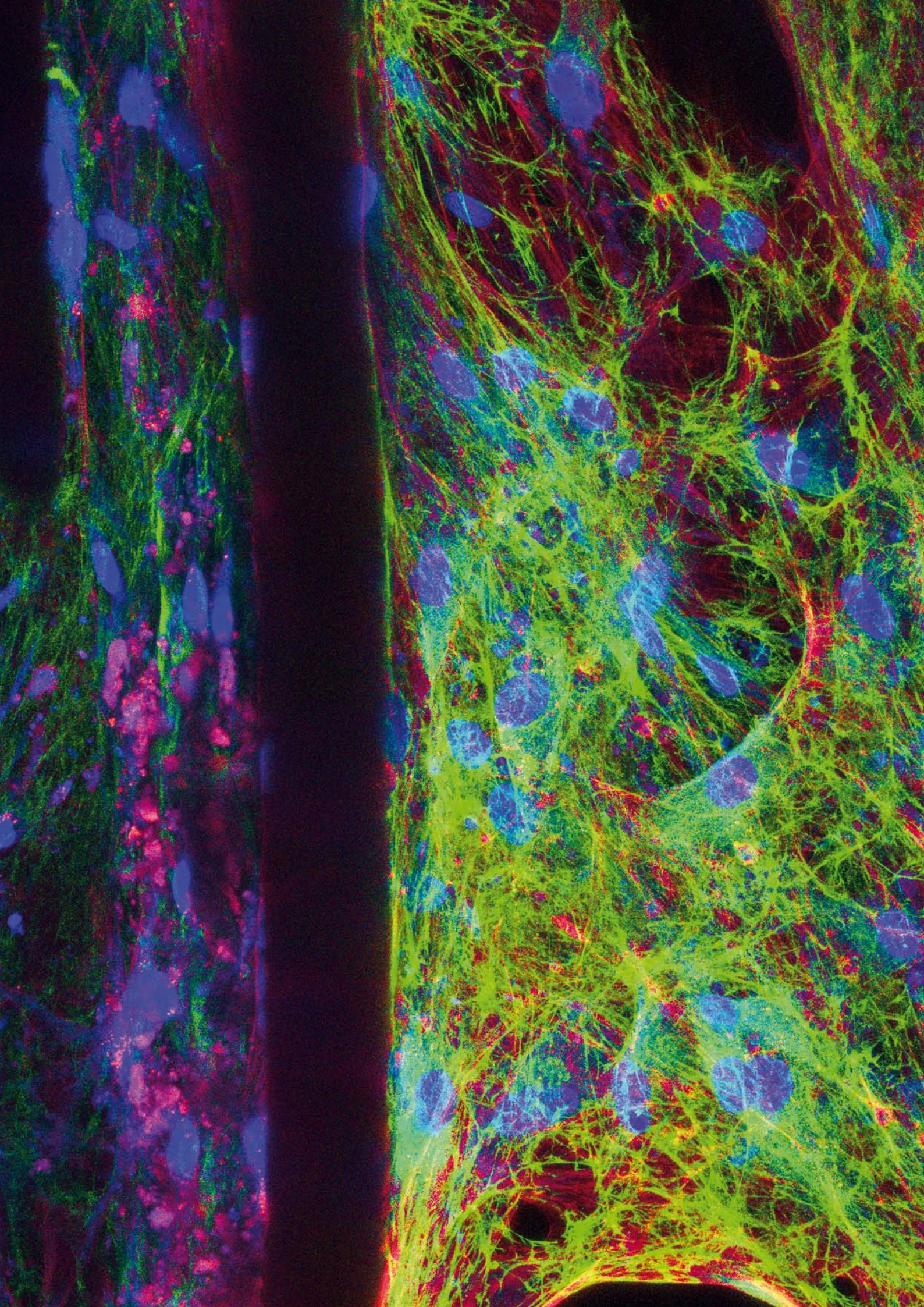


Clinical Cell and Tissue Engineering

Excerpts from an interview on Nov. 28, 2016

Interviewer: *Erica Gingerich*
Interviewed scientists: *Prof. Dirk Busch*, *Prof. Dietmar W. Hutmacher*, and *Prof. Stanley Riddell*

On the surface, medical therapies designed to treat diseased human tissue – cancer, for example – don't ostensibly have much in common with therapies designed to grow human tissue, such as the reconstruction of large bone defects. The TUM-IAS has brought together an interdisciplinary group of experts exploring how two different approaches to cell and tissue engineering – clinical cell purification, processing and therapy (CT) and tissue engineering and regenerative medicine (TE&RM) – might converge. In recent years, both CT and TE&RM have received increasing attention in the development of innovative and highly effective therapies for a growing range of illnesses and diseases.

Although coming from different research directions, leading experts from the TUM-IAS Clinical Cell Processing and Purification Focus Group and the Regenerative Medicine Focus Group have identified the synergies between their respective research areas, and collaborated for the TUM-IAS Clinical Cell and Tissue Engineering Focal Period for a workshop from September 20–22, 2016.

The major goal of the Focal Period group concept was to bring existing expertise at the TUM-IAS in TE&RM together with the TUM biomedical community in order to foster both fundamental and cutting-edge translational research in this rapidly emerging research field. With the establishment of its Graduate School of Bioengineering in 2015, TUM committed itself to strengthening its academic activities in this globally emerging research area. Biomedical engineering comprises one of the main pillars in this program, and TUM places a strong emphasis on translating technology developments into defined clinical applications.

TUM-IAS Cell Processing and Purification Focus Group (CT Focus Group)

Heading up the CT Focus Group, Hans Fischer Senior Fellow, Prof. Stanley (Stan) Riddell, is a professor with the Department of Medicine at the University of Washington (USA) and Director of the Immunotherapy Integrated Research Center at the Fred Hutchinson Cancer Research Center in Seattle (USA). His TUM host is Prof. Dirk H. Busch, the Chair of the Institute for Medical Microbiology, Immunology and Hygiene at the TUM School of Medicine. The CT Focus Group is working on the development of advanced and integrated cell processing platforms and the use of genetic modification of patient-derived immune cells to fight disease. In recent years, researchers have made major strides in the development of immuno-therapies that utilize a highly purified and genetically engineered T cells to combat certain types of cancer and infections. Focus Group researchers have already successfully demonstrated that they can treat life-threatening infections, like cytomegalovirus, that occur in patients undergoing bone marrow transplantation. Furthermore, the first applications of this approach for cancers, like B cell leukemia or lymphoma, have been undertaken by introducing a tumor-specific receptor into a patient's own T cells, and demonstrated very promising results. In many patients treated with these genetically enhanced T cells that recognize and attack cancer cells, "the tumor literally melts away," according to Stan Riddell.

TUM-IAS Regenerative Medicine Focus Group (RM Focus Group)

Hans Fischer Senior Fellow, Prof. Dietmar W. Hutmacher, is with the Chair in Regenerative Medicine and Director of the ARC Centre in Additive Biomanufacturing at the Institute of Health and Biomedical Innovation, Queensland University of Technology (Australia.) He shares leadership of the RM Focus Group together with Prof. Arndt F. Schilling, and Prof. Hans-Günther Machens, TUM Clinic for Plastic Surgery and Hand Surgery. The group's focus is tackling the lack of functional integration between tissue-engineered constructs (TECs) and surrounding host tissues, which poses a critical barrier that limits the effectiveness and clinical translation of current soft tissue interface graft technologies. The overarching goal of the RM Focus Group is addressing this challenge through the development of highly adaptable platform technologies in fields such as breast and lymph node tissue engineering, for example. Through this project, an international network spanning scientists, engineers, clinicians, industry and government will be established to accelerate the pace of regenerative medicine research that targets the reconstruction of complex soft tissue interface abnormalities and defects. The RM Focus Group also has the objective of developing a world-class TE&RM program that will be focused on additive tissue manufacturing (e.g. 3-D printing of tissue using human cells) for the regeneration of soft tissue interfaces, specifically for breast reconstruction.

Erica Gingerich, Dietmar W. Hutmacher, Stan Riddell and Dirk Busch

In an interview at the end of November 2016, *Erica Gingerich* (EG) with the TUM Corporate Communications Center and TUM-IAS science writer, caught up with *Dirk Busch* (DB) at his office in downtown Munich. He was joined by *Stan Riddell* (SR), who had just arrived from the U.S. to accept the accolade of TUM Ambassador from President *Wolfgang A. Herrmann*. *Dietmar W. Hutmacher* (DWH) stayed up into the early hours of the morning on the other side of the globe in Australia to join the conversation via Skype to talk about the Focal Period collaboration.

EG: Perhaps we could start with some background about your work together in each respective Focus Group – and about what convinced you to join forces for the Clinical Cell and Tissue Engineering Focal Period in September 2016.

DB: Looking at the CT Focus Group, my collaboration with Stan started with some very basic questions on how the immune system works, and subsequently, we asked how this knowledge could be used to develop targeted therapies for diseases such as cancer. The idea of using immune cells for therapy has been around for a long time – we consider this sort of therapy as having a “living drug” – unlike with a pill, chemotherapy or radiation, we use living lymphocytes, in our case, T cells. To make a long story short: What we’ve identified through our work together is that it matters what kind of T cell, what subtype, you use for a specific therapy.

Stan Riddell

Another topic we are working on is learning more about the characteristics a specific receptor has to have to offer the most effective targeted therapy. I think this field has seen dramatic developments in recent years. We nowadays have the ability to equip immune cells with receptors that can “see” only defined targets when introduced into the human body, for example, a cancer cell or an infected cell.

SR: At the beginning of the CT Focus Group, the idea was to take some of the technologies that Dirk had developed and see if we could apply them to the real world of clinical cell therapy. At that time, we were working on optimizing certain receptors that we could put into patient-derived T cells that would target some types of human cancer. What is remarkable is that over the course of the Focal Period, this technology has actually been applied in the first patients. We’re now treating patients with very defined cell populations, which is something that distinguishes the approach we’ve taken from other groups in the field. They’re not defining – at the level that we are – exactly what cell types they’re modifying to put into patients. The work in Dirk’s lab, particularly, has given us even deeper insight into the fundamental differences between the various cells we might use for therapy. This information will allow us in the future to be more selective about the cells that we engineer and improve outcomes – both in terms of safety and effectiveness.

EG: You mention that you’ve already introduced these cells into patients and successfully treated cancer that is resistant to other therapies – what are some of the results you’ve seen so far?

SR: We are treating patients who have blood cancers – leukemias and lymphomas – and we engineer the cells with a receptor that redirects them to recognize and kill those tumors. And as Dirk has said, this is a living therapy, so you may need to put in just a very small number of cells. And these cells will multiply in the patient until the tumor is eliminated. Using this approach, we’ve already treated more than 150 patients – patients who have failed all other treatments, and have no further treatment options. The remission rates – meaning complete elimination of measurable cancer cells – are as high as 90 percent for acute lymphoblastic leukemia. So this is really revolutionizing how we think about using the immune system to treat cancer. It comes back to the idea that by understanding the behavior and capabilities of the cells that you’re using in this therapy and their ability to LAST in the patient, we are entering a period in which we can examine expanding this cell therapy to many types of cancers. Dirk is already doing this in the clinic with infectious diseases, which doesn’t require engineering. He can select virus-specific T cells out of peripheral blood of stem cell donors, and use them to treat patients with certain viral infections after bone marrow transplants.

DWH: Ultimately, what really joins these two Focus Groups is that we are both using cells in our therapy concepts. And that's how Dirk, Stan and I actually got together. In our discussions, we were developing ideas about how to synergize our expertise from the fields of immune therapy and regenerative medicine to develop ground-breaking concepts – to really magnify both fields. The RM Focus Group approaches the topic from the perspective that you have a tissue defect – for example, a large bone defect based on a trauma or tumor removal. Or, from a soft tissue perspective, you have breast cancer that necessitates the removal of the breast – but then after the cancer is treated, you want to regenerate the tissue.

In regenerative medicine, we try to rebuild tissue by also using cells – yet with a different approach than the one which is currently being used in immune therapy. We combine cells with what is defined as a scaffold, because we really need the structural support to rebuild tissue, whereas with immune therapy based on the concept developed by Stan and Dirk, we inject T cells into the blood stream that subsequently proliferate and migrate to the site of infection or into the tumor tissue to destroy it. We start with something which is empty, and then we want to build up volume.

Our group specializes in using 3-D printing to fabricate patient-specific scaffolds. This technology allows us to run computer simulations to shape the volume as well as the form of the tissue we want to build. We use so-called additive biomanufacturing technologies to design and fabricate the scaffolds, and then add the patient's own cells with the goal of generating a lot of extracellular matrix. And then – similar to immune cell therapy – the cells and scaffold are implanted and interact with the patient's own body cells to regenerate the tissue. It is very important to have the surgical expertise to implant the tissue-engineered constructs (TEC) in the right way, as well as to provide optimal, post-operative treatment and to develop large preclinical models which mimic the human patient as closely as possible. That's why my Focus Group is actually hosted by the TUM Clinic for Plastic Surgery and Hand Surgery, and co-directed by my host department chair, Prof. Hans-Günther Machens.

EG: Let's delve into the Focal Period and talk about what you've accomplished – and what your scope of cooperation will entail moving forward.

DB: The Focal Period concept is a great idea developed by the TUM-IAS. The Institute has given us the unique opportunity to generate international collaborations with researchers at TUM. I believe the next step now is to further tap the synergies between experts working in very different areas – and develop something unique in the international research and clinical landscape. We may be using cells for therapy differently, but in the end, we all work with living drugs as the basis of our research. I think the other similarity we share is that we often have to start our work with mixtures of cells – and with cell mixtures that are often not very well defined, or that differ substantially from donor to donor. We believe that if we have better-defined cells, we can translate this into better therapy. And this is a need we recognized to be of major importance in different fields of cell therapy and regenerative medicine.

EG: Maybe we could talk about this interdisciplinary approach – what were some of the insights gained during the workshops?

SR: The real key was, maybe, gaining deeper insight into the research, and an introduction into the various issues that we're studying. I'm an immunologist – I study lymphocytes, and I'm trying to understand the behavior of those lymphocytes. Yet we also had participants who were experts in biomaterial sciences who gave us insight into the scaffolds they're using to grow specific types of cells, and how those scaffolds can influence cell behavior. Others were experts in new technologies for editing the genome. So I think a key takeaway from the Focal Period is this: As top experts in our respective fields, we had the chance to get to know each other better – and better understand how the work being done by others might fit into our own.

Stan Riddell and Dirk Busch skyping with Dietmar W. Hutmacher.

» *A TUM-IAS Fellowship is not just a three-year stint where we're here to develop a program at TUM and then return to our respective universities and do our own thing alone again. TUM has the ambition to be a global leader in the field of cell and tissue engineering, and they are willing, then, to give additional resources to bringing different experts from different Focus Groups together to develop new ideas. And then really follow those ideas up – remember that we are all based at a medical facility at our respective universities – to deliver new therapies and concepts for patients.* « *Dietmar W. Hutmacher*

DWH: TUM and IAS leadership have a great vision in the support of both Focus Groups in the pursuit of becoming a leader in this emerging field. Stan and I are both Hans Fischer Senior Fellows, which allowed us to work together with TUM faculty in our respective Focus Groups.

The interdisciplinary element is key, yet what is also of utmost important is the vision of President Herrmann – that such a commitment from stakeholders is a condition sine qua non to developing a truly sustainable program at TUM.

DB: Perhaps we should add here that by bringing together such a diverse group of international experts and TUM faculty, we identified areas in which we are particularly strong here in Munich. For example, in regenerative medicine, there are many groups in Munich working with cutting-edge technologies on induced pluripotent stem cells. We also realized that the research being conducted here in Munich on genetic engineering in combination with large animal models is clearly at the forefront of international research. And I think these are fields with high relevance for future research activities in the field of cell and tissue engineering.

EG: You talked a bit about some new revelations – surprising overlaps – that resulted from the Focal Period. Are there areas of research where you'd say that as the collaboration continues, they might be something you need to give more emphasis to?

SR: There are many challenges in the cell and tissue therapy field – even though it has been around for a while, it is a field in evolution and we are all very interested in developing clinical applications. For us, perhaps, realizing that in both of our respective fields [CT and RM], there are applications that are at very different stages in their development. They face some similar challenges, and also some unique challenges. And part of bringing people together is that you hope the insights you get from different perspectives will help solve some of those challenges and perhaps move things forward more quickly.

DB: Perhaps to pick up on an earlier point: via our efforts to collaborate, we have recognized that there is a need to make our cell products more defined. This also came up repeatedly during the discussions that we had at the Focal Period meetings. Obviously, this is a point we all have to give more emphasis to.

EG: I was surprised that for the general public, things that sound so close to the clinic – for example, that we can generate organs, tissues or whatever – that in reality, there is still a lot of work to be done.

SR: What Dietmar is doing with developing the right micro and macro environment for cells to grow into – I think we haven't solved enough about in our area of research. In our collaboration with Dirk, we explored many of these three-dimensional structures that use approaches from tissue engineering.

I think it will also be important for us to understand better how to culture, grow and regulate the differentiation of the cells we want to put into patients. The Focal Period emphasized that research is still at a very early stage. The ideas are there, but the synergies that will arise from bringing experts from these disparate areas together will allow us to take steps forward that, perhaps, we've so far never thought about.

DWH: I would also say another challenge we face is that what often happens after these kinds of meetings is that a lot of ideas are exchanged – yet then everyone goes back to his or her own institution, back to the routine and current national projects – and the follow-up never happens. So the challenge is to keep the flame burning – to now further develop the ideas which were exchanged at this symposium and workshop into real action plans in the form of grant proposals, exchanges of Ph.D. students and post docs. What's nice to see? That the flame is being kept alive. Not just between our respective Focus Groups and host departments at TUM, but also in ongoing discussions between three other groups which attended the workshop to design new experiments and also look for funding. It is rewarding to see that some of the activities we discussed are really taking place now. The three of us are confident that when we meet again in 2017, we'll have concrete progress to report upon – for example, that three or four of the groups of experts who joined us for the Focal Period meetings have designed new projects and are making progress in answering the questions and challenges which were raised at the workshop.

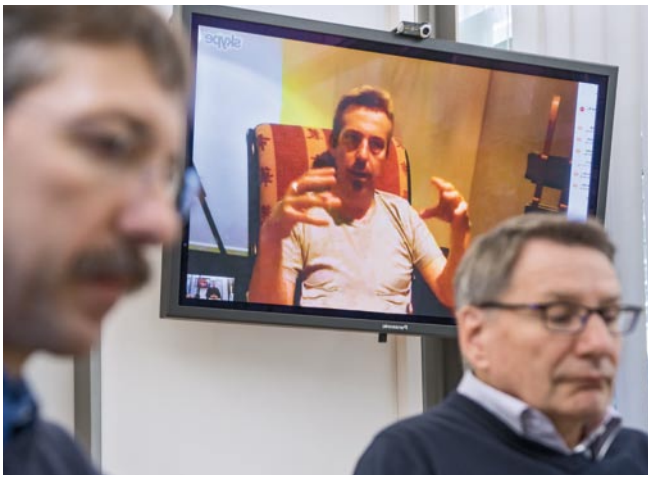
EG: Could you go into more detail about upcoming projects on the horizon that have resulted from the collaboration between your Focus Groups?

DB: One of the projects that TUM and LMU – Ludwig-Maximilians-Universität München – together with the University of California – are discussing centers around the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR – are segments of prokaryotic DNA containing short, repetitive base sequences) Cas9 system for genetic cell engineering, including clinical cell production as well as transfer to large animal models.

DWH: Another project where I'm involved in is together with Dr. Luca Gattinoni at the National Institute of Health – National Cancer Institute (USA); he's quite interested in culturing immune cells in a co-culture system with mesodermal stem cells. He's looking for a substrate for the scaffolding to accomplish that. One of my post docs will join his lab for two or three months to bring our technology – how we culture cells

on scaffolds – to his lab. In another project, Stan, Dirk and I are already doing some work on how to expand immune cells on innovative scaffold systems to replace the current technology, which is based on culturing those cells on cell culture beads.

EG: Prof. Riddell, you've already detailed how you've developed cells for therapy in humans – and Prof. Hutmacher has already developed an engineered structure that's been introduced into thousands of patients thus far. But you say we're still in the infancy of this kind of cell and tissue engineering and that the reality of where we're at with the technology and science differs from the expectations of the general public. Perhaps we could talk about that aspect – reality versus expectations?



Dirk Busch, Dietmar W. Hutmacher and Stan Riddell

DWH: Here is the big reality check – because what I try to explain to even a lot of my bioengineering colleagues is that we currently don't have the technological ability to print an organ or even a tissue. And perhaps we never will. There's a big misunderstanding about what we can do with this technology, as well as about where we need to direct the biology. The reality of what we can do: we can print scaffolds and can spike them with cells. But this is not yet a functional tissue. Cells form tissue, and therefore the whole concept will really only work when engineers, material scientists, molecular and cell biologists and clinicians work together, and everyone brings in his or her expertise. And again – when my engineering colleagues say they can print an organ – this is in Hollywood language – pure "La La Land" at this point in time!

So therefore, my approach is that with 3-D printing, we have a technology that allows us to print cells with what we call a very high spatial resolution. But then the biology needs to kick in and we need to guide the cells and produce an extracellular matrix, and after the matrix is produced in the right way, we can use it to form a tissue. And when the tissues are formed in the right way, then an organ is formed. But this can only happen inside the body – outside the body, we can print cells, and we can "free engineer" them by using bioreactors to direct cells to form a tissue-like architecture. This again underscores the importance of the synergies we're creating between my work and immune therapy research. For example, if I print a scaffold and I put mesenchymal stem cells onto the scaffold, and I transplant this, for instance, in a bone defect: even if I use the patient's own cells, there will be an immediate immune response to the cells on the scaffold. So if one can now harness Stan and Dirk's technologies by designing cells which we send through the body to the scaffold to modulate the environment, then the host cells reacting with that system might promote a microenvironment that we call a "proregenerative form." That would be a great advance, especially for the regeneration of large defects. And that is the direction we are moving in – converging these technologies, because both have certain unique features. By combining them, we have a much stronger therapy concept for the regeneration of tissue and, perhaps one day, organs.

EG: It seems that your respective areas of research dovetail at many different levels – some quite unexpected – in the development of therapies in your respective areas of medicine?

SR: They do. Using immune cells to assist with the tissue regeneration like Dietmar just outlined was not an application that we've thought about for immune cell therapy – and that was the beauty of bringing this together.

We've been focused on destroying tissues, particularly cancerous ones or infected cells. But we know that immune cells are very important in healing wounds – and what Dietmar is saying is that there may be ways of using immune cells and engineering them to go to these places where you're trying to initiate repair. I think that definitely is an area for future research. It illustrates the strengths of bringing the two groups together.

DB: I think we are already very excited to see that, yes, we have been able to take some of the principles that we've learned from basic research to try to translate them into clinical applications. For example, to treat an infection. Stan was already pioneering this area of research quite a few years back, but when he started, there was perhaps the general belief – for many reasons – that it would require us to generate large amounts of these cells in order to do effective therapy. And what we're now seeing is that there is a strong regenerative capacity within defined subtypes of T cells. What we have learned – and this might be of relevance for many other cell therapy approaches as well – is that if you start out with the right cells, you might only need to generate a very low number of cells for therapy. Furthermore, using a low number of cells for therapy could even have advantages over using more, especially with respect to side effects and acute toxicities. So, unexpected aspects from different fields – including regenerative medicine – came together, which is now helping us to facilitate the generation of the most effective therapeutic cell products.

SR: The reason I believe the field is still in its infancy – even though we're already having some success in the clinic – is that cancer is a big problem. And we're treating a very small number of types of cancers, and what we've got, really, is the first evidence that says you can engineer immune cells to recognize and destroy a tumor in a patient. But that doesn't mean you can engineer immune cells to destroy EVERY tumor in every patient. There are different types of cancer: you have to identify targets and understand the microenvironment of those cancers. So there's still a lot of work to be done.

Dirk Busch

A cancer is often referred to as a wound that won't heal. Because, in fact, some of the ways in which a wound evolves – those same processes are initiated by cancer. And some of that is actually to shut down the body's immune response. We need to learn from our colleagues who are doing tissue regeneration and trying to repair wounds that won't heal – to understand how best to do that. We're early on, but I think we do have what I say is really first proof of principle for clinical applications, of the concepts and principles that we've worked on through the Focus Groups.

DB/SR/DWH: In closing we would like to thank TUM and especially the Institute for Advanced Study for providing us the opportunity to work together on the quest to develop 21st century therapy concepts.