In Focus **Clinical Cell Processing and Purification**

Excerpts from an interview on March 18, 2011

Patrick Regan

Patrick Regan, Dirk Busch, Christian Stemberger, Stanley Riddell

*Although Hans Fischer Senior Fellow **Stanley Riddell** (SR) had flown in from the Pacific Northwest just a couple of days earlier, he already was so engaged with life at TUM that he had to be pulled away from a student journal club for our interview. His Host, Professor **Dirk Busch** (DB), explained that big-screen video conferencing has helped in developing a close-knit collaboration between Munich and Seattle but can't begin to substitute for time spent in each other's institutes. The exchange goes both ways and is transforming both labs. Busch, Riddell, and Carl von Linde Junior Fellow **Christian Stemberger** (CS) form the core of the Focus Group on Clinical Cell Processing and Purification – a name in which, as I was reminded repeatedly, every word has deep significance. Within the Focus Group, the TUM-IAS also supports two doctoral candidates, Jeannette Bet and Paulina Paszkiewicz, and postdoctoral researcher Stefan Dreher. Riddell is a pioneer in cell therapy, often relying on* in vitro *culture steps before suitable cells can be used for clinical applications; Busch and Stemberger have been pushing the limits of what can be achieved without culturing cells. Together, they are creating a technology platform that they expect will enable revolutionary advances: in cell-based therapies against cancers and infections, in regenerative medicine, and in fundamental biological research. (PR)*

PR: To provide a rough map of the area where your research interests meet, could you sketch out the state of the art in the therapeutic use of cells, its limitations, and its promise?

SR: Cellular therapies have been used for many years, for example the use of bone marrow transplantation for leukemia or various blood cancers. My early training in the 1980s was in bone marrow transplantation, and the process for transplantation really has not changed in 30 years. Bone marrow or peripheral blood stem cell preparations, which are now commonly used, are actually a complex mixture of cells, some of which are essential for the success of the procedure and some of which cause serious complications.

This Focus Group is looking at ways of being able to select out particular cells to rigorous purity so that you may improve the clinical outcome. So rather than giving a mixture of uncharacterized cells, some of which you know are necessary, you can purify each of the subsets that are in that mixture to make a better therapeutic.

The importance of cell purification for bone marrow transplantation is one example, but this extends into another field that we're all interested in, which is immunotherapy for infections or cancers, where selecting particular immune cells for therapy is critical to the success of the procedure.

What attracted me to this opportunity here in Munich is that the group at the Technical University is at the forefront of developing new technical procedures that would allow rigorous cell purifications to be achieved. There are very few places in the world where you could even contemplate simultaneously making critical technical advances and moving the work to clinical therapy. The purities that are achieved with the methodology that is being developed are extraordinary. It's not uncommon to have a selection where it's 99 percent pure, very close to 100 percent pure because you're looking at very, very low levels of contamination. That level of cell purity is something that I think will be very important for clinical applications.

PR: Is increasing the "yield" of a particular type of cell from a given sample a primary goal, or are other characteristics more important?

DB: I think the right term is quality. Yield is important, because you have a starting point, a mixture of cells, and of course what you can do with it will depend somewhat on how many cells I can purify out of it. But the fascinating thing is that there are examples where you can even titrate it down to a single cell, *if you have the right cell,* and you can still measure a therapeutic effect in a mouse and we think, potentially, also in a patient as well.

CS: It's such a broad field. We started with a focus on T cell-based immunotherapies especially, but the same rules apply to other cell types, for example also to stem cells. You have to make sure that you get exactly those cells that are best suited for a specific therapeutic application, or that science tells us are the best, and to dissect them from potentially harmful cells.

SR: We have a lot of evidence that the clinical applications will be better if we can do this. But there's also a lot to be learned about cell behavior, by being able to work with defined subsets of cells. The experiment that Dirk referred to, where essentially a single cell was able to mediate a major therapeutic effect, is teaching us a lot about the biology of individual cells and how they can respond, proliferate, and self-renew. But in order to be able to study these questions, either experimentally in animals or in the clinic, you have to be able to purify the cells at the highest level. And that's what the technology being developed is going to allow us to do.

PR: Let's focus in more closely on the technology itself. What are the key technical challenges, and what is distinctive about your approach?

CS: The challenges occur on many levels. The first level is that we are dealing with mixtures of cells. If you simply draw some blood, there is not only a single cell type in there, but there are, let's say ten major subtypes, and you want to have only, in the best case, one. And this needs to be precisely defined. To do this, a single marker – so let's say a surface protein in the

cell membrane, a receptor that can serve as a marker – in almost all cases is not sufficient. So you often need more than one marker.

DB: And you need to have methods that allow you to transfer these types of protocols not only to highest purity, but also to the clinic, which is a very different area from pre-clinical experimental studies.

PR: Because here we're getting into regulatory regimes?

DB: Absolutely.

SR: We must be prepared to deal with the regulatory aspects for clinical translation.

PR: Is that also a reason you in the Munich group have put such an emphasis on processing and purification without resorting to cell culture?

DB: As soon as a cell is kept in culture or changed from the way it was before, then the regulatory hurdles that you have to overcome are much higher. So this helps us, at least for certain applications, to process a cell in such a way that it stays "minimally manipulated," to make it easily accessible to bring it back into a patient, and also to make it meet the requirements of the regulatory process.

CS: I should add that it makes sense not only in the regulatory view, but it also in a biological view. If the labeled surface marker is, for example, a receptor that is essential for the function of the cell, even though the marker-binding itself might not directly harm the cells, the label still could block the receptor – and if you switch off its function, that is obviously not good for the cell.

PR: So how do you do it?

CS: We invented a technology that allows us to label the cells in a population of interest according to one or multiple markers, and afterwards we can retrieve the labels completely so at the end of the day we only have a cell without anything that's bound to the surface.

PR: That practically sounds like magic.

CS: It's been described as the painless fish hook.

SR: A very good description.

PR: How does it work?

CS: The way this functions is not that complicated. For instance, the cell type you are interested in purifying shares the same surface marker with another cell type that you don't want, but it also expresses an additional marker, and the combination of both markers is unique to the target cell population. So this shows us that for purifying the cells of interest out of this mixture, you need at least two markers. If you go in with the first marker, you fish both marker-positive populations out, and then after disengaging the first marker, you go fishing again with the second marker to home in on the desired cell type.

With the reagents we use to do this, we have one ligand that actually binds to the cell; the interaction is extremely weak. So if you only have a single one,

Christian Stemberger

you won't get a stable binding of that ligand to the cell. The trick now is, you take not only one of those weak binding ligands, but a few of them. And by doing that you increase the binding strength. So let's say if one goes away, there are still two or more bound to the cell, and that way you keep contact. This is called multimerization. You increase what we call avidity, the total binding strength, by multiplying weak interactions.

DB: It's a biological principle, which is used in many biological interactions to modify the strength of the binding. We learn from nature.

CS: So you can multimerize the ligands by using a kind of backbone molecule, and you can label the backbone molecule, for example with a fluorescence molecule or a magnetic particle that enables us to tear the cells out of the mixture. The multimer binds stably to the cell, but you can easily disrupt the binding to the backbone and break the whole complex apart.

DB: And that goes incredibly fast.

CS: Super fast.

SR: Less than a few minutes.

CS: Basically you just wash the cells, wait a couple of minutes, and the cell label will simply dissociate off. And that's how the cells lose not only the backbone but everything that you've bound to them. So this is the basic principle of our technology.

SR: Because you can now re-suspend the cells and come back in with a second (or third) reagent, the advantage is that you can do this sequentially, and very rapidly. There's no other technology that allows you to bind something and have it fall off so quickly that you can then come in right away with a second selection step.

CS: In basic experimental research, you could envision sorting target cell populations different ways, but it is not possible to transfer these strategies to the requirements of clinical cell processing.

PR: Is the problem the nature of the reagents?

CS: Well, yes and no. Yes, because conventionally available reagents simply stick to the cell and stay there.

Patrick Regan

SR: They would go back into the patient, and especially if you are using multiple reagents, you don't want to administer those back to the patient.

DB: Transferring a marker into a patient brings up a lot of regulatory hurdles. You have to show that the marker is not doing anything else, other than just pulling out the cell.

PR: If you can prove that your technique doesn't leave a marker, then, could that make it easier to get approval for use with patients?

DB: That's a very important issue. We are currently using this type of technology already in a first clinical trial, where we treat patients who are suffering from a chronic virus infection, specifically Cytomegalovirus (CMV) infection, after allogeneic bone marrow transplantation. We use one marker to pull out CMV-specific T-cell populations from the bone marrow donor. And indeed, because we were able to demonstrate to the regulatory authorities that we could completely eliminate the label for cell purification, they recognized the cell product as being "minimally manipulated," which at least for some applications is very advantageous.

An aspect of the technology that is very important is that we keep everything we are doing at relatively low temperatures. At physiological temperatures, for example 37 degrees C., a reagent that is bound to a cell can stimulate the cell, and potentially could transfer a signal to it. We can avoid this by keeping the temperature below 10 degrees, and preferably at 4 degrees.

SR: You want to take the cell from the blood and do the purification in such a way that in the end, the cell is unchanged. And you can do that by keeping the cells metabolically inactive through the whole process, then warming them up.

PR: That gives us at least the broad outlines of the innovation here, a purification or "positive enrichment" process that is extremely precise and reversible. Could you explain more about the range of clinical applications this technology could address, and the research challenges posed by different applications?

SR: There are many clinical applications. The potential here for human cell therapy is very broad. Right now the focus for us is primarily diseases that can be treated with T cells, because that builds on our collective scientific expertise.

DB: The immune system has mechanisms for developing specificity to an invader, such as an infectious agent or also some cancers. Besides antibodies, which are made by B cells, T cells are an important component of adaptive immunity. Those are cells that carry receptors that recognize a specific invader and have active functions that we are very interested in, such as destroying cells that are infected with a virus. Compared to antibodies, T cells have the major advantage of longevity, something we call immunological memory.

SR: Assuming you were vaccinated against the smallpox virus, for example, that will have induced specific T cells that recognize smallpox antigens, which are degraded components of the smallpox virus. Some of those smallpox-specific T cells will be in your body for the rest of your life, and will provide immune memory for the virus.

Stanley Riddell

DB: It is possible to do therapy with antibodies, and that is a very big area of research at the moment. But such antibodies have a relatively short half-life. This might be good in one situation but problematic in others. One of the major advantages of T cells, compared to a small-molecule drug or an antibody, is that they have this capacity to maintain themselves over extremely long periods of time. A lot of research, including our own, is aimed at better understanding the mechanisms of memory. But we are thinking mainly about how it can be used for therapy.

PR: How does this relate, for example, to the clinical trial that you mentioned earlier?

DB: In treating leukemia, because the cancer is sitting right within the immune system itself, you may get to the point where you eliminate the immune system, together with the cancer, and then build up a new one by giving a bone marrow transplantation. But within the time window of immune reconstitution, there's a serious threat that viruses that the patient's immune system normally keeps in check, especially members of the so-called herpes virus family, will cause very complicated clinical infections. If the bone marrow donor is positive for the same virus, then he will also have a population of virus-specific T cells, and those could be used to protect the patient. But in the peripheral blood of the donor you have a mixture of cells, with a small subpopulation of cells that is extremely useful for the patient and many others that could be harmful for the patient – for example, causing something called graft-versus-host disease, where T cells from the donor attack the tissue and organs of the patient.

SR: So by purifying the virus-specific T cells from the donor's blood and transferring them, you can protect the patient.

PR: Are there hybrid scenarios in which this approach to cell processing and purification would be combined with cell culture?

SR: Let's say you have a cancer. Suppose we take a memory cell out of your own blood – maybe it's specific for a virus – and then we engineer that cell to have a receptor that sees your cancer, and we put it back into your body. And because the T cell has the potential to be long-lived, to be able to proliferate,

Dirk Busch

it should work as long as there's a cancer cell there, until the last cancer cell is gone, and then it would enter a resting state – and survive even longer. One of our first clinical trials will test precisely this approach. That's a case where short-term culture comes in, because we can now introduce tumor-targeting receptors very rapidly. Because of the rigorous purity that you can get, you don't have to culture out the cell you care about; it's there to start with, and you can very rapidly manipulate it.

PR: And to what extent have you expanded your research beyond T cells?

SR: The group has developed reagents for selecting cells that have stem cell properties, so you could think of potentially purifying cells that you may want to use for transplantation, making highly pure products for example from umbilical cord blood. You could extend this even further and think about regenerative medicine, where we're talking now about stem cells that have the potential to become different tissues – again, being able to use markers for selection that may define the cells' ability to differentiate to a certain tissue.

CS: It was hard work, but we found a way to transfer the basic principle we learned from T cells to virtually any cell type that you can imagine being potentially useful for cell-therapeutic applications. The underlying principle of the broad extension of the technology is based on antibodies, or parts of antibodies that are called Fab fragments. Fabs are the parts of an antibody that recognize the target structure, or antigen, on the cells of interest.

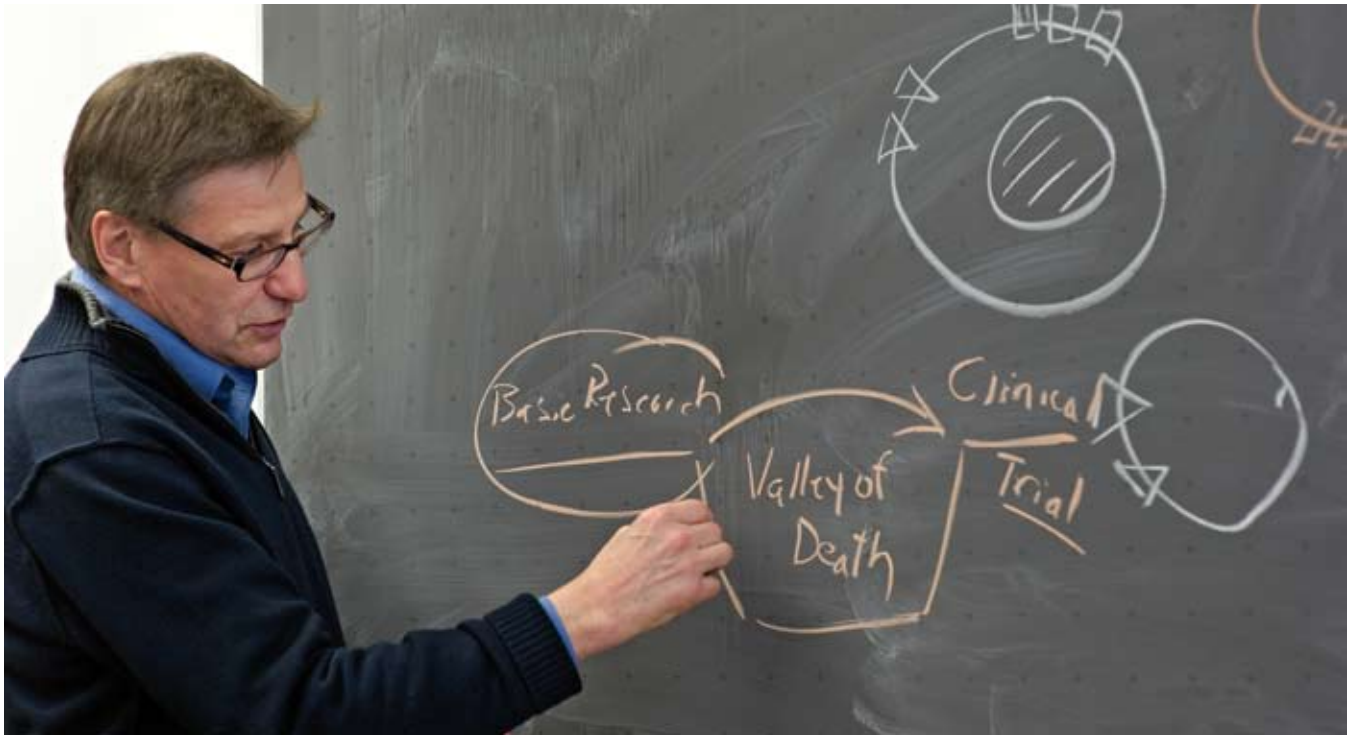
SR: In a normal antibody, the Fabs are always present in a multimeric form, either as a dimer or as a more complex multimer. But for our approach we engineer the Fab in a monomeric form.

DB: The basic principle.

CS: An antibody looks like a Y-shaped molecule; we are only using one of the arms. What we are doing is isolating the structure of the Fab fragment from nature and then engineering it to fit into a multimeric complex. But there is an important challenge. The most basic principle of our reversible agents is of course reversibility in order to eliminate the cell-selection reagent after purification from the cell, but by nature, antibodies are designed exactly the other way. They are often designed to bind extremely strongly. So we now have to go back to the bench and engineer or modify the molecule in such a way that it binds substantially more weakly to its antigen. Already, we have succeeded with fifteen different Fabs to engineer fully reversible Fab-multimers, and we would actually argue that we can succeed with any antigen for which we have an antibody sequence to start with – though Stan might laugh because he knows details about a really tough one.

PR: Is that a typical kind of interaction in this collaboration?

SR: The interactions can be contentious, but are meant to facilitate both the practical and scientific applications of the approach. Right now we have a GMP facility in Seattle, meaning "Good Manufacturing Practices," and we are doing clinical trials, and have expertise in the kinds of things necessary to perform a procedure for purifying cells that is compliant with that kind of facility. Here in Munich, they have the reversible multimer technology for cell selection but are just beginning to build a GMP facility. So I often find myself saying, that's wonderful, but how are we going to move it toward a clinical application?



Stanley Riddell

DB: Our Focus Group is more than collaboration, it's translation. In most cases scientists are talking about translation, but they are far away from ever doing it. And we are entering here an interface that not many researchers really reach. I can't emphasize strongly enough that Stan Riddell is a real pioneer. He was doing the first adoptive T-cell therapies with great success. And yet it's frustrating that although it was so successful, this treatment has not ended up in a commonly performed procedure. And this is the gap we have to close with our Focus Group.

SR: Right. We did the first cell transfer in 1990, but after 20 years it's not an approved product that patients can routinely get. In clinical translation, we often talk about a Valley of Death. I can draw it on the board – over on this side is basic research, which we all do, either to understand a biological process or some fundamental aspects of how human biology works; across here on the other side, we'd like to be able to apply that knowledge in a clinical application. And the gap from here to there is enormous, often for technical reasons. And what the TUM-IAS program is actually allowing us to do is essentially to make this leap across the Valley of Death, because we're developing technologies that have come from basic research and our understanding of how cells work, and being able to use this technology to move things into the clinic. You can spend your entire career on one side or the other, but to be able to bridge the gap is something special. I am very confident, even after just the first year of this Focus Group, that we will actually be using some of the technologies that Christian is working on in the clinic, before our program is finished.

PR: That's fast.

SR: That's incredibly fast. And I think it will include purified T cells that are cultured for brief periods of time to endow them with unique activities.

DB: We will both benefit in a couple of years in that we can set up a technology platform that allows us to do this clinical cell processing and purification jointly and in a similar manner.

SR: The idea is that this will eventually come together with platforms that are not just going to be useful for Munich and Seattle. We hope and believe

that these will be adopted widely in the immunotherapy community in many countries. And Dirk is working with other groups as well, as are we. But someone has to make the commitment to the technology development, and that's what our group is doing.

PR: "Platform" is a concept I understand intuitively. But getting down to the nitty-gritty, what will it mean practically?

DB: The idea is really to have on one side the mixture – you draw blood from the patient, or you have a cell sample – and you decide I need this or that population. We believe the simplicity of the basic concept even will make it possible to automate this process.

SR: We haven't worked through all of the steps, but my lab has experience with doing the genetic modification of cells and expanding them. Dirk and Christian have the experience in doing the cell selection. What we have to do now is combine these in a sequence of manufacturing processes. And it may mean having an instrument – one of the things being worked on is an instrument where the blood would go in and the desired cells would come out at the end.

DB: Whatever cell you want.

PR: It seems to me that papers about cell biology often include a section of statistics, basically to reassure you that the researchers probably were looking at the cells that they say they were looking at. And here it seems you have an answer to that, so that biologists would have a new way of being confident that the cells they are observing are exactly and only what they want to be studying. Would you like to see your platform become a tool for basic research as well as therapy and medical research?

DB: The basic principle that we're describing here, to find ways to purify, to isolate, a very defined cell population in a minimally manipulating manner, is something that could be incredibly valuable for basic research as well. Many data that are out there in the field have been generated in conventional ways where it's difficult really to purify cells. So you might be able to address very important questions in a much more sensitive way than we could do before. There are a lot of basic research applications that we currently envision.

Dirk Busch