



**Relevance of commitment:  
The outcome of the CoMMiTMenT project**

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CoMMiTMenT

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# Relevance of commitment: The outcome of the CoMMiTMenT project

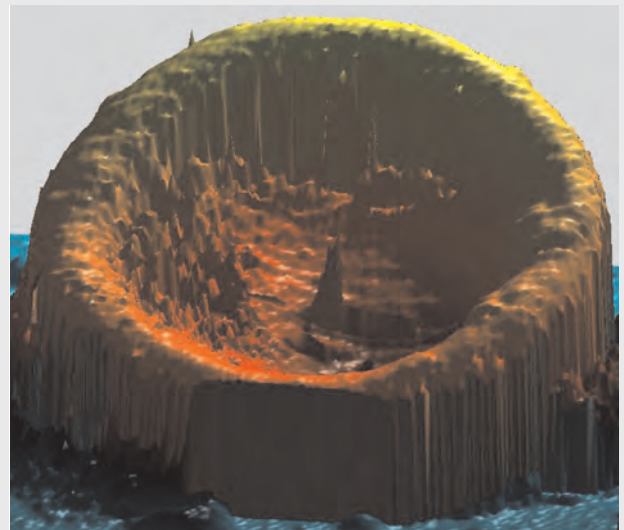
*Anna Bogdanova & Lars Kaestner*

## 1. Introduction

Anemia is a serious global health burden affecting 1.6 billion people worldwide. To tackle it EU has provided about 6 Mio funding to support a project in which nine partners from six European countries to address this challenge. The five years-long CoMMiTMenT project is a part of FP7 program initiated in October 2013 and will end in the fall of 2018. The acronym CoMMiTMenT stands for ‘**C**ombined **M**olecular **M**icroscopy for **T**herapy and Personalised **M**edication in Rare **A**naemia **T**reatments’. The project’s aim is to combine different types of advanced imaging technologies to diagnose rare and undiagnosed red blood cell related diseases and to allow personalised medication for patients suffering from them. Two clinical partners, two research laboratories and five industrial partners are committed to work together to identify new diseases, find modes for their diagnosis and come up with treatment concepts, which in the best case could be initially tested. Where do we stand after four and a half years of work?

## 2. General outcome

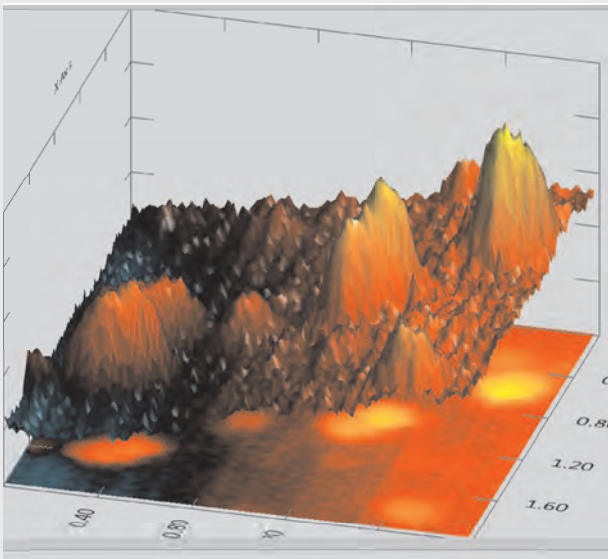
CoMMiTMenT project covers generation of intellectual property that may translate into a new high-tech product for blood analysis in the clinical and research laboratories and learning more about the fundamental mechanisms of hereditary blood diseases.



Healthy red blood cell  
Scanning Ion Conductance Microscopy

Initially we planned to combine **O**ptical **M**icroscopy based **C**ell **S**orting (OMiCS), introduced by Optorobotix AS (Denmark) with Scanning Ion Conductance Microscopy (SICM) developed by Ionscope Ltd. (UK) for analysis of unique properties of individual red blood cells assessing the frequency of occurrence of terminally damaged cells and heterogeneity of circulating red cells. Cells would flow through the microfluidic channel of the sorting chip provided by an expert company in microfluidics (EPIGEM, UK). When flowing, individual cells would be selected for further analysis by a machine-learning imaging system that recognises pathology based on the analysis of red cell shape. Such software was under development by a company specialized in image analysis (ARIVIS, Germany). Further scanning of cell surface and its further exploration such as detection of electric currents mediated by ions passing through the cell membrane using scanning ion conductance microscopy (SIMC) would provide

“Our findings revealed the existence of several “common denominators” of a number of rare anemias that were shared despite the fact that genetic causes of the diseases varied.”



Zoom 4x4  $\mu\text{m}^2$  in the internal part of the cell

information on the cause disease. It could also identify possible targets for pharmacological intervention or reveal how responsive the patient is to the therapy. The outcome of analysis using novel technology would be complemented by the readouts provided by the standard clinical laboratory analysis and unique method developed in research labs of the project partners. All together this information would represent a “fingerprint” of a rare hemolytic anemia if high diagnostic and prognostic value.

A combination of the approaches integrated into a single super-device got a name:  $\mu\text{COSMOS}$  (microscopic assay combining **SICM** & **OMICS**). The expectations of all partners on the project were high, but the initial plan underwent serious

modifications as the work progressed. Taking the lead in technological development EPIGEM Ltd came up with a new microfluidic-based device that allowed imaging red cell shapes in flow and monitoring responsiveness of individual cells to mechanical and chemical stressors. Impressive power of microfluidic approach allowed to come up with several microfluidic chip designs optimal for running simple, quick and reliable tests developed by research partners from the University of Zurich in which red cell membrane stability, amount of intracellular water and the stickiness of membranes were tested. Three new red blood cell analyser prototypes of a new device named MeCheM are now traveling across Europe and beyond to the clinical and research laboratories of partners where it is put on test and used for testing of blood of healthy people and patients with rare anemias.

Our findings revealed the existence of several “common denominators” of a number of rare anemias that were shared despite the fact that genetic causes of the diseases varied. This suggests that a combination of several drugs could potentially provide stabilization of red cells and improve the quality of lives of a larger group of patients making rare anemias more “attractive” for pharmacological companies looking for scaling the sales to match the investments into the drug development. One of such features is high intracellular levels of calcium ions. These ions are one of the core signaling control knobs in red cells regulating their ability to stay deformable and pass through narrow capillaries and the spleen, surviving for 100-120 days in the circulation.

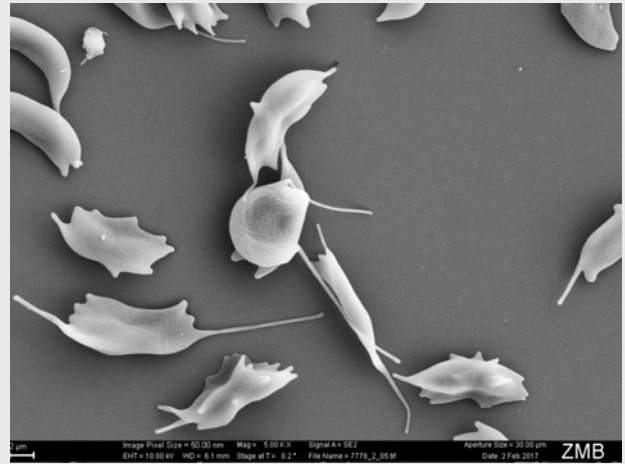
In the following paragraphs we present some particular outcomes that have the potential to be influential in the entire field.

### 3. Sickle Cell Disease and the Memantine pilot trial

Evolution of human genome is shaped by multiple environmental challenges including susceptibility to

malaria infection. Answering this challenges a particular mutation of a protein forming hemoglobin was selected and chosen as a life-saving option in four geographical locations. Being protective if inherited from one of the parents, it kills half of children born with this mutation inherited from both mom and dad before reaching the age of five. Searching for the course of severe pain crises and red cell damage in these patients sickle cell disease was the first hereditary anemia, which was acknowledged as a 'disease of one gene'. Despite the fact of that the genetic cause is known, very few therapeutic options are available for this vast group of patients (over a million worldwide) most of which live in African countries, but also in the US and European countries. The only few options available for symptomatic treatment include stimulation of fetal hemoglobin production by a cytostatic drug hydroxyurea, suppression of inflammation promoting hemolytic and pain crises and pain management. When pain hits the patient, blood transfusion is performed to prevent further escalation of the disease. At present attention of researchers and pharma companies is focused on development of novel affordable and effective symptomatic therapies. Development of such therapies poses a number of challenges such as searching for new targets for drug application within red blood cells, working on new markers and tests for monitoring responsiveness of the patients to new drugs in the clinics, and building new machines to perform these new tests.

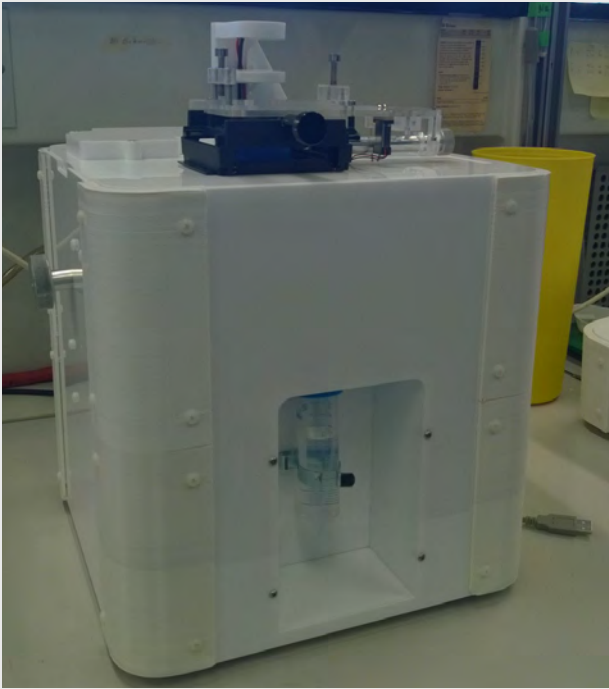
Such a new therapy was recently proposed by the 'Red Cell Research' group at the University of Zürich working on characterization of protein molecules involved in movement of calcium ions across the membrane of RBCs. One of such protein complexes discovered by the 'Red Cell Research' group in Zürich turned out to be an ion channel activated by the amino acids glutamate and glycine, known as NMDA receptor. These receptors are well-known to participate in memory formation in the brain. Abnormally high activity of these receptors is involved in progressive death of neuronal cells in the brain of patients with Alzheimer disease and other forms of dementia. Thus, drugs were developed to treat



**Scanning Electron Microscopy, Red blood cells of sickle cell disease patient**

patients with Alzheimer disease. Since mechanisms of red blood cell damage and death in patients with sickle cell disease share similarities with those of neurons in diseased brain, the anti-Alzheimer drug Memantine was used for preventive treatment of several adult sickle cell disease patients at the University Hospital Zürich. This approach, in which an existing drug that has proven itself safe for patients with one disease is used for treatment of a new disease (off-label application). It allows to save money at the developmental stage and enables faster delivery of a new therapy to the patients. This pilot clinical trial provided a perfect opportunity for the partners within the CoMMiTMenT consortium to define the new markers of disease severity and test the new methods of monitoring of the efficacy of new treatment targeting dehydrated RBCs that are overloaded with calcium ions.

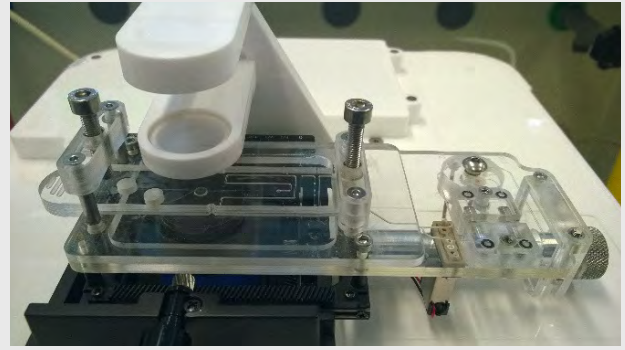
The trial has been closed in March 2017 and rated as successful. The set of new markers includes parameters characterizing heterogeneity in RBC density, hydration state, ion handling and stability of the RBC membrane. Based on the promising outcome a new trial has been initiated in Israel to further explore the efficacy of the NMDA receptor blocker Memantine. The new battery of tests will be implicated in this trial.



#### 4. MeCheM – a novel diagnostic device

One of the tests is performed using a prototype of the new device MeCheM developed by the CoMMiTMenT partners EPIGEM and University of Zürich. This device makes testing heterogeneity in responses of individual red blood cells to stress faster and more effective. It's using just a drop of blood that can be obtained by a single finger prick, a great advantage for neonatal patients in which, because of the hereditary nature, the disease manifests itself for the first time.

The MeCheM machine pushes the cells through a tiny channel of microfluidic chip where they get exposed to chemical or mechanical stress. Acidic conditions, shear and other stress factors to which the cells are exposed to in our body when flowing through blood vessels are causing cells to swell, shrink, adhere or burst when squeezing through the narrow passages. Responses of individual cells to stress at various points in time are monitored by a camera attached to the microscope. Analysis of these sets of pictures provides detailed information on the sub-populations of 'healthy' and 'diseased' cells that are either abnormally resistant or hyper-sensitive to stressors. For example, cells deprived of intracellular



MeCheM prototype

water do not explode when coming in contact with the stressor causing them to swell. These dehydrated cells are ideally suited to support polymerization of mutated hemoglobin in cells of sickle cell disease patients. Hemoglobin polymers make RBCs unstable and rigid. As a result their life span in our body is reduced from 100-120 days to 13 days. Thus, we may judge about responsiveness of RBCs to therapy by measuring the amount of dehydrated cells in blood of patients exposed to therapy. Recent findings of the clinical partners revealed the predictive potential of dehydration and changes in density of individual RBCs as a marker of disease severity for patients with hereditary spherocytosis and beta-thalassemia. 'Stickiness' of RBCs is another important indicator of blood diseases as a measure of vaso-occlusive propensity. MeCheM has a lot of potential as this simple device is very flexible and multiple testing protocols may be applied to challenge RBCs of patients revealing their hidden properties and validating their health and adaptive capacity.

#### 5. Gardos Channelopathy

Traditionally diseases are classified according to particular symptoms. Only recently we have the opportunity to screen the entire genome of a patient to find so called mutations, i.e. single errors in the blueprint of the proteins. With next generation sequencing the clinical partners of CoMMiTMenT, namely the University Medical Center Utrecht (Netherlands) and the Josep Carreras Leukaemia Research Institute in Barcelona as well as other groups

working on this subject identified point mutations in numerous patients. For instance in patients suffering from Hereditary stomatocytosis, different mutations were reported, e.g. in a mechano-sensitive ion channel called PIEZO1 or in a calcium activated potassium channel known as Gardos channel. Together with scientist of the Granda Ospedale Maggiore Policlinico in Milan (Italy), CoMMiTMenT partners investigated RBCs of patients with the Gardos channel mutation. It was found that the properties of these cells are somewhat different from other patients with Hereditary Stomatocytosis and therefore this disease is different and we named it Gardos Channelopathy. It was also found that the mutation of the Gardos channel results in an increased channel activity. Therefore it becomes almost obvious that a Gardos channel blocker would be appropriate to treat the disease. Also here we have a drug that was already in clinical trials: Senicapoc is an efficient Gardos channel blocker and it could prevent dehydration of RBCs in sickle cell disease patients. However, the trail was terminated because it had no benefit on the clinical symptoms, because in these patients Gardos channel activity is just one of many cellular responses induced by increased intracellular calcium.

## 6. Spherocytosis

In order to maintain membrane integrity and keep red cells “in shape” optimal for fulfilling their oxygen- and carbon dioxide-carrying duties, the red cell membrane is stabilized by a cytoskeleton made of a number of proteins forming the elastic hexagonal mesh underneath the lipid bilayer that is anchored to the membrane by super-complexes of multifunctional membrane proteins. They are essential architectural elements of the skeleton, but also participate in sensing mechanical stress, transferring CO<sub>2</sub> into bicarbonate to bring it to the lungs for exhalation, regulate cell volume by moving ions and osmolytes such as taurine across the lipid membrane, and provide it with nutrients. Mutations causing malformation or deficiency of any of these elements results in loss of stability in the whole cytoskeletal structure, membrane loss, increase in cell stiffness,

and premature red cell destruction. We have investigated the cells of patients with hereditary spherocytosis and identified new markers of poor prognosis for patients with this disorder. These markers may be used to follow the success of treatment and may give a clue for development of novel therapeutic strategies and test new drugs.

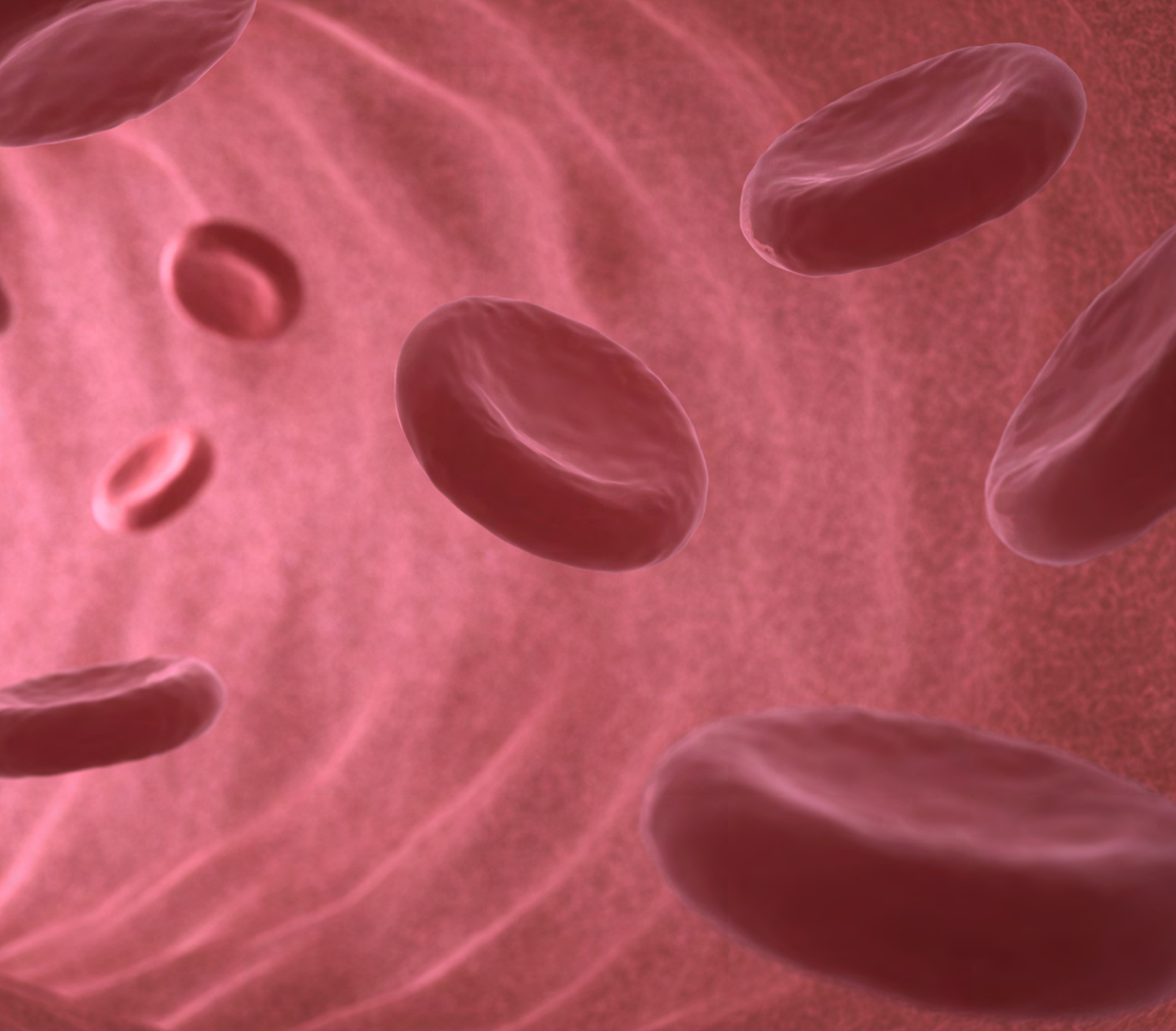
## 7. Lahn-valley erythrocytosis

In the early 1970ies Dr. Hans Pralle described a so far unknown erythrocytosis in a family from the Lahn valley, which was never registered. With complicated biochemical tests he could identify the activity of an alkaline phosphatase in the RBCs of these patients, which is absent in healthy RBCs. At Saarland University with the participation of Prof. Gregor Jung, a fluorescent probe for the activity of alkaline phosphatase could be developed. This ratiometric sensor allows quantitative measurements and can serve as an indicator in a diagnostic test of the Lahn-valley erythrocytosis.

## 8. Summary and outlook

As can be seen from the illustrative examples above, the CoMMiTMenT consortium made good progress within the past 4½ years. It can be regarded as a great success that the CoMMiTMenT members founded an Innovative Training Network named RELEVANCE (**R**egulation of red cell **l**ife-span, **e**rythropoiesis, **s**urvival, senescence and **c**lear**ance**) that educates PhD students in RBC research. So the strong interaction between CoMMiTMenT and RELEVANCE allows a direct transfer of the achievements of CoMMiTMenT to the next generation of scientists.

Well beyond CoMMiTMenT, RELEVANCE and other projects like the French initiative of excellence on RBC research ‘LabEX’, further big scale funding is required because we are left with new diseases without treatment and numerous open questions of the molecular regulation of rare anaemias.



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