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

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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

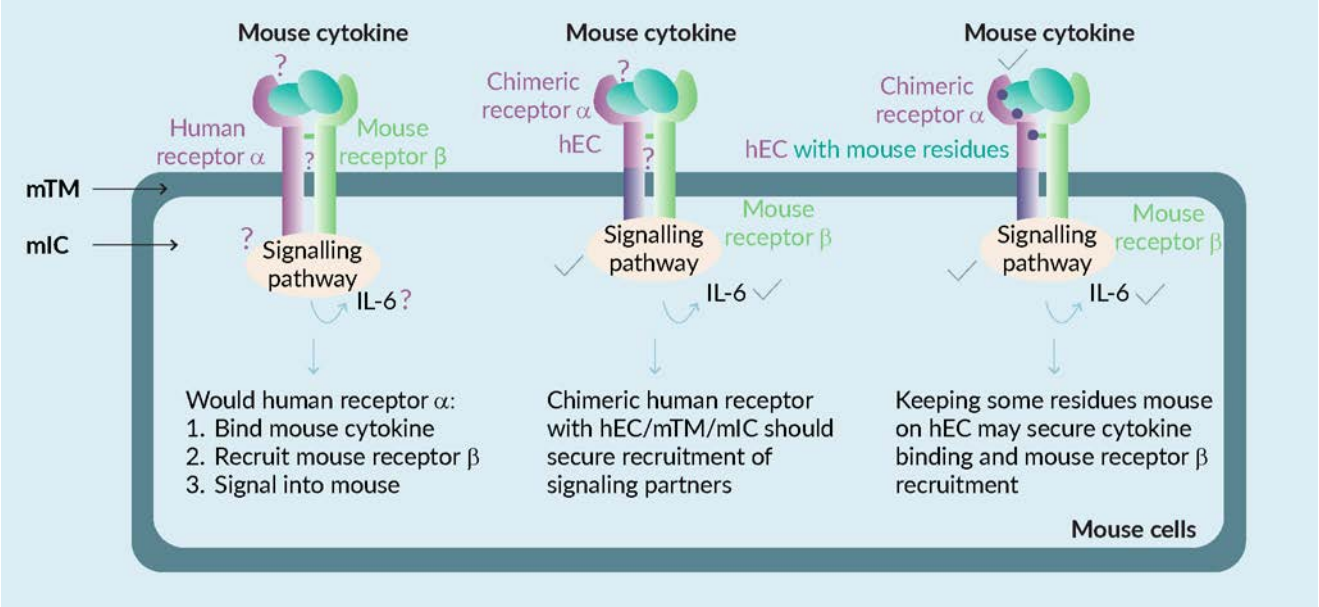
Several models are available in the field. The model discussed here, named BRGSF–HIS, has the advantage of exhibiting both human lymphoid and myeloid compartments.

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-γ), 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.

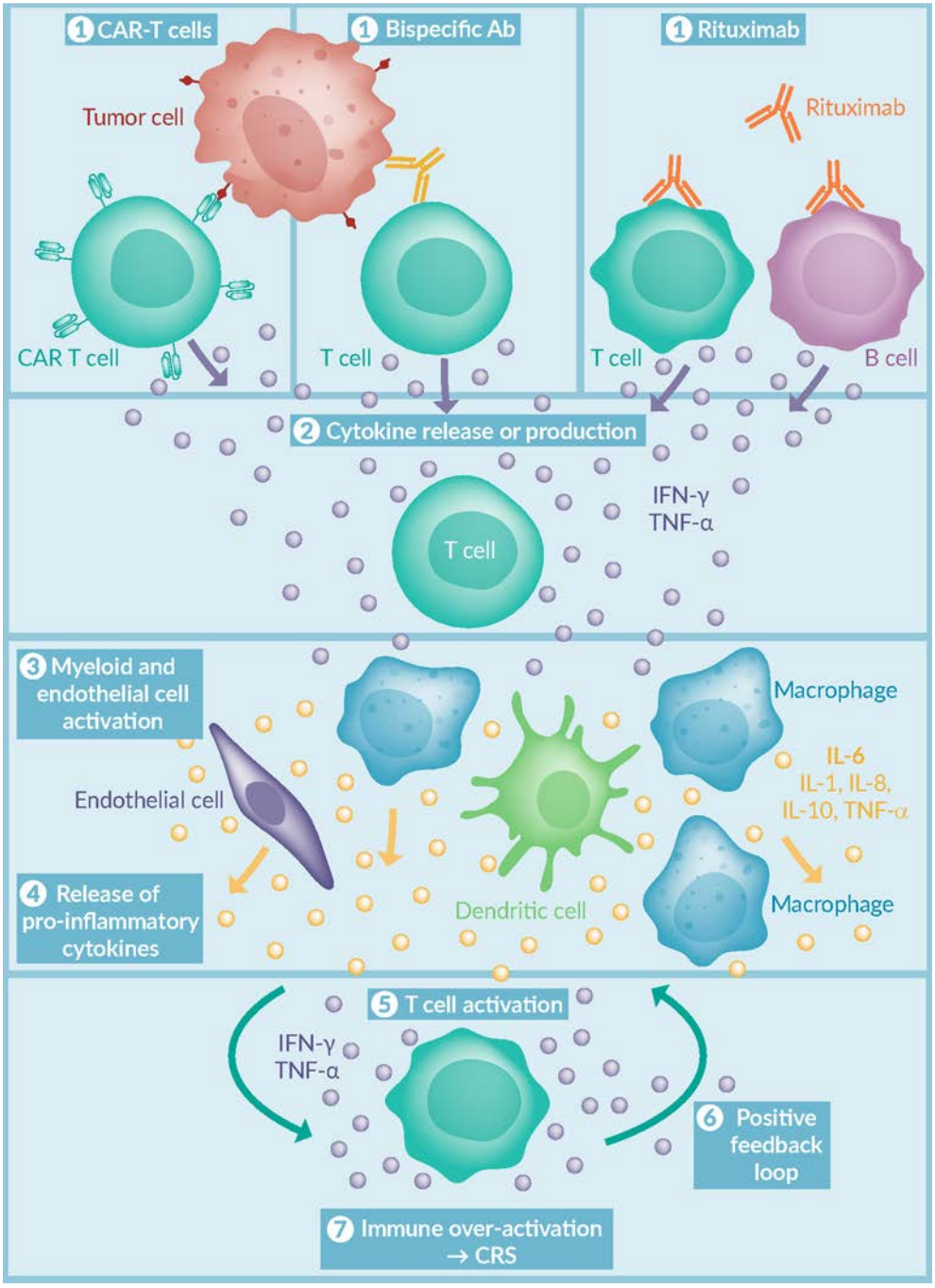
Pharmacokinetic studies were then performed in humanized VISTA KI mice developed by genOway and non-human primates (Figure 4). SNS-101 displayed a favorable single-dose pharmacokinetic profile, with no significant identified TMDD .

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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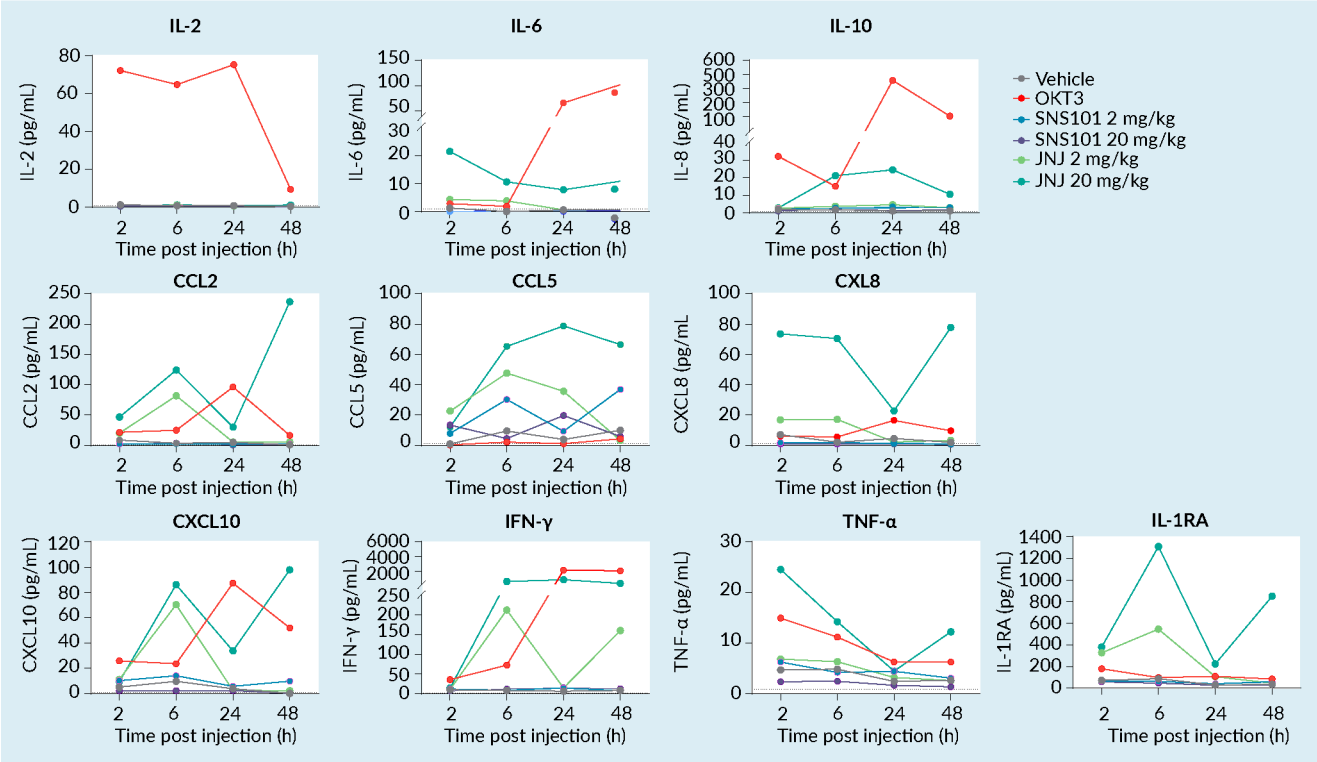
FIGURE 3

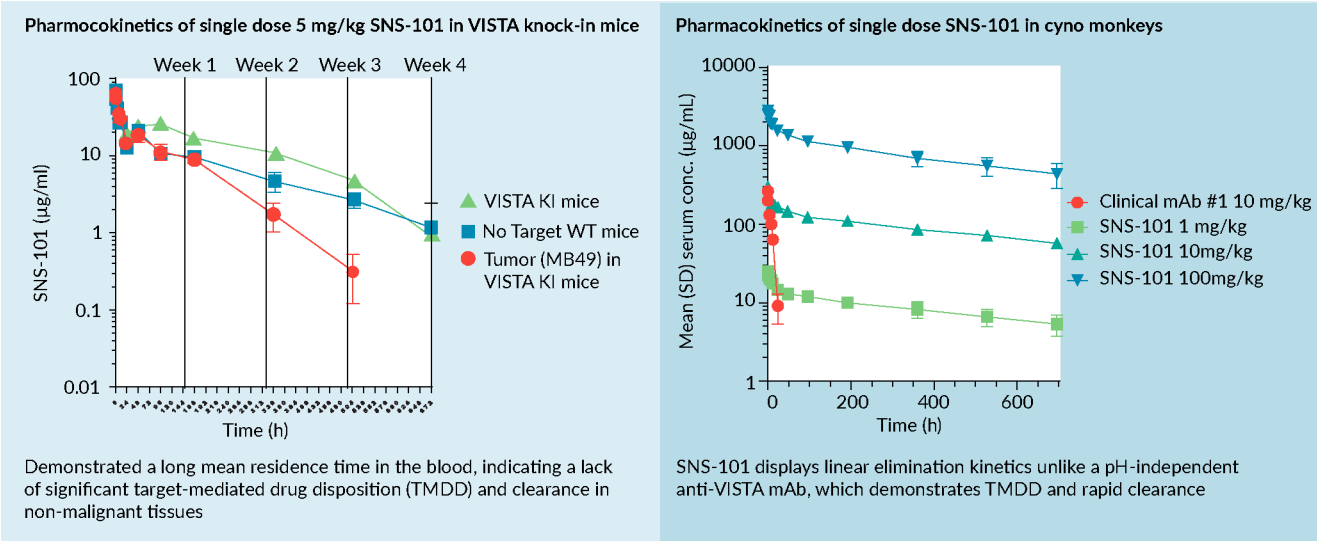
Induction of cytokines in myeloid-boosted BRGSF-HIS mice upon treatment with OKT3, SNS-101, and JNJ.



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

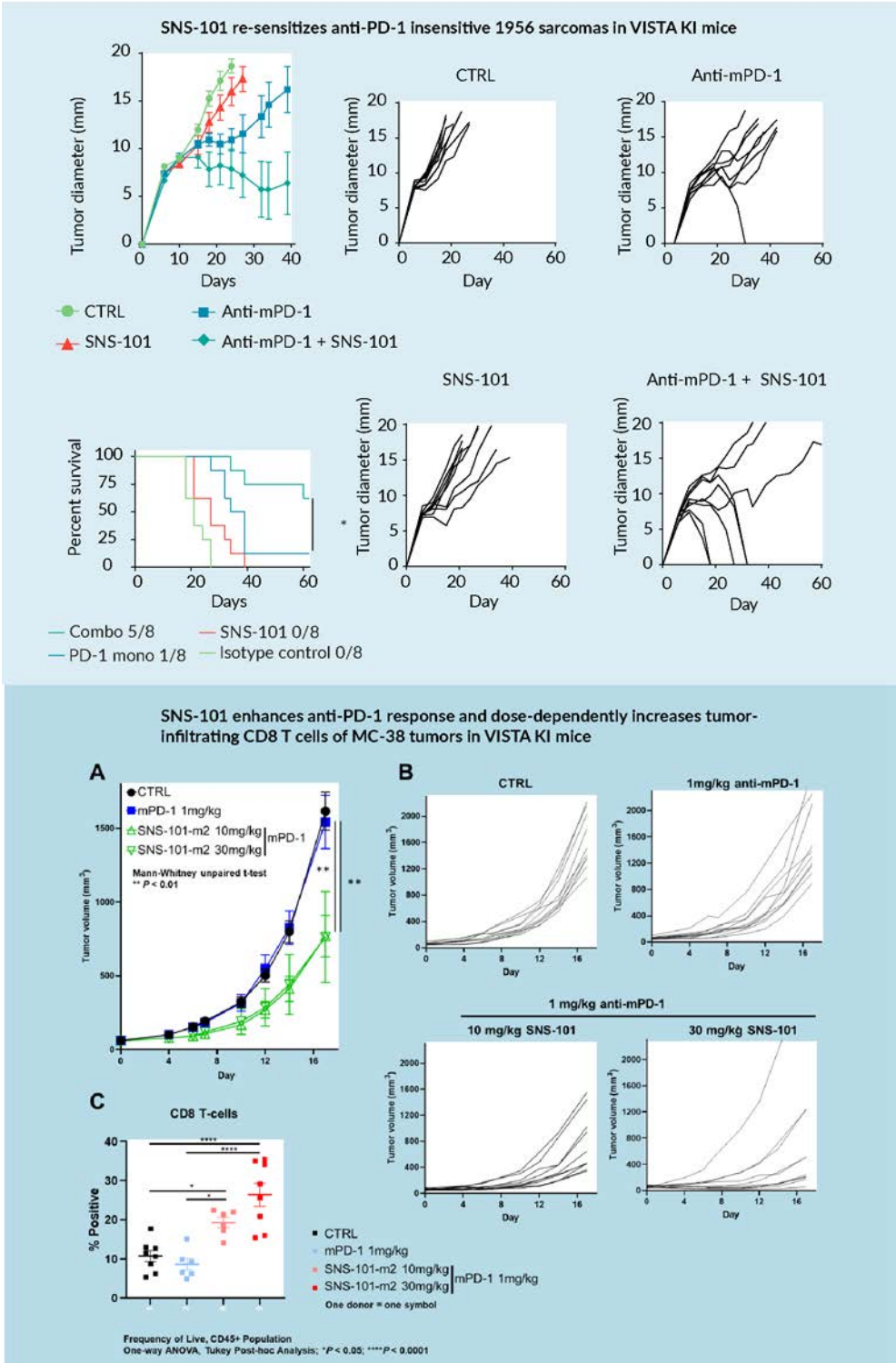
SNS-101 displays linear elimination kinetics unlike a pH-independent anti-VISTA mAb, which demonstrates TMDD and rapid clearance

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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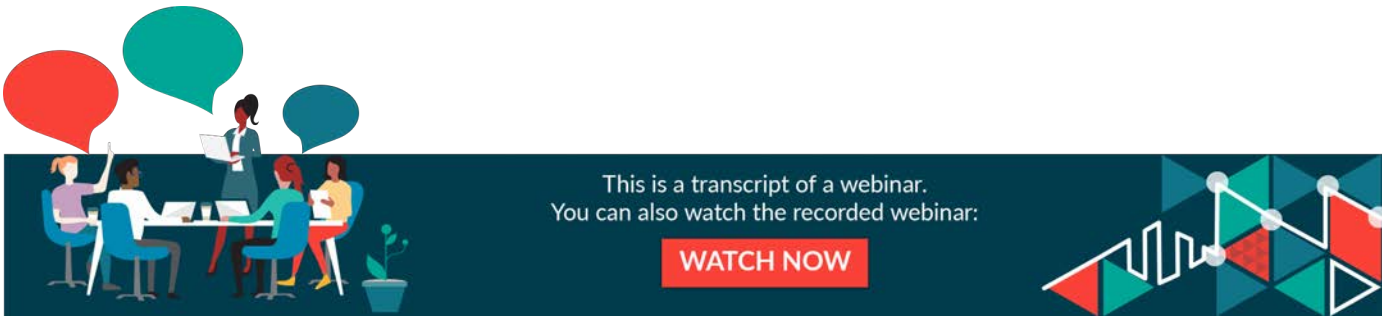

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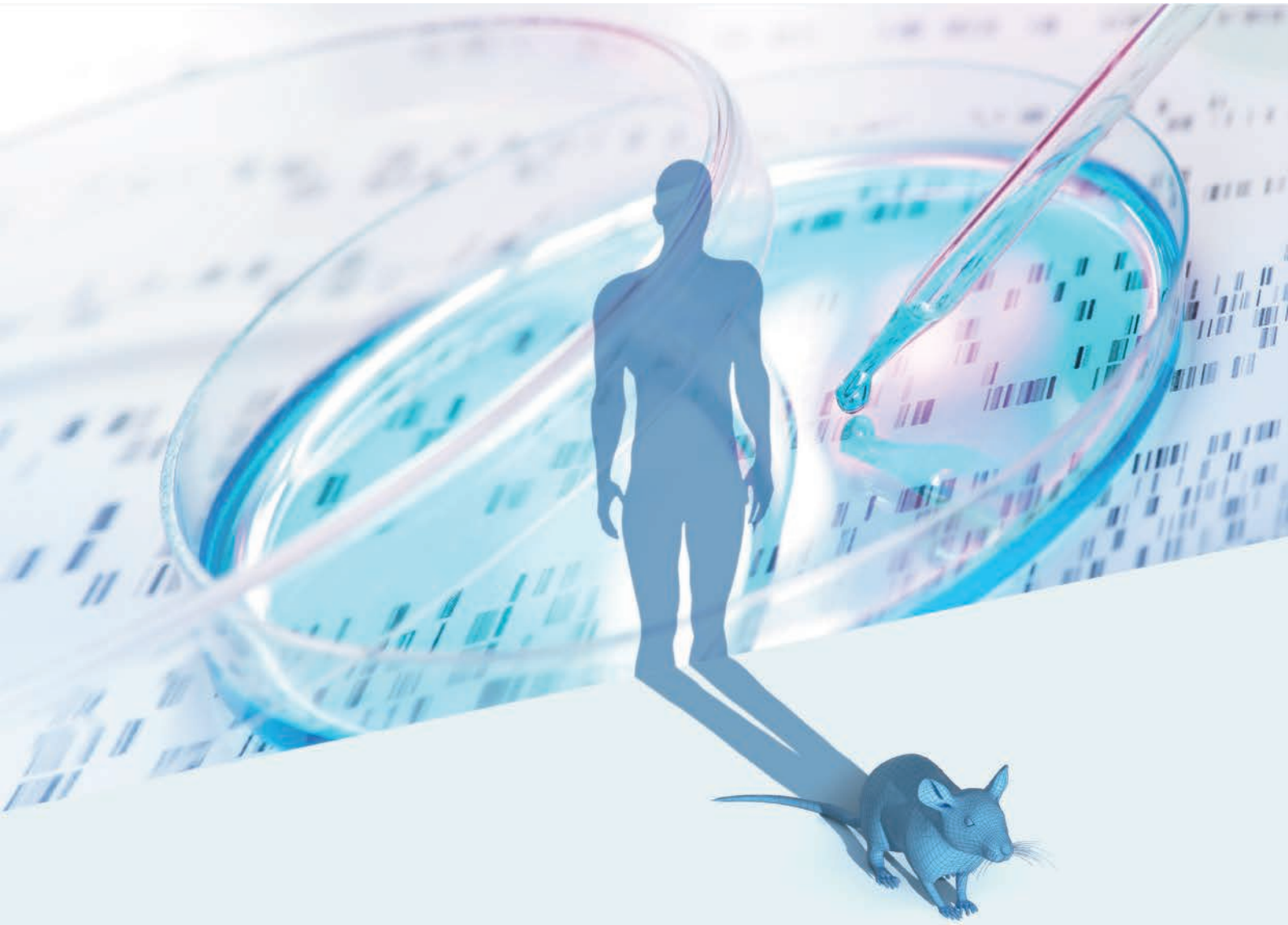

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