

INTERVIEW

Unlocking the Potential of CAR-T Therapies for Solid Tumors



TAMARA J LASKOWSKI received her doctorate degree in Human Molecular Genetics and Immunology from University of Texas Health Science Center where her work focused on genome editing of patient-derived stem cells to correct genetic mutations causative of Wiskott-Aldrich Syndrome, a immunodeficiency disorder that results in severe impairments to the immune system. Through her work, she demonstrated the ability to repair the underlying defects and restore normal immune function. Subsequently, Tamara joined Dr. Laurence J.N. Cooper's laboratory at MD Anderson Cancer Center as a fellow. Her work focuses on engineering stem cells with the goal of generating off-the-shelf NK and T-cell immunotherapies for targeting solid tumor malignancies. Dr. Laskowski also shares an interest in technology innovation, and has developed novel multi-plex assays for testing therapies against solid tumors. In 2017, this work led to an invitation to participate in the National Science Foundation Innovation Corps, an exclusive program through which she was trained on strategies for expanding the economic and societal benefits of innovative ideas which have commercialization potential. She was the sole recipient of an award for outstanding performance upon completion of the program. In addition to her research work, Dr. Laskowski also serves as a scientific communications coach and peer editor at MD Anderson, helping to promote effective communication between scientists and the public.

Q What features would an ideal off-the-shelf CAR-T therapy have, and what issues with current therapies would it address?

TJL: There are a few logistical issues with the current cellular immunotherapy approaches that have warranted research into an off-the-shelf strategy. One of the challenges with autologous CAR-T therapies is that the product is generated from the patient's own cells. It

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requires that the patient – who is often heavily pre-treated through chemo and radiation therapies – provides cells in sufficient numbers and of sufficient quality to be genetically engineered in the laboratory.

It is also a very personalized approach and therefore necessitates manufacturing in the lab for each and every patient. The time requirement for the manufacturing process – genetic manipulation, expansion and product validation – can become a significant obstacle to a patient who has fast-progressing disease.

The concept of off-the-shelf comes from the idea of having the drug ready to be used at the time of need, thus potentially overcoming some of these existing logistical challenges. Following the successes with CAR-T cell therapies, a great deal of thought has gone into finding a source of cells for off-the-shelf T-cell therapies, but an inherent challenge with T-cells is that they are able to recognize self from non-self and subsequently illicit graft-versus-host response in the patient.

Q If there is no dose material, how could the immune cells be created for CART or other immunotherapies?

TJL: The field has moved rather quickly into looking at potential cell sources for off-the-shelf therapies. There are several different alternatives under investigation at this time.

For T cell-immunotherapy there have been studies that show donor antigen-specific T-cells, such as Epstein Barr Virus (EBV)-specific, can elicit a response against EBV disease and tumors in sufficient levels to reduce disease burden, and with little-to-no incidence of GvHD.

More recently, with advances in genetic engineering technologies, such as the discovery of CRISPR-Cas9 system for genome modification, the idea of developing CAR T-cells that lack the genes responsible for graft-versus-host response has become feasible. A few groups have reported the development of what has been named “universal CAR-T cells”, which, by lacking the ability to induce GvHD, can presumably be used to treat a majority of patients in an “off-the-shelf” manner.

For NK cell-based immunotherapies there are several sources which have been shown as promising options for deriving NK cells capable of targeting and eliminating cancer cells. Healthy donor umbilical cord blood is a good source of NK cells, and our colleagues here at MD Anderson have seen very promising results with this strategy in pre-clinical and clinical studies

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treating blood cancers. In these studies, NK cells demonstrated significant anti-tumor potential with no evidence of GVHD.

Another source of NK cells is the CD34+ stem/progenitor cell compartment in the blood of healthy donors. These are the precursors that give rise to NK cells, and can be obtained from mobilized peripheral blood, or from umbilical cord blood. Once harvested, the stem cells can be genetically modified in the laboratory, and then exposed to the right conditions to allow the development of NK cells to occur. These NK cells, in turn, will carry the modifications in their genetic material, making them better-equipped to fight cancer. Another benefit is that these cells can be expanded in the laboratory to generate large numbers of modified NK cells, which can then be stored for use when needed.

There is a downside, however, to using healthy-donor-derived NK cells. A single cord blood or mobilized blood sample is not sufficient. Inevitably, multiple donor sources are required, and with that, variability of product may increase.

A third source for NK cell manufacturing is pluripotent stem cells. These are incredibly powerful cells which do not exist in the adult body, but can be derived from adult cells through a process called somatic cell reprogramming. That's my focus and specialty - looking at the power and utility of these stem cells for immunotherapy. Under the right set of conditions, pluripotent stem cells can be induced to generate NK cells. The advantage is that only a single source of genetically identical pluripotent stem cells is required. It can be expanded multiple times, and give rise to millions of NK cells, which in turn can be stored and used whenever needed, as an off-the-shelf drug.

NK cell lines have also been explored in the context of immunotherapy, and have shown promising results in pre-clinical and clinical trials. NK-92 cells are especially interesting. These were harvested from a patient with non-Hodgkin's lymphoma and, though abnormal in their genetic make-up, they can be sufficiently inhibited as to prevent their growth, but still maintain their anti-tumor power. So far, data have shown good safety and efficacy profiles when NK-92 cells were used for treatment of blood cancers. Being a cell line, these can be expanded to the large numbers necessary to create an off-the-shelf therapy that's available to multiple patients as needed.

Q Your work focuses on developing immunotherapies predominantly for solid tumors; how would those need to differ from those currently being devised for blood cancers for example?

TJL: This question is really central to everything we do. Solid tumors present many challenges. One such challenge is accessibility. Unlike

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blood cancers, in which tumor cells are dispersed within specific areas, solid tumours form three-dimensional masses comprised of cells organized in different spatial contexts. Immune cells must have the ability to kill cells in the outer perimeters (thus easier to come in contact with), but also cells that are embedded deeply in the tumor mass. To add even more complexity, cells located in different areas may have different properties, and may respond differently to therapy

Another, very significant challenge is the tumour microenvironment. In a solid tumour, the complex arrangement of cells creates an entire ecosystem which contains structures that interact with the tumour and support its growth. Moreover, this environment is often inhospitable to immune cells, lacking sufficient oxygen, and containing factors that suppress immune function.

Our current *in vitro* technologies do not closely recapitulate these challenges, so, in order to test new cellular immunotherapies, one must devise ways of specifically addressing these problems. We need to learn what the microenvironment looks like, what forces we’re going to be fighting against so that we can equip our immune cells with the necessary tools to counter these or be protected from their impact.

As part of my work, I develop assays that enable us to study how T-cells and NK cells tackle the challenge of encountering a solid tumor mass in an inhospitable environment, and what cellular functions we can enhance to help them overcome these challenges. It is important to consider how the cells will reach the tumor, how they will overcome the pressures of a hostile environment, and how they will ultimately attack and kill the tumor cells.

Q Has it been challenging to find technology and existing assays that allow you to study CAR-T or immunotherapies on solid tumors?

TJL: Yes, that’s certainly one thing I have found in the course of my work: many of the assays we use work really well for liquid tumors, but do not address the inherent challenges associated with solid tumors.

The need for more relevant technologies and methods is what led me to develop assays that can more closely recapitulate specific aspects such as the solid tumor and its microenvironment, tumor metastases, and how immune cells function in these contexts. My goal is to develop methods that allow us to interrogate the function and performance of lymphocytes in a relevant biological setting.

Q How is flow cytometry important to your research, and what are the limitations of classic flow cytometry techniques?

TJL: Flow cytometry is essential to what we do. My background in stem cell biology and immunology has taught me that even within a specific group of cells, such as NK cells, for instance, there are different sub-

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types, with different functions, and different biological properties. The ability to detect these differences and to characterize the biology and function of cells is very important. Flow cytometry is a great technology that allows us to answer many of these questions in a relatively short amount of time.

We encounter most challenges with flow cytometry when we run complex experiments that involve many questions. For example, when I look at multiple cell types in the same analysis, and need to interrogate multiple functions within each of these groups. The analysis panels become significantly large, involving multiple parameters. Moreover, the sample is often times small, requiring that all parameters are therefore analysed simultaneously. That’s when classic flow cytometry becomes challenging and where I think there’s an opportunity for improvements in the technology.

Another limitation is throughput. For some of the assays I have developed, the goal is to assess the function of lymphocyte populations that differ based on the genetic modification they possess. A typical experiment design often includes multiple doses for each therapeutic, triplicates for each condition, and measurements at several time points. Because of the limitations in classical flow cytometry, where analysis of one sample at a time is favored, an experiment such as this requires nearly an entire work day for completion. So, as an attempt to address this issue, I turned to high-throughput flow cytometry technologies, and have been successful with the Intellicyt® iQue Screener PLUS (Sartorius, USA) platform, through which different lymphocyte populations can be rapidly screened in a single-run,

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and multiple screenings can be performed in a timely manner. Using this platform, I have been able to develop multiplex assays designed to analyse multiple functions associated with different cell populations present in a given sample,

all done in only a few hours. Without a high-throughput flow cytometry approach, obtaining this type of information would require performing multiple experiments, and committing several hours, or even days, to be able to complete the work.

Specifically for my work with solid tumors, other types of data analyses are also relevant, especially when coupled with flow cytometry. Real-time imaging and quantitative analysis of cell death, for instance, are especially interesting. Recent platforms, such as the IncuCyte® Live-Cell Analysis System (Sartorius) allow for integration of live-cell imaging and fluorescence-based protein detection. We are now developing assays that can use this technology to report on important biological factors associated with solid tumor targeting.

The take-home message is that flow cytometry is essential to cell therapy work, but it does have limitations and will likely not be sufficient to answer all of our questions. Being cognizant of new technological developments and how those can be incorporated into your workflow is imperative for advancing your research.

Q How does the information gained from the flow cytometry data contribute to the next steps of the research process?

TJL: A well-planned flow cytometric analysis helps us gain insight into the biological properties of our cells. For instance, when screening our lymphocytes, we can assess which populations responded to the presence of tumor cells and activated killing mechanisms. This information will help us decide what populations we should move forward with, and the tests to perform next.

Specifically for my research into stem cell-based immunotherapy platforms, flow cytometry helps me to understand how these cells develop, respond to stimuli, and generate new cell types. This knowledge allows me to delineate the steps necessary to obtain the cells of interest, and gives me insight into how we can best influence biology and reach specific goals.

Q Do you anticipate the increase in screening capabilities and higher throughput will ultimately help reduce the cost of CAR-T immunotherapies?

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TJL: This is certainly knowledge outside of my field, but in my limited view, as a scientist working in this field, I can say that a big part of the research and development process is identifying and optimizing your lead compounds or therapeutics. Although cellular therapies have sparked a great deal of interest, there isn't quite the same precedent set for cellular-based therapies as there is for molecular drugs. But we still go through very similar processes: we identify potential leads, interrogate these leads, and take the most promising ones through several stages of validation. With each new stage, the requirements increase, involving more sophisticated experiments which are inherently more expensive.

I believe that if we are able to more efficiently screen for promising leads, we can identify the ones which truly show potential and thus merit further investigation. This reduces the number of leads that go through the more expensive testing and validation phases, which, in turn, translates into overall fewer resources and less personnel time required. Employing the right assays at each stage certainly plays an important role in this process.

Q How do you see your research evolving over the next 2 to 3 years?

TJL: Two to three years is quite a long time. This is a fast moving industry, which is really exciting. Every time you pick up a journal, you learn of a new discovery that is moving us closer to a cure. Helping to save the lives of people afflicted with cancer is a passion, and though we are making great strides, there is still a lot of work to be done. In addition to studying cellular immunotherapies for solid tumors, I have developed new methodologies that can address some of the unique needs of scientists in this field. I am currently refining these methodologies and platforms to share with the scientific community. My hope is that this knowledge will help move more discoveries from labs into the clinic, where they can impact patients' lives. As for what the future holds, I am keeping an open mind.

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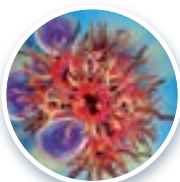
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