Transcript of Hallek Interview Part 3

Marti: The role of animal models in CLL. You mentioned the TCL1 or the TC1 from Croce.

Hallek: Yep.

Marti: Are there other animal models for CLL?

Hallek: Yes, there is. I mean, there is many of the so-called immunocompromised mice, NOD/SCID mouse or nude mice. I think at the present time, the TCL1 mouse is the best model because it the disease develops in the typical homing organs of CLL. Marrow, spleen, liver, lymph nodes. It is also—has a similar type of onset. It may reflect more the unmutated form of CLL although you see some mutated. I mean, John Gribben has done some analyses. And so, for all the models that you might think about, it's currently the best. And we wondered because TCL1 is a T cell oncogene, whether that has anything to do with CLL, but it is now turning out that we have a very relevant cofactor of B-CLL lymphomagenesis or leukemogenesis. Marco Herling in our group has analyzed TCL1. And it's strongly expressed in unfavorable patients in the bone marrow. Carlo Croce sees a interaction with essential pathways in CLL, like AKT. And so, in summary, by chance he may have discovered not only that this a valuable mouse model for CLL, but also that this is a very potent oncogene.

Some of the microRNAs that he has recently described are also binding and regulating to sites of the TCL1 translational machinery(?). So, they are targets for TCL1, or they are—their target is TCL1, I should say. And so, it comes up that TCL1 is relevant and TCL1, the mouse model, is the best that I currently know, and therefore we are using it for all our in vivo studies. Sometimes long, so you have to watch the mouse, as a CLL patient, for a year or more. It's not a quick experiment. But that is one of the challenges and the beauties of CLL. You have time to think before you do something. Yeah.

Marti: Sure, sure. It's my ignorance, we have no experience with the TCL1 model.

Hallek: Yes.

Marti: Our experience is totally with the NZB.

Hallek: Yep.

Marti: Which is more on an 18-month cycle.

Hallek: One cycle, yeah.

Marti: One year. There are some molecular changes that occur. But you actually then do use, try treatment strategies in the TCL1?

Hallek: We have done some. It's difficult because of the natural difficulties in getting drugs into mice. If you give it by the oral route, then you have to get them to swallow the drug. Injections are—have a different dosing regimen but yes, we did some and it worked quite nicely. It's one of the—we discussed it earlier also, when I was at Ohio State, it is really the major difficulty that we don't have a good preclinical model for testing drug combinations in CLL. TCL1 is maybe the best, or these models might work, but they don't really reflect what's going on later in the patients, still. So, we are right now using a combination of testing this, or some of the most interesting combinations in animal models plus use the cells, doing ex vivo studies.

Marti: Bendamustine. Bendamustine. Has that been tried in the mouse model?

Hallek: No. No, I don't know any study that has done that.

Marti: And I think you're probably right about the TCL1. It doesn't really have an early phase. It probably…

Hallek: Comes up very quickly. Yeah.

Marti: In that sense, a collaborator of mine, Elizabeth Raveche, has started using the term "MBL" with the NZB.

Hallek: So that may be more reminiscent.

Marti: It might be. I don't know. And also, the NOD, the N-O-D SCID mouse. No experience with it, just reading the literature, my impression is is that the CLL lymphocyte homes to that spleen in that animal.

Hallek: Yes.

Marti: But it doesn't expand.

Hallek: Not so much and it's more restricted to those sites and areas. The behavior of the NOD/SCID mouse is certainly not reflecting what's going on in [unintelligible] biology. It's a good model to start some pharmacokinetics for drugs, or also to get some hint on the…on some mechanisms of action, but when it comes to the correct interaction with microenvironment and all this complex complexity that is going on, I largely prefer these orthotopic models in immunocompetent mice because we need the immune system to actually get the CLL up and running.

Marti: But do we have any mouse models that meet that criteria?

Hallek: TCL1 is one.

Marti: That's what you mean by that, okay.

Hallek: Yes, because that—they have a totally normal T cell. I mean, until they get the disease—

Marti: Yes, yes.

Hallek: —they have a totally normal immune system.

Marti: Good point, good point. What about the cancer stem cell for CLL?

Hallek: Huh. One of the big mysteries. We don't know it and it needs to be defined. So, it—we would all guess that it should be there. It makes a lot of sense because all our failures of eradicating the disease could be easily explained by the existence of a stem cell that is not hit by current therapies. And what is needed is the same type of stringent experiments that John Dick and others have done for AML, to really identify then do repopulation experiments and clonal assays in mice. And here the NOD/SCID mouse model or others can serve well because you can see whether they keep the same leukemic phenotype. And until we don't have that, we don't have the markers, and so that's why I'm actually bringing this up. We don't have the markers to identify a true stem cell in CLL. I guess somebody is doing these experiments right now, and I don't know who, but I would expect that somebody in the field will.

Marti: We've made some attempts in the NZB, but the unfortunate thing with the NZB is that it has a very ongoing autoimmune hemolytic anemia. So, the hematopoietic stem cell is so elevated in that mouse that trying to look for a cancer stem cell in that population using that Hoechst blocking experiment, the so-called side population, the only thing in the side population is what everyone knows anyway, which is the hematopoietic stem cell. So, we have not been successful to date in locating it. I suspect that in thinking backwards that at least using the mouse model, and one of the benefits of mouse models, of course, is it lets you think in a way about humans that you can't right away. But for me, it's thinking about the aneuploidy is much easier to detect in the mouse model than it is in a human.

Hallek: Mmhmm.

Marti: And therefore, it would seem to me that if we could find the diploid tumor cell, the diploid clone, that that might contain some of the information as to whether or not that's the cancer stem cell. And for that matter, using 13q14 as our marker for aneuploidy, it's next to impossible to detect in flow in the human, but if we could sort those

two populations, the 13q14 aneuploid, or even the rare patient that has both alleles deleted and sometimes they have a mixture of some of the cells that just have one deletion and some that have two deletions, and then, of course, the diploid. We have not been smart enough to figure out how to sort, to detect, and then sort. If we could detect, sorting would be…I wonder what you think about looking for the diploid cell in the CLL clone, whether or not that might be a source.

Hallek: I think it would be a good choice because many of us think that this is the very likely initial event. And so, trying to figure out which of the early cells that have this phenotype, or have this genotype, I should rather say, is extremely promising. The question is how technically you can really get a small clone out of this larger mass of cells, but it should be solvable. And I would imagine that one could use Ulf Klein's mouse, in Dalla-Favera's laboratory, who has deleted this site in mice, it's in chromosome 14, and see whether they detect early cells, because you can do it more reliably in these mice at early time points by just deleting this part. And they get lymphoproliferative diseases. It's going to be published,[1] or is on the way of publication, I hope.

And if they are able to identify a B cell clone with specific markers, early on, that eventually might lead us to a definition of the CLL stem cell. So, I would imagine their mouse model, which might be the next best, or the better one, even better than TCL1, might help us to identify this type of stem cell in the mice and then give us hints to identify it in human beings. And I think the idea of doing it with this set of diploid changes is the right one.

Marti: I'm glad you mention that. I saw the abstract with the miR-15a, 16—

Hallek: Right. Exactly.

Marti: —I guess knockout.

Hallek: Yes.

Marti: And I don't know if it was miR-15a by itself and 16 by itself and then the two together, or whether there was another condition. I remember two or three conditions. But that's a good idea, to look for what the B cell stem cell might be in that mouse.

Hallek: They—I mean, it's probably worth talking to Ulf Klein. He's an extremely smart guy, and if he's not doing it yet, but in this model,

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[1] Klein U, Lia M, Crespo M, et al. The DLEU2/miR-15a/16-1 cluster controls B cell proliferation and its deletion leads to chronic lymphocytic leukemia. *Cancer Cell*. 2010 Jan 19;17(1):28-40.

you might actually have a clue on the very early populations coming up. And they should contain stem cell-like features.

Marti: You know, also in MBL, that there does seem to be a pattern of clonal evolution.

Hallek: Yeah.

Marti: And that should all precede the 13q14 deletion, because I think we all think that that's a late event.

Hallek: A secondary event. Right. Yeah.

Marti: A secondary event, if not a late event. Well, maybe leaving the mouse models and going back to treatment, you made some comments about, this morning about that the combination of Rituxan and fludarabine hadn't, hasn't really been compared in the same way that fludarabine and Cytoxan have. Would you enlarge upon that again?

Hallek: Yes. So, one of the important discussions that we currently have is over whether we need or not the Cytoxan added to the chemotherapy. There is two potential hypotheses coming out of this discussion. One is that you don't need the Cytoxan or cyclophosphamide because the combination of fludarabine plus rituximab does it all, and replaces the cyclophosphamide. Could actually also put it the other way around and ask, systematically, do we need the fludarabine and would it be necessary, sufficient to use the cyclophosphamide plus rituximab to get the effect? But we have good data for FR as well. This is basically produced here in this country, so there is a I think not so important discussion about this, whether we need it or don't need it.

The arguments for FCR, that there are three randomized trials all showing superiority. And one of them, now with more recent data, even in terms of survival. And that is important. And then there is, this therefore has been the building block for the novel treatment that again shows a survival benefit FCR. So, now you have a new standard which is not only producing better response rates and PFS which is a weaker end point, but also benefit in terms of survival. So, anything has to be compared against, and I would strongly encourage to do a trial of FCR versus FR to solve this question.

I'm not so sure, however, whether that's going to be a big advantage or big advance for the field, except to say that of course if you can spare toxicity, it will be always easier to combine novel treatment strategies in like, lenalidomide or novel antibodies or anything that might be, might create additional toxicity.

I mean, being a German, and I say this with a lot of irony towards myself, we are trying to be systematic, and therefore we would not go, leave out one step now that we have FCR, and say, "Well, FR is also as good because we never have proven it systematically." And therefore, I would say, until we have the scientific proof, we consider FCR being the standard. But I would love to get to one or two of these chemotherapeutic agents out of the regimen because I think chemotherapy is not intelligent anyway. So, it's a good question.

With bendamustine being the next step of that discussion, if that would prove to be equivalent, which we don't know, then I think this discussion is over anyway, because it will cause—it should cause less myelotoxicity and if it's equally potent and has the same results, then it should be over. If FCR is better than BR, then, one of the questions could be to go back and compare FR to FCR.

Marti: You showed such nice data this morning with minimal residual disease monitoring. And if I understood correctly, the blood was just as useful as the marrow for monitoring, which was good to learn. Makes sense. I got the impression that if someone was in MRD-negative after three cycles—

Hallek: Yes.

Marti: Would you continue to treat?

Hallek: Yes.

Marti: So, you would go six cycles?

Hallek: Yes. It's again a good question because we may overtreat already some of the patients, but this was not the trial question, so we treated all of them—

Marti: Sure.

Hallek: —and most of them got six courses. But I expect that getting an MRD-negative state after three courses is highly predictive and we may, in low-risk situations, think about tailoring down our treatment length or intensity in those patients responding well. But it's a scientific question.

We are more concerned about maintaining an MRD-negative state once we get it, because eventually they all relapse and if we could avoid this, it could make a difference. So that is our, my personal next step in—our two things, now we wish to combine another principle into the therapies that would be a drug that would act on the microenvironment, or something that would, well, act through the immune system. Lenalidomide combining into the FCR would be, or BR

would be a choice. And then the second important step would be maintenance or consolidation strategies for patients who are not MRD-negative. And that's—I mean, you can already see how our next trial would look like. Eventually, I have not decided yet, but these are the thoughts that we currently have.

Marti: I think I've pretty much run out of questions. One last area that I'm thinking of that we're starting to encounter in the clinic a little bit is that several years ago, Vietnam veterans that were exposed to Agent Orange, at least the Department of Defense in this country thought that there was a possible relationship to the causation of some non-Hodgkin's lymphoma, particularly CLL.

Hallek: Mmhmm.

Marti: We actually now have seen 4 or 5 patients, and I understand that in veteran hospitals around the country that it's even more frequent.

Hallek: Mmhmm.

Marti: Wondering about the role of just a natural history study of Agent Orange-associated, exposed CLL patients. How would you, how would one go about organizing that? And I'm thinking, I'm asking that question on the basis of your CLL study group experience, ERIC, your knowledge of the CRC.

Hallek: I mean, this is a extremely intriguing project. I'm, I'll tell you why. I had a patient last week or two weeks ago, just before I came, who was working as a professional climber to clean industrial plants, from the inside, basically to wipe out all the dust and dirt that is in there. Extremely young, but had CLL. And those young patients always ask, "Well, is it related to my professional activity?" And I said, "Well, in the literature, in the textbooks, we have plenty of hints that pesticides or any other agents, phenols, and so on cause the disease. But there is no proof." I mean, there is only this hearsay, I would almost say. Now if one could come up with a stringent registry of, let's say, all patients from the—I think you call it VA, here in the states?

Marti: Yes. The Veteran's Administration.

Hallek: From the VA, and look at those who have experienced Agent Orange, which is a clearly defined exposure, I would say, so much better. Of course, it is cynical to say, but it is a clearly defined exposure to an agent. And then, say, the—and you have the beautiful SEER data in this country, which we don't have. I mean there is so much—here is a clear strength of the American system, with a registry of a level. And you would see an increased incidence. That would be extremely interesting, because we could make a link between the molecular

causes that we know and the environmental changes that we don't know. But here we have a clearly defined exposure and we could say, "Well, when this happens, then you, let's say, double, or whatever, increase the number of patients having a CLL. And if that could be even matched like Peter Hillmen and Andy Rawstron did, with a larger screen for MBL, in the VA population, then compare this.

It would be a good project because it would solve one of the biggest enigmatic mysteries in CLL, pathogenesis, making, or going away from the hearsay to real facts, let's say. So, I think it would be a wonderful project. It will not—I think it can be easy, because you have some control, I would guess, over the—control in types of interaction from the NIH to the veteran's administration. They're at least probably closer links, although I can only guess. And it's maybe some of the things that can be done here much better than in any other place in the world. And the question is interesting, so I would hope yet that you would get the support to do that.

Marti: Hopefully we'll learn more with time. This also prompts me, another interest, is to ask about familial CLL in Germany. Any ongoing studies?

Hallek: No. That's—well, as we speak about complementary activities, we are even trying to recruit some of our families and patients into Danny Catovsky's registry.

Marti: Sure.

Hallek: The ERIC is trying to collect those data a little bit, and since we know that the UK and Danny and his group are so strong, we are trying to rather support him.

Marti: Right.

Hallek: And we have no independent activity at the present time, although I personally am convinced that this type of research is extremely helpful to learn about the original causes and pathogenesis of CLL.