IN SILICO TRIALS
FOR TUBERCULOSIS
VACCINE DEVELOPMENT

STRITUVAD

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The University Of Sheffield.

THE PROJECT

We want to fight Tuberculosis by developing an **innovative in silico approach** for testing the efficacy of new therapies through **computer modelling and simulation**.

We supervise phase IIb **clinical trial on tuberculosis patients in India**, and we perform **in silico trials on virtual patients** modeled on a computational platform that takes into account real data from recruited patients.

- **RUTI® adjuvant vaccine**, developed by the Spanish biotech company Archivel Farma, will be tested in the phase IIb clinical trial conducted at the All India Institute Of Medical Sciences of New Delhi and the Agartala Government Medical College of Agartala.

- **UISS-TB computational platform**, developed by the University of Catania, will expand the data from real patients with data from virtual patients through advanced modeling approaches and adaptive Bayesian methodologies developed by the University of Sheffield, to predict the effects of long-term combined therapy.

- **The model's credibility** will finally be assessed through verification and validation. New calculation verification strategies specifically designed for agent-based models were developed by the University of Bologna to identify and remove possible numerical errors associated to UISS-TB*.

*Curreli, C. et al., Verification of an agent-based disease model of human Mycobacterium tuberculosis infection, International Journal for Numerical Methods in Biomedical Engineering, 37(7), e3470

DURATION: 60 MONTHS

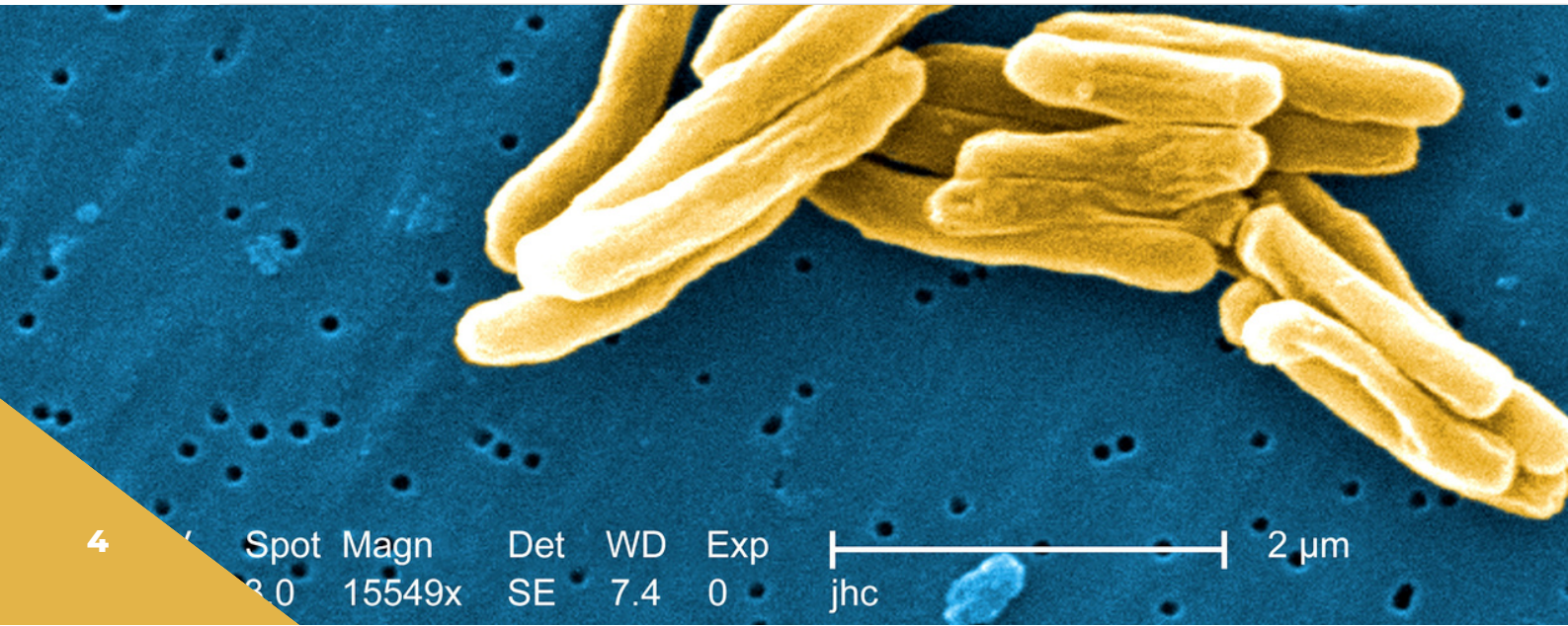
FEB 2018 **JAN 2023**

WHY TACKLING TUBERCULOSIS?

According to the World Health Organization, **Tuberculosis (TB) is still one of the top ten causes of death**. TB is present all over the world, but most of the people who fall ill with TB live in low- and middle-income countries: about half of all people with TB can be found in 8 countries: Bangladesh, China, India, Indonesia, Nigeria, Pakistan, Philippines and South Africa.
Data from 2020 show that 10 million people fell ill with TB and 1.3 million died from the disease. In India, the WHO TB statistics for 2021 give an estimated incidence figure of 2,590,000 million cases.

Tuberculosis is an **infectious disease caused by a bacterium** called *Mycobacterium tuberculosis*: it is highly contagious, as it is spread through the air when people with lung TB sneeze, cough, or sometimes also talk or sing.
When someone is infected, the bacteria tend to form in the lungs small spheres inside which the bacteria remain protected from the immune system, waiting for an opportunity to proliferate, so that the disease can remain dormant for a long time with little or no symptoms, but when the infection becomes active, it is severely debilitating and - if not properly cured - it is lethal.

Indeed, **despite being preventable and curable, TB still kills 1.5 million people each year** – being the world's top infectious killer.



MULTIDRUG-RESISTANT TUBERCULOSIS

The most alarming dangers are the drug-resistant strains of tuberculosis: **multidrug-resistant** (MDR-TB) and **extensively drug-resistant** (XDR-TB) tuberculosis, defined by resistance to at least two (MDR-TB) or four (XDR-TB) most commonly used drugs in the current first-line regimen.

Experts agree on these strains to be the result of many years of **wrong or improperly administered drug regimens**: if the antimicrobial therapy is interrupted too early, or if the drug has insufficient potency (for example because it was poorly preserved), there will be a selection of the bacteria carrying mutations that make them more resistant to the drugs. Patients infected with such strains will remain infectious, and when the disease manifests again, standard drugs will not work anymore.
Worldwide in 2018, the treatment success rate of MDR/RR TB patients was 59%.

The probability that an MDR-TB strain develops is proportional to the duration of the antimicrobial therapy, and inversely proportional to its efficacy: the more effective the TB therapies are and the more quickly they work, the more unlikely are the chances that an MDR-TB strain survives and diffuses.
The actual treatment for drug-resistant TB is very long, toxic, complicated and expensive.

THERAPEUTIC VACCINES

One very promising line of research for the treatment of MDR-TB is the use of **vaccine-like treatments as adjuvants of standard first-line therapies**, so-called therapeutic vaccines.

Preliminary evidence suggests that, in an organism heavily infected, **the inoculation of the vaccine can strengthen the immune response**, boosting the antimicrobial effect of the first-line therapies, reducing the duration of the treatment required for the full resolution of the active infection, and reducing the probability of a relapse.

WE NEED BETTER THERAPIES FOR TUBERCULOSIS, BUT WE ALSO NEED BETTER WAYS TO TEST NEW THERAPIES

According to a 2016 study*, the costs of drug development have increased exponentially in the last decades, reaching the amount of US$ 2.9 billion to bring a new drug to the market.
The cost of development, and especially the cost for clinical trials, contribute massively to the final cost of a drug. If we could **reduce the cost of development** while retaining the same level of safety and reliability, we would dramatically **reduce the final price of drugs**.

IN SILICO CLINICAL TRIALS

Pharmaceutical is not the only industry that produces potentially dangerous products, but in all other sectors, **the cost of safety testing has been reduced by using computer modeling & simulation** to virtually explore thousands of possible scenarios. Think about cars crash tests: nowadays 99% of car safety tests are done by computer simulation, and very few crash tests are performed to check that the models' predictions are accurate (model validation).

Back to the pharmaceutical industry, until 2016 it was impossible to produce primary evidence of safety or efficacy for a new drug using modeling and simulations; all evidence had to be obtained experimentally, on animals or on humans. **In silico trials** - that is the testing of new drugs with computer simulation - **could save in the future from 50% to 90% of the development costs.**

* DiMasi JA, Grabowski HG, Hansen RW. Innovation in the pharmaceutical industry: New estimates of R&D costs. J Health Econ. 2016 May;47:20-33. doi: 10.1016/j.jhealeco.2016.01.012. Epub 2016 Feb 12. PMID: 26928437.

Cost reduction is particularly important for a disease like **tuberculosis**, which mostly affects people living in developing countries, where **the final cost of the therapy is a critical factor**. In Europe as well, where universal health care models are predominant, there is a growing concern on the financial sustainability due to our aging population, and drugs are one of the main expenditures for most universal health care providers.

If the development cost is high, the final price for therapy will be as high, or even too high to be affordable for many patients living in lower-income countries.

The goal of the STriTuVaD project is to to **develop new technologies that make possible to test the reliability of adjuvant therapies** performing at least part of the clinical tests in silico, i.e. **through computer modeling and simulation**, which would dramatically reduce the costs involved, and ultimately provide more affordable therapies.

HOW DOES IT WORK?

When a pathogen invades the body of a patient, it seeds and starts to replicate. In most cases, the immune system detects the presence of the pathogen and sends specialised cells to search it and destroy it. A sort of race starts between the pathogen's rate of replication and immune system rate of "destruction".

The **UISS-TB platform developed Prof Francesco Pappalardo and his team at the University of Catania** simulates the pathogen invasion in an anatomical compartment; i.e. in the lungs for what concerns TB.

The race between the pathogen and the immune system is decided by a number of factors, which UISS-TB accounts for:

- the genetic structure of the pathogen;
- the initial pathogen load (how much pathogens the patient has been exposed to initially);
- the profile of the patient’s immune system

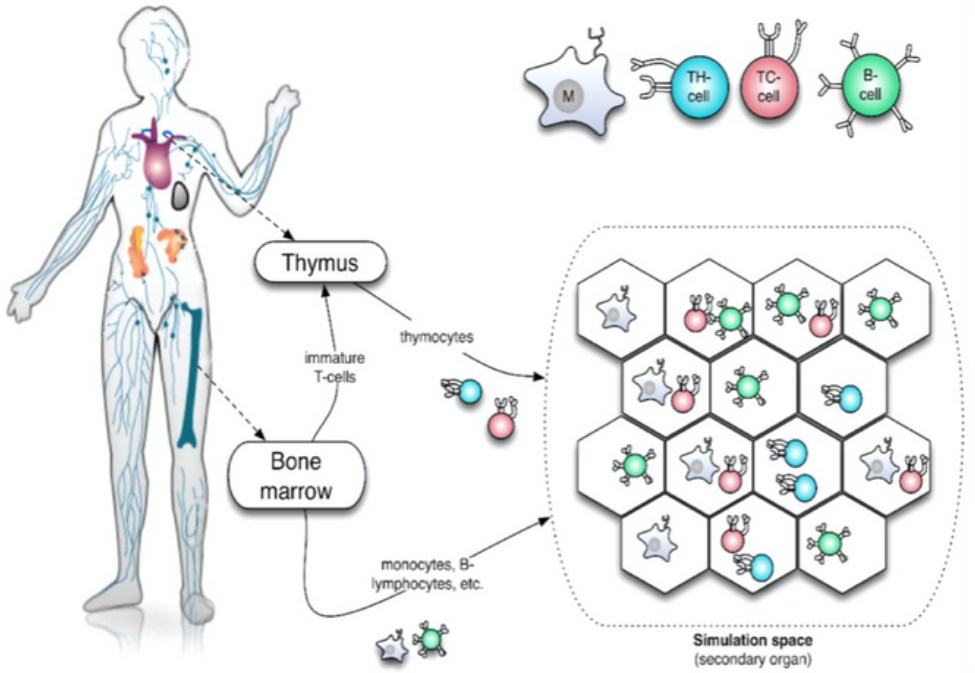
UISS-TB PLATFORM

UISS-TB (Universal Immune System Simulator for Tuberculosis) **is an agent-based disease model** because in the computer model, each bacterium is represented as an autonomous agent which can move, replicate and, if attacked, die.
Also, the cells of the immune system are modeled as autonomous agents, who can move, replicate, kill the pathogens, and die.

The UISS-TB model accounts for several factors, which include the genetic structure of the pathogen, the initial pathogen load (how much pathogens the patient has been exposed initially), and the profile of the patient's immune system.

One important information about this last factor is the Human Leukocyte Antigen (HLA), a portion of the patient genome that encodes many of the specific proteins that help the immune system to distinguish self (the host) and non-self (harmful agents), hence to recognise the pathogens.

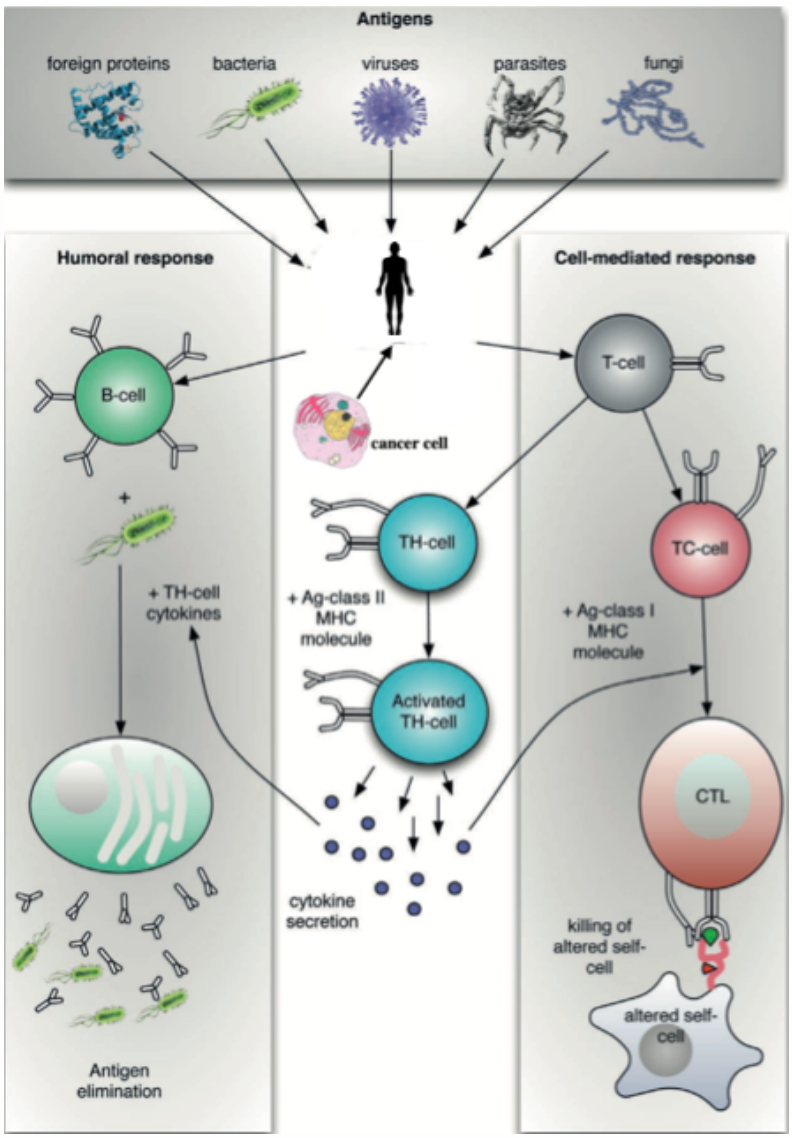
Knowing all this information about a patient, **it is possible to build a patient-specific model** capable of predicting how the pathogen will proliferate over a certain time.



In figure: UISS simulation space and machinery

In the case of TB, the best measure of the pathogen proliferation is probably the content of TB bacteria in the saliva or in the phlegm of the patient; by measuring the bacterial load today, and then measuring it again after some days, we can calculate a difference that gives us a measurement of the proliferation rate.

UISS-TB model is able to predict such proliferation rate. Comparing, for each patient, the actual proliferation rate against the model prediction, it is possible to measure the predictive accuracy of UISS-TB.



In some circumstances (for example in the case of a virulent strain of the pathogen), the immune system will not be able to fight the diffusion of the pathogen, and the bacterial load will increase over time.

However, if a patient is treated with an antibacterial drug, the proliferation would considerably slow down, and the immune system will be able again to clear the pathogen from the organism (except when we are faced with cases of multidrug-resistant TB).

In figure: The main scenarios UISS is able to model, in other words the physiological immune system response after a specific pathogen exposure.

IN SILICO AUGMENTED CLINICAL TRIALS

UISS-TB can also simulate the effect of different drugs and predict how the bacterial load after a given time will change on the basis of the drug used on that patient. Once more, by treating a patient with a drug, and then measuring the change in bacterial load, we can establish the predictive accuracy of UISS for that patient and that specific drug.

If we build many patient-specific models, to some extent we can consider these models as **virtual patients**, who when treated with a drug will respond likely as physical patients.
We can then create new virtual patients who are not copies of physical ones (i.e. not build on real patients' data), but who still have HLA, initial bacterial load, etc. that other patients could have.
One can test on these virtual patients a new treatment and see how effective this is, when compared to the current standard of care. Or one can simulate the effect of a standard treatment on special patients' groups, for example patients with co-morbidities, or exposed to a larger than usual bacterial load, or exposed to an MDR-TB strain.

Every physical patient we add to a **physical clinical trial** costs on average US$36,000. For a phase III clinical trial, one needs to enroll at least 1000 patients per each study. So, to study a new drug it is required $36 million; to study a different dose of the same drug another $36 million are needed; to explore if the drug still works on patients affected by other concomitant pathologies another $36 millions, and so on.

The main cost of an **in silico trial** consists of the initial development and the validation of the model.
We estimated that the development, validation, and regulatory qualification cost of UISS will be in the end €18 millions: this would be 50% of the cost of a single trial and will allow to use UISS-TB to test any new drug and their effect on a specific population, optimize the drug dosage for each subpopulation, and so much more. In special cases the savings could exceed 90% of the current costs.

RUTI® VACCINE

From its inception, RUTI® has been conceived as a **therapeutic vaccine** that increases the efficacy and shorten the duration of the current long and multiple antibiotic TB treatment. RUTI® is an inactivated vaccine that induces strong polyantigenic immunity, boosting the specific Th1 immune response both against secreted and structural antigens.

RUTI® is a liposome suspension of the drug substance with a charge excipient. The drug substance is based on cell wall nanofragments of *Mycobacterium tuberculosis*. It contains a wide mixture of antigens obtained by a *M. tuberculosis* growth under stress conditions.

The vaccine is presented as a dry powder for reconstitution with water for injections. The administration route of RUTI® is subcutaneous and of a single dose. **RUTI® has shown a good safety and wide immunological response in the clinical trials phase I and phase II in latent tuberculosis infection patients** (LTBI).

The proven ability of RUTI® to **activate the immune system** and **increase the efficacy of chemotherapy** offers an innovative answer for the treatment of multidrug-resistant tuberculosis (MDR-TB), whose incidence has grown alarmingly (next to 500,000 new cases in 2016 plus 100,000 cases of rifampicin-resistant TB) and that registers mortality rates of 40%. Additionally, patients with resistant forms of TB suffer a negative impact on their health due to the use of second-line antibiotics, increasing the time of treatment and side effects.

Archivel Farma focuses its strategy on the development of RUTI® to treat MDR-TB.

CLINICAL TRIALS IN INDIA

Last **October 2021**, partner Archivel Farma officially begun a clinical trial to test the safety and efficacy of the therapeutic vaccine RUTI®, which will be conducted at two hospitals in India and managed by Clinical Research Network India, an experienced Contract Research Organization.

The double-blind, randomized, placebo-controlled **phase IIb clinical trial** will investigate the efficacy of RUTI® as adjuvant of TB chemotherapy and will recruit 90 drug-sensible TB (DS-TB) patients and 50 MDR-TB patients.

The clinical trial is being conducted at two centers in India: the **All India Institute Of Medical Sciences** (AIIMS) of New Delhi, and the **Agartala Government Medical College** (AGMC) of Agartala.
The AIIMS was established by an Act of Parliament to develop patterns of teaching Undergraduate and Post-graduate Medical Education in all its branches. Twenty-five clinical departments including four super specialty centers manage practically all types of disease conditions with support from pre- and para-clinical departments.
The Agartala Government Medical College (AGMC) is situated in the G.B. Pant Hospital campus in Agartala, the capital city of Tripura.
It is recognized and approved by the Medical Council of India, Ministry of Health & Welfare, New Delhi, and affiliated to the Tripura University. It is completely run by the Government of Tripura.

"We are very satisfied that after many efforts we can begin this clinical trial in such prestigious Indian hospitals and in one of the countries most affected by tuberculosis disease. For Archivel it represents a great milestone since it brings the opportunity to get closer to new clinical findings that are expected to contribute to the future deployment of the RUTI® vaccine worldwide to improve the life of those affected by the disease" said Chief Executive Officer of Archivel Farma.

FROM CLINICAL TRIALS TO IN SILICO TRIALS

The group coordinated by **Prof Miguel A. Juárez from the University of Sheffield** developed and implemented a Bayesian hierarchical method for combining in silico and in vivo data into an augmented clinical trial using UISS-TB simulator.
The simulator produces in silico data from a number of biological entities and chemical species (e.g., cytokines) for an individual virtual patient, identified and characterised through an initial vector of 22 features (e.g. lymphocytes subpopulation count, bacterial load, body mass index).

From the virtual patient to the virtual cohort

To create whole cohorts of virtual patients, Prof Juarez and Dr Kiagias tuned a novel approach especially for UISS-TB, that samples the features mentioned above either at once or sequentially and, based on the joint distribution of the population characteristics, simulates each virtual patient using UISS-TB and the recorded endpoint of the clinical trial.
The goal of this strategy is to **reduce the number of real patients needed to test the efficacy of the adjuvant vaccine**. It aims at doing so by combining the information from the in silico data coming from the UISS-TB simulator with the in vivo data from the clinical trial that is taking place in India, and this is done using a Bayesian approach*.

The augmented clinical trials

Both sources of information, in vivo and in silico, are combined using a **novel statistical coherent Bayesian approach** capable of propagating the uncertainty from both sources of information onto the posterior distribution of the clinical endpoint. The contribution of the in silico experiment is controlled by a measure of compatibility with the in vivo data and weighted accordingly into the combined trial.

* Juárez, M.A., Pennisi, M., Russo, G. et al. Generation of digital patients for the simulation of tuberculosis with UISS-TB. BMC Bioinformatics 21, 449 (2020).

Indeed, it is important to balance the information between the two sources and to not overwhelm the information from the in vivo trial.

Thanks to this strategy, **UISS-TB successfully generated the first 26 virtual patients** based on real data from tuberculosis patients enrolled in the clinical trial ongoing in India. The results of the clinical trial for the first 15 patients were used to tune the UISS-TB platform and to design the first digital twins. The remaining ones were tested in silico to predict the effects of RUTI® vaccine and long-term combined therapy.

Using the information from in silico models to enhance clinical trials would allow to **decrease their size and duration**, that currently can reach between 5 and 7 years from Phase I to Phase III, and potentially speeding up the commercialisation of drugs or vaccines.
These improvements translate into a **lower final cost to the public**, a crucial aspect for those countries with the highest rate of incidence.

Release of Bayesian Augmented Clinical Trials - BACTS

BACTS is a suite of routines developed by Prof Miguel Juárez and Dr Dimitrios Kiagias from the University of Sheffield to analyse and augment the TB clinical trial with information from UISS-TB simulator.

The library implements the methods described in Kiagias* for **combining information from real clinical trials for TB therapeutic vaccination and computer simulations from UISS-TB**.
The library provides code to fit hierarchical Bayesian logistic models for dichotomous end points, with and without random effects. We use the former to fit the in silico data and the latter for the in vivo trials.
A second suite of routines combine the posterior distributions from these two sources of information into an in silico augmented clinical trial.

BACTS is available at www.github.com/kiagiasdim/BACTS and can be installed in R.

* Kiagias, D., Russo, G., Sgroi, G., Pappalardo, F. and Miguel A. Juárez, Bayesian Augmented Clinical Trials in TB Therapeutic Vaccination, Frontiers in medical technologies (2021).

Towards the Digital Patient

Towards the end of the project, the goal is to use the data collected from the Phase IIb Randomized Clinical Trial to generate the correspondent set of in silico models, in other words, the **virtual patients to represent each individual enrolled in the trial**, for a total of 140 profiles, 90 for the drug sensitive cohort and 50 for the multi-drug resistant cohort.

As of early January 2023, the partners from the University of Catania were able to generate the **first 90 in silico models corresponding to the 90 enrolled patients for the drug sensitive cohort**, by using the immunological parameters and the bacterial load detected through the active phase of the study.

These generated in silico models have been then used as input for the UISS-TB computational modelling framework to predict:

- **the percentage of patients with sputum culture negative** at time frame up to week 2;
- **the acid-fast bacilli smear grade** at time frame of the primary endpoint and at the end of the active phase.

The same process will be applied to to generate the remaining 50 in silico models corresponding to the MDR patients as soon as the data will be provided by the CRO handling the trial.

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