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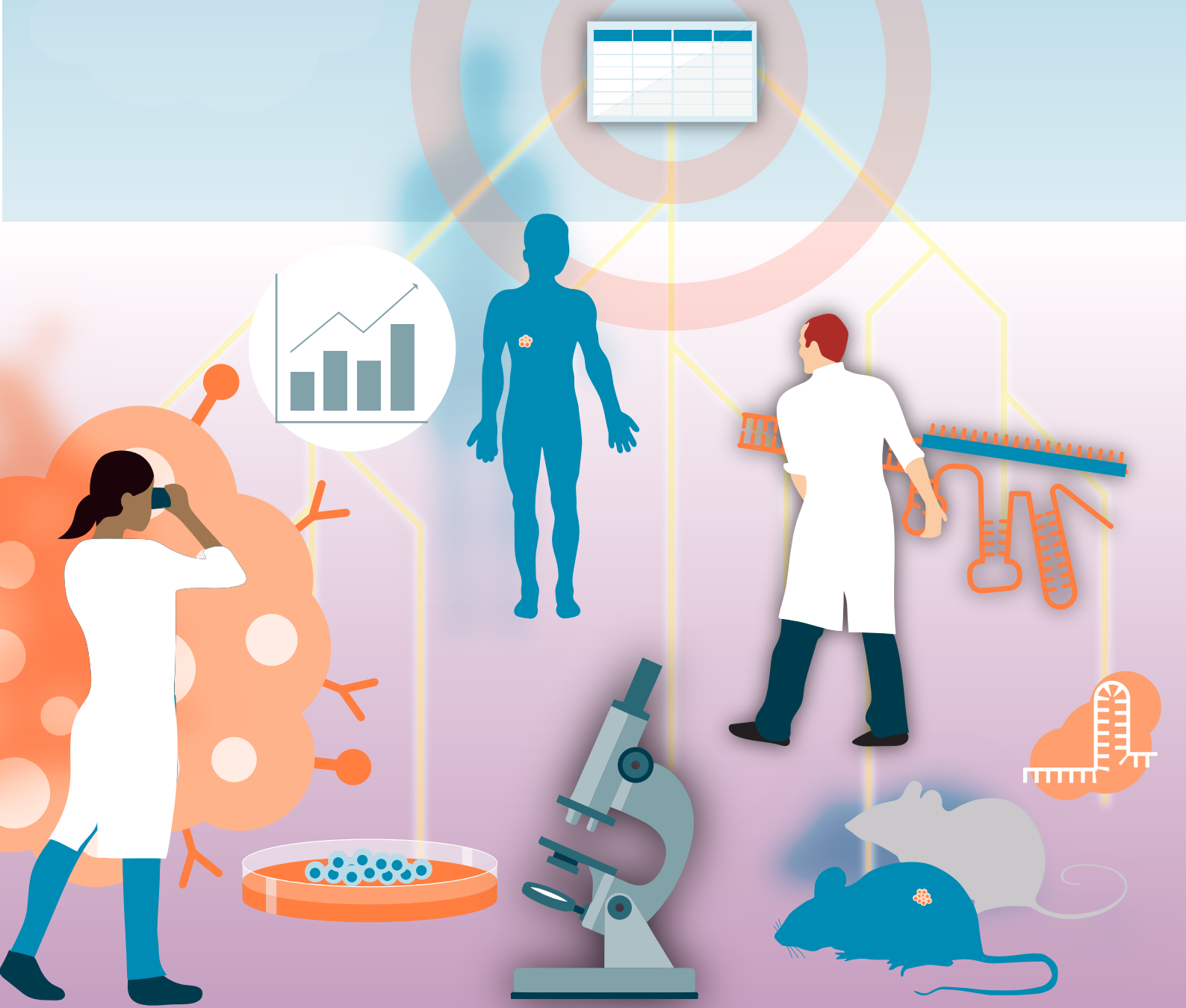
IMMUNO-ONCOLOGY INSIGHTS

SPOTLIGHT ON:

Nonclinical tools update: emerging technologies

Guest Editor: Pelin Candarlioglu, Chair of Industry Advisory Board at European Organ On Chip Society (EUROoCS)



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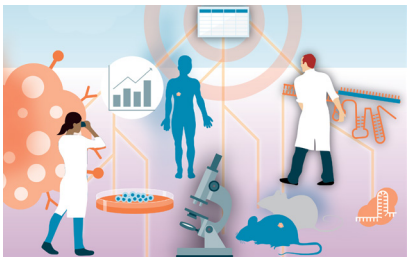

Nonclinical tools update: emerging technologies

IMMUNO-ONCOLOGY INSIGHTS

NONCLINICAL TOOLS UPDATE:
EMERGING TECHNOLOGIES

SPOTLIGHT

INTERVIEW

Looking for the bigger picture: current challenges & progress in preclinical tools for I–O

Róisin McGuigan, Editor, *Immuno-Oncology Insights*, speaks to **Pelin Candarlioglu,** Chair of Industry Advisory Board at European Organ On Chip Society (EUROoCS)

PELIN CANDARLIOGLU, PhD, is a tissue engineer by training, having received her PhD in the field from Imperial College London. She moved into oncology during her PostDoc position, working on circulating tumor cells at UCL. Her introduction to organ-on-chip (OoC) was during her leading a Cell Biology/Microfluidics lab in Cambridge at Enplas Corporation where she was developing a microfluidic chip system specifically designed for immuno-oncology applications. Currently, as part of Complex *in vitro* models (CIVM) group, she is leading a small team utilizing her expertise in microfluidics, tissue engineering and especially OoC to lead multiple initiatives both externally and internally to expand the complex *in vitro* model portfolio of GSK for immuno-oncology. Pelin is very active on the 3Rs initiative in GSK and supporting the reduce and replacement aspects. She also represents GSK globally in relevant organizations such as NA3RsC MPS Initiative, IQ-MPS, NC3R, OOACT in UK and as chair of industry advisory board at EUROoCS.

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Q How did you get involved in the cancer modelling space?

PC: My background is in tissue engineering, and previously my focus was in bone tissue engineering, which gave me a good understanding of the fundamental concepts you need to create really translational tissue outside of the body. I moved into oncology about a decade ago, and then began working on circulating tumor cells and eventually in immuno–oncology (I–O), particularly cell therapies. I have also worked in multiple different disease areas and organ types outside of oncology, but my main focus has been on developing complex *in vitro* models for cell therapy applications.

Q What models or tools do you currently work with most?

PC: I have been working specifically with various types of tumor-on-chip, as they lend themselves really well to cell therapy applications. All the key components are there: vascularization, recirculation (to a certain degree), and the ability to deliver the therapeutic cells directly through the vascular network, which is much more physiologically relevant.

Because of this I have been focusing on trying, testing, evaluating, and eventually adopting multiple different tumor-on-chip platforms, all in the area of cell therapy.

Q What are the unique considerations when developing models for cell therapy versus other immunotherapeutic approaches?

PC: When you are working with other modalities you may have the luxury of taking an almost pinhole-level focus, and looking entirely at just one aspect of the tumor. For example, when assessing efficacy for a small molecule, you might be very specifically looking at the metabolic aspects of the tumor without really thinking about other components such as vascularization. With cell therapies, you have to consider a wider context – it is not just about the tumor itself but the whole structure that it is in, because of the nature of the modality you are using.

For cell therapies the presence of a vascular network – and how well that vascular network is connected with the tumor – is very important. You have to consider whether you are getting the right shear stress in that vascular network, because that will affect your cell therapy efficacy. Chimeric antigen receptor T cells (CAR-T)s, for example, would be moving inside these vascular networks, and if you don't get it right, they might not move and infiltrate.

On top of that, you may need to get other aspects of the tumor microenvironment (TME) right. For example, if the type of the tumor you are trying to target with your CAR-T therapy is known to be hypoxic, you need to try to recreate that hypoxia. That will bring certain

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"The patient-specific TME is both extremely difficult, and extremely important to capture. There is also the histoarchitecture of the tumor microenvironment, and also the metabolic capacity."

limitations, and as such you have to have a certain size of tumor mass present in your *in vitro* model, otherwise you will not be able to capture that hypoxic element.

On top of that, if you can recirculate the CAR-Ts that is a great option to have. If that is not possible, one pass flow could still be utilized. But achieving a sustained flow of CAR-Ts in the way you see in the body is another challenge on one pass platforms – you want this to happen not for minutes or an hour, but ideally for several hours.

These are some of the aspects that you have to consider when selecting the right *in vitro* model for cell therapies, including the question of whether you can fully recreate these aspects or not. Compromises may be necessary, and you have to choose which compromises you can work with based on the scientific question you are asking.

Q What would you pick out as the key issues or challenges facing preclinical work in the I–O space?

PC: Firstly I would say translatability. When we look at the attrition rate in oncology and in I–O, it is pointing to the preclinical stage. The jump from preclinical from clinical is clearly not smooth, and the lack of translatability of the models is the main issue. This covers a range of issues – you can divide it to efficacy and safety, etc. but taken as a whole, this is the main overarching issue.

This is even more pronounced when it comes to I–O applications. We are trying to reprogram impaired anti-tumor immunity, but both the *in vivo* tools and *in vitro* tools we have are far from properly recapitulating the TME. All of us in the field are aware that there is a very intricate and patient-specific interaction between the tumor cell, the immune cell, the blood vessels, the TME, and also additional factors such as any underlying or background diseases the patient may have. All of these factors taken together result in the immune response that a particular patient will show to an immunotherapy.

In the past we used to apply traditional experimental disease models that focus only on the tumor cells. But that is too one-dimensional and we know it doesn't work. What we want to capture is the heterogeneity of the whole system. That includes the cellular heterogeneity, the genetic heterogeneity of the tumor, but also the cellular heterogeneity of the TME with all of its supporting cells. The patient-specific TME is both extremely difficult, and extremely important to capture. There is also the histoarchitecture of the tumor microenvironment, and also the metabolic capacity.

"I am very excited about the potential that organoids bring to the oncology field, including a tremendous improvement in capturing genetic heterogeneity. With some protocols, for a short term they are also able to capture patient-specific tumor infiltrating lymphocytes (TILs)."

When you list all of these, and potentially also add the blood vessels and vascular network, currently I believe there is no preclinical model that has the capacity to get all of these aspects right at the same time. We are essentially working in a reductionist environment, and making reductionist models, but putting them together in a complementary way to attempt to create a full picture before going to first-in-human. But to a certain degree, we are not getting a full picture until we reach the patient.

This is the biggest challenge we are facing, and it is undoubtedly an obstacle to creating more blockbusters like the results that we have seen with the immune checkpoint inhibitors, to expanding immune checkpoint inhibitors with new signaling pathways, or to increase their efficacy so that more patients can benefit. Being able to figure out why things work, and why they don't work, all boils down to this fundamental problem.

Q Looking to the future, is there anything new or emerging in the nonclinical tool space that you are excited about?

PC: Definitely, especially from the *in vitro* side. I am very excited about the potential that organoids bring to the oncology field, including a tremendous improvement in capturing genetic heterogeneity. With some protocols, for a short term they are also able to capture patient-specific tumor infiltrating lymphocytes (TILs). This is a huge bonus compared to what we had before, especially if you can put them into a three-dimensional form.

On top of that, we can look at taking organoids as one tool and combining them with stromal cells, endothelial cells, and autologous peripheral blood mononuclear cells (PBMCs), for example, on a tumor-on-chip. Now we have the flow and the vascular network. That suddenly starts ticking multiple boxes I mentioned earlier. Not all of them, but many more boxes than before for capturing the patient-specific environment.

Another popular and entirely justified approach to mitigating the issues I have listed is using precision-cut tissue slices. This is an old-school method, but reinvigorating it for I–O applications is opening new avenues to explore histopathology and histoarchitecture. When you complement this approach with other tools it can create very valuable and patient-specific datasets.

Additionally, we now have better tools to keep these slices alive for longer, as this has previously posed a problem. There are better media and little bioreactors or organ-on-chip models where you can put these tissue slices to create a bit more flow to maintain viability for a little

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bit longer. Doing experiments for up to a week with these has now become possible. There are some beautiful review papers that outline how to use tissues slices with organ-on-chip to increase their viability [1,2].

However, there are some challenges and important considerations with tissue slices. Sample freshness is still a problem – your lab ideally needs to be in the hospital so that you can start processing these instantly. Once you start freezing the tissue, that will usually compromise the quality of the data you will get.

Precision-cut slices also bring mixed pathologies. You see this even more when you start slicing from one end to the other – slices that are most distant to each other will likely show the most variation compared to slices that are next to each other. The inherent variety of the picture is exactly what you want to capture, but it means that you need to increase the number of experimental replicas in order to increase the statistical power and amplify your signal against the noise. However, the tumor size you can get from the patient may sometimes be limiting. Additionally, the system is generally very low throughput and very resource intensive due to it being highly manual

Q What would be on your 'wish list' for new developments that could help to accelerate the field?

PC: It comes down to the sample quality from the patient. If I had a magic wand, I would wish for an entirely autologous system – PBMCs that are matched with patient organoids, but also for example patient-matched induced pluripotent stem cells (iPSC) -derived stromal cells and iPSC-derived endothelial cells. This would allow us to build a whole microenvironment system that is entirely autologous for that patient, that has organoid heterogeneity and PBMCs, and also add fibroblasts, and a vascular network, all coming from the same patient. This would be a really powerful tool.

At the moment when we attempt to do something like that, there are four to five people on the same tumor-on-chip. Each cell comes from a different donor. We can try to go the extra mile and have them human leukocyte antigen(HLA)-matched, but that is the limit. When looking at other disease models, this doesn't necessarily pose a problem. These mismatches would create an issue *in vivo*, such as organ rejection, for example. That doesn't happen very often in the organ-on-chip, likely because we don't have the whole immune system there to trigger this reaction. However, in I–O we are specifically trying to capture the minute nuances of the immune response, so it is likely to be problematic if we do not pay attention to these aspects.

One final issue I want to raise is data sharing. This is a generic problem for everyone working in this field, and currently everyone is working in their own corners trying different strategies. It is entirely possible that we are trying the same strategies in different parts of the world. I would love to see a consortium that brings together complex *in vitro* model developers for I–O so that we can freely share data, learn from each other, and build this together. The practicality and feasibility of this is another discussion, but it would certainly help to accelerate progress in this field.

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NONCLINICAL TOOLS UPDATE:
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INTERVIEW

Considering the nonclinical toolkit in I–O: assessing safety, understanding the TME & working towards an animal-free future

Róisin McGuigan, Editor, *Immuno–Oncology Insights*, speaks to Genentech's **Kimberly Homan,** Director and Senior Principal Scientist, Complex *in vitro* Systems Lab, and **Aaron Fullerton,** Director, Investigative Toxicology, to take stock of the current state of play in the I–O nonclinical space and ask: where are we now, and where are we going?

KIMBERLY HOMAN directs the Complex *in vitro* Systems lab at Genentech, a core group focused on employing new predictive tools to enhance clinical translational outcomes. She has prior experience holding key leadership positions in two biotech startups, one of which she co-founded while in graduate school at UT Austin. As a co-appointed postdoc at Roche and at the Wyss Institute in Harvard, Kimberly invented methods to bioprint human tissues and use them to model drug disposition, mode of action, and safety. Kimberly holds a BSc degree in chemical engineering and PhD in biomedical engineering; she is also a former United States Marine Corps officer and veteran.

AARON FULLERTON obtained his PhD in Pharmacology and Toxicology from Michigan State University, where he focused on the role of inflammation and aberrant immune responses to xenobiotics in the etiology of hepatotoxicity. He continued this research focus as a postdoctoral IRTA fellow at the NIH (National Heart, Lung and Blood Institute) where he investigated the roles of the innate and adaptive immune systems in the pathogenesis of drug-induced liver injury. In 2014, he joined Genentech's Safety Assessment group and led a research team in the Investigative Toxicology Lab focused on identification of safety liabilities and elucidating mechanisms of toxicity in the areas of hepatotoxicity, immunotoxicity, and hematopoietic toxicity. Aaron currently leads the Investigative Toxicology Laboratory as they continue to support non-clinical safety assessment efforts for both small and large molecule drug modalities through the use of *in vitro* human and animal cell models.

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Q Could you both describe your respective groups at Genentech and the work they do?

KH: I direct the Complex *in vitro* Systems (CiS) group. We build complex models such as organoid and organ-on-chip technologies, also known as microphysiological systems, to support drug development. We sit within Safety Assessment and therefore prioritize safety applications, but we also support *in vitro* modeling broadly at Genentech, including applications in drug disposition, efficacy, and mechanistic studies.

AF: I lead the investigative toxicology group, which is also in our nonclinical Safety Assessment department. We are using many similar tools as the CiS group, but our focus is on applications to project portfolio support in the safety space exclusively. Those efforts span from assisting our research colleagues in the qualification of new potential immune–oncology (I–O) targets, through non-clinical development (i.e. lead candidate selection) and including reverse translation activities following adverse events (AEs) in clinical programs. We leverage various in-vitro methodologies to deconvolute mechanisms of toxicity in these scenarios and help develop mitigation strategies or inform backup programs. We utilize a wide variety of *in vitro* models within our group including those being developed in-house by the CiS group.

Q What unique challenges does the I–O field pose when it comes to developing preclinical tools, specifically for assessing safety?

AF: Early on, in terms of qualifying new targets, there is the issue of assessing differential expression for a target (i.e. tumor antigen expression relative to primary tissues). It's one thing to look at that using genomics data and claim we should have some order of magnitude difference between tumor cells and normal primary cells. It is another thing to actually characterize the functional implications of that expression difference, and how much of a margin is actually needed to enable the safe targeting of that specific tumor antigen. One of the gaps in that process is that typically we are conducting those early efficacy studies in mouse models using immortalized cancer cell line xenografts. These current in-vivo models do not adequately represent normal tissue expression in primary human cells.

We are getting to the point now where we are seeing the implementation of more complex human *in vitro* systems to address that need. These models profile test article activity against both tumor cells and normal primary cells in the same environment. We can look at what it takes to create a I–O therapeutic that truly has selectivity for cancer cells. It becomes more complicated when we start to consider what happens once we start killing cancer cells and there's potential bystander effect, or there's an inflammatory state that's induced. What does that alter in terms of the cell or tumor microenvironment (TME) phenotypes and presentation of on/off targets in the surrounding primary cells?

These are capabilities we are seeing come to fruition right now, as human *in vitro* models become complex enough to support that. There's a lot of background engineering that goes into it, whether it's adding the vasculature so that we can bring the immune cells into the correct cell-cell interaction context, or models that are more robust from a kinetics standpoint such that we can establish detailed time courses of events versus just looking at how effectively they kill cancer cells in a cell culture well at a twenty-four-hour endpoint.

KH: Happy to highlight two things here. Firstly, creating a therapeutic index (TI) window for a new target *in vitro*. One specific implementation of that is to make a cancer organoid and a healthy organoid (with associated on-target risks) and study them side-by-side. Looking at simple cytotoxicity readouts for both organoid types, one can start to estimate a TI. The second is translation – imagine a four-square box; we often think about two squares: *in vivo* animal studies, and how we translate that to *in vivo* human clinical responses. To enhance this translation, we need the other two squares in the box: *in vitro* human and *in vitro* animal. If we can build confidence from vetting *in vitro* animal to *in vivo* animal results, it will enhance our *in vitro* human to *in vivo* human predictions . There is quite a bit of effort in groups that we are a part of, like the IQ Consortium's Microphysiological Systems Affiliate (IQ MPS), to push for having animal *in vitro* models as well as human versions to enhance translational predictions.

Q What progress do you see being made in the solid tumor space?

KH: The TME is very challenging to model in its entirety *in vitro*. There are many reports in literature attempting to model immune desert, immune excluded, and inflamed

> "The challenge is that each unique cancer presents in a different way, even *in vitro*. Being able to represent the cell types properly, vascularize it properly, and make it immune-competent, are huge challenges for the field."
>
> – **Kimberly Homan**

tumors. The challenge is that each unique cancer presents in a different way, even *in vitro*. Being able to represent the cell types properly, vascularize it properly, and make it immune-competent, are huge challenges for the field. Immune-based therapeutics have proven effective in the liquid tumor space, but in the solid tumor space, we're still facing challenges *in vitro* and *in vivo*.

AF: Our ability to adequately predict a first-in-human (FIH) starting dose and the gap that needs to be covered during dose escalation (cohort by cohort) to achieve an effective dose in the heme-oncology space has been pretty good. However, in the solid tumor space, it has been more challenging to predict a dose where pharmacological activity will first be seen. This can generally lead to a bigger gap between FIH starting dose and a dose that provides patient benefit. We can only rely on those xenograft models so much, and as Kimberly pointed out, the models in the *in vitro* space are still being developed to appropriately understand immune transmigration out of a vascular environment into a solid tumor space. However, there is a lot of progress being made and many different groups are heavily focused on this.

Q What about progress in assessing combination therapy approaches?

AF: Practically speaking, of course there's a need to understand combinations from an efficacy side as well as from a safety side. For the most part we can assume that as we layer oncology treatments, we can often expect some gain in efficacy. But the real question is whether we maintain or increase the margin of safety for those same combinations.

Today we see an increasing emphasis on reducing animal use, and such combination tox studies would likely need to be done in NHPs because of lack of cross-reactivity in other species. However, we can increasingly leverage human *in vitro* models. Typically we know which organ systems we are looking at – we've looked at the single agent activity and we know that drug A and drug B have their own respective toxicities that are dose-limiting. Therefore we're not trying to do an entire animal-wide toxicology assessment. We can be very focused and ask when we combine these two therapies, do we get additive or synergistic toxicity? We can look at this in a liver chip, gastrointestinal chip, etc. or whatever the situation may call for.

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This approach is becoming a valuable tool for us in terms of understanding the relationship between enhancing efficacy in combination therapies, and the potential safety margin that we have to maintain to make them tractable treatments for patients.

KH: One of the hottest combinations is checkpoint inhibitors plus another treatment – chemotherapy, steroid, etc. I would love to test those combinations *in vitro*, but they are very challenging. You have to make an immune-competent model, and then test your checkpoint and your secondary therapy either concurrently or in sequence. Do you model the patient's tumor directly using a biopsy or try to piece together immune cells, cancer cells, and cancer-associated fibroblasts from disparate sources? While recent literature reports on the topic are encouraging, there remains a paucity of benchmarking data in this space.

Q How are current *in vitro* models helping to bridge gaps seen with more historical/traditional models in the I–O space?

AF: We have always relied heavily on xenograft models for the vast majority of our oncology portfolio. Usually there are high bar and low bar models, and we understand the boundaries of that in terms of translating to human efficacious dose. Where it gets trickier is when we try to optimize, in the lead candidate space, the true mechanism that's driving why one model might perform better than another.

This can require a large number of animals, and still only provides snapshots in time. It's laborious and time consuming, especially when you start thinking about doing dose fractionation studies and understanding drivers of efficacy, whether it's C-max or time over threshold, and so on.

All of these things could be more readily assessed kinetically in *in vitro* systems because we can take a snapshot under the microscope at any point in time, or even track live what's going on with immune cell transmigration to a tumor. You can sample almost as much as you want with the new technologies available to keep these systems alive, whether they are the traditional immortalized cancer cell lines or patient-derived tumor cells that we can keep happy in these more highly engineered environments.

This opens the door to asking more mechanistic questions and getting a better understanding of what's driving efficacy, but also of course, from our perspective, the safety considerations as well. Broadly speaking, what *in vitro* is bringing is the ability to look under the hood and take a much deeper dive into what's happening. We're still working on the translatability aspect, and how to benchmark that to historical models, but as far as moving to the next level and understanding why this therapy worked in this particular xenograft model, but not that one, there are more tools available in the *in vitro* space.

KH: There are two *in vitro* tools that have been quite useful in this regard. The first is a chip which can be used to assess which chemoattractants can increase T cell infiltration to the tumor area. These models are helping to narrow the lead candidate space for new cancer therapeutics.

In the bispecific antibody space, I am encouraged by the advanced microscopy techniques being applied to unravel how much a particular bispecific would cause target cell death, how fast it causes that death, and how fast the T cell can move from one target cell to the next. These new and very detailed parameters can go into a lead optimization formula or strategy that can be used to narrow lead candidate bispecifics in the future.

Q Why is reducing/limiting the use of animals in the nonclinical space an important priority for industry at the moment?

KH: Reducing animal use has been a priority in industry for quite some time because (1) animal lives are precious, and (2) we know animals fail to predict human responses in certain settings. Recently the FDA and other regulatory bodies have introduced and adopted the term NAMs – which they prefer to be defined as new approach methodologies, but other definitions such as new alternative methods, or non-animal methods are also pervasive. NAMs include *in vitro* approaches, which is what we're talking about today, but also *in silico* approaches and *in chemico* approaches that could all help increase our predictive power in human translation while reducing animal use. While you don't see as much replacement of animal studies in regulatory filings yet, I hope that with enhanced investment from pharma and recently funded NAM initiatives within the FDA, we'll begin to see a movement towards refining, reducing, and replacing animal use as in vitro and in silico models mature.

In addition, there are opportunities in pharma to reduce animal use that are out of the public view. For instance, pharma doesn't publish most of their early discovery or efficacy studies, but these are areas where animal use is decreasing. To give one poignant example, typically when we develop brain-penetrant molecules, mice are used to predict human brain penetration and it can be cumbersome to narrow in on lead candidate molecules. But now we have human *in vitro* models of the blood–brain-barrier that can help narrow that selection and drastically reduce the number of animals being used for that prediction..

Q Where are nonclinical tools going to have the biggest impact in the I–O space in the next few years?

KH: There has been a lot of funding in the space of *in vitro* immune modeling. A big program to highlight is the HOPE Program, which is funded by the Wellcome Leap Foundation. This program is working with a robust set of academics who are trying to solve complex problems like getting immune incorporation into parenchymal tissues, or getting immune incorporation into cancer. I believe this is the wave of the future.

AF: One of the areas we haven't had the tools to effectively look at until more recently is gaining a broader understanding of CRS. This impacts many I–O molecules, although the field has done a really admirable job of mitigating this through creative clinical design. While It doesn't become a showstopper for many of these therapeutics, it certainly is a

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limitation. With the capability to incorporate immune cells into some of these more complex models, and being able to monitor the activity of immune cells over longer periods of time, I think we're in a position now where we can leverage those tools to better understand what is driving CRS in some of these scenarios. We can even revisit problematic targets or modalities that have been deprioritized in the past, and understand how we might reengineer those therapeutics to avoid the AEs that were previously seen.

> "This field won't meaningfully move forward until we can align with the regulatory agencies on how to interpret and use some of this data, which is something various different groups are actively working on."
>
> –Aaron Fullerton

Additionally, what really drives patient-to-patient variability in this space is still largely an unknown, and as we are able to create these highly engineered vascular environments, we can generate more physiologically relevant cues and assess what's driving donor responses based on polygenic risk factors, immune cell composition, or prior immune activation status to understand factors predisposing patients to having a more exacerbated response.

I'm optimistic that we'll have those tools available to us in the next three to five years. We're already gaining a better understanding of what's driving this and also understanding the immune vascular endothelial response and how that plays into some of these scenarios. Considering that this is a safety liability that is uniform across most of this therapeutic area, I think it's a huge opportunity for us to leverage these tools and address it.

Q Looking to the future, what are your hopes and your predictions for this space? Conversely, what are your fears?

KH: I expect that we will continue to see robust development of NAMs which can both reduce animal use and enhance clinical translational outcomes for patients. We are actively driving in that direction across Genentech, and I can see other companies on the same path. It's very encouraging, but it's important to remember that it is not going to happen overnight. We need to qualify these tools and carefully benchmark them against known clinical outcomes. My biggest fear is that if we overhype what this technology can do right now, we could limit future investment in it. We need to take a measured look at where we are and where we are going, and properly fund this area in order to ensure it achieves its potential.

AF: This field won't meaningfully move forward until we can align with the regulatory agencies on how to interpret and use some of this data, which is something various different groups are actively working on. There is a lot of enthusiasm from health authorities to see this data, to have access to it, and to get a better understanding of it. This is

complicated by the fact that this is a rapidly evolving space. We can't necessarily take a snapshot in time and decide a model is qualified – the model we were using at one point may be substantially different from the more advanced version we'll be using next.

It's going to be a slow process, and it will involve a lot of teaching and sharing of information. Companies are already making a lot of internal decisions using these systems and my fear is that this doesn't disseminate out into the broader public scientific space, so that health authorities and other organizations can digest it and feel comfortable with the claims that can be made from these systems. It's all still happening in a somewhat siloed environment, although we're publishing as much as we can, and other companies are trying to do the same. I hope that that continues to happen.

However, there's a lot of good progress being made, so I think my fear is relatively minimized. The biggest change will be when the regulatory reviewers start seeing more of these case study examples. Because right now from our discussions, it is few and far between and typically only when there are no other animal options available that project teams are leveraging these models to great effect. We want to see this disperse out into more opportunistic use, rather than when it is the only option left.

KH: I would emphasize that industry and academia need to continue to publish in this space. To us, qualifying a model means ensuring that it's properly benchmarked across known outcomes from humans in the clinic. Not just with one proof of concept experiment, but with a whole set of compounds that allows you to properly calculate sensitivity and specificity inside of a particular context of use for a new model. This is a big burden and can't be left to academics alone – pharma and developers and startup companies all need to do this collectively. If we don't, and we keep publishing only proof of concept experiments with one compound or two, that won't move the bar with regulators or with pharma. While we also want to see new models developed, we need to see the current versions mature and show quantifiable predictivity. If we have that, we can more quickly move towards a new, animal-less preclinical testing paradigm.

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IMMUNO-ONCOLOGY INSIGHTS

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Assessing efficacy & MoA of mono & combo immunotherapies in preclinical humanized models

Kader Thiam, Arjun Surya & Edward van der Horst

The breakthrough of immune checkpoint-targeting therapies has unveiled new hopes for cancer therapy. However, subsets of patients who do not see robust responses to immunotherapy remain. To address this hurdle, combination therapies – coupling agents with distinct mechanisms of action (MoA) – appear promising to enhance treatment success against various cancers. However, a major challenge in the development of novel combination therapies is the unmet need for preclinical models to predict efficacy and tolerability.
Immunocompetent models featuring humanized immune checkpoints enable the assessment of human-targeted therapies in well-established syngeneic tumor models, allowing investigation with fully functional crosstalk among syngeneic tumor, immune, and stromal cells. While these models enable profiling evaluation of agents directed toward human targets, results still reflect mouse biology. Alternatively, immunodeficient mice reconstituted with a human immune system offer the possibility to investigate the efficacy and MoA of agents directed against human targets, with the advantage of exploring human biology using human tumor cell lines in a mouse model.
This article discusses examples of applicability and complementarity of syngeneic and BRGSF–human immune system (HIS) models to assess the efficacy and MoA of immunotherapies, either in combination with inhibitory immune checkpoints or as monotherapy.

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INTRODUCTION TO HUMANIZED IMMUNE CHECKPOINT & BRGSF–HIS MODELS

Currently, there are two different types of preclinical humanized models for the assessment of compounds modulating the immune response: syngeneic humanized immune checkpoint (ICP) mice in which a selective immune checkpoint has been genetically humanized, and BRGSF–HIS mice. The advantages and disadvantages of each model are outlined in Table 1.

HUMANIZED IMMUNE CHECKPOINT MOUSE MODELS

The main advantage of syngeneic humanized immune checkpoint models is that the immune system is fully functional. This allows for proper crosstalk among tumors, the immune system, and the stroma, and a plethora of well-calibrated tumor cell lines can be used in this model. These syngeneic models are available 'off-the-shelf', and they have been used for applications such as for understanding the MoA of targets for the rational design of bispecific antibodies, and assessing the efficacy of different compounds.

To ensure the overall performance of genetically humanized models, it is particularly important to identify how the genetic design may impact the target's expression, regulation, and binding with partners. Thus, the target's biology must drive the choice of genetic strategy to guarantee its functionality.

Some human targets, like CD28, have several isoforms – a canonical one and a shorter one which has been reported to act as an amplifier of CD28 engagement. A humanized knock-in (KI) model expressing only the canonical isoform will thus most likely result in a biased assessment of the effect of an agonist compound. Having a model with two isoforms would be more relevant. Additionally, if the intracellular domain is different between mouse and human, there could be an amino acid that triggers specific downstream effects, such as the secretion of inflammatory cytokines, in humans but not in mice (as it is for CD28). Depending on the construct selected

TABLE 1

Pros and cons of two types of preclinical humanized models for the assessment of immunotherapies.

	Pros	Cons
Syngeneic humanized ICP mouse 	Fully immuno-competent Proper cross-talk between stroma, tumor microenvironment and immune system Access to plethora of mouse tumor cell lines Efficacy assessment toward the human target Suitable for biologics, including bi-specific – same agents will be used in patients No extra costs/time to develop a surrogate	Read-out: mouse immune response
BRGSF–HIS 	Exhibits functional human lymphoid and myeloid components Reflects an overall human immune response translatability: • Mimics clinical observations and heterogeneity Versatility of assessment of broad spectrum of combination therapies Enables: • Therapeutic assessment onto human cells • Safety	Not fully immuno-competent-interaction between tumors and microenvironment could be partially defective May not be appropriate to investigate drug with high impact on tumor microenvironment

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for model generation, it may not mirror the inflammatory response that CD28 agonists would have triggered in human cells. Thus, a KI model could be well suited to assess efficacy but not safety and toxicity, or the other way around, depending on the target.

When using a KI model, properly de-risking the target's functionality in mouse cells is recommended. Figure 1 summarizes an approach for testing of a cytokine receptor's functionality, considering a receptor with two subunits: alpha and beta.

Several strategies can be considered: the humanization of the entire receptor, of the extracellular domain, or this same chimeric version in which key amino acids have been kept murine to ensure proper interaction with partners. *In vitro* testing of these constructs is the only way to identify the optimal design, and the most functional receptor for *in vivo* assessment of biologics specific to the human target using syngeneic models. Physiologically relevant expression of the target gene should also be maintained.

Once the KI mouse model is generated, the target's functionality should also be confirmed *in vivo,* including ability of the model to respond to known therapeutic agents.

The limitations of syngeneic humanized models include their inability to fully reflect an overall human immune response (the immune system remains murine) and being restricted to targets that have an ortholog in mice. As such, translatability towards the clinic may be limited. Therefore, there is a need for translational preclinical models that exhibit a human immune system, namely HIS mice.

BRGSF–HIS MICE

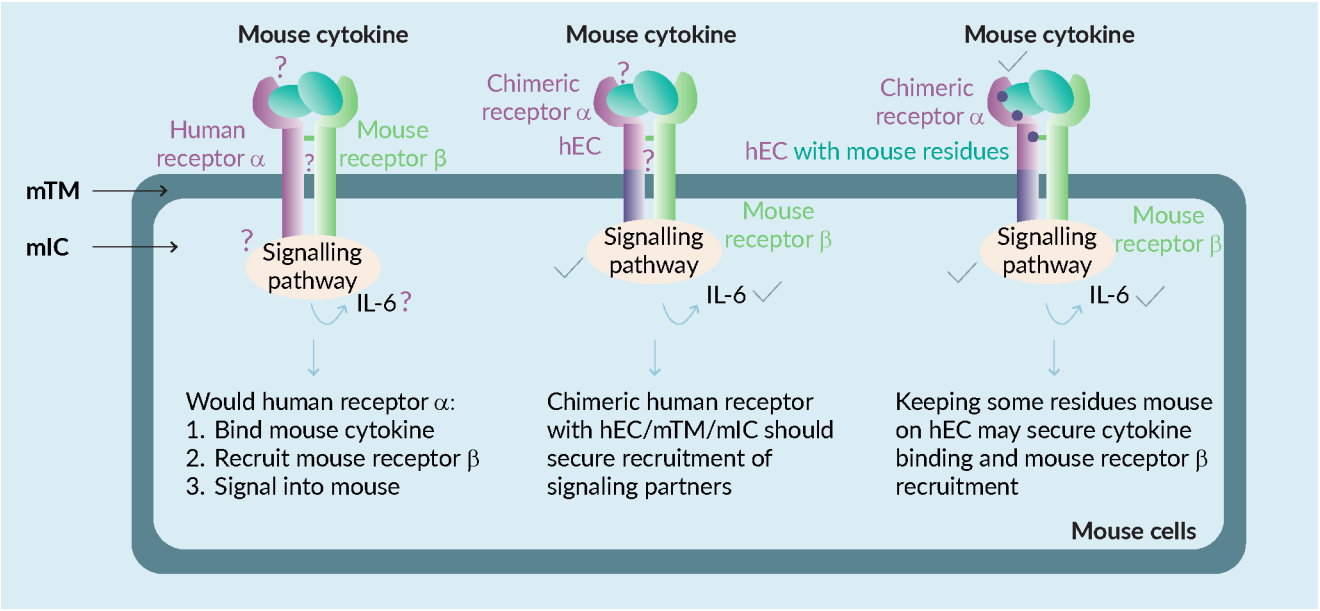
Several models are available in the field. The model discussed here, named BRGSF–HIS, has the advantage of exhibiting both human lymphoid and myeloid compartments.

BRGSF–HIS mice have no mouse T, B, or natural killer (NK) cells. They display a mutation in the Flt3/Flk2 gene that results in a reduced mouse myeloid compartment. This frees up a niche for the human myeloid compartment to develop upon reconstitution with human CD34+ cells.

BRGSF–HIS mice have a human immune system dominated by human lymphoid cells. Upon Flt3 treatment, the human myeloid compartment is boosted. As this human myeloid compartment develops after a transient

FIGURE 1

De-risking human target substitution of a mouse counterpart *in vitro*.



treatment, these mice do not develop side-effects reportedly related to myeloid cell development in other models, such as strong anemia or reduced life span.

BRGSF–HIS mice develop human T and B cells, monocytes, NK cells, classical dendritic cells (cDC), and plasmacytoid dendritic cells (pDC) upon reconstitution, and the engraftment lasts for up to a year. The T cell subsets in BRGSF–HIS mice are a standard subset of human T cells, including gamma-delta T cells. The majority of B cells in the blood are transitional B cells, which can be maturated into major B cells by IgG^+ and IgM^+ cells. Upon Flt3L treatment, human cDC and pDC numbers are increased in the blood and spleen. This treatment also significantly enhances the frequency and number of human monocytes and NK cells.

This reconstituted model has been used to assess therapeutics targeting immune checkpoints, either as monotherapy or as decision tools for combination therapies. It has been shown to reflect the chimerism of response observed in humans to certain immune checkpoint inhibition, in different types of tumors. It has also been used to assess the efficacy of myeloid-targeting therapies and could be used to assess the safety of T cell engagers.

The limitation of this model is that although the immune system is human, some of the interactions between the immune system and the stroma may not be fully competent, which may impact the MoA of certain drugs.

ADDRESSING THE CHALLENGE OF COMBINATION THERAPY MODELS

When developing new immunotherapies, the initial approach must prove safety in advanced/metastatic settings with the novel therapy as a single agent. A key challenge faced by drug developers is that a single agent is highly unlikely to reverse these patients' conditions, with even PD-1 and CAR-T therapies only working in a fraction of patients. Tumor-specific standard of care in patients limits combination options. In addition, clinical development is a long and expensive process, as each combination trial can take years. Thus, predictive models for combination therapies are an urgent unmet need.

As previously mentioned, syngeneic models are the current best way to test an immune therapy. Indeed, these animals are immunocompetent, display rapid tumor growth upon inoculation, and are easily manipulated, making syngeneic studies generally reproducible. In addition, 'real-life' cancer features such as genomic instability are recapitulated in these models.

Other problems that need resolution can come from species selectivity. For example, Curadev Pharma has used genOway's human STING knock in technology to advance its STING agonist program into clinical development. The clinical asset does not activate murine STING, so genOways' human STING KI mice were used to demonstrate the anti-cancer activity of the compound. STING is an innate immune sensor triggered by the presence of cyclic dinucleotides and is well represented within the tumor microenvironment (TME). Cyclic dinucleotides act as an agonist or a warning system for STING, which triggers the type I interferon response.

Curadev's IV-administered STING agonist compound activates STING in the same way. This enables the maturation of DCs, which eventually leads to T cell activation and tumor degradation. When an IV-administered STING agonist dose was given to STING KI mice, increases in many cytokine serum levels, such as IP-10, interferon-(IFN) α, β and γ, and IL-6, were observed (data not shown). Combinations with epigenetic and immune checkpoint-targeting therapies for Phase 1b/2a are now being explored.

CONDITIONALLY ACTIVE ANTIBODIES FOR IMMUNO-ONCOLOGY

Sensei Biotherapeutics is an immuno-oncology company focused on the discovery

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and development of next-generation therapeutics for cancer patients. They develop conditionally active therapeutics designed to disable checkpoints and other immunosuppressive signals selectively in the TME to unleash T cells against tumors.

Sensei's lead investigational candidate is SNS-101, a conditionally active antibody designed to block the V-domain Ig suppressor of T cell activation (VISTA) checkpoint. VISTA is a potent T cell-inhibiting checkpoint extensively expressed on myeloid cells. It is a B7 family member that suppresses T cell function. Targeting VISTA means targeting 90% of the immune system, and this potency poses a challenge in the context of pharmacokinetics, and from a safety perspective. VISTA interacts in a pH-dependent manner with the receptor PSGL-1 on T cells, aiding T cell suppression.

Clinical development of anti-VISTA antibodies has been challenging due to three major factors: a lack of clarity on the identity of the critical counter-receptor responsible for T cell suppression, observed cellular activation and cytokine release syndrome (CRS) in humans at sub-therapeutic doses, and high clearance via target-mediated drug disposition (TMDD) by VISTA+ neutrophils and monocytes at physiologic pH.

As VISTA is broadly expressed on myeloid cells, and considering the involvement of these cells in deleterious immune responses such as CRS (Figure 2), it is of particular interest to develop an anti-VISTA compound that limits myeloid cells' activation.

The pH-sensitive SNS-101 antibody was designed to selectively target active $VISTA^{pH6}$ over $VISTA^{pH7.4}$ and is designed to block VISTA's interaction with PSGL-1 and all other T cell receptors at pH 6.0. Investigational New Drug Application (IND) filing for the fully effective competent IgG1 format is due to commence in or before April 2023. SNS-101 was found to inhibit VISTA:PSGL-1 interactions and potential binding partners at pH 6.0 in an *in vitro* assay. Importantly, no significant binding of SNS-101 was found to monocytes, neutrophils, NK cells, or T cells in whole blood at physiological pH.

SNS-101's potential toxicity was further investigated using genOway's myeloid-boosted BRGSF–HIS mice for CRS assessment. SNS-101 was compared to the clinical stage, non-pH-selective anti-VISTA antibody JNJ. Sera were collected at different time points and cytokines were quantified (Figure 3). The positive control anti-CD3 (OKT3) efficiently induced CRS. SNS-101 was found to only mildly induce chemokine CCL-5, while JNJ induced a dose-dependent secretion of IL-6, IL-10, CCL-2, CCL-5, CXCL-8, CXCL-10, IFN-γ, tumor necrosis factor alpha (TNF-α), and IL-1RA.

An experiment was also conducted to assess the impact of SNS-101 on monocyte activation. Mice were sacrificed at 24 and 48 h and immune cell proportions in the spleen were evaluated by flow cytometry. The non-pH sensitive antibody JNJ induced monocyte activation ($CD86^+$) at 24 h, followed by a decrease in monocyte proportions at 48 h. SNS-101 was found to have no significant impact on monocyte activation. SNS-101 did however induce significant expansion of CD4 and CD8 T cell subsets, and favored memory CD4 and CD8 T cells over effector phenotypes.

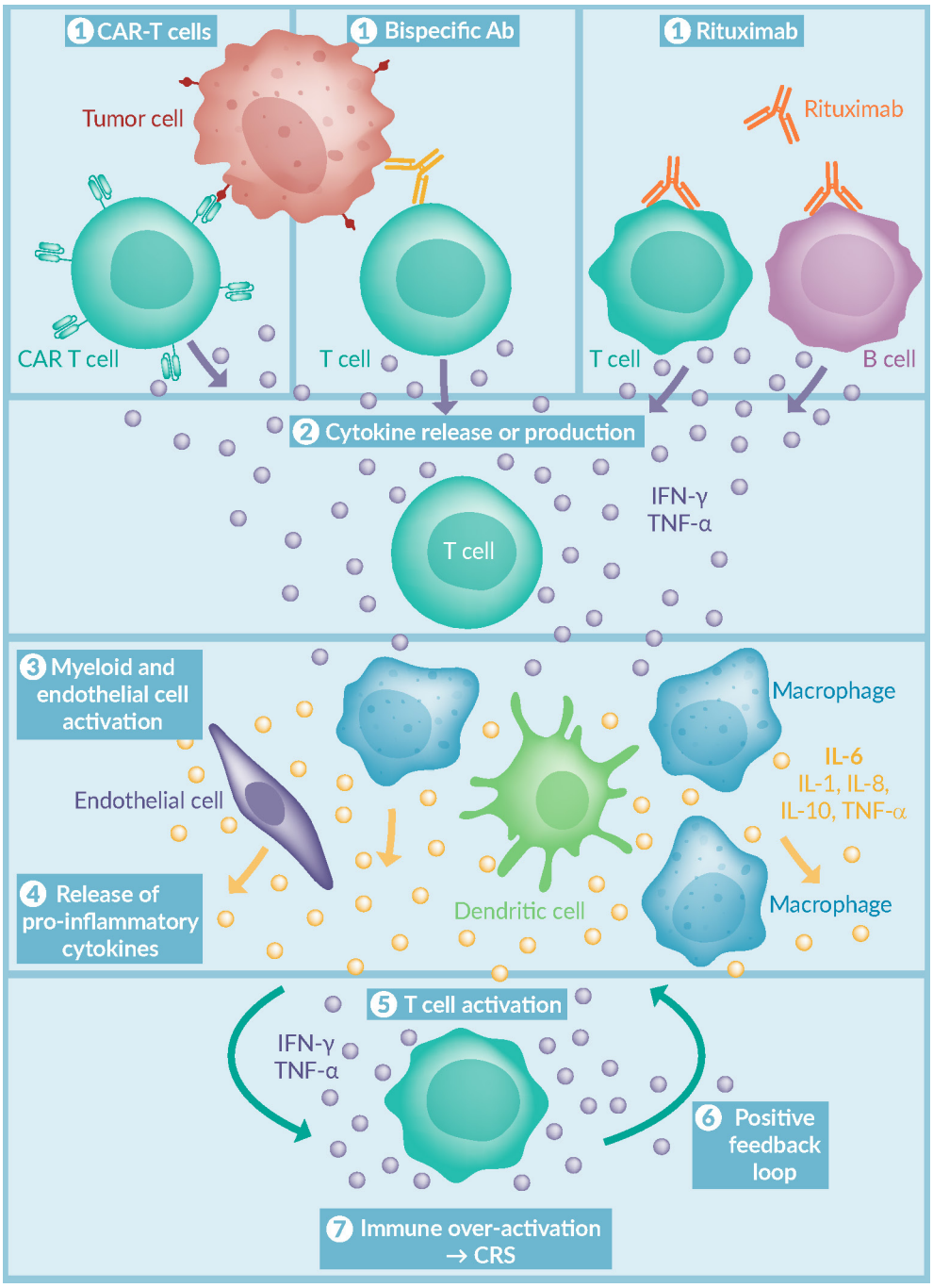
Pharmacokinetic studies were then performed in humanized VISTA KI mice developed by genOway and non-human primates (Figure 4). SNS-101 displayed a favorable single-dose pharmacokinetic profile, with no significant identified TMDD .

Indeed, a tumor implanted into hVISTA KI mice showed a growth drop-off, as TME acidity decreases while the tumor grows. As myeloid cells infiltrate the tumor, SNS-101 binds to $VISTA^+$ cells under acidic conditions exclusively, and is therefore eliminated from circulation. Findings in cynomolgus monkeys were consistent with TMDD of VISTA antibodies under physiological conditions.

SNS-101 was also found to significantly enhance the anti-tumor effects of PD-1 blockade in humanized VISTA KI mice (Figure 5). Established tumors were treated

FIGURE 2

The role of myeloid cells in the pathophysiology of CRS.



Ab: Antibody; CAR-T: Chimeric antigen receptor T cell; CRS: Cytokine release syndrome; IFN: Interferon; IL: Interleukin; TFN: Tumor necrosis factor

with either isotype control, anti-mouse PD-1, SNS-101, or a combination. Five out of eight animals receiving the combination treatment completely rejected the tumor versus only one in the anti-mouse PD-1 arm, thus increasing survival. The combination therapy increased the amount of infiltrating CD8 T cells within those responsive tumors, whereas anti-PD-1 alone did not, and was also correlative with anti-tumor effects.

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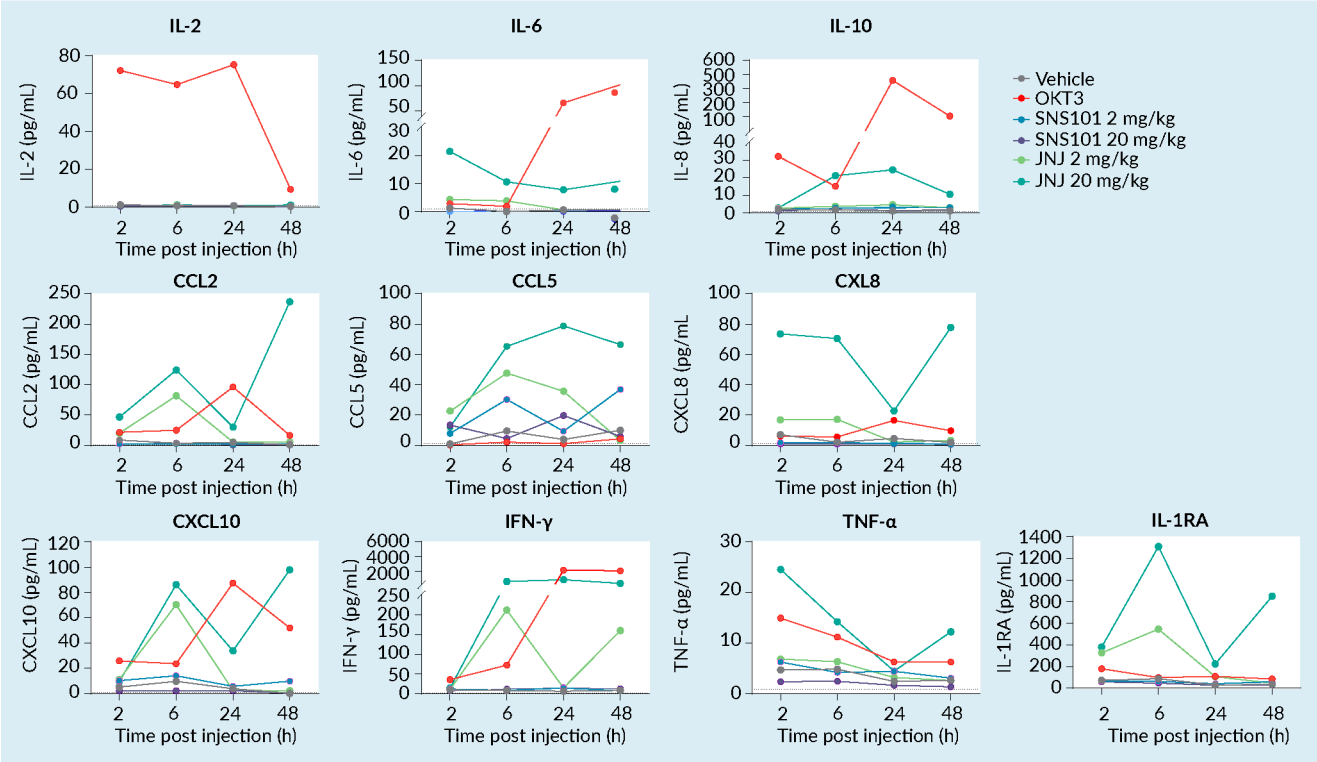
FIGURE 3

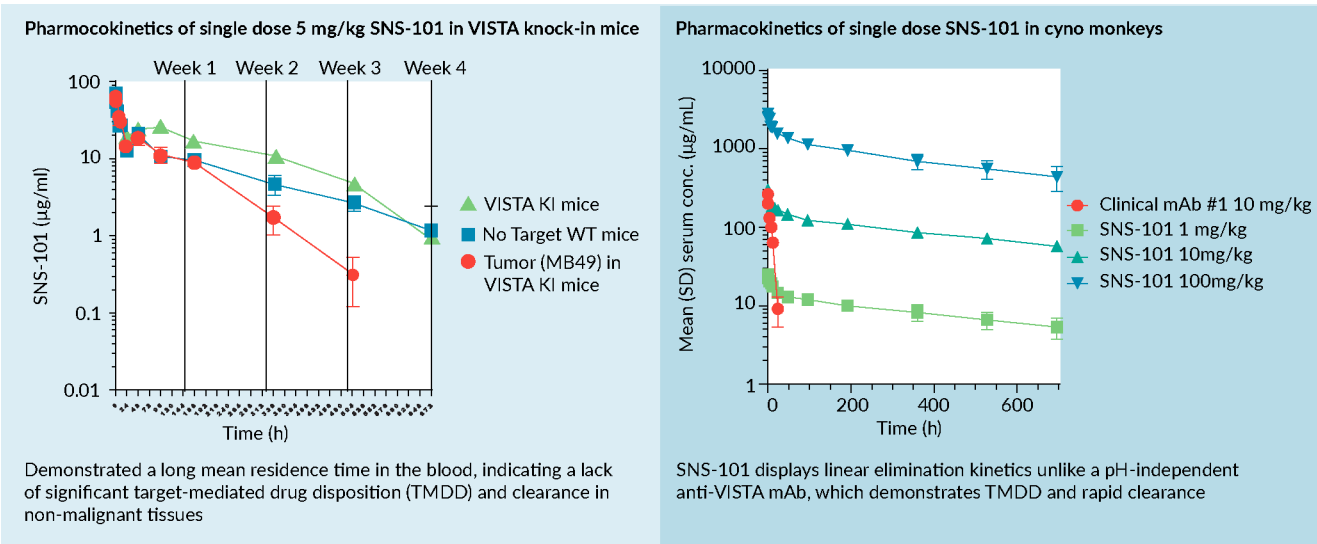
Induction of cytokines in myeloid-boosted BRGSF-HIS mice upon treatment with OKT3, SNS-101, and JNJ.



Together, these data demonstrate that SNS-101's exceptional selectivity for active, protonated VISTA has the potential to abrogate TMDD and lower CRS risk, while significantly enhancing the anti-tumor effects of the PD-1 blockade.

FIGURE 4

Pharmacokinetic profile of single dose SNS-101 in syngeneic hVISTA KI mice versus cynomolgus monkeys.



Demonstrated a long mean residence time in the blood, indicating a lack of significant target-mediated drug disposition (TMDD) and clearance in non-malignant tissues

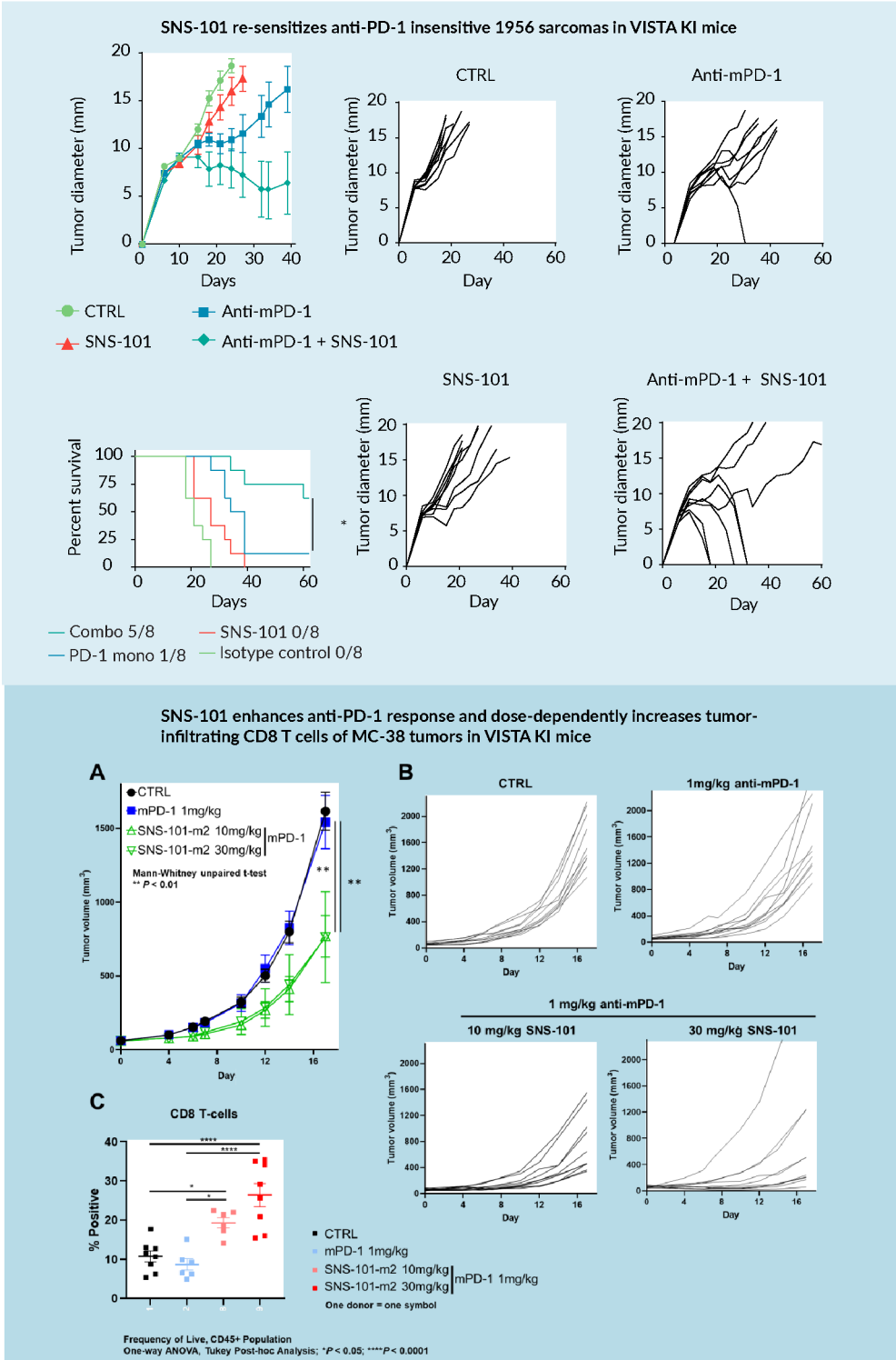
SNS-101 displays linear elimination kinetics unlike a pH-independent anti-VISTA mAb, which demonstrates TMDD and rapid clearance

CONCLUSION

As shown here, humanized preclinical models are useful tools for the assessment of mono and combo immunotherapies' efficacy, safety, and MoA. Syngeneic humanized and BRGSF–HIS mice represent promising and supplementary approaches, with both

FIGURE 5

Effect of SNS-101 on anti-PD-1 insensitive sarcomas (left), and on anti-PD1 response and infiltrating CD8 T cells of MC38 tumors in syngeneic hVISTA KI mice (right).



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displaying advantages and disadvantages. Understanding targets' biology, regulation, and binding to partners is key to choosing the optimal preclinical model for a specific application. Nevertheless, challenges in these models' applicability and complementarity do remain. Preclinical innovations including IV-administered STING agonists and VISTA-targeting could prove critical approaches in fulfilling the pressing need for predictive preclinical models for combination therapies.

BIOGRAPHIES

KADER THIAM received his PhD for work at The Pasteur Institute, Lille, in cellular immunology and infectious diseases before becoming a fellow in its Department of Peptide Chemistry. There, he focused on regulating immune response using recombinant viruses and synthetic agonists of cytokines, and on intracellular delivery of lipopeptides modulating pharmacological targets. Kader Thiam joined genOway in 1999, serving first as Scientific Consultant and Head of Immunology, and later as Director of Transgenic Technologies, driving R&D programs to develop alternatives to overcome transgenesis limitations and new models for immune response monitoring and better predictability toward the human situation. He is currently Senior Vice President Discovery – Preclinical Models & Services, overseeing the feasibility, design, rationale and accuracy of genOway's preclinical models.

ARJUN SURYA has more than 30 years of research experience and has held leadership positions of increasing seniority in the industry. He founded Curadev along with his partner Manish Tandon in 2010. In less than a decade Curadev has emerged as one of India's rare drug discovery success stories with a slew of first in class small molecule patents that have emerged from its unique approach to target selection and drug discovery. Two of Curadev's lead drug candidates and programs have been licensed to Top 10 Big Pharma companies. He has extensive experience in the assessment of drug molecules against several target classes across a range of therapeutic areas and specializes in building high performance research teams. His past organizations include SmithKline Beecham, Ranbaxy and TCG Lifesciences where he played a founding role in establishing the biology and drug discovery teams. Dr Surya has an integrated Masters in Physics from IIT Kanpur and a PhD in Biophysics from Syracuse University.

EDWARD VAN DER HORST is a molecular pharmacologist with a strong focus on antibody drug development across diverse target classes in oncology. He has 20 years of research and development experience from Zenith Epigenetics Ltd., Igenica Biotherapeutics Inc., OncoMed Pharmaceuticals, Tularik, Inc. (now Amgen), and U3 Pharma GmbH (now Daiichi-Sankyo). Dr van der Horst's contributions and discoveries have led to the clinical evaluation of several novel drug candidates at Igenica Biotherapeutics and OncoMed Pharmaceuticals, as well as to the first clinical stage anti-HER3 antibody at U3 Pharma GmbH. He received his postdoctoral training at Tularik, Inc., earned his PhD in biochemistry from the Max-Planck Institute of Biochemistry and conducted his master's thesis at Max-Planck Institute of Neurobiology. He graduated with a MSc of chemistry from the Ludwig Maximilian University of Munich.

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AUTHORSHIP & CONFLICT OF INTEREST

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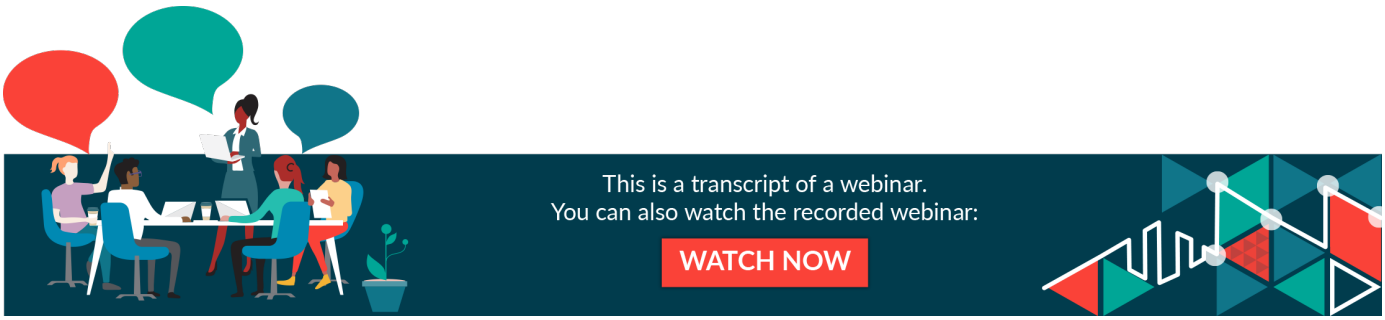

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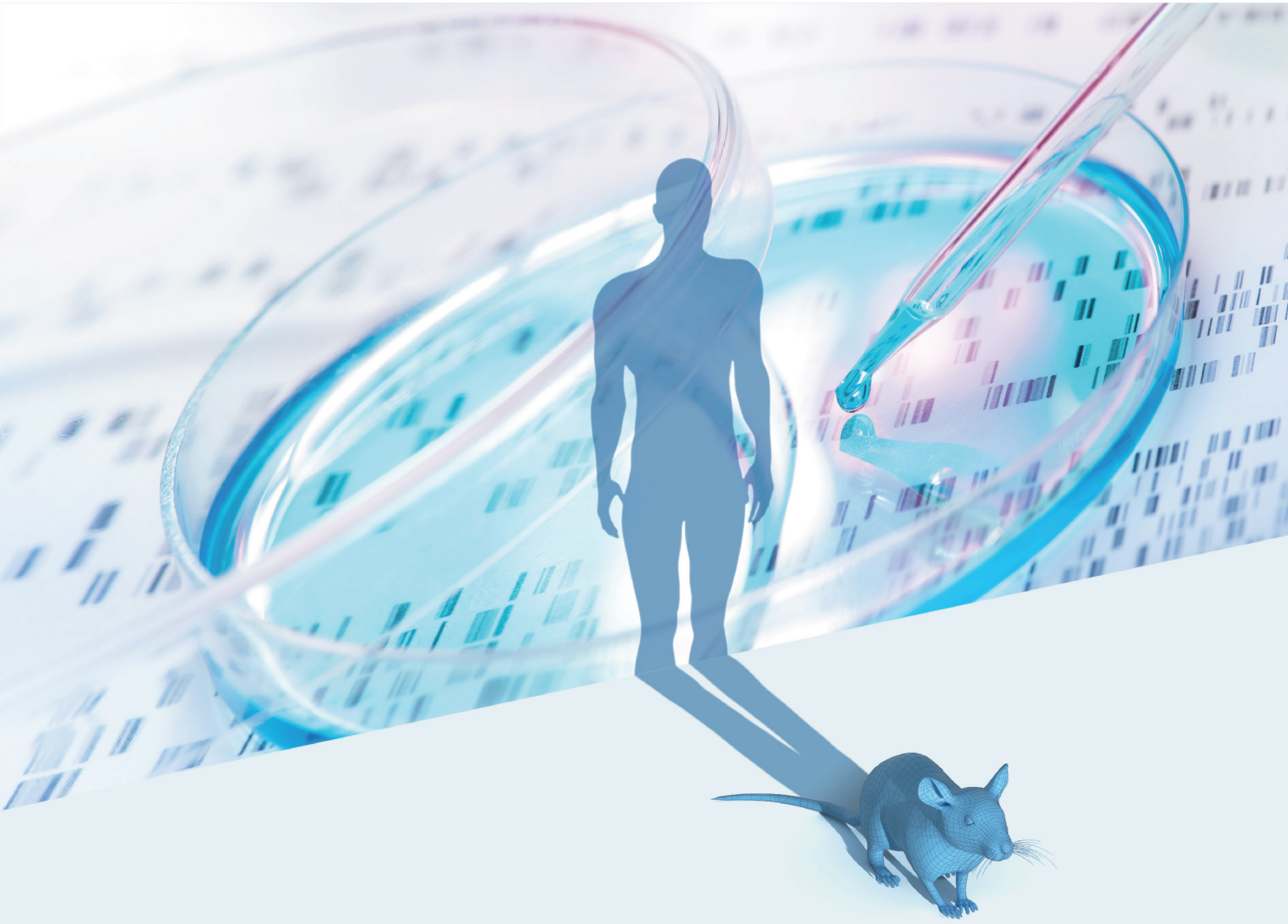

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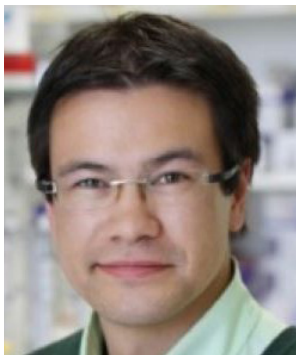
INTERVIEW

Addressing the complexity of the TME with organoids



Roisin McGuigan, Editor, *Immuno-Oncology Insights,* speaks to (pictured) **Etienne De Braekeleer,** Senior Research Scientist in Discovery Biology, R&D, AstraZeneca

The highly complex tumor microenvironment (TME) represents something of a 'black box' to the I–O space, and successful translation of therapies for solid tumor applications remains a challenge for the field. In this interview, Etienne De Braekeleer, Senior Research Scientist in Cell Biology and Immunology, AstraZeneca, discusses current progress and future applications of organoids to help recapitulate the TME — along with the importance of building more comprehensive datasets from patient samples.

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Q What are you working on right now?

EDB: I am the scientific lead for the organoid derivation project we have in collaboration with The Royal Papworth Hospital, The Wellcome Sanger Institute, and The Human Cancer Model Initiative. We receive tissues from the Royal Papworth Hospital, and we derive them in organoids, characterize them and share them back to the scientific community. With the organoids that we derive from this work, we try to initiate,

develop different pipelines in relation for specific projects where we think organoids will provide a significant advantage, and more information will be gained by using them compared to 2D cell lines.

We are using lung organoids, prostate organoids, and gastric organoids as our main models. Organoids are especially useful when we have a requirement to mimic the tumor microenvironment (TME) and when we need a certain complexity that has to be incorporated, either coming from the project or in the specific question that is asked. When we want to validate that the therapeutic effect is better defined in a 3D system, or for example, into a patient with a more context-related situation, then organoids are well-fitted for this.

Q What are the key barriers that the TME poses to developing and potentially translating new therapies?

EDB: The TME remains a bit of a black box. We are aware of it, and we know that it has a very profound effects on most of the therapies that we are using. If we consider I–O or cell therapy approaches, we know that when they are used against liquid tumors like lymphoma, or certain type of leukemias, they can be very efficient. However, not all patients respond to these therapies, and in particular translating the success of cell therapies in blood cancers to solid tumours has proved challenging. It is clear that there are aspects of the TME that we still don't know enough about.

We do have some initial knowledge — for example, that it has a specific metabolic profile. We also know that it has a certain immunosuppressive component to it, but the exact role of the tumor stroma is still unclear. There are still many different questions around what it does, how it supports the tumor, and how it blocks the immune compartment or even reduces compound effects.

For example, we and others have shown that in the TME you have much higher secretion of lactate. This lactate is not metabolized by T cells, but it is metabolized by immunosuppressive regulatory T cells (Tregs). We can visualize this gradient of a high concentration of lactate in the TME, and then a gradient of lactate with glucose at the invasive front, and then mainly glucose in the stroma (Figure 1). This remains a physical barrier for T cells to be able to infiltrate that microenvironment. The lactate is metabolized by Tregs and helps in recruiting the Tregs, and then you have different elements that are secreted from the tumor cells or the cancer associated fibroblasts (CAF) like transforming growth factor(TGF)-beta, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-10 and many others that polarize the different immune cells to become immunosuppressive. You have the tissue-resident macrophages being polarized into M2 macrophages or tumor-associated macrophages (TAMS). You have the myeloid-derived suppressor cells (MDSCs) and Tregs — all these different immunosuppressive types of cells that are reducing the efficacy of T cells in the immune compartment.

There is a very good paper from Rachel Rosenthal from Charles Swanton's team at The Francis Crick Institute, where they analyzed the immunosuppressive compartment in several lung tumors. They found that almost every tumor has very different tumor microenvironment

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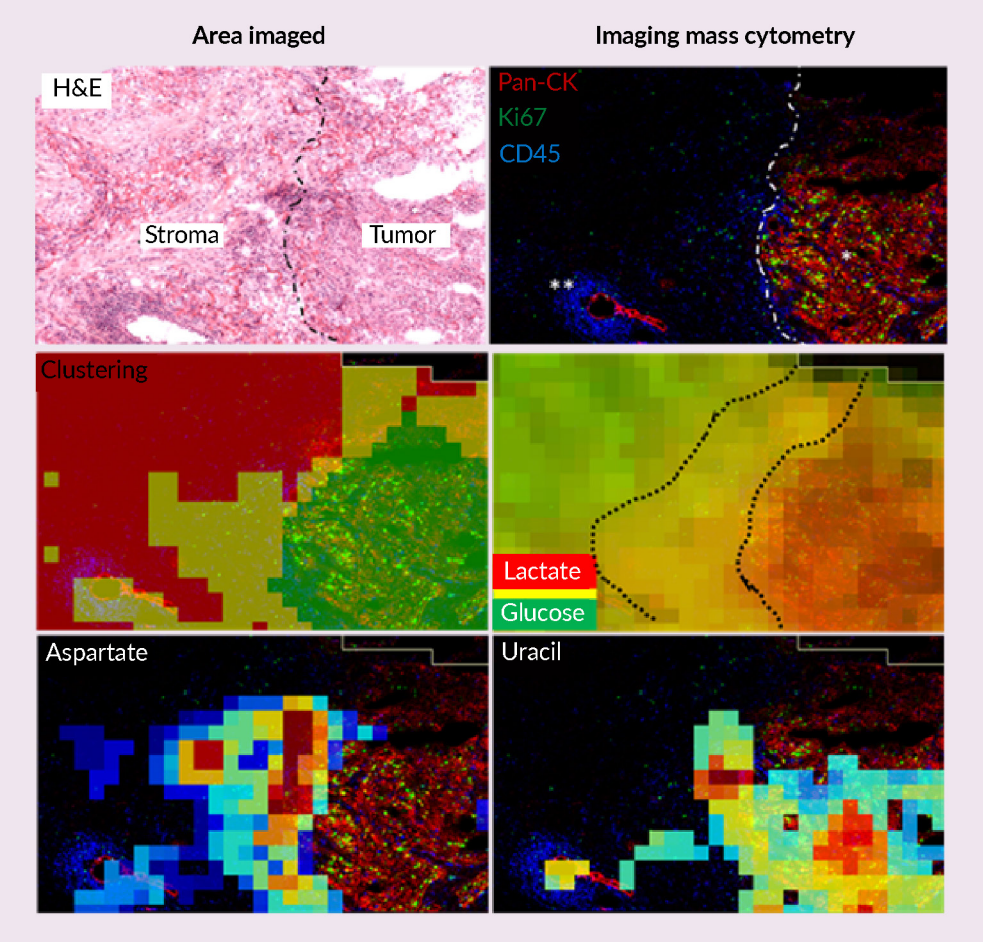
FIGURE 1

Spatial integration of the metabolome and proteome, cellular markers on tissue to discover new biological insight.



cellular types, and that the localization of these immunosuppressive cells is not uniform. Some parts of the tumor have a much higher concentration of immunosuppressive cells than others. That adds an extra layer of complexity to the TME. We are trying to add selectively one after the other — for example trying to understand the effect of adding just Tregs. We have seen that it reduces the efficacy of immunotherapies; we see the same thing with TAMs and MDSCs. But what is the right mixture of these cells? We know that from patient to patient it is going to be very variable. In turn, what are the effects of these variabilities on cell therapies?

Q What for you is the current state-of-the-art when it comes to the current tools we have to help understand the TME?

EDB: One approach is using single cell sequencing to be able to decipher what kind of cells you have in a tumor. You have the expression profiles of the specific type of cells, but then you lose the spatiality of the different cells and the depth of sequencing. The

"I envision more tailoring and better armoring of cell therapies with the ability to bypass those immunosuppressive barriers and metabolize metabolites that are present in the TME, so they can perform properly in that environment."

spatial component is therefore another important aspect to measure, using tools like CyTOF or imaging mass cytometry. Then you have the spatial transcriptomics or the spatial proteomics, and also everything linked to the metabolomics of the TME. All of these different elements let you assess different aspects of the TME. We need to better understand what shapes a particular TME, in order to understand how it reacts, and in turn how we would be able to then tailor a therapy to be able to respond to that specific TME.

Q How do you see therapeutic approaches evolving as our understanding of the TME continues to improve?

EDB: I envision more tailoring and better armoring of cell therapies with the ability to bypass those immunosuppressive barriers and metabolize metabolites that are present in the TME, so they can perform properly in that environment. A lot of the current cell therapies exhaust quickly, so they have a very short lifespan in the TME. Having an approach that can help these therapies to survive longer in these harsh conditions will be an important consideration when developing new therapies.

Q What should be the next specific goals for the field in terms of further improving our understanding of the TME?

EDB: We need to create a TME database to try to better understand the different elements that are in play and how they interact with each other. A lot of people are predicting that in the long run, we will have more and more tailored therapies, especially if we can improve on the ways we derive organoids. One vision would be to take a biopsy from the patient and grow it *in vitro*, then perform the screening of mono or combination therapy on the tumor sample of the patient to determine the right combination of therapies, before applying that tailored treatment to the patient.

There is a theory that the issue with the way we are treating tumours currently is that many standard of care treatments use one inhibitor, one compound, or one chemotherapy to kill the majority of the tumor cells. With this approach you favor the generation of resistant cancer cells, or you are creating so much stress that the stem cell-like part of the tumor goes into a

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dormant state and becomes resistant to that kind of therapy. The ideal approach is to attack the tumor in different ways or attack different facets, so that even if the initial compound will lead to a form of resistance, we already have something being introduced in the initial therapy that will block those tumors from developing any form of resistance or dormancy.

Q What would you consider the biggest challenges facing the cell therapy space today, particularly when considering the nonclinical toolkit?

EDB: It is a nascent field in a way, we and others have progressively realized that it's not a straightforward process. There are several limitations to chimeric antigen receptor (CAR)-T cell therapy, including limited efficacy against solid tumors, inhibition and resistance in B cell malignancies, antigen escape, limited persistence, poor trafficking and tumor infiltration, and the immunosuppressive microenvironment,

We are working to find ways to make treatment more efficient, but it is akin to a puzzle with parts that are very complicated to piece together, and you have limited time to solve it. At the same time, it is rewarding work because we know that for all the things that we do in the lab *in vitro*, there are patients who would potentially benefit from it in the long run. We aim to be as accurate as possible while also working as quickly as we can.

Another issue we have is that the quantity of material that we have to work with is quite limited. This is especially true if we want to be able to take a tumor sample, plus the normal adjacent tissue to have a normal counterpart, and then have everything matched to it — so having the peripheral blood mononuclear cells and being able to then use them to create Tregs, TAMs, and MDSCs, and then also create the CAR-Ts that would mimic the live patient context. This is very challenging. Some people say that we are able to recreate the TME in mice, but it's not truly feasible using a mouse immune system. If you are putting a human CAR-T in it will not be facing a proper TME. For example, if you take some patient samples and create patient-derived xenografts, we know that the stromal part of those tumors are very quickly replaced by the fibroblasts of the mice. The same thing occurs with the immune compartment — you will not have the immunosuppressive compartment of the mouse starting to try to protect the human tumor. You are creating a biased system. However, we are working on this to see if we would be able to have more of a human tumor with the immunosuppressive compartment, and the human stromal compartment, in a mouse setting.

Q Are there any emerging tools or approaches that you think will help address some of the current challenges?

EDB: Getting more patient data would be fantastic because that will pave the way forward for understanding how to develop more efficient therapies. In addition to acquiring data, it is also about collecting it in a uniform way. Everybody is focusing on their

field of research and this results in the data being heterogeneous, which makes it difficult to compare and/or combine different datasets.

Additionally, some people are only going to be doing only part of the omics research, and so, will have only part of the data. But since it is coming from a patient sample, you cannot go back to it because when it is used, it is used. Trying to maximize the information we can get from the samples that we have, and having more communication and standardization between different groups, will enable us to create the most comprehensive database possible.

Some more complex models are being created to try to mimic the tumor and the TME by adding the different immunosuppressive cells, the cancer associated fibroblasts, the stromal compartment and the vascularization. At the polar opposite, you can use a tumor tissue and dissociate them into small tumoroids that can be then screened for different therapies. They have retained most of the initial tumor properties, but you are very limited in the amount of assays that can be performed and the type of tumors since you are dependent on patient samples coming from surgery.

BIOGRAPHY

ETIENNE DE BRAEKELEER has received his PhD from the Medical Faculty in Brest (France) and was awarded a postdoc fellowship from the German Cancer Center in Heidelberg where he joined Andreas Trumpp's lab. During his second postdoc at the Wellcome Sanger Institute and at the Cambridge Stem Cell Institute, with George S Vassiliou and Kosuke Yusa, he has performed several genome wide CRISPR screens and validated several potential therapeutic targets. Now in AstraZeneca, as a senior Research Scientist, Etienne is the scientific lead of the lung organoid program. He has developed several organoid capabilities to support various oncology and biopharma programs.

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