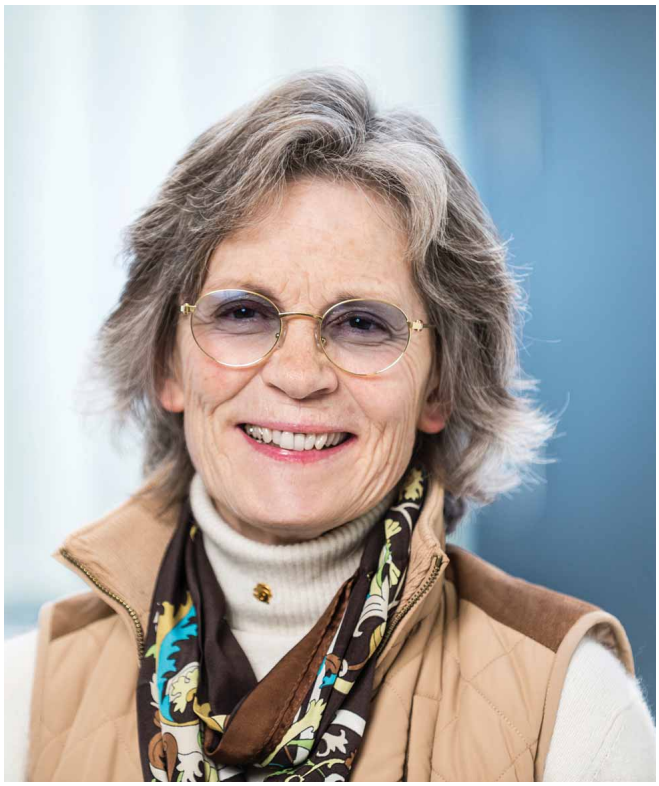
cabmm
center for applied biotechnology and
molecular medicine



Brigitte von Rechenberg

Prof Dr med vet, Dipl ECVS
Head of the Musculoskeletal Research Unit (MSRU), Head of the Competence Center for Applied Biotechnology and Molecular Medicine (CABMM)

The Competence Centre for Applied Biotechnology and Molecular Medicine (CABMM) is a cutting-edge and innovative medical research organisation, working within both the VetSuisse and Medical Faculties of Zürich University, to provide novel therapies for humans and animals. It provides a platform for interdisciplinary research across the fields of regenerative medicine, experimental medicine and surgery, applied biotechnology and molecular medicine. Because of an interdisciplinary and collaborative approach, the CABMM is also able to pioneer translational medicine, using research findings to create medicines and treatments for diseased and dysfunctional tissues.

Over the past year, the organisation has presented some of their groundbreaking research in Pan European Networks' publications. This research covers a wealth of topics from the CABMM's broad range of research interests, but is united by the shared goal of improving human and animal health. Some of the best of the CABMM's articles are now collated in this booklet, highlighting a few key aspects of the varied and important work that the organisation is undertaking.

Here, the centre writes on the development of new methods of transcutaneous treatment for skin problems, cartilage lesions and deep wound infections; the creation of new therapies for the treatment of chlamydial infections in both humans and animals; and an investigation into the possible role that certain proteins found in genomic regions of cat chromosomes could have on obesity. Each of these articles presents new innovations or advancements in medical research which could have a profound impact upon the future of medicine, therapy and patient care.

As well as presenting research and results, the CABMM has written a thought provoking piece on the ethics of animal testing, proposing the introduction of better frameworks across Europe that could co-ordinate pan-European research and prevent unnecessary animal experimentation. Another article explains the importance of understanding and applying the principle of translational research. Implemented effectively, translational research offers to revolutionise how human and animal testing is carried out, involving careful planning from the preclinical tests through to human clinical trials, ensuring that the results of this testing go from 'bench to bedside and back'.

This booklet contains only a selection of the CABMM's publications, and many more are available on Pan European Networks' websites. The organisation has written on an even wider variety of topics than are included here; further articles explore the science and ethics of anaesthesia and the technology of Bonewelding®, which allows for the anchorage of implants in bone. The collected body of the centre's research speaks for itself, demonstrating the CABMM's role as an organisation at the pinnacle of experimentation, and at the forefront of developing new medicines for humans and animals alike.

Gene genies

The University Children's Hospital Zurich discusses gene therapy for inborn immunodeficiencies

Gene therapy as a novel therapeutic approach was developed a few years ago for the treatment of severe inherited human diseases, caused by single gene defects. The first successful applications of gene therapy in human beings were lifesaving for babies with the most severe form of inborn immunodeficiency, severe combined immunodeficiency (SCID). This disease is usually lethal within the first year of life if the transplantation of blood-forming stem cells from a healthy donor or gene therapy cannot be performed.

Immunodeficiencies

The healthy immune system protects humans from the numerous harmful microbes that continuously invade the body by detecting and eliminating them. Impaired function of cells or organs of the immune system leads to diverse clinical manifestations, ranging from susceptibility to infection, to exaggerated inflammation, and autoimmunity (an attack of the immune system against the own body). Defects in the genetic code, so-called 'genetic mutations', account for more than 300 known inherited immunodeficiencies with varying degrees of severity. The most severe diseases are lethal unless the patient receives a transplant of blood (and immune system) building stem cells (hematopoietic stem cells, HSCs) from a foreign, sibling or unrelated, healthy donor.

This HSC transplant (HSCT) works best if the cells of the donor and the recipient patient share significant similarities of human leukocyte antigen (HLA) cell surface markers. Based on this requirement, around 25% to 33% of patients with severe immunodeficiencies will need other forms of treatment. This motivated physicians and scientists to develop gene therapy as an additional treatment option.

Principles of HSC gene therapy

Stem cell gene therapy is an alternative to HSCT, which exchanges the diseased HSCs of a patient by healthy HSCs from a foreign donor. In contrast to conventional HSCT, stem cell gene therapy relies on correction of the patient's own HSCs: first, a patient's HSCs are isolated from his bone marrow, then the mutation leading to immunodeficiency is corrected within these cells in a highly specialised laboratory, and the genetically corrected cells are given back to the patient via an intravenous injection. These cells then recolonise the bone marrow and build up a new functioning immune system (Fig. 1).

Gene therapy is called an autologous procedure, as opposed to HSCT from a healthy foreign donor, which is called an allogeneic procedure. The advantage of this approach is that autologous cells from the patient cannot cause graft-*versus*-host disease – a severe and potentially life-threatening attack of the newly built up immune system of donor origin against the patient's body. Also in patients transplanted from HLA-mismatched donors, delayed immunological reconstitution and graft rejection are of concern.

Present gene therapy is a gene-addition therapy, i.e. a healthy copy of the diseased gene is inserted into the genetic code of the patient's HSCs, without excising the defective gene. The activity of this newly added, corrective gene (a so-called 'transgene') compensates the missing function of the mutated gene, leading to cure of the patient. This transgene is inserted into the genetic code by engineered transport vehicles derived from retro-viruses, called gene therapy vectors. A feature of these gene therapy vectors is that they can adhere to HSCs and release the corrective transgene into these cells. Within the nucleus of the HSCs the transgene is then stably integrated into the genetic code of the patient and transmitted to all progeny cells deriving from the corrected HSCs. This will in turn result in the synthesis of functional protein and thereby correction of the immunodeficiency of the patient.

This approach has been and is currently being used in several clinical trials for patients with inherited immunodeficiencies, X-chromosomal SCID, ADA-SCID, Wiskott-Aldrich syndrome and Chronic Granulomatous Disease. Gene therapy for further immunodeficiencies is currently in development.

Chronic Granulomatous Disease

In healthy individuals, microbes are detected and taken up (phagocytosed) by specialised immune cells termed phagocytes (from the Greek *phagein,* meaning 'to eat'), i.e. granulocytes, monocytes and macrophages. After phagocytosis of microbes these cells produce bleach-like acting reactive oxygen species (ROS). ROS have a direct and indirect toxic effect on microbes, resulting in the killing of microbes and termination of the inflammatory reaction of the immune system.

Chronic Granulomatous Disease (CGD) comprises a group of inborn immunodeficiencies of phagocytes manifesting with recurrent and potentially life-threatening bacterial and fungal infections. About half of CGD patients may additionally have severe inflammation, mainly in the gastrointestinal tract (colitis), which is often misdiagnosed as Crohn's disease, if occurring as sole manifestation. CGD is caused by single gene defects in subunits of the so-called NADPH (nicotinamide adenine dinucleotide phosphate) oxidase of phagocytes. This protein complex consisting of several subunits is responsible for the production of ROS required for the killing of microbes and the

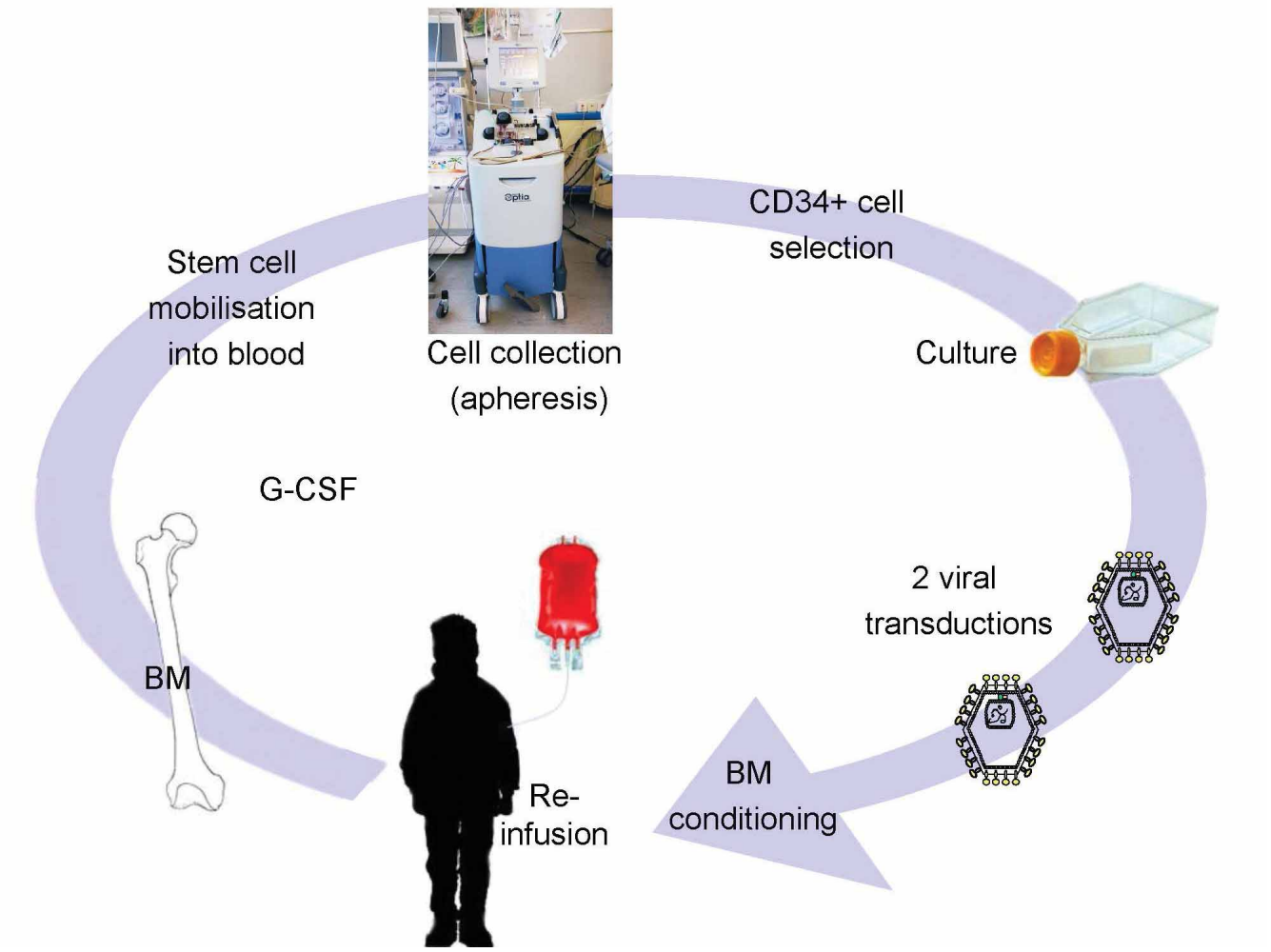


Fig. 1 Gene therapy to cure immunodeficiencies: After treatment with granulocyte-colony stimulating factor (G-CSF), hematopoietic stem cells (HSCs) are released from the bone marrow (BM) into the blood of the patient. HSCs are then collected from the blood by apheresis, selected by surface marker CD34 and cultured in vitro. *Next, patient HSCs are incubated twice with the gene therapy vector (transduction), by which the therapeutic gene is introduced into the genome of the patient's HSCs. In parallel, the patient is treated with chemotherapy (conditioning) to provide enough space for the genetically corrected HSCs to grow in the BM. As corrected HSCs give rise to immune cell subsets, the therapeutic gene is disseminated to these subsets upon differentiation of HSCs*

termination of inflammation. In Western countries, in about 60% of all CGD patients the malfunction of the NADPH oxidase complex is caused by mutations within the gp91^{phox} subunit, encoded by the *CYBB* gene. In a further 30% of CGD patients the disease is caused by mutations within the p47^{phox} subunit, encoded by the *NCF1* gene. Defects of other subunits are less frequent in Western countries, but more frequently encountered in consanguineous populations.

As CGD phagocytes are unable to synthesise ROS and therefore cannot kill invading bacteria and fungi, phagocytosed microbes persist within them. This leads to persistent inflammation and accumulation of these cells (granuloma formation). The full mechanism leading to persistence of inflammation is however only partially understood. We have shown that CGD hyper inflammation is linked to hyper-active inflammasome in the monocyte and macrophage subtypes of phagocytes.[1] The mechanism of granuloma formation in CGD is currently investigated in our laboratory.

In addition to the above mentioned intracellular killing defect, CGD phagocytes have a dysfunction in extracellular killing of microbes: Together with Professor Zychlinsky's research group from the Max-Planck-Institute in Berlin we have shown that in contrast to healthy cells, CGD phagocytes are unable to kill fungi by traps formed from DNA and antimicrobials. These web-like structures are called neutrophil extracellular traps (NETs). CGD patients cannot form NETs due to deficient ROS production, explaining the susceptibility to severe mould infection[2-5] (Fig 2). CGD can be cured with good results and few side-effects by HSCT if an HLA-compatible donor is available. For CGD patients without compatible donors, stem cell gene therapy might be an option to cure the disease. We could show that NET formation is restored by gene therapy and leads to clearance of severe fungal infection in CGD patients (see below).[2,3]

Gene therapy clinical trials in our own division

Embedded in a collaboration network with partners in Germany, our division of immunology at University Children's Hospital Zurich conducted a first clinical gene therapy trial several years ago, aiming to cure gp91^{phox} deficient CGD (X-CGD) patients (see www.clinicaltrials.gov): Between 2004 and 2007, four CGD patients lacking HLA-matched HSC donors were treated by gene

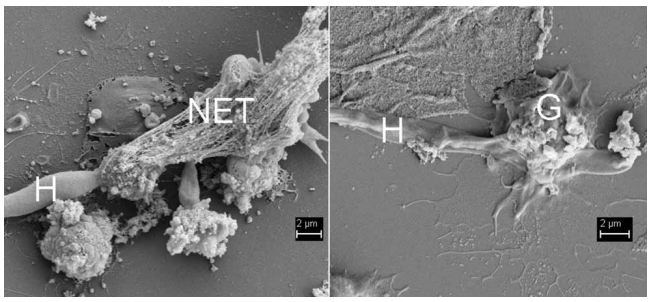


Fig. 2 Electron microscopic visualisation of neutrophil extracellular traps (NETs). Upon contact with fungal hyphae (H), healthy granulocytes are able to form NETs (left), whereas CGD granulocytes (right) are unable to form NETs

therapy using a first-generation gamma-retroviral vector; two adult patients in Frankfurt and two children in Zurich. All four overcame their pre-existing treatment refractory infections.[6-9]

On the one hand, this trial showed that CGD can be treated by gene therapy as the killing of bacteria and fungi by phagocytes was successfully restored after gene therapy. On the other hand, as in other first-generation gene therapy trials, the side effects related to retroviral vector-mediated activation of growth-promoting genes (oncogenes) were observed, and prompted intense investigation into the mechanisms of this side effect, as well as the development of novel safer genetherapy vectors.

Gene therapy vectors developed and currently used in a next generation clinical gene therapy trial in our division, are lentiviral vectors, engineered from the human immunodeficiency virus 1 (HIV-1) virus. These vectors are called self-inactivated vectors, as the expression of the corrective transgene is not driven by a viral control element (promoter), but by an internal tissue-specific promoter. For X-CGD gene therapy, gp91^{phox} transgene expression can be restricted to phagocytes, by the use of a synthetic chimeric promoter.[10]

Such a vector is now applied in a next-generation, EU FP7-funded clinical trial (www.net4cgd.eu) in study centres in Frankfurt, London, Paris and Zurich. In parallel, our laboratory developed a gene therapy vector for the second most frequent form of CGD caused by p47^{phox} deficiency, which is currently tested pre-clinically. This vector relies on the use of a micro-RNA miR223 internal promoter which restricts p47^{phox} expression to phagocytes, thus conferring a safety measure, and which is resistant to silencing.[11-13]

Outlook gene therapy

With recent progress in a number of clinical trials, stem cell gene therapy is about to establish its potency as a novel treatment option also for other diseases than immunodeficiency, such as defects of metabolism, cancer, or neurodegeneration. While gene addition approaches are state of the art in clinical gene therapy, next generation gene therapy will follow a targeted gene repair approach. Methods for this repair were developed recently and rely on the use of nucleases, such as Zinc-Finger nucleases, TALE nucleases, or CrispR/Cas9. The efficiency of correction using these nucleases varies between different cell populations and is unfortunately very low in HSCs, which are the target cells for gene therapy in immunodeficiencies. Our laboratory as well as others are therefore working on improvement of these gene repair systems in terms of better efficiency.

Bibliography

1 Meissner F, Seger RA, Moshous D, Fischer A, Reichenbach J, Zychlinsky A. Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease. *Blood.* 2010

2 Bianchi M, Hakkim A, Brinkmann V, *et al.* Restoration of NET formation by gene therapy in CGD controls aspergillosis. *Blood*. 2009;114(13):2619-2622

3 Bianchi M, Niemiec MJ, Siler U, Urban CF, Reichenbach J. Restoration of anti-Aspergillus defence by neutrophil extracellular traps in human chronic granulomatous disease after gene therapy is calprotectin-dependent. *J Allergy Clin Immunol.* 2011;127(5):1243-1252 e1247

4 Romao S, Gasser N, Becker AC, *et al.* Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. *J Cell Biol.* 2013;203(5):757-766

5 Romao S, Puente ET, Nytko KJ, Siler U, Munz C, Reichenbach J. Defective nuclear entry of hydrolases prevents neutrophil extracellular trap formation in patients with chronic granulomatous disease. *J Allergy Clin Immunol.* 2015;136(6):1703-1706 e1701-1705

6 Ott MG, Schmidt M, Schwarzwaelder K, *et al.* Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nat Med.* 2006;12(4):401-409

7 Stein S, Ott MG, Schultze-Strasser S, *et al.* Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. *Nat Med.* 2010;16(2):198-204

8 Stein S, Siler U, Ott MG, Seger R, Grez M. Gene therapy for chronic granulomatous disease. *Curr Opin Mol Ther.* 2006;8(5):415-422

9 Siler U, Paruzynski A, Holtgreve-Grez H, *et al.* Successful Combination of Sequential Gene Therapy and Rescue Allo-HSCT in Two Children with X-CGD - Importance of Timing. *Curr Gene Ther.* 2015;15(4):416-427

10 Santilli G, Almarza E, Brendel C, *et al.* Biochemical correction of X-CGD by a novel chimeric promoter regulating high levels of transgene expression in myeloid cells. *Mol Ther.* 2011;19(1):122-132

11 Brendel C, Hanseler W, Wohlgensinger V, *et al.* Human miR223 Promoter as Novel Myelospecific Promoter for CGD Gene Therapy. *Hum Gene Ther Methods.* 2013

12 Jiang Y, Cowley SA, Siler U, *et al.* Derivation and functional analysis of patient-specific induced pluripotent stem cells as an *in vitro* model of chronic granulomatous disease. *Stem Cells.* 2012;30(4):599-611

13 Wrona D, Siler U, Reichenbach J. CRISPR/Cas9-generated p47phox-deficient cell line for Chronic Granulomatous Disease gene therapy vector development. *Sci Rep.* 2017;7:44187

Co-Author

Professor Janine Reichenbach, Co-Head Division of Immunology, University Children's Hospital Zurich, Steinwiesstrasse 75, CH-8032 Zurich, Switzerland: janine.reichenbach@kispi.uzh.ch, Phone: +41 44 266 7311, Fax: +41 44 266 7914

Ulrich Siler
Division of Immunology
University Children's Hospital Zurich, Zurich
Switzerland and Children's Research Centre Zurich

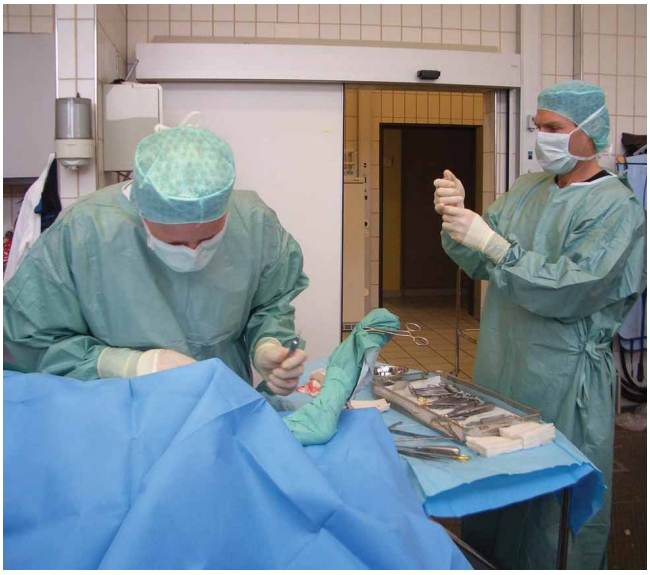
http://www.cabmm.uzh.ch/en.html

Everyday ethical considerations

The ethics of animal testing should be a consideration that is woven into the everyday business of researchers

Let's put it straight from the very beginning: no living, ordinary person could be enthusiastic about using animals for research and making them suffer in the laboratories of the world to advance medicine. But the serious researchers among us know that we cannot find new insights and new therapies for humans and animals without using them, and this is for several reasons. Among them are basic insights to studying disease mechanisms and thereby understanding where the chain of events is broken in cases of pathology. Cell and organ cultures can tell us a lot and are widely used; however, they cannot explain everything. For this, living organisms are too complex to just be quickly mimicked in cell culture wells or bioreactors. Therefore, animal experiments cannot be avoided, since on the other side of the coin weighs the suffering of both human and animal patients desperately awaiting cures for their diseases.

Although we divide research into basic and applied research, in reality these borders are not so clear and distinguishable – it's a transient business. How can we find good strategies for therapeutic regimens if we don't know where to intervene? To give you an example: millions of animals give their lives to study cartilage resurfacing with very limited success and why? Certainly hyaline cartilage is a tricky thing to repair to start with. However, one of the main reasons is that nobody has yet found out what the normal regeneration and repair mechanisms of hyaline cartilage are, and how this is connected to the underlying subchondral bone.



Granted, this is difficult to find out, but if we can't understand this fundamental physiology of our joints, there is little hope that we will ever be able to regenerate new cartilage after a defect occurred on the surface. This is true for humans and animals. Dogs and horses have a lot of joint problems and wait for new therapy strategies as humans do. For horses this is one of the most common reasons to be euthanised or slaughtered. This shows impressively that animal experiments are not just for humans but also for animals – to save their lives.

Ethics and philosophy

The ethical and philosophical aspects of why animal experiments can be justified in science are widely discussed and are not the focus of this essay. There is also the issue of the famous 'three Rs' (3Rs), which stand for Reduce, Replace and Refine, leading to more conscious use of animals in research, and this issue is mainly left to the philosophers. One of the main problems, however, is very often neglected, but is instrumental for animals in research and directly connected to the individual animal's wellbeing or suffering. It's about the quality of how animal experiments are conducted and who conducts them. Some aspects are covered by legislations within the different countries. They are mainly related to infrastructure and environment of animal facilities. It also includes personnel with their training and very rudimentary documentations.

Most of the FELASA accredited laboratories nowadays include veterinarians for staff members. However, these are often not veterinary specialists for either small rodent facilities or large animal surgery, respectively anaesthesia. Nowadays, this should be unacceptable in all laboratories. It should be required by law that veterinary specialists with board certifications of the European or American Colleges of Laboratory Animals (ECLAM/ACLAM) are leaders of such facilities. It should also be the law that specialists of the European or American Veterinary Colleges of surgery (ECVS/ACVS) and of anaesthesia and analgesia (ECVAA/ACVAA) must be involved in every single surgery for experiments with larger animals like sheep, goats, pigs, calves, heifers, dogs and cats or primates.

Medical doctors, biologists or other basic scientists should not be allowed to be alone at the table in future when surgeries on these animals are conducted. Surgeries should only be permitted in collaboration with veterinary specialists, who in the case of larger animals, should have both ECVS/ACVS and ECVAA/ACVAA diplomas. The latter are just as important for conducting correct anaesthesia and analgesia regimens. Too many serious errors happen and have been witnessed around surgeries and aftercare by the author, when specialists are not involved.

Quality

If ethical concerns are taken seriously, it starts right there: with the quality of the experiments performed at the table with each individual animal, and not only at the drawing board or with administrative legislation. Involving true specialists would reduce incidents that include administration of wrong human dosages to sheep, for instance with muscle relaxants, heparin, or other incompatible drugs, leading to experiment-unrelated death of hundreds of experimental animals in the world, some including serious suffering of animals (bleeding or suffocating to death).

Quality in animal experiments also includes their standard documentation and considerations of regulatory affairs if therapeutic approaches are studied and are part of the project. This goes hand in hand and should be part of initial planning in animal experiments to avoid repeating them later. This is imminent for reducing animal numbers right from the start (3Rs). Regulatory affairs relate to accreditation of preclinical experiments by either European agencies (TUV) or the FDA (Food and Drug Administration) in the United States, a prerequisite for allowance of clinical trials, Phase I-III and later registration of any medication, medical devices or combination products. Depending on the study and the technology tested, preclinical experiments should comply with Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP).

Once clinical studies are attempted these have to be conducted according to Good Clinical Practice (GCP). Different to the United States, where projects can be conducted according to GLP given the correct audits, in Europe the facility has to be officially acknowledged by the authorities (e.g. Swissmedic in Switzerland) prior to any GLP experiment being conducted. In most countries GLP or GMP approved facilities are private companies without the academic background of universities and other academic institutions (e.g. Fraunhofer institutes in Germany), where the expertise for most projects would be considerably broader.

This major gap between academia and later industrial needs is responsible for many animal experiments that have to be repeated, or are not accepted by the regulatory bodies due to the animal model used and missing or incomplete documentation. Therefore, it is advisable to include companies that should be involved in production and upscaling of test items, and contact regulatory bodies right from the start. To include these considerations in scientific translational and also basic projects means applying ethical considerations for animal experiments in everyday business of researchers.

Roadmaps of Horizon 2020 consider regulatory affairs in connection with animal experiments an important aspect for translation from bench to bedside.

The official Competence Center for Applied Biotechnology and Molecular Medicine (CABMM) is a unique professional network at the University of Zurich, Switzerland, for translational medicine where medical problems are investigated literally from 'bench to bedside' (see http://www.cabmm.uzh.ch/index.html). The expert members of the CABMM deal with either: a) experimental medicine or surgery; b) molecular medicine; c) regenerative medicine; or d) applied biotechnology. Basic researchers focus on molecular regulation mechanisms, and material scientists place their emphasis on developing new (intelligent) scaffolds/matrices used for tissue engineering, one of the modern backbones of modern regenerative medicine. *In vitro* generated tissues are studied in preclinical experimental animal studies, where biocompatibility, integration and functionality tested before clinical trial phases in humans can be initiated.

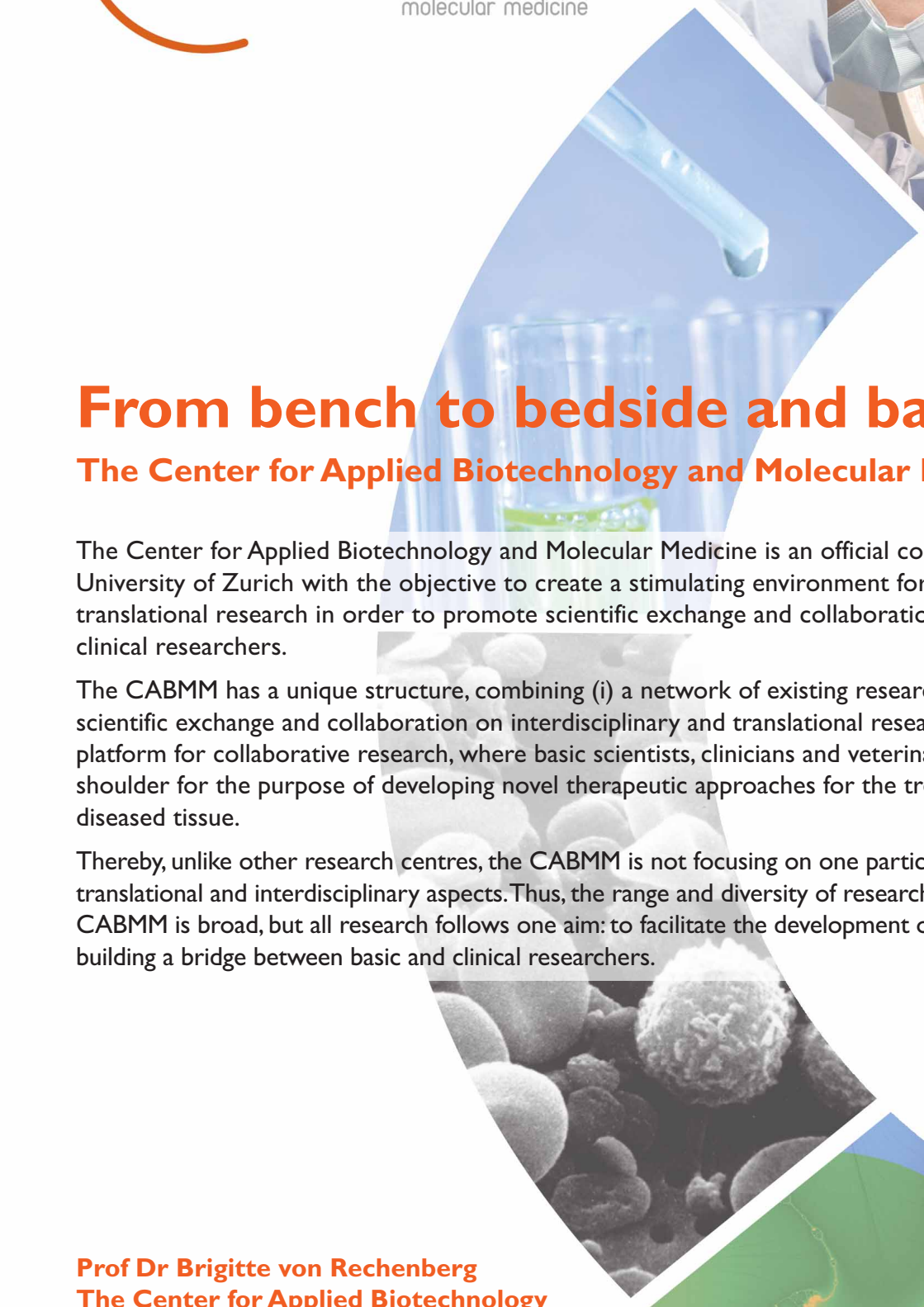
The main strategic goal of the CABMM is the promotion of translational research based on excellent interaction between basic research and clinics, academic institutions and industrial partners. Through consolidation and optimisation of an already excellent infrastructure, the methodical knowhow is continuously improved and the expertise of all members and their national and international research partners allows the development of products and appropriate technology transfer. Through the uniqueness of the CABMM, the University of Zürich is the only European university with official accreditation in Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) and Good Clinical Practice (GCP). These are required to get products registered at the FDA for clinical application.

Brigitte von Rechenberg, Prof Dr med vet, Dipl ECVS
Head of the Musculoskeletal Research Unit (MSRU), Head of the Competence Center for Applied Biotechnology and Molecular Medicine (CABMM)
Dean of the Vetsuisse Faculty
University of Zurich, Switzerland

+41 (0)44 635 8410

bvonrechenberg@vetclinics.uzh.ch
http://www.cabmm.uzh.ch/index.html

cabmm
center for applied biotechnology and molecular medicine



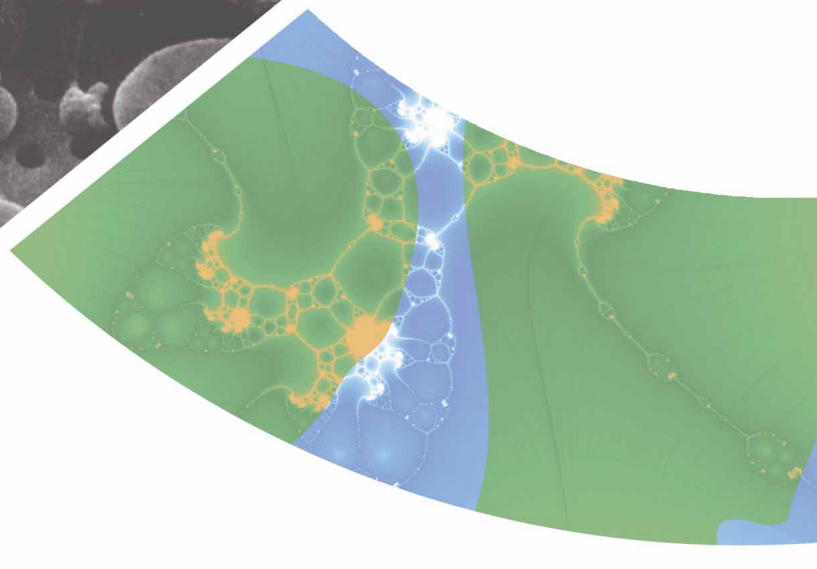
From bench to bedside and back again

The Center for Applied Biotechnology and Molecular Medicine (CABMM)

The Center for Applied Biotechnology and Molecular Medicine is an official competence centre of the University of Zurich with the objective to create a stimulating environment for interdisciplinary and translational research in order to promote scientific exchange and collaborations between basic and clinical researchers.

The CABMM has a unique structure, combining (i) a network of existing research groups interested in scientific exchange and collaboration on interdisciplinary and translational research projects and (ii) a platform for collaborative research, where basic scientists, clinicians and veterinarians work shoulder to shoulder for the purpose of developing novel therapeutic approaches for the treatment of dysfunctional and diseased tissue.

Thereby, unlike other research centres, the CABMM is not focusing on one particular medical field, but on translational and interdisciplinary aspects. Thus, the range and diversity of research being conducted within the CABMM is broad, but all research follows one aim: to facilitate the development of new treatment regimes by building a bridge between basic and clinical researchers.



Prof Dr Brigitte von Rechenberg
The Center for Applied Biotechnology and Molecular Medicine (CABMM)
University of Zurich

Tel: +41 44 635 8410
bvonrechenberg@vetclinics.uzh.ch
www.cabmm.uzh.ch