CELL & GENE THERAPY INSIGHTS

PRECLINICAL & TRANSLATIONAL INSIGHTS

SPOTLIGHT

COMMENTARY

Complexity be damned: the need to better use biology to achieve more impactful cell therapies

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Biological complexity is both a benefit and a bane of cell therapy. An astute partnership with nature is required to achieve a reliable clinical outcome from cell therapy. To accomplish it, we must dig deep enough to acquire knowledge crucial to translation, while maintaining a perspective that will prevent misdirection. Not every bit of information will be of equal importance, yet we can't skip over or miss what could be pivotal. Dealing with the biological complexity surrounding cell therapy may seem like a risky balancing act. However, luck favors the prepared, and there are practices we can employ to reduce translational risk and form a clinically impactful collaboration between science and nature.

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BIOLOGICAL COMPLEXITY IS A FORMIDABLE CHALLENGE, BUT IT IS NOT AN EXCUSE TO IGNORE IT. INSTEAD, WE HAVE TO UNDERSTAND IT AT THE MOST INSIGHTFUL & USEFUL LEVEL

Biology is complex but goal-oriented and, in humans, remarkably determined to fulfill evolved developmental, physiological, and protective objectives. Yet we want to correct genetic abnormalities, persuade nature to do things differently to achieve a better outcome,

and give the immune system an advantage against cancer. To form an effective partnership, we have to understand the objectives of the cell population, the more complex tissue, and the functional organ. They provide vital context to make wiser decisions. If we think we are smarter than mother nature, we'll lose. To be effective, we must appreciate that we are never really the controllers but rather the enablers. Therefore, one of the most powerful ways to work with complex biology is to find a way to foster a process and then, preferably, get out of the way to let innate programs add the details. For example, a cell therapy healing a chronic wound interacts with the patient's tissues at multiple levels that we are only beginning to understand through genomic expression and network analysis [1,2] even though we've observed its functional impact in the clinic for over 20 years in the USA.

The beauty of cell therapy is that we don't have to get deep into the weeds to produce an effective treatment. It doesn't mean we don't need enabling knowledge, which can still be substantial. If we understand the processes and systems at work for us and against us, we are more likely to gain understanding we can use to design biologically savvy therapies, robust manufacturing processes, and achieve more reliable and impactful clinical applications. The more we can work with nature, at all levels, the better chance we have to be able to improve outcomes.

TECHNOLOGY IS ONLY AS GOOD AS THE STRENGTH OF ITS APPLICATION

Genetic engineers may think that cell behavior is not their problem. We know that single genetic mutations can have far-reaching consequences. If we can fix it, then we cure the disease. However, the questions then become: where is the correction of the mutation needed, can we reach it, and at what efficiency? For decades we have known how we might cure Duchenne's muscular dystrophy (DMD). Yet correcting the genetic deficiency with the capability to create a lasting clinical impact is a significant challenge. Despite advances in the clinical application of gene therapy for DMD and knowing what to target, the corrected dystrophin levels achieved are disappointingly low, suggesting that some biological obstacles may still exist. However, cures are achievable when there are enough enabling knowledge and experiences, e.g., in the use of hematopoietic stem cells and the process of hematopoiesis. In my own experience, the development of a therapeutic organotypic skin construct over 20 years ago, was enabled by a preceding decade of epidermal cell research that created a useful scientific foundation. In attempting to cure DMD, is there something we are inadvertently missing about satellite cells and muscle fiber generation that might better enable a DMD gene therapy? The same type of scrutiny is probably overdue for other applications of cell and gene therapy.

The idea that we'll get to a definitive therapy faster by getting into humans earlier without crucial foundational work is wishful thinking and, worse, slows real progress. We have to guard against premature leaps to development that plague many attempted applications of cell therapy. It does not mean we shouldn't be bold and innovative, just smarter and more willing to identify, illuminate, and face the limitations head-on. New science and technology have exciting therapeutic possibilities until reality sets in as we attempt to translate it in a safe and clinically meaningful way. We know the reasons why biological hurdles are often back-burnered: careers, ability to publish, limiting dogma, grants, appeasing venture capitalists, patents on the technology and so on. However, to achieve the best technology can offer and biology will allow, and deliver it to patients in the fastest way, we have to deal with reality early and often. Also, the practices and institutions stymying this must change to allow it and support a fast fail approach.

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WE CAN HAVE TECHNOLOGY PROGRESS WITHOUT A THERAPEUTIC ADVANCE

There are now many ways to induce pluripotency, design a chimeric antigen receptor (CAR), or print cells into a 3D form. The limitations beyond the technological innovation lie in the biology: challenging us to reliably differentiate pluripotent cells into functional cells with high fidelity, direct T cells engineered with CARs to curative solid tumor targets, or clinically translate 3D printing.

IT IS ONE THING TO HIGHLIGHT THE CHALLENGES & SUGGEST THAT WE HAVE TO DO BETTER, BUT HOW CAN WE DO IT?

I offer a few suggestions for how we can achieve more fruitful translation:

Work to an applied standard, which is focused on gaining actionable knowledge

There is a misconception that applied research is simply the application of knowledge gained through academic research. Working to an applied standard is much more; it is a more demanding research approach and use of knowledge. Genetic characterization can now dig deeper and be better understood using network analysis tools. It becomes even more powerful combined with functional measures at different levels of experimentation, from single-cell analysis, the culture of a single population in a dish, organotypic, or organoid culture, to grafts in animals. Each level gives us a different insight into the dynamics of a cell population, its interaction, and its innate genetic program. Attention to processes and tissue objectives provides insight that can lead to practical application. Relying on the academic process, which is driven by the need to publish, can be slow and inadequate. Academic data on tumor biology is particularly problematic. Ideally, to speed translation, industry and academic colleagues should work in concert to create research plans that are complimentary – guided by the right questions, a broader integration of information, and a deeper dive in the right places.

Maintain a proper perspective

While there is a need to dig deep to tackle challenges, we also have to step back from the data to gain perspective on the processes at work. Think of an impressionist series like Monet's Japanese Bridge, which depicts a bridge over a pond – the defining element being the bridge. Focusing too closely on a brushstroke or its color is not very informative. However, when we step back, we now see what the brush strokes and their color are trying to achieve even as the bridge in the series becomes increasingly obscure. The colors impact our ability to see the bridge, but the composition is more informative than a single color. Do elements in the rest of the garden add information and impact to the painting? Yes, but the bridge defines the series and, once we see it, we understand that it is needed to cross a pond. How can we apply these principles to big data?

Use biological priorities to hone therapeutic objectives

Cell and gene therapies to eliminate cancer, correct biochemistry, administer hormones, cure metabolic diseases, support or redirect failures in regeneration, and repair get muddy in search of key elements as our access to big data increases. The biology of our tissues and organs has evolved to be interactive. An organ's differentiated parenchyma, stromal component, and its vasculature each have a role to play. Those roles set biological priorities. The parenchyma that defines the function of the organ is the most important component and, through my experiences,

the most self-contained or self-directed. While a stromal response leading to fibrosis may limit epithelial regeneration, it does not drive the parenchyma. Likewise, while angiogenesis enables regeneration, it does not drive the regenerative response in the parenchyma. That means that if the problem lies within the parenchyma, that is the primary concern. Then, if the lack of persistence is due to inflammation working against us, the inflammatory response is the next priority. Successful engraftment and establishment of functional parenchymal cells enabled by the control of inflammation then work against fibrosis.

Interpret wisely

The challenges of working with complex biology lie in the gray areas, where many things have some effect. We're rarely entirely wrong, but frequently a bit off the mark. It is challenging to stay on the most direct and most effective path. Part of this stems from how we interpret a biological result. An example, and a potentially far-reaching one, is in the interpretation of the meager therapeutic effectiveness of adoptive cell transfer in solid tumors. The microenvironment, T cell biology, and fibrosis, much of it rooted in stages of the wound healing process, have been proposed as reasons for disappointing results. However, first and foremost, we lack good targets for the tumor cells, particularly with CAR-T, which requires surface molecules of reliable expression and biological significance. The microenvironment, myeloid cell composition, and T cell biology do have a role and an impact, they just aren't the first step. Also, it suggests that technological advances of CAR-T processes while needed will only go so far. The primary shortfall, in this case, will not be a technological inadequacy but more a biological one. If it turns out that surface targets are all that biology permits for a certain type of cancer, then, by all means, we should pull out the stops where we can, cognizant of their limitations. However, it shouldn't replace dealing with first things first.

Form a comprehensive knowledge base for savvy product design & strategy

Effective translation will mean administering cells at the right point of their lineage commitment or differentiation to achieve functional significance in the patient. It requires knowledge of their developmental program, reaction to regenerative challenge, and attention to cell lineage and resulting heterogeneity. A reluctance to dig deep where there are inconsistencies, gain proper perspective, and integrate what organogenesis, normal injury, regeneration, and repair (nature) telegraph will continue to stall clinically meaningful advances.

NO WEAKLINGS ALLOWED: ROBUSTNESS IN THE FACE OF NATURE'S ODDS IS KEY TO A CELL THERAPY'S CLINICAL UTILITY

When do we know our efforts are ready for clinical translation? Experiences with the development of wound healing therapies teach us that even the 'same' cellular components, delivered differently, can cause one approach to fail when another succeeds. The use of dermal fibroblasts and keratinocytes to change the course of a venous leg ulcer is an example. To date, there have been many approaches, yet only one has succeeded clinically in this application. Sometimes, the seemingly 'small' things matter. In the bilayered organotypic skin construct Apligraf® (Organogenesis Inc., Canton MA, USA), preclinically, the development of a barrier was pivotal to its ability to engraft and persist on an athymic mouse [3], suggesting it was something to pay attention to. Also, the physical strength of the stratum corneum facilitated the handling of the material in the clinic. Thus, the stratum corneum was likely enabling in ways from physiological to physical. I believe it added a critical level of robustness that contributed to the material's clinical utility in the chronic wound. Yes, it required the skill to create an epidermal cell

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population with sufficient regenerative capacity and protocols to manufacture the construct reliably. However, the differentiation program of the epidermal keratinocyte and how it was used, made it an effective therapy.

BEWARE OF A 'GOOD ENOUGH' MENTALITY; NATURE DOESN'T CARE WHAT IS EASIER OR HARDER FOR US TO ACHIEVE

To redirect or activate a biological course of events in a patient will require all the robustness we can muster in our design.

- The less required of the cells to get to a state that helps the patient, the more reliable and robust the results will be;
- The more directly a therapy connects to the primary element of the problem, the more potent the treatment will be;
- The more 'natural' the design, the more enabling it will be for cell function, survival, and effect.

No matter what your expertise is in 3D printing, you are unlikely to form an organized tissue as well as the right cells can through growth, lineage progression, and interaction, so be sure to enable that with or without a 3D printer.

For cell therapies that require the implantation of a stem cell or progenitor cell, we should look to how processes in the body will enable or thwart their development. However, first and foremost, we need sufficient insight into the lineage and behavior of the cell population, gained through experimentation at several levels (alluded to earlier). The more we can develop a cell population along the path to the desired function as far as is feasible, the more robust the therapy will be, and the more reliable the outcome will be. The development of a pancreatic islet transplant is a good test case for those considerations.

QUESTIONS FOR THE FUTURE MIGHT BE

- Can we leapfrog the limitations that chronic inflammation or fibrosis place on regeneration by engineering a robust regenerative phase through the administration of the right progenitor population at the right time?
- Could we enable more effective impact or engraftment where necessary with anti-inflammatory or anti-fibrotic agents?
- Can we improve the efficiency and permanent integration of genetic modifications through a more robust use of biology?

Let's curb the urge to do premature 'product development', acquire enabling knowledge to an applied standard, maintain a proper perspective, be mindful of biological priorities, and use some brass tacks to nail things down.

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