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INTERVIEW

The changing landscape of cancer vaccines: developing *in situ* vaccination

Abigail Pinchbeck, Assistant Editor, BioInsights, speaks to **Joshua Brody,** Director, Lymphoma Immunotherapy Program, Tisch Cancer Institute, Mount Sinai

DR JOSHUA BRODY runs the Lymphoma Program at Mount Sinai. He received his BA in Biochemistry from Harvard, MD from SUNY Stony Brook, completed an internal medicine residency at Yale-New Haven Hospital, and postdoctoral fellowships at Stanford in the labs of Ed Engleman, Jonathan Pollack, and Ron Levy. He came to Mount Sinai in 2011 to develop a translational cancer immunotherapy lab and build the Lymphoma Program. The lab has been highly productive, translating novel therapies from mouse models into early phase clinical trials and publishing in high impact journals, such as Nature Medicine, Nature Communications, Journal of Clinical Oncology, Blood, and Cancer Discovery. The lab has been fortunate to receive funding from the NIH, the Damon Runyon Cancer Research Foundation, the Cancer Research Institute, the Lymphoma Research Foundation, the Follicular Lymphoma Foundation and several industry collaborators.

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Cancer vaccines could prove a valuable addition to the immunotherapy repertoire, but they are yet to reach their full potential. To bring these medicines to patients, several developmental hurdles must be overcome. In this interview, Joshua Brody, Director of the Lymphoma Immunotherapy Program at Tisch Cancer Institute discusses his work on *in situ* vaccination, and how personalized cancer vaccines could become the next immunotherapy frontier in 2023, and beyond.

Q What are you working on right now?

JB: We are working on several different things in the lab and in our clinical translational research unit. We are working on ways to improve *in situ* vaccination as an approach to cancer therapy, and most importantly, to understand how it works. The purpose of *in situ* vaccination is to allow tumor antigens to be cross-presented to anti-tumor T cells, and we call those T cells cross-primed. T cell priming can be done in different ways. First, there is direct priming, where T cells respond to the antigen found on the tumors. However, rather than energizing the T cell, it puts it to sleep. By contrast, cross-priming is when dendritic cells take up those antigens and present them in a more immune stimulatory context. Over the past 10 years, models in the lab which use dendritic cell-deficient mice, most famously Batf3 and WDFY4 knockout mice, have suffered from failed immunotherapies suggesting the importance of cross-presentation of dendritic cells. We are trying to discover what the transcriptomic signature of a cross-primed T cell is and to characterize it in simpler ways.

Q The therapeutic vaccine approach your group pioneered – your *in situ* vaccine – is currently in Phase 1/2 clinical trials. What are the most significant results so far, and what's next for this work?

JB: Our team is trying to understand how *in situ* vaccination works so that we can try to improve it and as we do not have pharmacodynamic readouts for many immune therapies, we are trying to invent a metric to measure whether we are effectively cross-priming T cells. In the lab, we are trying to understand the vaccine by inventing immunodynamic metrics like a cross-primed signature.

There are three main components of the vaccine: something to mobilize, something to load with tumor antigens, and something to activate the dendritic cells. Currently, we are loading and mobilizing dendritic cells with FLIT3-ligand and are loading them with tumor antigens using radiation therapy. We are activating them using toll-like receptor (TLR) agonists, such as poly-ICLC. We are continually finding ways to improve our processes. For example, instead of using synthetic pathogen receptor recognition (PRR) agonists such as poly-ICLC, we have taken vaccine pathogens such as measles, mumps, and rubella which can activate immune cells as well as natural pathogen receptor recognition (NAPRR) agonists. We are characterizing all the NAPRR agonists in all the standard FDA-approved pathogen vaccines and discovered that some of them are even more potent than synthetic PRR agonists. In theory, we would replace the TLR agonist from the *in situ* vaccine and in parallel, we are finding ways to replace both the dendritic cell activator and the dendritic cell antigen loader, the radiation, and the poly-ICLC. For this, we have used an oncolytic virus, however, this is not yet fully approved by the FDA. Only T-VEC, an intra-tumoral oncolytic virus, has been approved for melanoma so far. We have studied an oncolytic virus called Newcastle disease virus (NDV) and observed that it was able to both load dendritic cells with tumor antigens by killing some tumor cells, and then activate the dendritic cells.

Overall, our main focus is to understand how the *in situ* vaccine works and how to improve on it further. We are studying patients both with lymphoma and breast cancer and studying it in combination with the now common standard immune therapy of anti-PD1

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antibody. We have received positive results and patients in the advanced stages of cancer have had a great response to the vaccine.

Q Your recent paper described cancer vaccines as the next immunotherapy frontier. What do you predict for the future of cancer vaccines, and where do the challenges in this field remain?

JB: One of the greatest challenges is not a scientific challenge at all – we have a psychological challenge to overcome after many years of insufficient successes in cancer vaccine trials. We have one FDA-approved cancer vaccine called Provenge, which is a prostate cancer vaccine. That vaccine, now approved almost a decade ago, had some benefits including prolonged survival in patients with prostate cancer. Despite that, it was still a failure overall because the benefits were quite small. Furthermore, it was difficult to track the benefits it had for the patients, although the results did show that patients who received it lived longer than those who did not. In parallel with its approval, the release of a number of new advanced-stage prostate cancer agents created a competitive landscape for that one approved vaccine.

There have been other successful cancer vaccines, many of which are not well remembered. One cancer vaccine for melanoma, Gp100, was overall not a great vaccine, but it did show some promise. A clinical trial gave the vaccine alongside a high-dose therapy of interleukin-2 (IL-2). In that context, the vaccine provided a marked survival advantage. However, due to the severity of high-dose IL-2 therapy, not many people want to prescribe it, as it often leaves patients in need of intensive care. Gp100 was a conceptual success, but a practical failure.

We would not have had the COVID vaccines had it not been for BioNTech and Moderna already having worked on these cancer vaccines for several years prior to the pandemic. It was only because they were making cancer vaccines that they were able to quickly pivot and make the vaccines that I got in my shoulder, and probably you did as well. There is an exciting history of promise now, because of the success of COVID vaccines, which will bolster these companies to do more cancer vaccine trials.

The advancements in cancer vaccinology have moved from using common antigens towards a more personalized approach. Past vaccines would use common antigens despite the heterogeneity among individuals with cancer, which has been a big part of the insufficient success in the past. Moving forward involves integrating a more personalized approach. This is broken down into personalized, pre-defined cancer vaccines, and personalized, anonymous vaccines. The most famous among these personalized, pre-defined vaccines are neo-epitope vaccines. They pose challenges of required biopsies from each individual patient, which is a time-consuming process. Alternatively, anonymous vaccines involve injecting immunostimulants directly into a tumor. These frequently rely on intra-tumoral therapies, injecting immune stimulants into one of a person's many tumors.

"Moving forward involves integrating a more personalized approach. This is broken down into personalized, pre-defined cancer vaccines, and personalized, anonymous vaccines."

In anonymous vaccines, the names of the antigens we are vaccinating against are unknown. Pre-defined antigen vaccines face the obstacle of everyone's tumors being different. For each patient requiring a personalized vaccine, we need to take a biopsy and sequence the exome and transcriptome, and complete *in silico* computer work to find the desirable antigens with which to produce the vaccines. That process can take many weeks or even months. This is a big hurdle, as patients in these settings cannot wait that long.

We have been lucky to have some great success so far with personalized cancer vaccines. Some other groups are also having successes, but we have seen some of the more drastic responses in lymphoma and breast cancers so far. I think the future of cancer vaccinology is moving away from the history of using common, shared antigens, like Gp100 and Provenge, and moving towards personalized vaccines.

Q Can you tell us about your work in bystander T cell killing, and how these findings could be translated?

JB: The most common immunotherapy approaches use anti-PD1 antibodies and anti-PD1 therapies are used in a majority of cancer types. In our vaccinated patients, we can observe a common shortcoming of most cancer immunotherapies, which is antigen escape. This is caused by major histocompatibility complex (MHC) class I downregulation and causes failure in a lot of anti-PD1 therapy. Although everyone has different antigens in their tumors, they are all presented on MHC I to be seen by T cells. A common approach for treatment has been MHC I downregulation.

It is easier to see antigen escape with CAR-T cell therapy or other types of T cell therapy. If you make a CAR-T cell against CD19, if and when patients relapse, they relapse with CD19-negative tumors as these would have lost CD19, and we will select for those antigen-escaping cells. An approach to try and solve this is to attack CD20 or CD22. However, this approach has problems to overcome as sometimes a tumor may lose all these molecules simultaneously. They de-differentiate, losing CD19, CD20 and CD22 completely. As we move beyond B-cell lymphomas and leukemias, we do not have a plan B, because we do not have a second target.

Our proposed solution is to utilize bystander killing whereby once activated, T cells kill the cell immediately next to the target cell. Bystander T cell killing does already happen but to a very limited degree – we want to increase this. We believe that bystander killing is inherently geographically localized or limited, although the geographical limitations of this phenomenon are unclear. We are currently characterizing this in the lab to find out more. We hypothesize that if you were in a tumor of ten billion cells, if there is one antigen-negative cell, it is statistically most likely surrounded by many thousands of antigen-positive cells. Therefore, instead of exploiting the antigens, we can exploit geography. In other words, when fighting cancer, bad guys are usually in bad neighborhoods.

We published a paper last year in *Cancer Discovery* highlighting that the mechanism of bystander killing appeared to be very different from on-target killing. It appears to be Fas ligand-mediated, meaning the Fas ligand on the T cell touches Fas on the tumor cell and causes it to die. Most T cell killing is not accomplished that way but instead by perforin/granzyme, which is a bit different.

We can take advantage of this Fas signaling, as signaling cells have built-in regulators which can be deactivated. Some examples of Fas signaling are protein inhibitors of apoptosis

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(IAP), such as B cell lymphoma 2 (Bcl-2). We could inhibit some Bcl-2 family member proteins, and overall, inhibitors could improve Fas-ligand signaling. In our paper, we stated the use of different types of inhibitors to potentiate fast signaling and increase bystander killing. In the lab, we are working on how this prevents antigen escape. Many of those potentiators of Fas signaling are already FDA-approved medicines, such as Panobinostat, a histone deacetylase inhibitor. Recently, we put these FDA-approved medicines in a combination study alongside immunotherapies. The results have proved that preventing antigen escape is critical and can be prevented by increasing bystander killing.

Q As we look to 2023 and beyond, what are your key goals in your research over the next few years?

JB: Our goals are to ensure our patients with advanced stage cancers live longer by virtue of teaching their immune systems to hate their cancer as much as we do. We are trying to continuously do this in our patients during these early-phase clinical trials, and will hopefully move those into more advanced-stage clinical trials as we are lucky to have success. Moreover, we are working in the lab to understand how these immunotherapies like *in situ* vaccination work, and thereby how we can improve them.

AFFILIATION

Joshua Brody, MD
Director, Lymphoma Immunotherapy Program,
Tisch Cancer Institute, Mount Sinai

AUTHORSHIP & CONFLICT OF INTEREST

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INTERVIEW

Strategies to enhance immune cell activation & cancer cell killing

Abigail Pinchbeck, Assistant Editor, BioInsights, speaks to **Karine Breckpot,** Director, Laboratory for Molecular and Cellular Therapy (LMCT), Vrije Universiteit Brussel

KARINE BRECKPOT obtained a MSc in Biomedical Sciences in 1998 and a PhD in Medical Sciences in 2004, all at the Vrije Universiteit Brussel (VUB, Belgium). Following a postdoctoral stay at the Division of Infection and Immunity at the University College London (United Kingdom), she became a group leader and Assistant Professor in 2010 at the Laboratory for Molecular and Cellular Therapy (LMCT), VUB. She became a Tenured Professor in 2014 and Full Professor in 2019 at which point she became director of the research group LMCT. Her research is focused on cancer immunology and immunotherapy, designing novel immunotherapies focused on T cell activation using technologies such as mRNA, lentiviral vectors and antibodies, including camelid single domain antibodies. She was laureate of the Fund for Biotechnology award in 2005, received the Dr Karel-Lodewijk Verleysen award of the Belgian Royal Academy of Medicine in 2011, the Melanoma Research Alliance young investigator award in 2017 and the BCLAS award in honor of Jean-René Maisin in 2019. She is (co)promotor of 15 defended and 16 ongoing doctoral theses and author on more than 135 Q1 publications.

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Technologies such as mRNA, lentiviral vectors, and nanobodies are vital tools in the immuno-oncology arsenal. In this article, Karine Breckpot, the director of the Laboratory for Molecular and Cellular Therapy (LMCT), explains how she applies the latest technology to her work in engineering dendritic cells for immune activation and T cells for cancer cell rejection – and her predictions for 2023 and beyond.

Q What are you working on right now?

KB: I am looking into two types of immune cells and how we can exploit them to recognize and kill cancer cells: dendritic cells and T cells, which work hand in hand. The work that we have been doing is mostly in the context of melanoma and aggressive skin cancer. What we have learned in terms of cancer immunotherapy strategies used for melanoma can also be translated to other solid tumor types, such as lung and colon cancer.

Dendritic cells are cells that search our body for foreign invaders. If they encounter a virus, bacterium, or damaged cell, they can sense whether it is a potential threat to our system. If it poses a threat, the dendritic cells will travel to the secondary immune organ which will interact with T cells and present what they have encountered to them using antigens. If the T cell specifically binds to those proteins, a reaction can be triggered. Cancer cells express proteins that are not present on the healthy tissues in which they arise. These proteins can be presented by dendritic cells to activate T cells and are expressed on the surface of the cancer cell. Once the dendritic cell has educated the T cell, the T cell can then search for cells that express and present that same protein and, in that way, can recognize and kill them. Figuring out how dendritic cells can be employed to activate T cells is one part of our work.

The other part we are working on is the T cells themselves. We know what dendritic cells can do with an antigen, but we need to know which stimuli are required by dendritic cells to activate T cells, and how they support T cells once they are activated. When T cells are activated they will kill virus-infected cells or cancer cells, but at a certain point there is negative feedback. In cancer, we have identified a number of proteins that are expressed on cancer cells and in the environment that prematurely give negative feedback, so that T cells mistakenly receive information that the threat is dealt with whilst cancer cells are still present. To avoid T cells becoming prematurely paralyzed or dysfunctional, we have looked into the signals that are provided to T cells to investigate how we can block them, in order to ensure the T cells continue to be active.

Q What advantages do dendritic cell-based immunotherapeutic approaches offer for cancer treatment?

KB: Dendritic cell vaccination is a cancer immunotherapy designed to activate the immune system and ensure that it can specifically recognize and kill cancer cells. The big advantage is that once the immune system has been warned, it forms downstream

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memory. This memory means that if the patient is cured, but cancer cells arise again and have the same identification markers, their immune system is primed to immediately react and respond to the cancer cells. This is advantageous compared to classical approaches, where you deal with disease initially, but if the disease comes back you have to go through surgery, chemotherapy, or radiotherapy again. In our case, patients should never relapse from the same cancer. This is a benefit of cancer immunotherapies as a whole.

"To determine the role of certain molecules, such as CD83 and CD70, we employed immature dendritic cells and engineered them to express the marker that we are interested in, so that only that marker is present on the cell."

Q What tools and approaches are you using to better understand dendritic cell biology, and the signals that lead to priming of effector T cells?

KB: The idea in the beginning was that a dendritic cell has two life stages: an immature life stage and a mature life stage. We knew from the literature that at the immature stage, the cell is perfectly equipped to take on different antigens from viruses and bacteria, but is not equipped to activate T cells. Once it matures, it changes what it expresses, both on the surface as well as the molecules it secretes, i.e. cytokines and chemokines.

To determine the role of certain molecules, such as CD83 and CD70, we employed immature dendritic cells and engineered them to express the marker that we are interested in, so that only that marker is present on the cell. To engineer cells that express a particular protein, we use two technologies. The first is lentiviral vectors to deliver cargo and integrate it into the genome of the dendritic cell, to enable it to express the protein encoded in the viral vector. The second is using mRNA to deliver cargo to dendritic cells. In this instance, we used an *ex vivo* approach in which the dendritic cells are in a culture dish and using electroporation, the mRNA passively migrates into the cell. The mRNA can then be translated into the proteins that you want to evaluate and express.

These two strategies have been employed since the early days, though we have moved towards other methods such as RNA interference to delete certain proteins rather than over express them. Recently, other new technologies have emerged to allow the deletion of proteins from cells such as CRISPR/Cas9. Moreover, we are employing single-domain antibodies or nanobodies, which are antibody fragments that originate in camelids to bind to proteins and activate or inhibit them, allowing for the evaluation of the function of the protein. In the case of dendritic cells, they allow the study of T cell interactions. This can also be utilized in therapeutic settings, as nanobodies can be used as tracers to identify proteins present in tumor cells and therefore can be used as an entry point for cell therapy. Nanobodies

have come into play as a useful tool for different approaches that can help improve cancer immunotherapy.

Q How do lentiviral vectors and mRNA compare as approaches for cell modification?

KB: During my early work in the lab, I was not familiar with the best strategies for modifying dendritic cells. At that time, lentiviral vectors and mRNA received lots of attention and we studied both approaches. Lentiviral vectors allow stable modification of cells and as cells divide, the daughter cells will also contain the cargo that was incorporated by the lentiviral vector.

In terms of dendritic cells, division is not an issue because we start with monocytes as a source to generate our dendritic cells in the laboratory and these do not proliferate. Therefore, one monocyte can give rise to one dendritic cell, and that one immature dendritic cell can give rise to one mature dendritic cell. Whether you use lentiviral vectors or mRNA for dendritic cells you achieve the same thing. However, we continued to focus mostly on mRNA based on its practicality as it does not require the specialist facilities that lentiviral vectors do. Furthermore, stable integrations also have a theoretical risk of generating a cancer cell because if the insertion into your host DNA is close to an oncogene or a tumor suppressor gene, it can have negative effects. mRNA can also be used as a template for protein engineering and can produce any protein of interest. Overall, mRNA has become our molecule of choice to engineer these dendritic cells because of the many advantages it has in terms of safety, the working environment required, and its ease of delivery.

Q Can you expand on your work evaluating strategies for direct immunomodulation of the tumor environment?

KB: When T cells enter the tumor environment, they encounter immune checkpoints which interfere with their activity. We have investigated how we can interfere with these immune checkpoints and ensure that the negative signals received by the T cells can be blocked. Most companies work with monoclonal antibodies. However, in the case of large tumors, they can only penetrate to a certain level and never reach the core of the tumor. This can be problematic in the sense that you become dependent on the cells that are at the periphery of the tumor to break down the tumor before the next round of therapy – a monoclonal antibody – can reach it. Research focusing on cancer imaging has shown that as nanobodies are much smaller, they can reach the center of these tumors. We began discussions with our colleagues in the *in vivo* cellular and molecular imaging laboratory about whether nanobodies could be used in the context of immune checkpoints as a therapeutic.

We decided to start up a collaborative project, and as a first test case we used a programmed death ligand, PD-L1, which deletes and paralyzes T cells. We hypothesized that if

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you can make these nanobodies go deep into the tumor, you can make them bind PD–L1 and block this interaction. We showed that these nanobodies have a dual potential; immune checkpoints are one aspect. We have generated nanobodies against PD–L1, and we have also published papers describing using nanobodies against another immune checkpoint, lymphocyte-activation gene 3 (LAG-3). We have shown that you can use these in combination.

We have also looked at what is present in the tumor environment, because these immune checkpoints can be expressed on tumor cells, and also on other cells like macrophages, monocytes, and neutrophils that easily infiltrate the tumor. We know that once these cells arrive in the tumor, instead of acting against the tumor, they are corrupted to work in favor of it, such as by producing growth factors to produce blood vessels. Another example of the activity of these myeloid cells is interacting with T cells and interrupting their activity. We tried to understand why once a myeloid cell arrives in a tumor, it becomes such a suppressive, T cell-countering cell and how we can manipulate that. Together with colleagues of the cellular and molecular immunology laboratory, we found that nanobodies are an excellent tool to manipulate myeloid cells and to push them towards a T cell-promoting cell.

Q What immuno-oncology tools and technologies would you like to see further develop to aid your work in the field?

KB: For me, it would be in bioinformatics. Bioinformatics in the context of dendritic cell vaccination will become tremendously important because much of the work that has been done with vaccination has a basis in classical cancer-associated antigens, which are not the best choice. First, they are not unique to the tumor so there is a risk of collateral damage, and second, T cells only bind to cancer-associated antigens with a low affinity.

Bioinformatics can help because cancer cells accumulate errors in their DNA causing it to continuously proliferate. The mutations, insertions, and deletions that occur in DNA give rise to new proteins, known as neoantigens, that your immune system can recognize, and as these proteins have never been presented to T cells, they react against them and have a high affinity for binding to cancer cells. These neoantigens are unique for every patient, meaning bioinformatics and good algorithms are needed to identify them. One of the major achievements has been setting up these algorithms. There is a strategy through sequencing where you can compare the genome of cancer cells to the genome of healthy cells, for instance white blood cells, and based on the differences, you can identify neoantigens, mutations, insertions, and deletions.

For vaccination, identification of neoantigens and predicting whether T cells will have an immune reaction against them is going to make a tremendous difference. This is one of the latest projects that we have been working on, which has led to the formation of a spin-off company, Persomed. Together with three companies, myNEO, Antleron, and Quality by Design, we are looking into new antigens, how we can identify them, and how we can produce them in an efficient way. Then, we can deliver the mRNA encoding for these new antigens to dendritic cells. Together with the university hospital of Antwerp, we are preparing

"To summarize, we are working on a combination of personalized therapies on the dendritic cell level and the immune checkpoint level, with vaccination and imaging to detect which immune checkpoints are present in the tumor environment and blocking those, so that activated T cells have the full potential to exert their function."

for a Phase 1 clinical trial to evaluate new antigen-loaded dendritic cells as a vaccine for colorectal cancer.

Another strength of bioinformatics involves identifying gene signatures in the tumor microenvironment. Once sequencing within the tumor is complete, and algorithms have identified the gene signatures present, we can identify which gene signatures are predictive of the expected patient response. This is beneficial for predicting the potential outcomes of immunotherapy, and will also be more cost effective, which is tremendously important.

Turning again to nanobodies, these can be used for imaging, and using gene signatures, we could find out which proteins can dictate a patient's reaction. A strength of these nanobodies is that you can generate them against any protein and they can image in a non-invasive way, in the same way that PET scans are being used for [^{18}F]fluorodeoxyglucose. Overall, bioinformatics and nanobodies can be used as strategies to stratify which patients are eligible for certain therapies, and personalized therapies are beneficial as we can tailor therapy to the needs of each patient.

Q As we move into 2023, how do you predict that your work and the field will develop over the next year and beyond?

KB: I strongly believe in personalized therapy. Our work will continue to focus on strategies to identify the main markers in a tumor. For vaccination, we will focus on identifying which neoantigens are present in a tumor, and develop dendritic cell vaccines so that new antigens are inserted in the dendritic cells and will be stimulated against T cells. One hypothesis is that even a relatively low number of neoantigens, once they are immunogenic, can stimulate T cells, which can then find the cancer cells and express the neoantigens. These tumor cells are killed, setting off a cascade where antigens are released and can again be taken up by antigen-presenting cells that are present in the patient. These can then activate new T cells.

We also want to exploit nanobodies to evaluate immune checkpoint expression. Cancer vaccination can jumpstart an immune response, but you must ensure that once the T cells have been activated, they remain functional in the tumor environment, and immune checkpoints act as accelerators to keep the immune response active.

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To summarize, we are working on a combination of personalized therapies on the dendritic cell level and the immune checkpoint level, with vaccination and imaging to detect which immune checkpoints are present in the tumor environment and blocking those, so that activated T cells have the full potential to exert their function.

AFFILIATION

Karine Breckpot
Director, Laboratory for Molecular and Cellular Therapy (LMCT),
Vrije Universiteit Brussel

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INTERVIEW

Shifting paradigms & advancing technology to find I–O biomarkers

Abigail Pinchbeck, Assistant Editor, BioInsights, speaks to **Mustafa Khasraw,** Deputy Director of the Center for Cancer Immunotherapy, Duke Cancer Institute

MUSTAFA KHASRAW MD, is a professor of medicine and neuro-oncology and Deputy Director of the Center for Cancer Immunotherapy at Duke University, USA. He is leading several clinical and translational programs with significant laboratory collaborations and is the principal investigator on first-in-human Phase I immunotherapy clinical trials in solid tumors with a special interest in CNS cancers.

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The discovery of relevant biomarkers in immuno-oncology is crucial to match the right patients to the right treatments. In this interview, Mustafa Khasraw, Deputy Director of the Center for Cancer Immunotherapy at the Duke Cancer Institute, highlights the current challenges in this space – and potential approaches to overcome them.

Q What are you working on right now?

MK: I am a medical oncologist with a focus on brain tumors, clinical trials but also across the spectrum of preclinical, drug development, and translation into early-phase clinical trials and biomarker development. This includes going back from the bedside to the lab to test tissue and other specimens from humans. Our team and I undertake a very broad spectrum of research.

Q Why is the need for robust, reliable biomarkers in the personalized I–O space so urgent?

MK: Even in diseases where I–O is effective, most patients still do not reap the advantages of it. If you look at the survival counts of immunotherapy trials, it exemplifies how patients do not benefit from them. If you have reliable, predictive biomarkers, then you could identify those who will benefit from a specific treatment and those who will not. An advantage of this is that it can save time in the long term as alternative treatments can be used.

In the immunotherapy space, when the first breakthroughs were made in diseases like melanoma, people began conducting immunotherapy trials in a variety of diseases. However, biology is complex, and something that works in one disease is not necessarily going to work in another. Initially, the same immune checkpoint inhibitors that were successful in immunologically 'hot' tumors that have more immune cells in the tumor, were tested in other 'cold' cancers that have very few or no immune cell infiltration. We know that some diseases, such as glioblastoma and pancreatic cancer, are immunologically 'cold', which means that the same approach that works in other diseases is unlikely to work.

Different end goals and mechanisms of action are required to overcome immunosuppression, whether this is through generating antigens, making the tumor more immunogenic through cellular therapies, using cancer vaccines, or giving therapies in combination with radiation. There are a variety of scientific discoveries, but translating them into meaningful clinical benefits is challenging and is a big barrier in the field.

Q What for you represents the cutting edge in terms of tools and technologies being applied in clinical trials?

MK: I am personally interested in changing the way that we perform clinical trials as there is so much heterogeneity not only across patient populations, but also in the biology of the tumor. This includes heterogeneity of both the tumor histologically and in the immune response in patients over time. Integrating biologic assessments

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"...we are coming up with new tools to acquire biospecimens at multiple timepoints to learn how the tumor responds to treatment and if there is a negative response, to understand why."

into clinical development is key, so that we do not use the same paradigm of testing a specific treatment in a hundred or a thousand patients to see if it works. We must be more specific and individualize the treatment to each patient. That involves serial collections of biospecimens including tumors, blood, cerebrospinal fluid (CSF), and any tools such as novel imaging to help us better understand the biology, and why some patients benefit from particular treatments and others do not.

Biologic assays and sequencing technologies, whether it is at the exome, genome, or single-cell level are dropping significantly in price. These are technologies that were unaffordable 10 years ago but can now be covered within the budget of a typical government grant, which is quite exciting. In addition, liquid biopsy technologies are making significant progress. It is great to see that they are being increasingly incorporated into the design of clinical trials to help us understand how a tumor behaves at multiple timepoints.

Q Turning to your work on immunotherapies for central nervous system (CNS) malignancies, what have been your most significant findings so far?

MK: Historically, the brain is considered an immune-privileged compartment and is known to have a small population of immune cells. However, that has been challenged as there are many cases of patients who have developed autoimmune diseases in the brain, for example multiple sclerosis, which exemplifies that the immune system is active within the brain. Moreover, the same strategies used in other diseases may not be successful in this organ. A significant degree of immunosuppression is observed in the brain, and this has evolved to protect it from external danger. Simultaneously, the interaction between the CNS compartment and the extracranial space, including for example the deep cervical lymph nodes, the bone marrow and other bodily compartments can be key in driving and stimulating the immune response in the brain.

Another challenge involves drug delivery due to the Blood–brain barrier (BBB). There are new insights on how we can overcome those barriers, and we are working on strategies, either through vaccination or cellular therapies that can aid in this. This may involve administering immunotherapies directly into the tumor or the CNS space, which can minimize the challenge of the BBB. Additionally, we are coming up with new tools to acquire biospecimens at multiple timepoints to learn how the tumor responds to treatment and if there is a negative response, to understand why.

Q Could you tell us more about the tools you are developing to better understand CNS malignancy?

MK: The current paradigm – patients being treated and then, if they live longer, or if their MRI looks better, the treatment is declared successful – has major limitations. One limitation is that in the absence of a control, you do not know if improvements in an MRI are meaningful. Sometimes, there are changes on an MRI that do not represent disease activity but may reflect radiation necrosis or other changes that have led to contrast uptake. These changes may not 100% represent an actively dividing tumor. Some patients may, if they do not have a tumor but have necrosis taking up contrast, experience necrotic mass that reduces in size over time. If that patient is in a clinical trial, and you do not have access to the tissue, you would declare that treatment promising, even though the mass or abnormality was destined to shrink in size regardless of the treatment you have administered.

What we are advocating for is to give treatment and then follow that by resection or biopsy of the tumor to examine biologic measurements that can reflect the pharmacodynamic effect. We are also looking at strategies to collect CSF serially after we give the treatment, because that can give you a window into the immune environment, cellular characteristics, and other aspects to help you understand what is happening.

The other paradigm shift we are working on is to test more treatments during the course of disease, because as cancer evolves over time, it becomes more heterogenous and chaotic, and less likely to respond to any treatment. We are part of a large international consortium called the Glioma Longitudinal AnalySiS (GLASS) consortium, and we have collected large numbers of matched pair tumor samples from primary tumors and from the timepoint when the tumor relapses. It has been observed that tumors are more chaotic during relapse, which reduces the likelihood of treatments being successful. This means that trials in recurrent disease are less likely to lead to a positive outcome and that we need to more drug development to an earlier stage during the course of the disease.

The challenge of introducing any experimental therapy to patients is that results can be hampered by the need to combine it with standard chemotherapy. While traditional chemotherapy is proven to help patients, it is not a curative treatment. There are some patients, for example those with unmethylated MGMT, a DNA repair enzyme, who are known not to benefit from chemotherapy, therefore it is justifiable to not to give them the standard treatment and offer them a promising experimental therapy as an alternative. Although there are caveats and we must be careful when omitting standard of care, in a disease that has not seen a shift in survival curves for decades, I think it is worth considering.

Q As we move into 2023, what are your key goals for your work over the next year and beyond?

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MK: We have a number of new clinical trials that we are hoping to come into fruition over the next 12 months, and some of them involve the new ways of doing things that I have spoken about. There are many steps we have to go through, including approvals, reviews and securing funding. It is certainly happening steadily, and I am looking forward to the next steps.

AFFILIATION

Mustafa Khasraw
Deputy Director of the Center for Cancer Immunotherapy,
Duke Cancer Institute

AUTHORSHIP & CONFLICT OF INTEREST

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