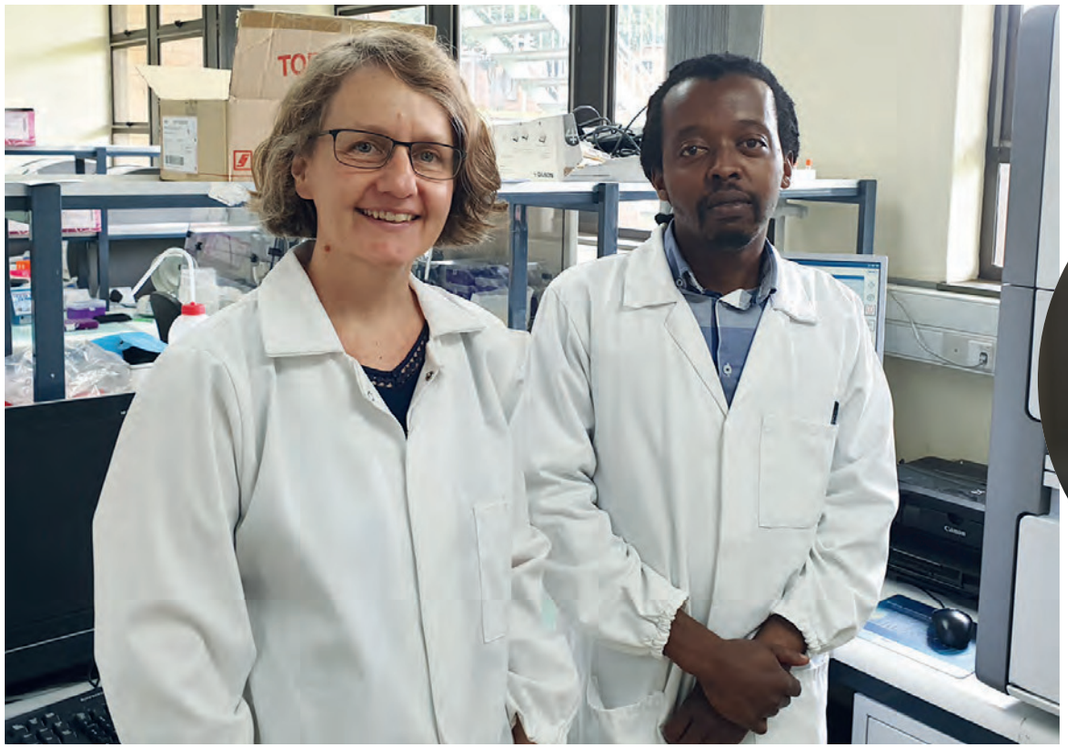
Among other factors, virulence of pneumococci can be attributed to the polysaccharide capsule that defines the pneumococcal serotype and of which more than 90 different serotypes exist. The polysaccharide capsule forms the basis for the manufacturing of vaccines, which play a large part in today's health by protecting people of all ages against diseases including IPD. In 2009, the 7-valent pneumococcal conjugate vaccine (PCV) was introduced for children in South Africa. Two years later, in 2011, it was replaced by PCV13. The introduction of these vaccines has led to a decline in vaccine-serotype IPD in all ages. However, while the prevalence of vaccine-serotypes is decreasing, increases in non-vaccine serotypes have been observed. A major reason for this is that currently all vaccines against *S. pneumoniae* target the polysaccharide capsule and are therefore specific for a fraction of the serotypes circulating in the human population. This gives a selective advantage to those serotypes absent from the vaccines. For this reason and to ensure that vaccines continue to be efficient in protecting against diseases, continued improvement and design of new vaccines is necessary.

Interestingly, some serotypes, referred to as invasive serotypes, are often associated with disease. Other serotypes are less likely to cause disease and are referred to as colonisers as they can be found colonising the nasopharynx of healthy adults and

Dr Lucy Hathaway and Annelies Müller in the laboratory in Bern, Switzerland.

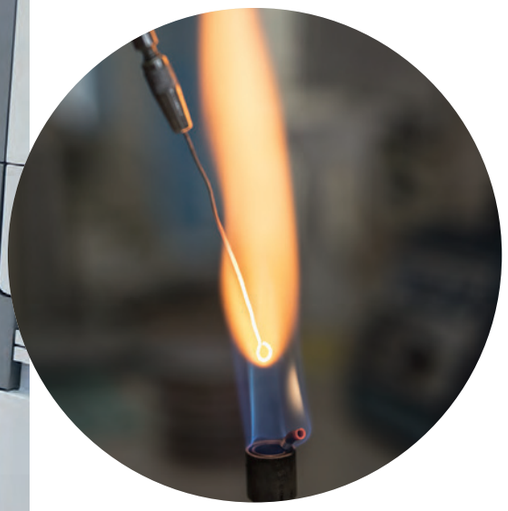


Pneumococcal colonies growing on a blood agar plate.



Photograph courtesy of Prof A von Gottberg

Professor Anne von Gottberg and Thabo Mohale, an MSc graduate, at the National Institute for Communicable Diseases laboratory.



An inoculation wire loop is sterilised in preparation for lab experiments.

children. It has been shown, however, that when these normally colonising pneumococci invade the body, they tend to cause particularly severe disease. Consequently, certain serotypes are clinically relevant depending on their invasiveness and the severity of disease they cause. Additionally, previous publications by the research group in Switzerland have shown that the pneumococcal capsule may play an important role in the severity of disease. These publications form the basis of this project.

The GERMS-SA enhanced surveillance program in South Africa and the expertise of the group in Switzerland in basic research, will contribute towards understanding the relationship between serotype and severity of disease. The knowledge gained from the project is important to help inform the design of future vaccines and will also contribute to the understanding of pneumococcal virulence in general.

The challenge involves coordination of 15 different GERMS-SA enhanced surveillance sites for CSF collection and organisation of transport logistics to ensure stability of inflammatory markers.

TIMEFRAME

2017 – 2021

Research Domain
Communicable Diseases

Innovation Sector
Pharmaceutical

Cross-cutting Domain
Biotechnology

TOTAL PROJECT BUDGET
SSAJRP

CHF 487 274

ZAR 7 518 643

POSTGRADUATES

1 PhD 0

EXCHANGES

0 TO SWITZERLAND

1

1 TO SOUTH AFRICA

WORKSHOPS

0 1

SWITZERLAND GLOBAL JOINT
SOUTH AFRICA TOTALS

Role of TRIM5 and CypA in the HIV-1 transmission and pathogenesis of a well-characterised South African cohort

UNIVERSITY PARTNERS

University of Geneva
Professor Dr Jeremy Luban
University of KwaZulu-Natal
Professor Thumbi Ndung'u

A study of HIV-1-infection-prone individuals has revealed several genetic factors that protect some individuals from infection and disease progression. The research team investigated mechanisms of natural regulation of the HIV-1 restriction factors and HIV-1 cellular co-factors. Elucidation of these mechanisms will enable scientists to create novel vaccines and therapeutic interventions against this disease.

When these novel vaccines are rolled out into the global market, it could result in a significant global decrease in the infection rate. The innovation of novel HIV therapy will represent a major advance on a global scale.

During a study of the roles of CypA (cyclophilin A) in HIV-1 regulation, the research team discovered the tripartite interaction motif containing protein 5 (TRIM5) as an additional factor that could explain this disparate clinical phenotype. While searching for such factors among a specific cohort, they generated evidence to indicate that differences in TRIM5 and CypA correlate with different rates of infection, or different rates of disease progression in this cohort. The collaborators investigated the causes of the different clinical outcomes of HIV-1-infected people in this specific cohort with respect to the variants in the TRIM5 and CypA proteins.

The contribution of type 1 interferons (IFNs) as antiviral factors to HIV pathogenesis is not completely understood. The researchers carried out investigations to determine if the increased expression of a select type 1 IFN and TRIM isoforms is associated with a significantly lower likelihood of HIV-1 acquisition and viral control during primary HIV-1 infection. They found concordance between type 1 IFN (INF-1) and the TRIM22 isoform, the latter which is thought to act as an antiviral effector *in vivo*.

To test the hypothesis of the dysregulation of TRIM E3 ligases (TRIM5, 11, 19, 22, 36), CypA and IFN-1 factors upon HIV infection, they tested the peripheral blood mononuclear cells (PBMC) of HIV-positive subjects (within one year of infection) against those of HIV-negative subjects.

They also tested the hypothesis that there is a reduced likelihood of acquiring HIV-1 if innate

HIV PCR preparation workbench.

Members of Professor Thumbi Ndung'u's research group: Dr Kavidha Reddy and Sharon Khuzwayo.

Professor Thumbi Ndung'u

Preparation of reagents for whole genome HIV PCR.

and intrinsic immunity factors such as TRIM E3 and IFN-1 are expressed in increased quantities. Matched samples, i.e. PBMC from non-seroconverter subjects versus subjects who were recruited while HIV-negative and who later contracted the disease, were used. Conversely, they hypothesised that the likelihood of HIV contraction is increased by a high baseline expression of CypA. Results have shown that the expression of these factors indicates the progression of the disease (viral load and CD4+ T-cell counts).

This project gave answers for a number of emerging questions, some pertaining to the relative susceptibility of a well-characterised cohort of high-risk South African individuals to HIV-1 infection being due to expression levels of factors such as TRIM E3 ligases, cyclophilin A, and type 1 interferons in PBMC, and whether these factors correlate negatively with viral loads and positively with CD4+ T-cell counts. Insight into the HIV-1 target cells that express TRIM E3 ligases, and cyclophilin A and a positive *in vitro* demonstration of enhanced HIV-1 replication upon knockdown of particular TRIM E3 ligases, were also questions that pave the way for novel vaccine and therapeutic intervention creation against the disease.

TIMEFRAME

2008 – 2012

Research Domain
Communicable Diseases

Innovation Sector
Pharmaceutical

Cross-cutting Domain
Biotechnology

TOTAL PROJECT BUDGET
SSAJRP

CHF 407 599

ZAR 6 289 254

PUBLICATIONS

2

POSTGRADUATES

0	MSc	1
0	PhD	2

HDI

1	MSc	0
1	PhD	0

SWITZERLAND GLOBAL JOINT
SOUTH AFRICA TOTALS

INNOVATION
No innovation was achieved during the project period but had the potential for the creation of novel vaccines and therapeutic interventions against the HIV-1 pathogen.

Analyses of geographical patterns of malaria transmission and mortality in Africa using Bayesian spatio-temporal modelling

UNIVERSITY PARTNERS

Swiss Tropical and Public Health Institute
Dr Penelope Vounatsou
University of the Witwatersrand
Professor Kathleen Kahn

Photograph courtesy of Paul Weinberg

Fieldworkers collecting Geographic Information System and mortality data at the MRC/Wits-Agincourt Health and Demographic Surveillance System (HDSS) site.

In South Africa, mortality in most age groups has been increasing. Estimating the geographical patterns of mortality and assessing spatio-temporal trends is important in identifying vulnerable population sub-groups, introducing interventions, evaluating the effectiveness of interventions and progress in achieving the Millennium Development Goals.

Malaria, caused by *Plasmodium falciparum*, the most severe of the parasite species to infect humans, is the most prevalent human parasitic disease. The geographical distribution of malaria is not well defined in South Africa, and the risk of contracting malaria varies across the risk areas. It is thus essential to determine the transmission of malaria to define the areas with greatest risk and to facilitate appropriate control strategies for these regions. Key factors affecting the transmission of malaria should be identified. A map outlining the malaria transmission in South Africa can help with intervention strategies to optimise human and financial resources.

This project enabled South African scientists to acquire the relevant skills and training in disease mapping and risk factor analysis of geographical data in order to contribute towards efforts to understand determinants of mortality and to reduce malaria risk. It builds on the long-standing collaboration between the Swiss Tropical and Public Health Institute (Swiss TPH), Wits School of Public Health and the South African Medical Research Council (SAMRC) in Durban. The partnership drew on data available within the three institutions, and expertise in Bayesian spatio-temporal modelling, malaria epidemiology and mortality, to address key research questions of major concern across much of Africa.

The first objective was to investigate mortality by identifying risk factors related to cause-specific mortality for defined age groups, producing maps of cause-specific mortality and assessing long-term temporal changes of all-cause and cause-specific mortality. The second objective was to investigate malaria transmission. This was achieved by estimating and mapping malaria seasonality in Africa, assessing spatio-temporal patterns of malaria transmission and producing regional- and continent-wide transmission maps adjusted for

Drawings courtesy of Mark Collinson

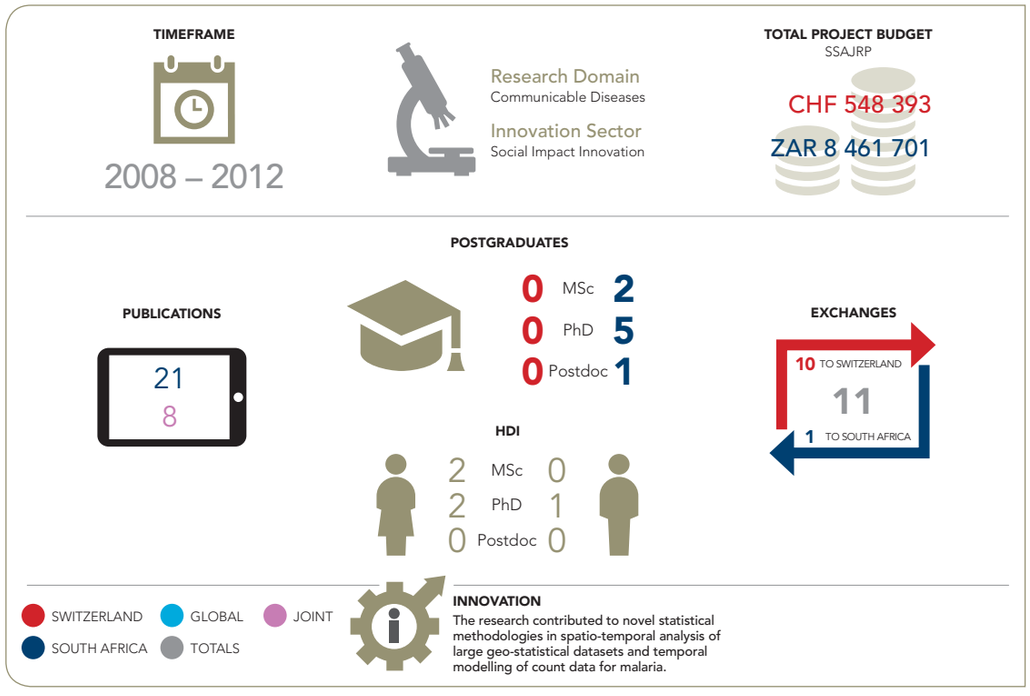
Location of the MRC/Wits-Agincourt HDSS site.

Professor Kathleen Kahn

age, seasonality and climate factors and to develop models for forecasting malaria case data.

This research contributed novel statistical methodologies in spatio-temporal analysis of large geo-statistical datasets and temporal modelling of count data. Application of this work to mortality and malaria data from Africa, together with state-of-the-art existing Bayesian modelling approaches in the analysis of spatio-temporal data, will contribute to understanding the risk factors, geographical patterns and spatio-temporal changes of mortality; maps of malaria seasonality and malaria transmission; and malaria forecasting models. This provided estimates of burden of disease and its distribution, which will contribute to public health policy and programmes and evaluation of interventions.

This project assisted with capacity building in South Africa. Statistical capacity was developed by nesting doctoral students and postdoctoral fellows from both South Africa and Switzerland within this project. The project supported South African doctoral students to be trained on Bayesian spatio-temporal modelling, disease mapping, Markov chain Monte Carlo simulation methods, and mathematical modelling of malaria transmission.



Medicines from marine microbes

UNIVERSITY PARTNERS

Swiss Federal Institute of Technology Zurich
Professor Jörn Piel
University of Western Cape
Professor Marla Trindade

The objective of the project was to employ metagenomics technologies to identify and characterise novel pharmaceutically applicable prokaryotic biosynthetic pathways and to use gene transfer technology to express the biosynthetic pathways identified in easily cultured bacteria for the production of larger quantities of the compounds necessary for the preclinical and clinical phases.

In South Africa the project obtained 940 extracts from 479 marine invertebrate-associated bacteria that were screened on various platforms for activities against a number of pathogens and diseases. The genomes of the 20 most promising bacteria were sequenced. The sequence information was used to link antimicrobial activities to biosynthetic gene clusters and to establish heterologous expression systems. Three biosynthetic gene clusters were transferred into either *E. coli* or *Pseudomonas putida*. An NPRS cluster expresses in *E. coli* and confers antimicrobial activity against *B. cereus* and *S. epidermidis*.

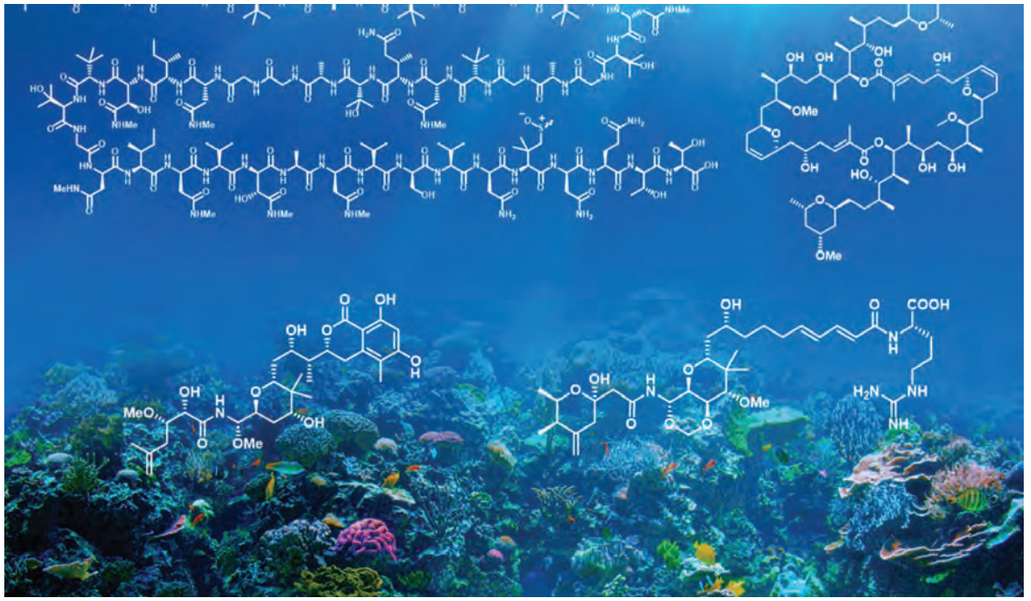
The compound was isolated and is currently being characterised. A rhamnolipid pathway was successfully expressed in *P. putida*. Rhamnolipids have antibiotic bio-surfactant properties that make them suitable for application in a number of different industries. The integration of a rhamolipid pathway on a GRAS-status bacterium genome has never before been successful, which is required for industrial development.

Jointly, the Swiss and South Africa laboratories investigated two *Thalassomonas* species through bioactivity-guided fractionation. Initial fractionation indicated bioactivity against *P. putida* and a highly multidrug-resistant *E. coli*. These efforts, combined with electron microscopy, indicated that these two species produce tailocins with antimicrobial activity, and further analyses are underway to complete the investigation.

The Swiss laboratory focussed on the sponge-associated bacterium which produces a new antibiotic with nM activity against Mycobacterium tuberculosis. The compound is structurally novel and can be synthesised in a straightforward way. MoA studies and generation of analogs are underway to further evaluate its potential for TB therapy. In studies on uncultivated producers of sponge

Ms Shanice Adams, SA MSc student and Professor Marla Trindade.

Professor Jörn Piel



Representative bioactive natural product families isolated from the marine sponge Theonella swinhoei, *which are produced by a bacterial symbiont.*

Photograph courtesy of Prof Piel

QPix robotic colony picker, for the high throughput screening of marine metagenomic libraries for bioactive compounds.

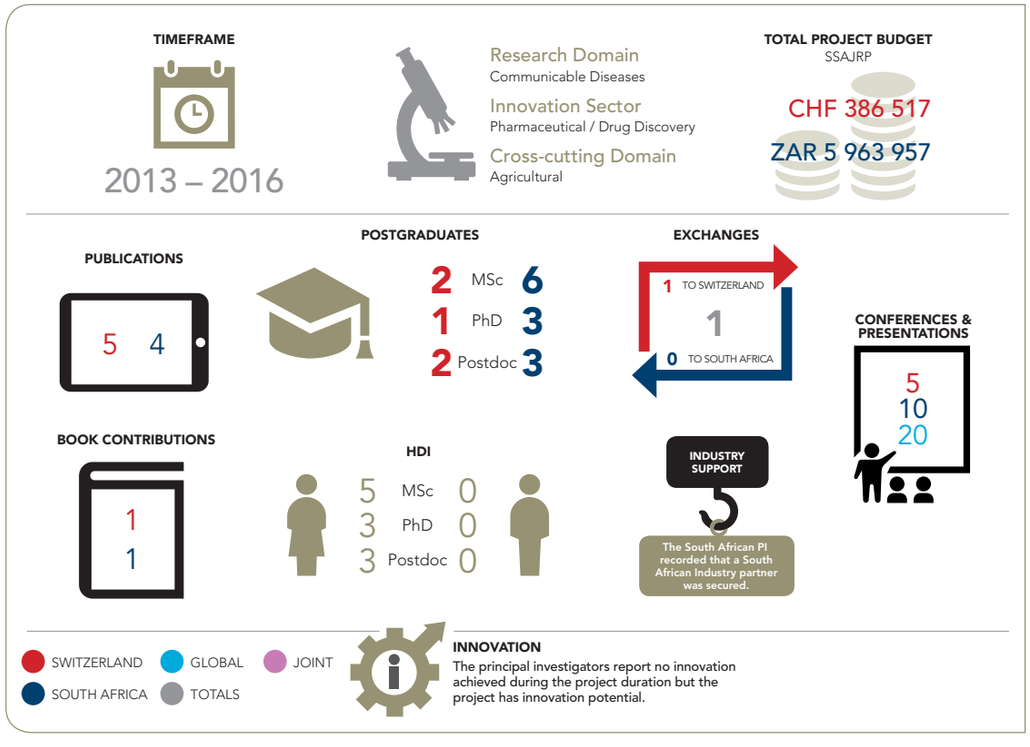
pharmaceuticals, uncultivated symbiotic producers from five different sponges were identified and genomically characterised. These belong to at least five different candidate genera, thus validating the chemical potential of uncultivated microbes. Dozens of biosynthetic gene clusters are now available. For one of these, encoding biosynthesis of the highly cytotoxic and complex polytheonamides, a new bacterial expression platform was established that produces these compounds within one to two days and also generates engineered variants.

The project presents novel opportunity to evaluate the mode of action, chemistry and the development of pharmaceutical products in the South African marine invertebrate isolates. The project has partnered with the H3-D centre in collaboration with Dr Digby Warner to further screen the libraries against TB.

The project enabled the potential for the discovery of completely novel structures with potentially valuable bioactivity properties for pharmaceutical development. Beyond drug discovery is the economic opportunity for bio-surfactants and pigments to be used in cosmetics and other personal medicines, antifungals for agricultural pesticides, and as antioxidants and additives in foods. This project therefore has both a social and economic impact as many enzymes and compounds from marine sources are being used as the basis for many biotechnology applications, a multi-billion dollar industry. This, however, is a long process but proves that the South African biodiversity is "hot" and holds much promise for future commercialisation objectives.

Photograph courtesy of ETH Zurich

Transmission electron microscopy of tailocin preparations from T.viridans.



Gene engineering HIV-resistant cells

UNIVERSITY PARTNERS

University of Zurich
Professor Roberto Speck
University of Pretoria
Professor Michael S Pepper

The project objective was to identify and target host elements in order to render CD4+ T-cells (cells that play a major role in instigating and shaping the adaptive immune responses) resistant to HIV and to prevent viral escape.

The collaborators' gene engineering approach was assessed *in vivo* in humanised immune system (HIS) mice infected with HIV as pre-clinical proof-of-concept. They directed their work at optimising techniques to expand haematopoietic stem cells for use initially in the HIS mouse model and ultimately for Phase I clinical trials. Subsequent work has been directed at optimising the microRNA approach (not part of the projects funded by the SSAJRP).

The Swiss research team was in charge of testing whether the microRNA to CCR5 – as generated by the team of Professor Karl Heinz Krause from the Geneva-based laboratory-generated an HIV-resistant immune system *in vivo*. Key findings and expertise gained were: efficient delivery of the lentiviral construct into haematopoietic progenitor and stem cells (HSPCs) demonstration of multi-lineage haematopoiesis of gene-engineered HSPCs; and functional cure of HIV achieved when humanised mice transplanted with gene-engineered HSPCs were generated (pre-clinical proof-of-concept).

The work in the South African laboratory has focussed on optimising techniques for expansion of haematopoietic stem cells, and on establishing the susceptibility of human haematopoietic stem cells (HSCs) to HIV infection. With regard to the first, a combination of *in vitro* experiments and *in vivo* experiments in NSG mice – one of the most immune-deficient mouse strains – have confirmed the effect of stemregenin-1 on expanding the primitive sub-population of HSCs.

In addition to the above, all three groups are preparing for a Phase I clinical trial based on the data generated over the three years of the project.

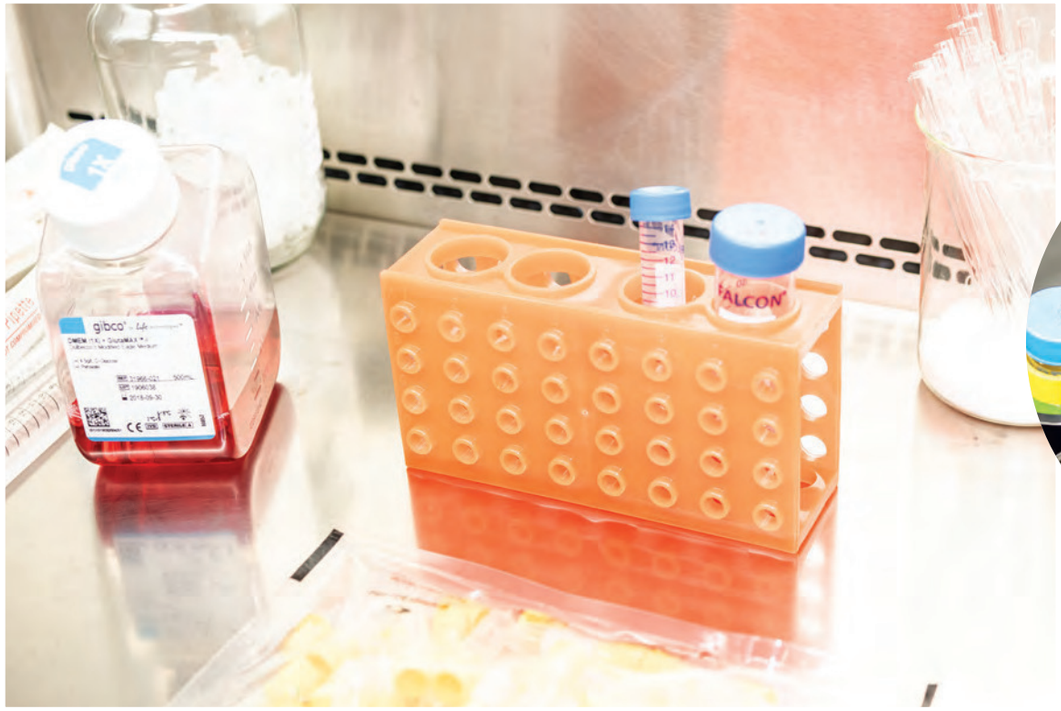
As the team gets closer to utilising the technology they have developed in patients, initially as the Phase 1 clinical trial, there is likely to be a significant impact on patients (society) and on industry, the latter initially in the form of a start-up biotechnology company.

Professor Michael Pepper

Professor Roberto Speck

Photograph courtesy of University Hospital Zurich

Pipettes used for laboratory work.



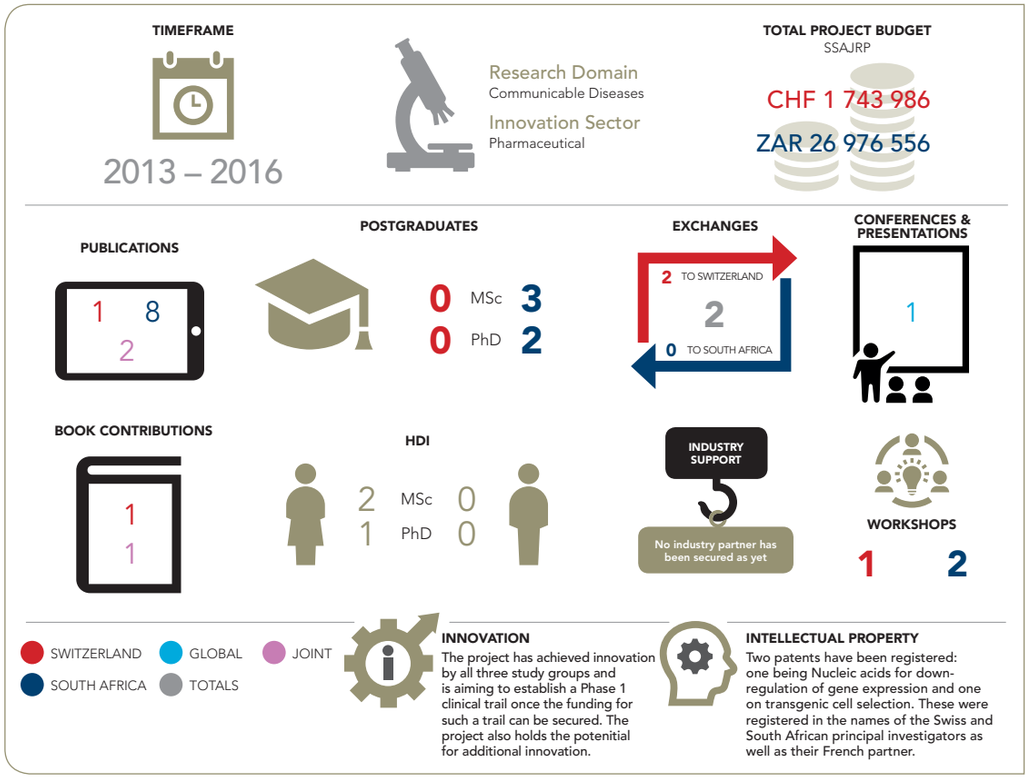
Centrifuge for research work.

Tissue culture setup.

The project has cemented a very strong relationship between the University of Geneva, University of Pretoria and the University of Zurich participating groups. The funds provided by the SSAJRP have allowed for an excellent and ongoing collaboration between these universities. Very importantly, the approval of this project intensified the collaboration between Switzerland and South Africa with yearly workshops either in Switzerland or in South Africa.

The funds provided by the SSAJRP established a platform from which additional funding was obtained from two competitive calls released by the SAMRC. The first was the prestigious SAMRC University Flagship Programme; the second was the SAMRC Extramural Unit award for Stem Cell Research and Therapy.

Finally, two patents emanated from this project, with one of them forming the core element of a start-up company that is registered in Switzerland.



Cultivation of marine algae for the development of valuable pharmaceutical agents

UNIVERSITY PARTNERS

Swiss Federal Institute of Technology in Lausanne
Professor Jean-Paul Schwizgübel
University of Western Cape
Dr Rolene Bauer

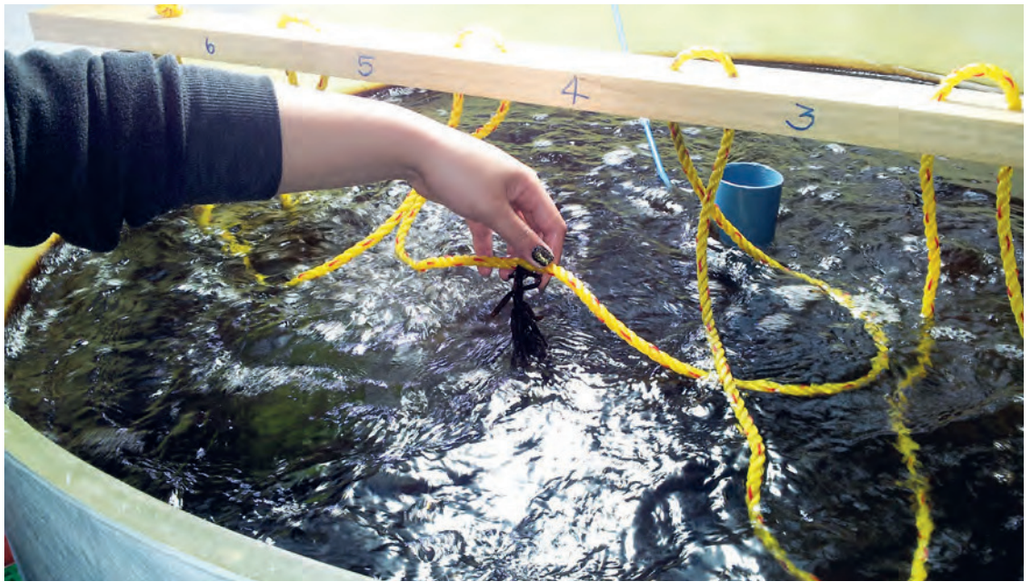
Algal extracts have been shown to exhibit potential pharmacological actions in mammalian systems, many with novel complex structures. Marine algae have a very high protein and/or polysaccharide content making them ideal for biomass production. The study was aimed at establishing cultivation platforms for micro-algae (Switzerland) and macro-algae (South Africa). Potentially valuable pharmacologically active molecules produced by these algae were targeted.

Numerous macro-algal species, endemic to South Africa, were implicated in production of polysaccharides with activity against breast cancer cells. Of particular interest was a brown algae (Splachnidium sp.) characterised by cylindrical branches filled with mucus (cellular bags of highly valuable fucoidan). Sulphated polysaccharides, such as fucoidan, have potent anticancer and antithrombotic activities due to the ability to imitate patterns of sulphate substitution.

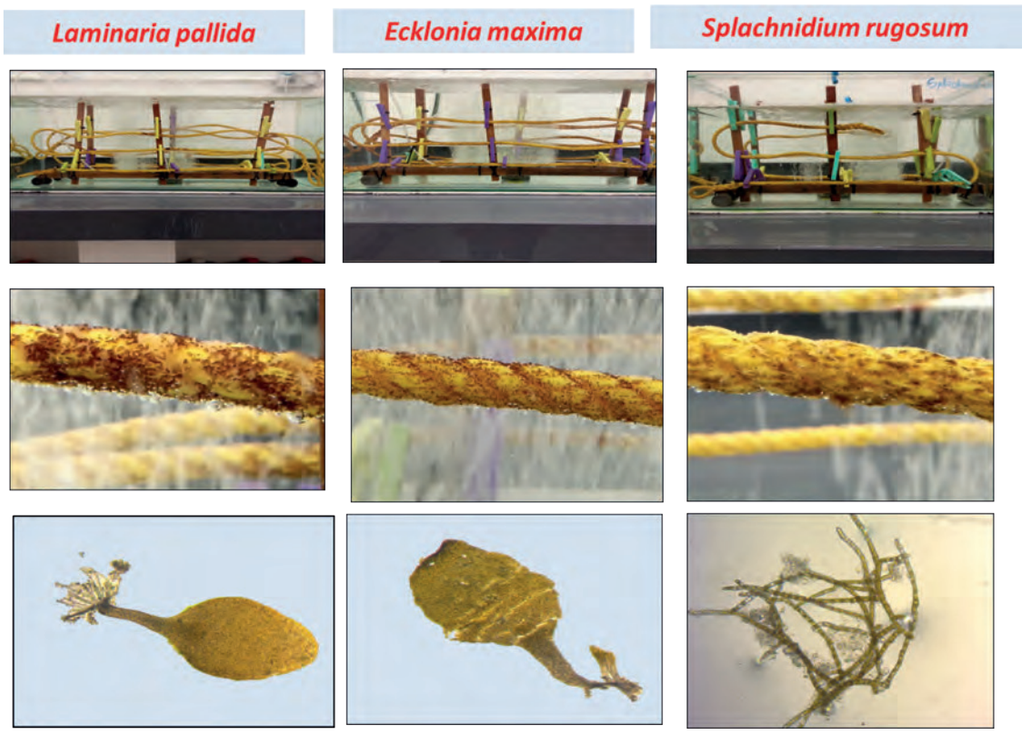
Short-term objectives of the project included cultivation of algae under controlled conditions and the elucidation of the structure of purified bio-active molecules. A long-term objective included bulk production of pure pharma-compounds for clinical trials and bio-medical applications.

The project resulted in the establishment of a cultivation platform for selected species of macro-algae endemic to South Africa in the open ocean (West Coast) and is now used and maintained by the algal research team of the Department of Agriculture and Fisheries (DAF). Collaboration with researchers at EPFL (Lausanne) and student exchange (Stephen Mackay, PhD) resulted in one joint high-impact publication.

Micro-algae are a valuable source of high-value products, lipids and biomass. However, several energetic limitations restrict sustainability. One of the major limitations to micro-algae large-scale production in open ponds has been the harvesting of micro-algae which can make up 50% of the operational cost. Stephen Mackay published a joint study on a newly described lichen co-culture that shows potential for application as an energy-efficient harvesting method. The method is based on bio-flocculation of filamentous fungal pellets that form large dense lichen pellets with micro-algae that could be easily harvested.



Development of marco-algal spores on ropes in tanks.



Dr Rolene Bauer

Grant January (MSc student) harvesting macro-algae.

Splachnidium rugosum.

All photographs courtesy of Rolene Bauer

Growth of macro-algae on ropes in the open ocean.