Since I'm not running for political office, I thought I would take the opportunity in this *Journal Club* to be brutally honest. I dropped the ball. Yes, I said it, I dropped the ball. Two months back, Dawn suggested that I discuss two recent research papers relevant to the use of leucine in treating DBA. Since I figured I already knew what was in these papers and didn't see what the big deal was (I'll come back to this point below), I pushed instead to cover the ribosome synthesis meeting which was fresh on my mind and filled with exotic new information I felt might be relevant to DBA. Then, last month with all the excitement and effort put into the Chase Charity effort, it was decided to forego September's newsletter. So here we are two months later, and I have finally gotten around to taking up the leucine story.

So I rolled up my sleeves and did a pubmed search of Diamond Blackfan anemia and what did I see? - **"Leucine alleviates Diamond Blackfan anemia,"** an article published in the premier hematological journal *Blood* (Kamimae-Lanning & Kurre, 2012). This article is a commentary on two primary research papers published in the same issue of *Blood* (Jaako et al, 2012; Payne et al, 2012). In addition to these articles, there was a fourth entitled, "Activation of the mTor pathway by the amino acid L-leucine in the 5q- syndrome and other ribosomopathies;" where again the effect of leucine in DBA is highlighted. So it's hardly surprising that this flurry of manuscripts stimulated a great deal of interest in the DBA community, so again, my apologies for not discussing this subject sooner.

Editor's Note: Steve's August 2012 Journal Club article highlighting the Ribosome Synthesis Meeting was an important and exciting article and deserved the priority status!

I mentioned that I didn't see what the big deal was with the two primary research articles that stimulated this firestorm of interest. It is not that there is anything wrong with these articles, in fact they are quality research carried out by some of the leaders in the field, it's just that they don't change the underlying fact that the only way we are going to find out if leucine alleviates DBA "in humans" is with a carefully controlled clinical trial; and if you read the fine print in any of these articles, that is the underlying point each makes.

Unfortunately, this latter point doesn't always come across in the titles we choose. **Leucine alleviates Diamond Blackfan anemia**... certainly suggests that taking leucine would alleviate the underlying clinical features of DBA, leading many, I suspect, to wonder why their physicians aren't prescribing leucine. But is that what the authors really meant? Reading a little further into the fine print, it becomes clear that the commentary is on two research articles that discuss the effect of leucine in ameliorating erythropoiesis in animal and cellular models of DBA. So the question then becomes how good are these animal and cellular models of DBA in recapitulating the disease observed in humans, and then depending on the answer to this question, we can make a better assessment of the relevance these studies may have on the use of leucine in a DBA patient.

The research article I choose to emphasize for the rest of this *Journal Club* is entitled, "Dietary L-leucine improves the anemia in a mouse model of Diamond-Blackfan anemia." This title is certainly an accurate assessment of what is embodied in the manuscript. Now, in defense of Drs. Kamimae-Lanning and Kurre, *Blood* severely limits the number of words authors can use in Commentaries, and also requests that the titles be gripping; to catch the casual reader's eye and direct the reader to the primary research article. Well, eye-catching their title certainly was, but not everyone has access to the body of manuscripts published in *Blood*, and so some may be left with only the titles, and wonder why their child isn't being treated with leucine.

Let's go ahead and look at the studies in the mouse model of DBA. The mouse model was created in the laboratory of Dr. Stefan Karlsson in Lund, Sweden. It is remarkable the lengths they had to go through to create this mouse. An early mouse, where one of two copies of the RPS19 gene was inactivated, so similar to the situation in 1 in 5 DBA patients, showed no effect on red cell production (Matsson et al, 2004). Knocking out both copies of RPS19 in mice was lethal shortly after conception, so neither situation reflected DBA in humans, leaving the DBA community without a mammalian model for many years. In the current mouse, the Karlsson lab put both copies of the RPS19 gene under the control of a compound which can be included in the drinking water. This compound when ingested then shuts down expression of RPS19, and so the thought was by titrating the amount of the compound the mouse ingests,

it may be possible to reduce Rps19 levels to a point that triggers a phenotype akin to what is observed in humans. And in this respect, the authors were successful. The mice developed a mild macrocytic anemia. But it should be pointed out, that these mice do not need transfusions and more importantly, with time, they eventually begin to compensate for the defect in erythropoiesis. Whether this compensation reflects the remission observed in humans is unclear at this point, but the fact that all mice display this compensation indicates that the mechanism may be distinct from what is observed in humans. Nevertheless, there is a transient window of time where the mice display a macrocytic anemia which gave the authors a chance to evaluate the effect of leucine in ameliorating the erythropoietic defect.

The results were encouraging. The authors saw an 18-19% increase in both hemoglobin and the number of red blood cells; an increase they demonstrated was significant. But what is the meaning of the term "significant?" Here, it is meant to show the results are statistically significant, which means that relative to a commonly accepted norm of statistical significance, the results obtained in the presence of leucine differed from the results in its absence (well for you statistical purists, they actually excluded the null hypothesis that the results were the same). But how do we transfer these statistically significant results in mice to whether leucine might be clinically significant in humans? Well, one would be the magnitude of the effect... would a 19% increase in hemoglobin values dramatically influence transfusion dependence or at least prolong transfusion intervals in a human patient? Perhaps. But importantly, in my mind, it also depends on how leucine is having an effect in mice. If leucine is having its effect by influencing the poorly understood compensation mechanism observed in mice, it may or may not be relevant to humans.

Many important strides have been made in the use of model systems to investigate aspects of DBA. But models systems for DBA still remain limited in recapitulating critical aspects of the human disease, and so not everything observed in a model system will translate into improved therapies. And no, I am not ignoring the second manuscript published in *Blood*entitled "L-leucine improves the anemia and developmental defects associated with Diamond-Blackfan anemia and del(5q) MDS by activating the mTOR pathway"(Payne et al, 2012). While not implicitly stated in the title, this manuscript also addresses the effect of leucine in model systems, either zebrafish or human cellular models, where many of the arguments I made above for the mouse model still hold.

Please don't get me wrong. I am not ruling out that leucine may have a positive effect in alleviating Diamond Blackfan anemia "in humans." I am just pointing out that regardless of what is observed in model systems, the final answer to this question will only be obtained from carefully controlled clinical trials using DBA patients. The single publication reporting a DBA patient who had a positive response to leucine is clearly not a definitive study (Pospisilova et al, 2007).

A logical question then becomes-where does the clinical trial assessing the effect of leucine on alleviating Diamond Blackfan anemia "in humans" stand? This trial has gone through various fits and starts, while the investigators have gone through various funding and regulatory hurdles. Let's hope that any remaining hurdles in getting this study started can be overcome soon, so we can get the role of leucine therapy in DBA resolved once and for all.

Jaako P, Debnath S, Olsson K, Bryder D, Flygare J, Karlsson S (2012) Dietary L-leucine improves the anemia in a mouse model for Diamond-Blackfan anemia. Blood 120: 2225-2228

Kamimae-Lanning AN, Kurre P (2012) L-leucine alleviates Diamond-Blackfan anemia. Blood 120: 2157-2158

Matsson H, Davey EJ, Draptchinskaia N, Hamaguchi I, Ooka A, Leveen P, Forsberg E, Karlsson S, Dahl N (2004) Targeted disruption of the ribosomal protein S19 gene is lethal prior to implantation. Molecular and cellular biology 24: 4032-4037

Payne EM, Virgilio M, Narla A, Sun H, Levine M, Paw BH, Berliner N, Look AT, Ebert BL, Khanna-Gupta A (2012) L-leucine improves the anemia and developmental defects associated with Diamond-Blackfan anemia and del(5q) MDS by activating the mTOR pathway. Blood 120: 2214-2224

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