ISCT TALKING WITH GIANTS

"I have been involved in these fields of research for up to 49 years and have never lost my interest or enthusiasm, even when work in the laboratory was at a low point."

International Society ISCT Cell & Gene Therapy®

5 Questions with Hal E. Broxmeyer, PhD

Patients around the world can thank Hal Broxmeyer, PhD for saving their lives. His work helped found and nurture the field of clinical cord blood transplantation, establishing human cord blood as a source of transplantable hematopoietic stem cells. With a team of clinical investigators, his research translated to thousands of cord blood transplants, saving patients with life-threatening malignant and non-malignant diseases. He is Distinguished Professor, Mary Margaret Walther Professor Emeritus, and Professor of Microbiology and Immunology (full time) at the Indiana University School of Medicine. He received a PhD from New York University, then did post-doctoral training at Kingston General Hospital, Queens University Kingston, Ontario, Canada. He worked at Memorial Sloan Kettering Cancer Center (MSKCC) in NYC working up from the rank of Associate Researcher to Associate Member. To date, he has published 795 scientific papers which have been cited 62,800 times with an h-factor of 119/i10 index of 575 (Google Scholar).

Dr. Broxmeyer has been recognized with a number of honors including the Mellor Award (2nd prize 1976; 1st prize 1977) and Boyer Award (1983) from MSKCC; Special Fellow (1976-1978) and Scholar Award (1978-1983) from the Leukemia Lymphoma Society; Merit Award, NCI (1987-1995); Karl Landsteiner Award, AABB (2002); E. Donnall Thomas Prize and Lecture, ASH (2007); Donald Metcalf Award, ISEH (2011); elected Fellow, AAAS (2012); Honorary Professor, Peking Union Medical College (2014); NHLBI Outstanding Investigator (R35) Award (2018-2025); and Lifetime Achievement Award, Cord Blood Association (Sept. 2019). He is past president of ISEH (1991) and ASH (2010).

1 How is the cell and gene therapy field different now compared to when you started? How has it evolved?

While I have worked in the field of regulation of blood cell production (hematopoiesis) since my PhD thesis work at New York University starting in the late 1960's, I have also worked with gene vectors for gene transduction to study regulation of hematopoietic stem (HSC) and progenitor (HPC) cell functions for over 25 years, and am currently a PI on an NIH T32 training grant on: "Basic Science Studies on Gene Therapy of Blood Diseases". So, my comments can cover both areas, but with an emphasis on cellular therapy. The progress made in both areas of research has been nothing short of truly amazing. Consider that we can now rigorously phenotype HSCs and HPCs, have functional assays for these cells to study their regulation, and that there are now different viable sources of HSCs and HPCs for successful hematopoietic cell transplantation (HCT). These were not available when I started. Other areas not available when I started include use of cytokines (e.g. G-CSF) and small molecules (e.g. AMD3100=Plerixafor) for efficient mobilization of peripheral blood (mPB), and use of cord blood (CB) for HCT. Both have been used as life-saving procedures to treat, and in many cases cure, malignant and non-malignant disorders in adults and children using HLA-matched and HLA-disparate grafts from siblings, related donors, and especially from unrelated donors. Bone Marrow (BM) HCT, while initiated in the late 1950's by Nobel Laureate, E. Donnal Thomas, was not really ready for prime-time until more than a decade later when the HLA system was beginning to be defined and used to choose appropriate donors so that there was decreased life-threatening graft vs. host disease. There was the purification of proteins, and then through use of recombinant technology the production and subsequent purification of numerous cytokines and chemokines, now numbering in the hundreds. Many of these have been and still are used for therapeutic benefit. There was the development of monoclonal antibodies and exotic cell-analyzers and -sorters, and the use of monoclonal antibodies for study and clinical advantage. Now there is the emerging field of precision medicine, which I personally believe still has a very long way to go. Precision Medicine is a new addition in the medical field, but is this being performed correctly when the cells being tested for gene expression profiles and responses to drugs outside the body, have been removed from their natural in-vivo microenvironment in context of oxygen tension (low *in vivo* (1-5%) but high (~21%) when removed into an ambient air atmosphere) and regulatory stromal cells? We need to be aware and cautious of current hype for success of immune therapy, as a final frontier. This includes checkpoint inhibitors and tyrosine kinase inhibitors (TKIs). The very successful use of TKIs to treat chronic myelogenous leukemia was a "glowing light" but not all patients with cancer presently being treated with checkpoint inhibitors or TKIs respond, and the responses may not be long-lasting and can be associated with serious side effects that can linger after cessation of treatments. While immune therapy is an exciting area, there is still much to be learned. We now have a variety of viral and non-viral vectors available, some of which show promise for the still emerging field of gene and immune cell therapy. This is not meant to be an all-inclusive list of the truly amazing discoveries, that most of us probably did not even envision when I started working on my PhD thesis in the late 1960's. I seriously doubt that many of the current scientific and clinical investigators truly understand how rapid and amazing these advances have been, as most might not read the literature beyond the past 5-10 years, or even one year, as part of their training. These advances might previously have been considered science fiction at the best when I started. In 1968, I was finishing my Masters of Science (MSc) Research at the Brooklyn Center of Long Island University after receiving a BSc degree from Brooklyn College, which is now part of the City University of New York. My MSc research was on RNA synthesis regulation in bacteria, which I pursued while working a full-time job as a laboratory specialist at Midwood High School in Brooklyn, New York where I prepared the teacher demonstrations for the faculty in the chemistry and physics department. At the same time, I was also pursuing 9 credits a semester at NYU, and began dating Beth, the woman that I have now been married to for 50 years. Beth was pursuing a MSc degree at NYU in neonatal erythropoiesis and explained to me all the recent exciting work from the late 1950's, early 1960's on the emerging field of hematopoietic stem and progenitor cells by McCullough and Till from Toronto, Bradley and Metcalf from Australia, and Pluznik and Sachs from Israel. There was little of this work being done in the United State at that time. As part of a PhD course I took on cell kinetics at NYU I had to write a paper for part of my grade in the course. I chose to do it on all the early work published until 1970 in this newly emerging stem/progenitor cell field. I was really taken by and enthused with this early work. At the beginning the research was all very descriptive. What Till and McCullough called stem cells were actually not the long-term repopulating HSC, but rather a cell identified by the *in vivo* spleen colony forming cell assay (CFU-S) as more differentiated but with self-renewal capacity. It was not until much later that this distinction in HSC hierarchy became apparent. Pluznik and Sachs and Bradley and Metcalf, developed in vitro colony assays for what were originally termed committed stem cells, and later called HPCs. The growth medium for the in vitro colony cells was made up of serum, culture media, and crude mixtures of cell products such as cellular conditioned medium (CM). No one really knew what was in these CM, but they stimulated colony formation. Not all CM was equally potent for stimulation of the colonies. The activity in the CM was called colony stimulating activity (CSA). It was not until later that that factors involved would be called colony stimulating factors (CSFs), which were later found to a family of proteins including what we now know are granulocyte-macrophage-CSF (GM-CSF), granulocyte (G) CSF, macrophage (M) CSF (or CSF-1), erythropoietin (EPO), and thrombopoietin (TPO). The potent co-stimulating factors: Stem Cell Factor (SCF, also called kit ligand) and Flt3-ligand were identified much later. The colony forming unit-macrophage (CFU-M) and -granulocyte macrophage (CFU-GM) assays were defined first, followed by an erythroid colony forming cell assay (CFU-E; now considered an erythroid precursor, rather than progenitor, cell). Then the erythroid burst forming unit

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(BFU-E, an erythroid progenitor cells) assay, followed by the megakaryocyte (Meg)-CFU, and the multipotential (CFU-GEMM) assays were developed. These assays are still being used today to great advantage by my laboratory and other laboratories. The long-term culture assay was developed by Michael Dexter from Manchester, England when others until his work could not sustain hematopoiesis in cultures for more than a week or two at most. This was followed by the sophisticated ex-vivo HSC/HPC expansion assays currently in use today and studied for clinical advantage when donor HSC/HPC numbers are sometimes in limiting supply, such as for CB HCT. It is amazing to realize what we were able to learn with such original rudimentary knowledge and reagents. In the 1970's, in my first, first-author publication I showed with serum collected from mice injected with varying dosages of E. coli lipopolysaccharide that the "factors" that caused the release of blood cells from the BM were different from those that stimulated colony formation in vitro. This was done in a small project I set up myself during my PhD thesis tenure and published over a year after I received my PhD degree. Prior to that such factor(s) were considered to be the same. Arguably, I was the first to truly demonstrate a role for negative regulators as part of my two year post-doctoral time at Queen's University, Kingston, Ontario, and in my 1 year advanced post-doc and then early faculty position at the Memorial Sloan Kettering Cancer Center. I first used crude CM, followed by purified molecules, and then purified recombinant preparations of lactoferrin and H-ferritin. At the time, few if any investigators believed in negative-regulators and -regulation. We now know that here are numerous negatively acting regulators, most of which I have worked with and published on. In the early days, it was felt that if the "regulatory" molecules didn't have extremely well-defined specificity, they couldn't possibly have physiological relevance. We now know that this is far from the truth, and that most "regulators" have multiple functions. In the 1960's, 1970's we had little or no idea of how the factors, cells, and cell interactions mediated their actions mechanistically through specific cell surface receptors and the intracellular signaling pathways being activated or how these manifested in physiological or pathological outcomes. How incredibly far this field has come. Unfortunately, it is not likely truly appreciated by the younger generation of investigators all that has led to our current state of knowledge. Consistently we are being treated to new information, but what is not at all clear is how all the different events link or do not link to each other. I just wrote a Preview to a paper being published in Cell Stem Cell demonstrating a role for Ca2+ in maintenance of HSCs in culture. In this Preview, I made a case for investigators to get together to think-tank trying to decide how to put all this knowledge together for a clear understanding of the regulation of HSCs, HPCs, and hematopoiesis. This information may allow for more precise clinical therapies with fewer side effects of the current treatments modalities. All treatments have side effects, some that can be as devastating as the disease being treated. To know where we need to go, we must understand and appreciate how we arrived at our current stage of knowledge. We have come amazingly far, but I anticipate the fields moving "light-years" ahead in future efforts to improve health care.

2 Where do you see the cell and gene therapy field in 5 years?

While it is very difficult to predict the future, with the speed that the scientific and clinical advances are being made in both the cell and gene therapy fields, I would think that the following predictions are possibilities, or that at least we will move significantly towards them. In the cell therapy field we should have a better indepth mechanistic understanding of cell-cell and cytokine-cell interactions, and how oxygen levels and the in-vivo microenvironment regulate the functioning of hematopoietic stem (HSC) and progenitor (HPC) cells and hematopoiesis over-all. This includes more in depth insight into immunological cell interactions and their control, which can be used for enhanced treatment protocols for malignant and non-malignant diseases. I envision that cord blood (CB) hematopoietic cell transplantation (HCT) will be vastly improved, assuming that the present newer methods for collection of increased numbers of CB HSCs and HPCs can be tested for clinical utility, that ex-vivo HSC/HPC procedures are improved such that the expanded cells are truly normal and of high potency, and that the homing capacity of HSCs is enhanced. This will likely increase the use of CB cells for HCT, and in the process enhance CB banking efforts for both public and private CB banks. I envision that there will be improvements in efficiency of collecting mobilized peripheral blood (mPB) especially in the hard to mobilize donor populations. Bone marrow (BM) HCT will still be used, but perhaps at a lesser frequency than at present compared to that presently, in comparison to CB and mPB HCT. I am not yet sure where halpoidentical HCT will be in 5 years. Right now it looks very promising, but what will the long-term results of patients receiving haplo-identical HCT be? Will there be more graft vs. host disease that might negate the upfront quicker engraftment and thus the initial benefits of this form of HCT? Hopefully we will have a better understanding of events involved in regenerative medicine, and what we can actually do with the cells involved. Most of what is known in this field is still in its infancy in terms of treatment potential. There are many unscrupulous persons "milking" this field in very bad ways by setting up unproven "stem cell" clinics, when in fact they are not dealing with stem cells at all. At best these stem cell therapies have shown no real therapeutic advantage, and at worse present real harm to the patients. We should know more about the actual cells involved in regenerative medicine, and what they can and cannot do. CB is currently being tested for effectiveness in non HCT procedures. We should know if in fact CB or other sources of cells can or cannot have positive therapeutic effects in these conditions (see recent New York Times article on this: *https://www.nytimes.com/2019/04/18/well/live/the-lifesaving-power-in-stem-cells.html*). Immune cell therapy (including tyrosine kinase inhibitors and checkpoint inhibitors) have had some successes, but in most cases have been effective in only a minority of cases, and effects are not necessarily long-lasting and have been associated with significant side effects. I envision much improvement in understanding and use of immune therapies, and anticipate lessened side effects, which are sometimes more devastating than the disease being treated. There will likely be significant increases in gene therapy for a variety of disorders, but this will require better vectors, and increased knowledge of gene transduction and the best target cells for these vectors. In fact the vehicle cell carrying these new genes, and how these cells engraft and function is likely as important as the vectors (viral or non-viral, integrating or non-integrating) that will be used. It is hoped that the safety profiles of the vectors and cells carrying these vectors will have substantially improved for increased therapy and health benefits. All these hoped for endpoints 5 years from now and beyond require extensive and rigorous scientific and clinical efforts. This means an influx of increased funding for these studies, and making sure that we encourage not only current investigators, but the next generation of new, young and "hungry" investigators. Cooperation between investigators is also of importance to realize our future goals and aims towards enhancing cellular and gene therapy efforts.

3 Why are you passionate about working in the cell and gene therapy field?

I have been involved in these fields of research for up to 49 years, and have never lost my interest or enthusiasm for them, even when the work in the laboratory was at a low point. I look forward each day to the possibility of finding new information and insight into the regulation of hematopoietic stem (HSCs) and progenitor (HPCs) cells and hematopoiesis that might someday be used for therapeutic treatment and health benefit. While my laboratory is a very basic scientific endeavor, I never lose track of the possibility for potentially bringing what we learn to the clinic for testing in collaboration with clinical investigators. I try to remind my lab members (pre-doctoral students, post-doctoral fellows, and clinical fellows) whom over the years I have had the privilege of mentoring as well as those not directly associated with my lab who I have helped mentor, that while they are working on mechanistic insight to understand cell regulation, that they need to always keep an open mind to the potential of using their laboratory work for clinical translation. Over the years I have been very fortunate to have been associated with a number of different clinical trial efforts, some of which was initiated through our

laboratory findings. This includes use of AMD3100/Plerixafor to mobilize HSCs and HPCs, and to synergize with G-CSF to enhance HSC/HPC mobilization. The original mouse studies that set the stage for the clinical studies were done in my laboratory, and we helped to evaluate and provide data on HPCs for the original clinical trial in this area. I am also extremely proud of our studies on cord blood (CB) HSCs and HPCs which led to initiation of the field of CB Hematopoietic Cell Transplantation (HCT) which we worked on with outstanding clinical investigators. We continue to try and nurture this field through our laboratory studies that seek to find ways to enhance clinical CB HCT. This includes efforts to collect more HSCs through hypoxic collection and processing of the cells, ex-vivo expansion efforts, and through increasing the homing capacities of the CB HSCs. We always first consider simple means to enhance these procedures, based on our philosophy that simple is better, and that the simpler the procedure the more likely that it will result in rapid translation to clinical utility. It is a pure joy to work in this area, and especially to interact with and collaborate with so many outstanding scientific and clinical investigators, whom I am proud to call frients. The learning process never ends and I hope that those we interact with learn as much from us, as we have learned from them. I truly look forward to work each day, wondering what new and exciting things we will learn from our own efforts, and from the findings of others. Not everything we do will result in positive or useful information, but when this does happen, it is a priceless experience.

4 What are the biggest challenges facing the development of new cell and gene therapies?

The development of new cell and gene therapies is only limited by our visions, desire, and enthusiasm to pursue new and as yet untested/unknown experimental procedures. That being said, we are limited by the numbers of investigators presently working in these fields, funding to pursue these studies, and recruitment and mentoring of the next generation of bright, motivated and dedicated young investigators. None of these efforts can result in instant fixes, and will require a very concentrated effort on the part of those already involved in these fields. There are many well-deserving projects and investigators that unfortunately do not meet the cut-off for funding by the NIH and other granting agencies. It is especially difficult for new investigators to get funded without having the extensive accomplishments of the more established investigators. We all need to do our part to find and mentor these new investigators, and provide them with the time and resources to succeed and publish their results in respected peer-reviewed journals so that they can compete for external peer-reviewed funding on their own. This is not an easy task, and is time-consuming, but is well worth the effort. We have to also be able to collaborate with other investigators for maximal output and productivity taking the time to work together in collaborative efforts and to think-tank current problems and how best to begin to resolve them. These are not insurmountable problems, but as noted above there are not easy fixes at present to accomplish these goals. The sooner we begin, the sooner we will likely be able to generate new ideas and experimental results for laboratory testing and for eventual translation for clinical benefit.

5 For people just starting out in this field now, what would be the one piece of advice you would give them based on your experience?

There is obviously more than one piece of advice to be given. One simple piece of advice is to love the area you are working in, and if not move on to another area. Pursue your chosen area of research with enthusiasm and dedication. Understand that not everything you try will provide you with positive results that can be further investigated. You may have more perceived failures than successes, but even one success has the potential to sustain you for a long time. Do not give up on your work, but be open to the possibility that perhaps you are at a "dead end", and you need to move on. One of the best pieces of advice I ever received, that I have never forgotten, was at the end of my qualifying exam to continue in the PhD program at New York University. One of the committee members who was very impressed with the enthusiasm of my presentation if not so impressed with the data I provided, told me to expect many ups and downs in my career. He told me to understand that when I have ups, enjoy them, as they might not last long. When I have downs, they will not last forever, and will be followed by ups. These are some of the truest statements I have learned over many years. The PhD thesis work I pursued that was suggested and "guided" by my PhD mentors and which I spent over 3 years on day and night never resulted in publishable findings. However, on my own, while pursuing their suggested work that turned out to be non-productive, I designed my own experiments and pursued them over a period of a few months each. It was these experiments, done on my own, that eventually resulted in 5 first-authored papers. I had to submit these papers after receiving a PhD degree "for effort" and while I was pursuing a 2 year post-doc. I had left copies of these five papers on my mentor's desk, but he did nothing with them for almost 2 years. I finally fixed their English presentation up and submitted them by myself, making sure to include my mentor as a co-author and other co-authors who had provided only very minimal input for suggestions or showed me how to do a colony assay, even though I thought of and designed and did all the experiments and writing myself. A few years later when I was invited to give a talk in Paris, that my main PhD mentor also attended, my mentor approached me and asked how I could have submitted the paper without his permission. I told him that he had had the papers on his desk for 2 years, and every month I would ask him when he would provide me with comments on the papers, but he never responded. I told him that I gave him numerous opportunities to respond with suggestions and that he was hurting my career by holding up his comments and submission of my papers. I finally got so frustrated that I sent these paper out, and all 5 got accepted with minimal or no revision requests from the journals. I am extremely proud of these papers even though by today's standards they would likely be considered sub-par, but at the time that was the state-of-the-art of science. These papers got accepted along with 2 first authored papers from my 2 year post-doc at Queens University in Kingtons, Ontario that were published in the same issue of Blood. I am as proud of these papers as I am of all the subsequent papers we have published, even though they were not of the sophistication and intense rigor of my later papers. They were relatively simple studies, but to my mind provided important new and still highly relevant information for regulation of hematopoiesis. So my final advice is to find the "right mentor(s)" and solicit that mentor's advice when needed. This means finding a mentor who is productive (publishes and has NIH or comparable peer-reviewed funding), and who you can get along with. I, myself, am a relatively hands-off mentor, as was my later mentor, Malcolm A.S. Moore at the Memorial Sloan Kettering Cancer Center for my 1 year advanced post-doc position and then as a faculty member in his department for the next 7 years where I was able to compete successfully, first for a Leukemia Lymphoma (LLS) Special fellow Award (1976), and then for their Scholar Award (1978), and for my first NIH R01 grant (1978). I have been fortunate to have been funded continuously since 1978 by NIH grants from the NCI, NHLBI, and NIDDK, and most recently by an HL R35 Outstanding Investigator Award that goes to 2025. This funding has not always been easy to attain, but fortunately I had another grant when one failed to reach a funding priority score. Persistence is very important, and while hard, it is best to not take negative critiques on your papers or grants too personally, but rather to use them to improve. I am always available to provide advice, but am relatively "hands-off" and do not sit over the day-to-day work of my pre-doctoral graduate students, and post-doctoral fellows. I ask them to find a project themselves that they would like to pursue, as long as it is in relative areas of my expertise so that I can offer help if they need it. I also make sure that they solicit help from other lab members, and students and faculty outside my laboratory. I want them to know that what they accomplished, was in large or complete part due to their own efforts. This way they will have confidence in their future abilities to succeed. This type of mentorship is not for everyone, so it is important to find what type of mentor works for you. It is also important that your mentor provide you with a mentoring committee, as not just one person has all the answers, and it helps progress if you get advice from different perspectives.