# Q&A: John Dick on Stem Cells and Discoveries

The cancer stem cell concept captures essential properties of malignancy, such as sustained self-renewal and phenotypic plasticity. Capturing such cells experimentally has been "like finding a needle in a haystack," says John E. Dick, PhD, FRS, who pioneered the isolation and characterization of normal and leukemic human hematopoietic stem cells. Dr. Dick and his lab personify how "blood cancer research leads the way," having discovered core biological and clinical features of stemness and created tools that inspired and enabled decades of research far beyond hematology. His work is currently aimed at elucidating the origin and nature of cancer and developing new approaches to cancer therapy. John E. Dick is a Senior Scientist at the Princess Margaret Cancer Centre, and Professor at the Department of Molecular Genetics, University of Toronto. Dr. Dick has been distinguished by numerous awards including this year's Inaugural AACR Award for Outstanding Achievement in Blood Cancer Research. Speaking with *Blood Cancer Discovery*'s Tanya Bondar, he offers historic, biological, and clinical perspectives on stemness in cancer.

**How has the definition of a cancer stem cell been evolving?**

The minimal definition is that the cancer stem cell is the cell in a tumor that can keep the tumor going. Papers in the 1990s envisioned a rigid hierarchy: there was a leukemia stem cell distinct from all other cells, and these properties were hardwired. In the 2000s, we did clonal tracking experiments that suggested there was more than one kind of leukemia stem cell. Some propagate the tumor on a serial passage, others repopulated the primary transplant but didn't have enough self-renewal capacity to repopulate secondary recipients. But there was also a minor, latent population staying dormant for several transplantation cycles and then eventually becoming dominant. Dormancy is a powerful state harnessed in malignancy. And for normal human hematopoietic stem cells as well, there is diversity in self-renewal capacity and entry out of dormancy. Now with single-cell multi-omic analysis, we're coming to learn the molecular underpinnings of how that's governed. The other change in my own thinking is the plasticity of stemness in some other tumors, where cells are transitioning between a stem and nonstem state.

**Does this mean that the cancer stem cell as a concept is more relevant for some cancer types than others?**

The cancer stem cell model is relevant only if there's heterogeneity amongst the cells; if all tumor cells are equal then the model is not wrong, just irrelevant. In leukemia, we've shown that hierarchy changes between diagnosis and relapse. At diagnosis, hierarchy can be deep, with few stem cells and many more non-stem cells—whereas years later, that same cancer becomes more stemlike. In cancer evolution, there's a drive toward primitiveness. Cells become more uniform; the hierarchy flattens out, becoming shallower.

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"There are many ways for a cancer cell to be abnormal from the cell surface all the way through to the nucleus, but very few ways to be a stem cell," *John Dick says.*

Some would argue that in some tumors, cells have equal capacity and function, and there is no discrete cancer stem cell population. I think it depends on the assay system. Models used for ease of research, by definition, are more uniform. Cell lines are much more homogeneous than a primary tumor. Primary tumors are typically late-stage when they are accessible from patients. If there is not much hierarchy in a late-stage cancer, which is aggressive and genetically aberrant, that doesn't necessarily mean a hierarchy wasn't there earlier.

**Are there cancer stem cells in lymphoma, myeloma?**

Malignancies reflect the tissue from which they came. The dissociation between self-renewal and stem programs is not as strong on the normal B lymphoid side, and I think that is reflected in the tumors that arise. In contrast to the myeloid side, even mature B cells like memory B cells can be dormant for decades and then become activated again. By definition, these are stemlike properties.

**How do you decide which big question to study? How did you come to develop xenograft models and stem cells in cancer?**

I'd like to say it was all part of a large, greater plan that comes from one's brilliance, insight, and literature reading. Sadly, in my case that was not true! In most cases, big insights come from serendipity. Our work tracing leukemia evolution in acute myelogenous leukemia (AML) is an example where we were studying something else: how tumors change between diagnosis and relapse. To answer this, we set up genetic sequencing of archival AML samples from a biobank, but needed a germline control. We thought of T cells, as they are not part of the myeloid lineage. And it worked beautifully for most mutations we were sequencing, except Liran Shlush came to me and said, "in four samples T cells are positive for mutant *DNMT3A*." Having ruled out contamination, the only way T cells and leukemia cells can have an identical mutation is if there is a common ancestor. And we know in the blood, normally the only cell which is an ancestor of a T cell and a myeloid cell, it's a stem cell. That caused us to look at stem cells.

How did we come up with xenografting? I did my postdoc in an era of retroviral gene transfer and showed how one could put a gene into a mouse stem cell. As I was starting my lab in the same city as my mentor Alan Bernstein, I thought to differentiate myself by putting genes into a human stem cell. In the mouse system, stem cells were defined by repopulation assay, and started thinking about how to study human stem cells at this level. Robert Phillips, one of the pioneers of immunodeficient mice,

was my colleague. And I just said, "Bob, how about if we put human cells in your mice?" He said, "We tried that 20 years ago and it doesn't work because the mice still have innate immunity." And I said, "Well, I'll try it, we'll see." And then he said, "John Roder across the street has an antibody that we could maybe give to suppress the NKT and NK cells." As we managed to put that all together, I remember thinking to myself: This is probably not going to work, but if it does work, nobody will be crazy enough to think about this idea and I will have terrific research area to start my lab. It took us eight months to get our first data back. It was already shocking that it worked, but the other shock came as I was telling one of my colleagues about it. He said, "this is weird. I was just having coffee with Irv Weissman in California, and he's been putting human cells in a mouse." It was just one of these crazy moments and, again, part of that was serendipity.

And how did we get to the leukemia stem cell? I wanted to understand the making of leukemia. Another colleague of mine, Tony Pawson, was an expert on oncogenes. The second project I wanted to start my lab on was to put oncogenes into viral vectors, then put these vectors into normal human cells and watch the process of leukemia. We tried that but there was not any outgrowth in culture. After talking to colleagues, I realized that the idea of putting an oncogene in human stem cells might work, but the problem may be in our assay system: Even though primary leukemia cells grow rampantly in a person, it is very hard to get them to grow in culture. As we had already started putting normal human cells in mice, we thought, let's just put human leukemia cells in mice. And lo and behold, it worked—we got leukemia cells growing in a mouse. But the frequency of a repopulating cell was one in a million, whereas the frequency of a clonogenic progenitor in AML is one in a hundred. So, either xenografts were a poor assay of clonogenic progenitors or an assay for a different cell. Purification was the only way to resolve this. Using CD34 and CD38 markers, we separated a pot of cells that had clonogenic activity. But that pot never made leukemia in a mouse, whereas a 1% population of another phenotype did. So, none of this was planned. It was just like putting one step in front of the other one.

*"For the first time, we can begin to think about preventing AML."*

**Was it hard to convince everyone that you are looking at stem cells with the xenograft assay?**

For the normal cells, people were skeptical, because the level of engraftment was so low. But we were aided by competition. The Weissman lab, the Mosier lab and us did it in different ways. In the fall of 1988, the three papers came out. As the most junior member of that group, I benefited greatly from the imprimatur that these more senior people had on establishing the credibility of xenografting.

Xenografting, while an artificial model, can predict important things. From xenograft-based stemness assays, we have identified the LSC17 gene signature, which predicts survival outcomes across a thousand patients. Every one of those patients has their own private cancer genome with its own diversity, and yet it is remarkable that something as artificial as whether a cell can graft a mouse or not can predict outcomes.

For the leukemia stem cell work, I had the good fortune to interact with Barney Clarkson, a towering giant in the field. He told me a lot about what life was like in the early 1960s, and he gave me access to things he had published; they were often monographs that were hard to find. By kinetic labeling of cell division, he and others identified a slow-cycling cell population in leukemia patients. And in this literature, he said, accurately, that these cells are likely to resist therapy, and that we could think about these as leukemia stem cells. So, the concepts were there, and when we came up with the actual proof, people were happy it was finally there, but it wasn't a shock or a surprise. By contrast, it took 10 years until the concept extended beyond the blood cancer field, when Michael Clarke and Peter Dirks published the first solid tumor work in 2004. But much remains to be understood and some controversy of relevance still exists until today.

**How typical is it for discoveries made in blood cancer research to cross-pollinate other fields?**

Mel Greaves said, "blood cancers lead the way." Huge advantages of the blood system are the ease with which one can study a whole tissue and get easy kinetic experiments just by putting a needle in the bloodstream. The development of clonal assays by Till and McCulloch 60 years ago enabled identifying single cells and understanding relationships between them. That's how we built our tree picture of the healthy blood system. The same principles and assays were then applied to understand leukemia, and back, and forth. We now have a very deep understanding of both, which are like two sides of a coin.

There are very few other tissues in the body where we have this deeper understanding of the normal developmental processes. An important thing for cancer research is to build that. Particularly in this single-cell omics era, we need that information of the normal to understand how a developmental program has been subverted in cancer. A fundamental principle in that game, developed in the 1970s by Barry Pierce, is that tumors are caricatures of normal development. So, we need to understand what normal development is before we can understand what that caricature looks like.

In our new study, we investigated the structure of the leukemias by cellular hierarchy, asking how a patient's hierarchy differs from the normal architecture. And we found that hierarchy is a more powerful predictor of clinical features and of response to therapy than any existing gene-expression program. In another ongoing study with Peter Dirks and Catherine O'Brien, when we define stem cells as multiple different states, we are uncovering a lot more convergence of stemness signatures among widely differing cancers including AML, colorectal cancer, and glioblastoma. With this approach, we can begin asking what the core elements of stemness are. There are many ways for a cancer cell to be abnormal from the cell surface all the way through to the nucleus, but very few ways to be a stem cell. And perhaps there is an opportunity for therapeutic targeting common across tumor types.

**Is it possible to target cancer stem cells without also killing normal stem cells?**

That may be a challenge because normal and leukemic stem cells share many programs. But leukemia, there's some evidence

that noncycling leukemia stem cells are in an activated, or poised, state—whereas in the normal stem cell pool, there's a subset in deep dormancy. The poised state could be a therapeutic window.

**Could the stemness gene signature potentially be applied to leukemia screening?**

Remember that finding we stumbled across when looking at diagnostic blood samples, that the normal T cell in the tumor shared the initiating *DNMT3A* mutation with leukemia? We then went on to ask whether individuals in the normal population have these initiating mutations. Remarkably, in our cohort, we could identify patients with the mutations in their blood 10 years before they developed AML. In the meantime, others had looked in the general population and had discovered clonal hematopoiesis. Now the challenges for the field are to understand: why *DNMT3*A, *JAK2*, and other mutant cells gain a clonal advantage? And is it targetable? For the first time, we can begin to think about preventing AML.

*"My advice to trainees is to find a good question, big enough to last you a career. Stay focused on where you're going but be open to unanticipated results that might challenge dogma. Look to the past to guide the future. Otherwise, you're going to end up reinventing the wheel."*

**Is stemness a function of the environment to some degree?**

Stem cells are exquisite sensors of the environment. By studying the wiring of the sensors, both in the environment and within the stem cells, we'll begin to understand the selective forces that drive clonal advantage of mutations in preleukemia.

**What is the role of tools versus ideas in discovery?**

Technology shows what's possible. The human stem cell is like a needle in a haystack, and after thirty years we managed to find it with high resolution sorting and single-cell transplant assays. Thirty years later, we can look at gene expression, proteome, epigenetics, methylation, and chromatin accessibility in these cells, all at single-cell resolution. The question that we've been working on for 30 years, and which we got from Till and McCulloch, is what makes a stem cell a stem cell and how does it go bad in cancer. We can answer it with much more precision than back then, but it still drives us today. So, my advice to trainees is to find a good question, big enough to last you a career. Stay focused on where you're going but be open to unanticipated results that might challenge dogma.

**Was that the advice you got from your mentors? Can you talk a bit about your training?**

My PhD was in a small university, and I didn't have very much access to molecular biology, which was just being founded at that time. I didn't know anything about the blood system. Then I moved to Toronto and worked with the Bernstein lab. There was my first exposure to molecular biology and viral vectors and the blood system. What I really learned in that period is how they did science. It was to ask big questions and stay focused. In the middle of the 1980s, gene transfer was the rage. Gene therapy was going to cure everything. It was very competitive. A trainee can get caught up in thinking "who is chasing me, how can I get to a finding first." Bob Phillips and Alan Bernstein grounded me to say: don't look over your shoulder, just stay focused on where you're going. If a research direction is worth taking, if someone gets to a finding before you, you can just move forward to a bigger goal with that new information in hand.

The other thing I learned was to look to the past to guide the future. Otherwise, you're going to end up reinventing the wheel. At lab meetings we would come up with an idea and Bob Philips, who trained with Till and McCulloch, would look back and say, "well, in 1964 they did this and this experiment...." It helped me appreciate the historical development of an area. I often say that most of my career is redoing in the human system the clonal assays, functional assays, and studies of lineage relationships between cells that Till and McCulloch published in the 1960s and 70s. Principles are generally the same.

The other part was the social environment of doing research in Toronto: a lot of collaboration and collegial interactions, giving advice or helping in an experiment without asking for anything in return. It was a generous way of doing science. And it continues to exist today. People rather collaborate than compete. You can take that for granted until you go to other places and realize that that's not necessarily the norm everywhere.

**How many people have you trained, and how many of them became independent scientists?**

Depending on how you count, about 180 postdocs, graduate and undergraduate students. At least 80% are in some aspect of academia. I view the lab very much as a family, and I think we all benefit from that interaction. One of the good fortunes that I have is that we travel quite a bit, and so I get to see my trainees on a regular basis in their own environment, their own homes, with their families. And I find it one of the most special privileges of this job. I have these relationships with all of them including my earliest trainees, Suzanne Kamel-Reid, Tsvee Lapidot, and Francoise Pflumio and Josef Vormoor and other people who were in the lab in the early 1990s. There is a special sense you have when you see a paper from one of your trainees, a warm feeling seeing your children flourish and thrive, and knowing you had some role to play.

**Having met some of them, when I find out that they trained with you, it made sense—I could see that lineage.**

It's a real lineage.