iPS-Cell Based Cell Therapies for Genetic Skin Disease

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Introduction:

Dr. Wernig is a Professor and Co-Director at the Institute of Stem Cell Biology and Regenerative Medicine at Stanford University. For over a decade, he and his close collaborator Dr. Anthony Oro have pioneered research towards what could be the world's first widely applicable curative treatment for Epidermolysis Bullosa (EB). This rare genetic disease causes chronic and incredibly painful skin wounds that often leads to an aggressive form of skin cancer and eventual death. While various cell-therapy approaches have been attempted and are on-going, Dr. Wernig and collaborators identified the need for induced pluripotent stem cells (iPSCs), and further deciphered how they could be utilized to treat EB in a more efficient, broadly applicable, and commercially viable manner. While there are still hurdles to climb before a cure is fully realized, Ajinomoto is proud to support this pioneering research with our StemFit media.

To provide an update and first-hand account of this exciting research, we invited Dr. Wernig to an interview covering topics from inception through to future challenges. Please enjoy!

Abstract on Epidermolysis Bullosa (EB) Research: The Disease and Their Approach

Ajinomoto (AJ): *So, where did it all start? Can you give us some background on EB?*

Dr. Marius Wernig (MW): We got together with Tony Oro's group over ten years ago. We were thinking how to find a definitive cure for this rare genetic skin disease called Epidermolysis Bullosa. This disease is caused by mutations in collagen 7 gene type, which is known to be the molecular glue between epidermal layer and dermal layer. When collagen 7 is missing, the upper, epidermal layer of the skin is loosely attached and comes off in blisters. The effect is patients are born with normal skin, but as soon as they experience any sort of trauma on their skin surface, they develop blisters that then develop into wounds. These wounds cannot heal, yet are very painful, so you can imagine what a horrific painful life these patients have to endure. These wounds then become chronic, which leads to permanent chronic inflammation and attempts

by the local epidermal skin stem cells to repair the damage but because the local stem cells are mutant, they can't repair anything and proliferate in vain. This leads to an increased proliferation rate of these stem cells in an inflammatory environment which eventually leads to cancer. In fact, most of the patients develop a very aggressive form of squamous cell carcinoma, which is often the cause of death of these patients.

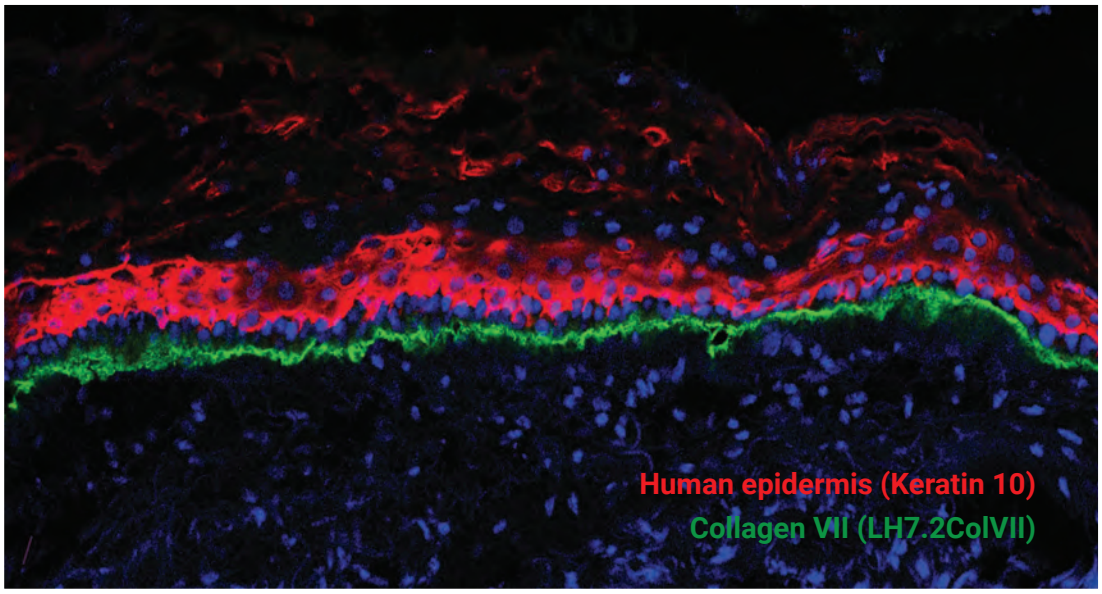

Fig. 1. 3-week xenografts on skin of NSG mice

> **This figure shows the xenograft to be transplanted onto patients in the original approach mentioned at Stanford, described in the paragraph below.**
> *Sebastiano, Zhen et al., 2014 Sci. Transl. Med.*

AJ: *What is novel about your approach to cure EB?*

(MW): There are several approaches that attempt to treat this condition. The Stanford dermatology team had already worked out a cell-based therapy based on conventional gene transfer. The idea was to take a primary skin sample, grow out keratinocytes form patients and infect them with a virus to deliver the therapeutic gene. And then, develop grafts, literally sheets of corrected epithelia, and… transplant these skin sheets onto the patient's wounds…

While this approach was an important first proof-of-concept and continues to be a refined, there are also several shortcomings that limit the applicability. First, the approach relies on using viruses, which are randomly integrating into the genome and therefore are risks to activate oncogenes. Second, it turns out that, at least for some patients, scalability is problematic. In order to replace large areas of the body surface you need to grow and expand these somatic skin cells to enormous numbers, which is not always possible for every patient. Especially in chronic patients, you can imagine these skin stem cells are prematurely aged and potentially also depleted by the time you harvest the skin due to their continues attempts to proliferate and repair wounds.

So, that's where iPS cells come in. Essentially the idea is to insert a step in between that allows you to more reliably scale the cells. In addition, by going through an iPS cell intermediate you can, of course, accomplish a genetic correction in a much more defined way while avoiding the risky virus.

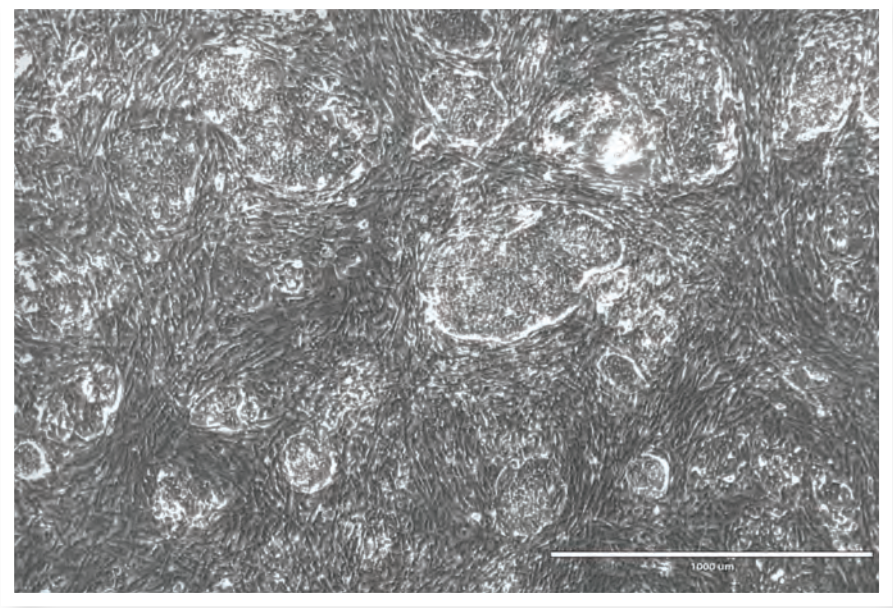

Fig. 2. Patient EB125 fibroblasts, 10 days into reprograming

> **This figure shows EB patient derived iPS-cells that were reprogrammed from primary fibroblast cell samples and will be expanded for genetic correction.**
> *Koji Tanabe, iPeace Inc.*

AJ: *Once conceptualized, how did you develop the iPS-cell approach?*

(MW): Well, this was a long time ago. Back then there was no CRISPR, so we did work on various ways to accomplish gene correction. We used conventional approaches by introducing long stretches of DNA and we explored AAV mediated gene targeting, which was actually very successful, in some cases on par with CRISPR. Another critical aspect was the cell differentiation. And this was really the goal of Tony Oro's lab, which did a fantastic job of devising efficient ways to turn iPS cells into engraftable skin-equivalents. This setup was a great synergistic team effort, my lab focused on reprogramming and gene engineering and Tony's lab on cell differentiation and grafting. A key moment to get us off the ground was when we were fortunate enough to receive one of the first large disease team grants by the California Institute for Regenerative Medicine (CIRM).

That brought us a long way, but it was clear that our initial idea, which was to reprogram to iPS cells then do gene correction, was quite an involved procedure… Along the way you have various QC steps, right, so first to reprogram you need to make sure those are high quality iPSCs cells, then you do gene correction and have to do the entire QC again to make sure the cells have not lost their properties or have become genetically abnormal, then you differentiate them, [and] along the way you have to bank cells…

When CRISPR came around we thought, well, if the gene correction efficiency is high enough, we could potentially shortcut much of the cell manufacturing by reprogramming and correcting the patient cells in one step. Thanks to the effort of our labs, and additional funding, we eventually succeeded in this effort, and it actually worked better than we had even hoped it would. With this new approach, half of all these time consuming and expensive QC steps could be eliminated. I am, of course, telling you the story from hindsight. When we were still in the trenches, as always, there are a lot of potholes and results that could have misled us easily, but gladly we overcame all these challenges.

One aspect we were always concerned about is that you always have to be very careful with genetic engineering, and with CRISPR correction many unexpected genetic events can happen. This is because CRISPR is essentially just introducing a precise cut in the genome, but it is left to the cells to repair this DNA break and there are many different ways the cells fix this DNA damage and only one of these many ways is the one that we desire. I'm not talking about potential off target mutations, that is yet another issue, but how the DNA is re-connected after the cut. But luckily, I can say this: the bottom line is this approach works, it works very well and very robustly.

Beginning to think about the clinic

(MW): As we have been continuing to develop this engineering, Tony on his side has further optimized the differentiation controls, which got better and better including a cell purification step of clinical scale and cell production using clinical-grade reagents. His lab is [still] fiddling around with some of these aspects, as we are optimizing the genetic engineering simultaneously. Yea, and what I described is essentially our project that is again generously funded by CIRM by a translational award.

"One guide, one IND"

AJ: *What did the FDA think of your approach?*

(MW): The idea was of course that we would switch out the guides [guide RNAs] for each mutation. The COLLAGEN 7 gene is large and there are many different mutations that can cause the disease. The disadvantage of CRISPR is that the repair can only occur mutations very close to where the CRISPR cuts the DNA.
The so-called guide RNAs are the components of the CRISPR that direct the enzyme to the specific sites in the genome. For this reason, we wished to obtain an IND, in which for every mutation we wanted to fix we would switch out the guides. But the FDA very clearly got back to us and said, 'one guide, one IND', and that was quite a disappointment for us… But you know… It's actually perfectly understandable. The FDA's main concern is safety and CRISPR technologies are still very new with little clinical experience. For example, early on it was not so clear how strong the off-target rate is in practice. It could have been prohibitively pronounced, posing additional safety risks. Luckily, now we have learned from several groups that this risk can be manageable, and in our hands, it is in fact very very low risk. Naturally, the FDA needs to see this type of data before they can relax their conditions. The one guide-one IND policy is, though, a formidable hurdle to bring such CRISPR therapies to the clinic.

While for now we will have to find ways around it, we are confident that in years to come more encouraging safety data will become available that will allow the FDA to change the policy and qualify the swapping out of different guides as a reagent change and not consider that a change of the entire therapy requiring a separate IND application.

But I don't know, sort of the more down to earth people, people who are thinking to commercialize it… they said, well, this is too risky. We need a genetic engineering approach that the FDA would approve now, with one IND. If you have to file INDs for every patient, this is not feasible. And that makes total sense...

Overcoming the regulatory challenge

AJ: *What's next then?*

(MW): The problem we were facing now was that the one-guide/one-IND policy was simply too expensive to commercialize this therapy, which in turn is needed to bring it to the clinic and make it available for all patients. For that reason, we were, again, challenged with coming up with new ideas. But being challenged is not a bad thing, in fact it was fun, to be forced to make the therapy even better! And we came up with a really cool strategy, to replace essentially the entire gene in one gene editing step with a normal copy. When we first thought about it, we thought this idea is crazy, but our newest data suggest that we may have a good shot at literally replacing the entire locus with a new sequence. If this approach works, it would be a genetic fix for 100% of DEB patients. We know already that we can make a 2.7kb exchange and that would cover about 30% of all patients, at least in the Stanford registry. That makes us hopeful that we may even be able to replace the whole locus. But this is ongoing research, so the future will tell whether the approach will be reliable enough for clinical development.

Experience with StemFit media and the challenges it solved

AJ: *Could you share how our StemFit media has helped in your research?*

(MW): Luckily, I heard about StemFit quite early in the process, which solved a critical problem early on and ever since I don't think about this problem anymore!

Early on, the issue was quite a critical struggle and key challenge in the first CIRM grant that we had, the early disease team grant as it was called. I vividly remember the annual meetings with our scientific and regulator advisors who (rightly so) pounded on the importance of genetic stability of our cell product. Yet, no matter what chemically defined iPS cell media we tested, invariably we saw karyotypic abnormalities develop, sometimes earlier, sometimes later, but it happened every single time. Back then, the only way we could grow iPSCs cells with a normal karyotype over longer periods of time was on mouse feeder cells with serum! This combination of mouse cell coculture and undefined bovine serum set our advisors on fire because it would be just so hard to perform this in compliance with FDA safety standards. Yea that's where we were...

And then… I forgot how we learned about StemFit... but [once we tested it] in the lab, we quickly realized that this simple solution allowed us to safely expand the cells and the rest is history. We had been so thankful to be provided with the media [by Ajinomoto] since at the time it was not commercially available yet. It was a game changer for us. I remember in our next interaction; the FDA did not comment on the issue and apparently had no concerns. Ever since [switching to StemFit], this issue was no longer a topic of discussion.

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