INVESTIGATOR'S BROCHURE

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DOCUMENT HISTORY AND SUMMARY OF CHANGES

Document history

Edition	Release Date
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A high-level summary of changes from Edition 3.0 to Edition 4.0 is presented below:

Section	Change
1 Summary 5 Effects in Human 6 Guidance to the Investigator	Reorganize by subsection and add emerging data from the two ongoing clinical studies study SBT777101-01 in RA and study SBT777101-02 in HS.
7 Reference Safety Information	• Moved from Section 6.4.1
General	• Update list of abbreviations • Minor clarifications and alignment across document • Use consistent terminology (e.g., participant, study) throughout the document

LIST OF ABBREVIATIONS

Table 1: Abbreviations and Specialist Terms

Abbreviation	Description
ACPA	Anti-citrullinated protein antibodies
ACR	American College of Rheumatology
ADA	Anti-drug antibody
AE	Adverse event
bDMARD	Biologic disease-modifying anti-rheumatic drug
°C	Degrees Celsius
CAR	Chimeric antigen receptor
CCP	Cyclic citrullinated peptides
CD	Cluster of Differentiation
CFSE	Carboxyfluorescein succinimidyl ester
CIA	Collagen induced arthritis
Cit-Prot	Citrullinated proteins
CMV	Cytomegalovirus
CRES	CAR T-cell related encephalopathy syndrome
CRP	C-reactive protein
CRS	Cytokine release syndrome
csDMARD	Conventional synthetic disease-modifying anti-rheumatic drug
CTV	CellTrace™ Violet
CV	Citrullinated vimentin
DAS	Disease Activity Score
DLT	Dose limiting toxicity
DMARD	Disease-modifying anti-rheumatic drugs
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EC_{50}	Half maximal effective concentration
EGFR	Epidermal growth factor receptor
FDA	Food and Drug Administration
FOXP3	Forkhead Box P3
GFAP	Glial fibrillary acidic protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GRP78	Glucose-regulated protein, 78-kDa
GvHD	Graft versus host disease
HLA	Human leukocyte antigen
HS	Hidradenitis suppurativa
HSP70	Heat shock protein 70
ICANS	Immune effector cell-associated neurotoxicity syndrome
ICH	International Conference on Harmonization
IFNγ	Interferon-gamma
IgG1	Immunoglobulin G1
IL	Interleukin
IND	Investigational New Drug
IV	Intravenous
JAK	Janus kinase
LN	Lymph node

Abbreviation	Description
MSC	Mesenchymal stem cells
MTX	Methotrexate
NET	neutrophil extracellular traps
NETosis	regulated neutrophil cell death
NOAEL	No observed adverse effect level
PAD	Peptidylarginine deiminase
RA	Rheumatoid arthritis
RCL	Replication-competent lentivirus
RNA	Ribonucleic acid
SAD	Single ascending dose
SAE	Serious adverse event
scFv	Single-chain variable fragment
SLE	Systemic lupus erythematosus
T1D	Type 1 diabetes
TCR	T-cell Receptor
TNF	Tumor necrosis factor
Teff	Effector T cell
Treg	Regulatory T cell
tsDMARD	Targeted synthetic disease-modifying anti-rheumatic drug
TSDR	Treg-specific demethylation region
USA	United States of America
VICM	Citrullinated and matrix metalloproteinsase-degraded vimentin

1. SUMMARY

1.1. Investigational Product

SBT777101 is a cryopreserved ex vivo expanded autologous $CD4^{+}CD127^{lo/-}CD25^{+}$ regulatory T cell (Treg) preparation that has been transduced with a lentiviral vector encoding both a chimeric antigen receptor (CAR) specific for citrullinated proteins (Cit-Prot) and a modified epidermal growth factor receptor (EGFR) tag. The Sponsor intends to target Cit-Prot in the extracellular matrix at sites of inflammation using SBT777101 as a novel approach for Treg therapy in autoimmune diseases.

1.2. Nonclinical Overview

The nonclinical studies documented the Treg phenotype of SBT777101 via FOXP3 expression, FOXP3 Treg-specific demethylated region (TSDR) analysis, cytokine profiling, and regulatory/immunomodulatory functions in vitro. Studies also demonstrated that the SBT777101 CAR specifically recognizes Cit-Prot and does not exhibit off-target binding. An immunohistochemistry-based tissue cross reactivity study showed that the SBT777101 CAR mostly stains cytoplasmic and nuclear elements in multiple cell types and that it is associated with membranous staining only in rare epithelial and mononuclear leukocytes in various tissues. Staining of extracellular material was also observed in various tissues. The nonclinical assessment of SBT777101 demonstrated in vivo that SBT777101 does not cause adverse events towards normal tissues including under proinflammatory conditions, that SBT777101 is not activated in vivo within normal tissues, that SBT777101 exhibits a stable Treg phenotype under pro-inflammatory conditions, and that SBT777101 exhibits an immunoregulatory activity in vivo that is similar to the activity of untransduced polyclonal Tregs. Insertion site analyses exhibited a multi-site integration site profile with no dominant integration site observed, which is consistent with numerous published studies of lentiviral vectors similar to that used in the SBT777101 vector. The SBT777101 CAR Treg cells did not show any abnormal growth activity in the absence of exogenous interleukin (IL)-2.

1.3. Clinical Overview

The clinical development program for SBT777101 includes two ongoing open-label Phase 1 studies, each evaluating single ascending doses (SAD) of SBT777101 administered intravenously (IV). REGULATE-RA (study SBT777101-01) is enrolling participants with moderate to severe rheumatoid arthritis (RA) and REGULATE-HS (study SBT777101-02) is enrolling participants with severe to moderate hidradenitis suppurativa (HS).

2. INTRODUCTION

2.1. SBT777101 Mechanism of Action

SBT777101 is a cryopreserved ex vivo expanded autologous $CD4^+CD127^{lo/-}CD25^+$ Treg cell preparation that has been transduced with a lentiviral vector encoding both a modified EGFR tag and a chimeric antigen receptor (CAR) specific for immunodominant post-translationally modified citrullinated proteins (Cit-Prot); see Section 2.2.1. SBT777101 is administered intravenously and is anticipated to traffic to the site of immune-mediated inflammation where it is anticipated to encounter antigen in the extracellular matrix. Upon binding of Cit-Prot to CAR Treg cells (SBT777101), these cells will be activated and exert functionality through direct and local bystander suppression. Tregs, and thus SBT777101, have an inherent ability to traffic to inflamed tissues (Campbell, 2015) and expression of the CAR for the citrullinated antigens overcomes the need for antigen presentation to support localized expansion of SBT777101 and Treg activity. This multifaceted functionality of Tregs to mitigate inflammation includes suppression via cytokine production (e.g., IL-10); expression of inhibitory checkpoint receptors (e.g., CTLA-4); increased CD25 expression, which acts as an IL-2 sink that starves T effector (Teff) cells, and metabolic reprogramming through indoleamine 2,3-dioxygenase (IDO) and adenosine generation. Together these activities should result in reduced numbers and function of inflammatory cells. Finally, Treg cells have been shown to produce factors, such as amphiregulin, which can mediate tissue repair contributing to the multifaceted activity of CAR Tregs (Arpaia et al., 2015; Lei et al., 2015).

The net result of the adoptive transfer of expanded Tregs is expected to result in long-lived and persistent activated Tregs that maintain their phenotype, resulting in the reduction of inflammation and resolution of symptoms. This persistence and phenotypic durability are hypothesized to result in long term improvements of disease and durable response.

2.2. Scientific Rationale

2.2.1. Citrullinated Proteins and Autoimmunity

There is strong scientific evidence for the presence and contribution of Cit-Prot in the autoimmunity of RA and HS (Darrah and Andrade, 2018; Fox, 2015; Holers, 2013; Byrd et al., 2019). Autoimmune diseases that have chronic activity demonstrate strong staining of Cit-Prot specifically at the sites of inflammation. There is well-documented evidence of the presence of deposits of Cit-Prot in the joints and tissue of RA patients and HS skin (Fox, 2015; Byrd et al., 2019). While the exact mechanism of pathogenesis is not fully elucidated, it has been shown that citrullinated protein autoantigens can in some individuals induce T-cell -dependent B cell activation (Szili et al., 2014; Sokolove, 2019). This activity leads to anti-citrullinated protein antibody (ACPA) production by autoreactive B cells and activates pro-inflammatory mediators, which subsequently may lead to chronic inflammation. Cit-Prot can be highly immunogenic, and some patients with RA or HS make ACPA early in the course of their disease, implying that autoimmunity may be present long before the development of overt disease manifestations (Kroot et al., 2000; van der Linden et al., 2009; Renner et al., 2014). Data have shown that ACPA can react with several Cit-Prot including collagen, filaggrin, fibrinogen, α enolase, and vimentin (Aggarwal et al., 2009; Sokolove et al., 2012; Frew et al., 2020a). Citrullination can happen independent of ACPA positivity (Won et al., 2021) demonstrating that citrullination is part of the pathology of disease independent of ACPA formation.

Citrullination of proteins by peptidylarginine deiminase (PAD) enzymes is an irreversible posttranslational modification. The citrullination pathway impacts protein structure that regulates histones, the cytoskeleton, and function of secreted proteins (Witalison et al., 2015). It has been shown that PAD enzymes are strongly expressed in myeloid cells, including macrophages and neutrophils. PAD enzymes play an important role in neutrophil extracellular traps (NETs), a phenomenon that externalizes autoantigens and immunostimulatory molecules (He et al., 2018). During NETosis (a regulated form of neutrophil cell death that contributes to the host defense against pathogens), neutrophils externalize citrullinated autoantigens, releasing Damage Associated Molecular Patterns (DAMPs) as innate immune activators, which are implicated in driving RA pathogenesis, including but not limited to vimentin and α-enolase (Khandpur et al., 2013). While expressed intracellularly, aberrant PAD activity can lead to the deposition of extracellular Cit-Prot in many tissues, including the joints, lungs, lymph nodes, skin, and periodontal tissues in patients with inflammatory disease (Musaelyan et al., 2018). Thus, Cit-Prot are a common component of the inflammatory milieu in RA and HS.

2.3. T Cells and Autoimmunity

T cells are lymphocytes developed from bone marrow-derived stem cells that are selected and differentiate in the thymus and play a key role along with B cells in the adaptive immune response (Alberts et al., 2002). T cells have many functions, including mediating cell death of virally infected and cancerous cells, producing cytokines and chemokines to recruit other immune cells, and activation of B cells, including antibody production and immunoglobulin class switching (Alberts et al., 2002). These T cells are referred to as T effector (Teff) cells when active. They consist of $CD4^+$ and $CD8^+$ subsets. The $CD4^+$ T cells are considered helpers in promoting both CD8 T cells and B cell responses, whereas the $CD8^+$ T cells are often more cytotoxic and not very efficient in helping $CD4^+$ T cells and B cells. While important for preventing illness from pathogens and cancer, self-reactive $CD8^+$ T cell responses can lead to damage of healthy tissue, and the development of autoimmune disease. Thus, immune homeostasis, commonly known as immune tolerance, requires a counterbalance to regulate immunity.

Peripheral immune regulation is governed by a specific T cell subset, regulatory T cells (Tregs), that function as the master controller of autoimmunity. Tregs, defined by the expression of the transcription factor FOXP3 and the expression of self-reactive T cell receptors (TCRs), are critical in controlling the homeostasis of the immune system both systemically and during localized immune responses (Ochs et al., 2007; Rudensky, 2011; Ramsdell and Ziegler, 2014). Patients deficient in FOXP3 function and thus, Tregs, develop IPEX disease, an often-lethal disease characterized by systemic autoimmunity.

Tregs use multiple mechanisms to balance immunity: directly through interactions with the same antigen presenting cell of the Teff cells, and indirectly via bystander suppression where a self-antigen promotes Treg activity, including cytokine production, to dampen a response of nearby Teff cells reactive to different antigens. Treg cells can also inhibit innate immune cell activation. Finally, Tregs can influence other cells recruited to the inflamed microenvironment to become regulatory cells, so-called "infectious tolerance" reinforcing the regulatory response (Vignali et al., 2008). This multifaceted activity of Tregs provides a means to reduce inflammation with one cell type but via many different mechanisms. In addition, Tregs also promote tissue repair by releasing various tissue repair and stem cell factors. These varied activities are dependent on the activation of the Treg by its respective antigen. Thus, Treg and Treg-supportive immune therapies are being developed to promote immune therapeutic effects to promote immune homeostasis in a variety of autoimmune and transplant related diseases.

2.3.1. Nonclinical Antigen-Specific Treg Studies

Preclinical studies have shown that polyclonal autologous Tregs, as well as those with selective alloantigen specificity have the potential to treat systemic inflammation and organ injury (Bluestone and Tang, 2018).

Transfer of Treg cells in mouse models of RA disease has been shown to suppress disease in vivo (Sun et al., 2018). Adoptive transfer of antigen-specific Treg cells generated in vitro by culturing $CD4^+$ T cells from established collagen-induced arthritis (CIA) mice was shown to reduce clinical scores over time and to reverse CIA progression compared to untreated control animals. There are no established preclinical models of HS.

Similar studies have been performed in a variety of autoimmune-prone rodent models. For instance, expanded islet antigen specific Tregs prevent the transfer of diabetes by spleen and lymph node (LN) cells in a spontaneous mouse Type I diabetes (T1D) model (Tang et al., 2004). Antigen-specific Tregs from the autoimmune-prone non-obese diabetic (NOD) mice were expanded in vitro using a combination of anti-CD3, anti-CD28, and interleukin 2. Expanded polyclonal NOD Tregs and activated Teff cells were then co-transferred in vivo to NOD.RAG (recombination activating gene) mice. The data showed that transfer of antigen-specific Tregs reversed diabetes in the mice compared to controls. Importantly, the expanded antigen-specific Tregs were far more efficient than polyclonal NOD Tregs in preventing the onset of diabetes.

These studies demonstrated that antigen-specific Tregs are strong therapeutics agents when transferred in autoimmune models. In preclinical models, the antigen is often known and/or the model is of syngeneic mice which removes allogeneic and major histocompatibility complex (MHC) barriers that are present in the clinical setting antigen-specific TCR therapies. One way to overcome these barriers is to introduce a chimeric antigen receptor (CAR). The binding domain of the CAR is not restricted to MHC and provides an additional co-stimulation signal not provided by a TCR (Dawson et al., 2020; Salter et al., 2021). This allows the CAR Treg cell to circumvent the need to interact with an antigen-presenting cell and the need to discover autoantigens that are presented efficiently in disease.

Preclinical studies with CAR Tregs have also demonstrated activity in several inflammatory models. One of the earliest CAR Treg studies showed that Tregs expressing a CAR specific to 2,4,6-trinitrobenzenesulfonic acid (TNBS) suppressed colitis only when TNBS was present (Elinav et al., 2008). This study also demonstrated that CAR Tregs were more potent than polyclonal Tregs, which are historically strong regulators of colitis in mouse models. In a mouse model of islet allografts, CAR Tregs specific to the major histocompatibility complex (MHC) of the islet donor prevented rejection whereas a similar dose of polyclonal Tregs did not promote survival (Pierini et al., 2017). Lastly, in a model of asthma, CAR Tregs specific to an epithelial antigen expressed in the lung reduced IgE and disease pathology in the lungs whereas polyclonal Tregs only conferred minor protection compared to the CAR Tregs (Skuljec et al., 2017). These preclinical studies with mouse CAR Tregs demonstrate improved anti-inflammatory activity of adoptively transferred CAR Tregs over polyclonal Treg therapy.

Studies performed with human CAR Tregs in humanized mice (mice with human PBMC or skin grafts) demonstrate similar results to syngeneic mouse studies in the prevention of allograft rejection (Noyan et al., 2017), reduction of graft versus host disease (GvHD) (Dawson et al., 2019) and (Dawson et al., 2020) and prevention of autoimmune antibody responses (Yoon et al., 2017) . As with the immune competent mouse studies, CAR Treg specific to human leukocyte antigen A2 (HLA-A2) prevented skin allograft rejection whereas polyclonal Treg transfer did not demonstrating that endowing a Treg with a CAR to create a functional antigen-specific response provides a benefit over treatment with polyclonal Tregs.

Collectively, these preclinical experiments demonstrate the conferring Tregs with antigen specificity via TCR or CAR expression, provides therapeutic benefits greater than that demonstrated with polyclonal Tregs.

2.3.2. Clinical Treg Studies

Polyclonal Treg therapies have been tested as a potential therapy in patients with autoimmune conditions, including Type 1 diabetes, kidney and liver transplantation, Crohn's disease, systemic lupus erythematosus, pemphigus, and graft versus host disease (Bluestone et al., 2015; Brunstein et al., 2011; Chandran et al., 2017; Dall' Era et al, 2019; Desreumaux et al., 2012; Di Ianni, 2011; Marek-Trzonkowska et al., 2014; Mathew et al., 2018; Roemhild et al., 2020; Todo et al., 2016; Trzonkowski et al., 2009; NCT03239470, 2022) (Table 2).

Table 2: Summary of Key Clinical Polyclonal Treg Studies and Case Reports

Study ID	Phase	N	Product	Dose	Study Status	Safety
				Type 1 Diabetes		
(Bluestone et al., 2015) NCT01210664	Phase 1	14	Autologous $CD4^+CD127^{lo/-}CD25^+$ polyclonal Treg	5×10^6 to 2600×10^6 cells	Completed	Infusions were well tolerated. No cytokine release, infusion reactions, or infectious complications.
(Marek-Trzonkowska et al., 2014) ISRCTN06128462	Not reported	12	Autologous $CD4^+CD25^+FOXP3^+$ Tregs	Up to 30 x 10^6 cells/kg	Completed	Adverse events (AEs) of flu, gastroenteritis and sinusitis resolved No serious adverse events (SAEs).
				Liver and Kidney Transplantation		
(Mathew et al., 2018) NCT02145325	Phase 1	9	Autologous $CD4^+CD25^+CD127^-$ $FOXP3^+$ Tregs	500×10^6, 1000×10^6, 5000×10^6 cells	Completed. Results reported	No clinical AEs (infection/rejection) De novo donor specific antibody development (n=2; associated with suboptimal immunosuppression due to drug intolerance and overt noncompliance)
(Chandran et al., 2017) NCT0208893	Phase 1	3	Autologous $CD4^+$ $CD127^{lo/-}CD25^+$ polyclonal Tregs	~320 x 10^6 cells (319, 321, and 363.8 x 10^6)	Completed	No infusion reactions No infections or malignancies observed over one year. One Grade 3 leukopenia, possibly related to study drug, resolved spontaneously. No treatment related SAEs 100% patient and graft survival 1 year, with no episodes of graft dysfunction or malignancy
(Roemhild et al., 2020) NCT02371434 EudraCT:2011-004301-24	Phase 1/2a	17 (11 treated)	$CD4^+CD25^+FOXP3^+$ Tregs	0.5×10^6, 1.0×10^6, $2.5\text{-}3.0 \times 10^6$ cells/kg	Completed	No treatment related AEs

Table 2: Summary of Key Clinical Polyclonal Treg Studies and Case Reports (Continued)

Study ID	Phase	N	Product	Dose	Study Status	Safety
(Todo et al., 2016) UMIN-000015789	Phase 1/2a	10	Allo-antigen-reactive CD4+CD25+FOXP3+ Tregs	23.3 x 10^6 to 143.8 x 10^6 cells/kg	Completed Results reported	One AE of CMV hepatitis without prophylaxis. Two AEs of CMV antigenemia without clinical manifestations. One patient with diabetic nephropathy required continuous hemodialysis post-transplantation. One AE of transient mild alopecia following CYC in 1 patient No SAEs
Autoimmune Disorders						
(Desreumaux et al., 2012) Crohn's disease Eudract no. 2006-004712-44	Phase 1/2a	20	Autologous ova-Tregs	1 x 10^6 to 1000 x 10^6 cells	Completed Results reported	Injections of ova-Tregs were well tolerated 54 AEs (2 related to study drug) 11 SAEs (3 related to study drug, all recovered)
(Dall' Era et al, 2019) Systemic Lupus Erythematosus NCT02428309	Phase 1	1	Autologous polyclonal $CD4^{+}CD127^{lo}CD25^{high}$ Tregs	100 x 10^6 cells	Completed Results reported	Not reported
Pemphigus NCT03239470	Phase 1	4	Polyclonal Tregs	1.0 x 10^8 cells	Completed Results not reported	No AEs of Grade 3 or higher No SAEs
Graft Versus Host Disease						
(Di Ianni, 2011) CEAS Umbria Protocol No 01/08.	Not reported.	28	HLA-matched non-autologous $CD4/CD25^{+}$ Tregs	Up to 260 x 10^6 cells if use weight of 65 kg	Completed	At a median follow-up of 12 months (range, 19-31), 12/26 (46.1%) patients were alive and disease free. Overall, 13/26 patients died due to venoocclusive disease (3), multiorgan failure (1), adenoviral infection (1), adenoviral infection and GVHD (1), GVHD (1), bacterial sepsis (1), systemic toxoplasmosis (1), fungal pneumonia (3), and central nervous system aspergillosis (1) during long-term follow up.

Table 2: Summary of Key Clinical Polyclonal Treg Studies and Case Reports (Continued)

Study ID	Phase	N	Product	Dose	Study Status	Safety
(Trzonkowski et al., 2009)	Not reported	2	Ex vivo expanded $CD4^{+}CD25^{+}CD127^{-}$ Tregs	0.1 - 3 x 10^6 cells/kg	Completed	For the case of grade IV acute GvHD Treg therapy only transiently improved the condition, for the longest time within all immunosuppressants used. No AE or SAE reported due to Treg therapy.
(Brunstein et al., 2011) NCT00602693	Phase 1	23	$CD4^{+}CD25^{+}FOXP3^{+}$ Tregs	0.1 × 10^6, 0.3 × 10^6, 1 × 10^6, 3 × 10^6 cells/kg (one cohort with 3 × 10^6 cells/kg on Day 15	Completed	No dose limiting toxicities (DLTs) were observed. 2 patients had grade 3 hypertension, one after infusion of a fresh and 1 after infusion of fresh then cryopreserved product, with all resolving with standard clinical management. 2 patients with Grade 2 neurologic changes prior to infusion, attributed to previously prescribed narcotic medication

All of the recipients in these studies tolerated the infusions well. There were no reports of infusion related reactions, cytokine release syndrome or neurotoxicity. Most events were non-serious and resolved without sequelae.

A Phase 1 study investigated the utility of autologous polyclonal Treg therapy as a treatment of patients with Type I diabetes (Bluestone et al., 2015). In this study, Tregs were isolated from patients and expanded ex vivo prior to being returned to patients as a single infusion of expanded cells. The study included four dose escalation cohorts ranging from 50 x 10^6 to 2600 x 10^6 expanded Tregs. The expanded Tregs retained their T cell receptor diversity and were well tolerated. Additionally, while the primary objective of the study was not to assess efficacy, nor was it powered to be able to interpret results in the context of age- and disease duration-dependent progression of disease, some of the diabetes-related clinical biomarker secondary endpoints suggested potential for efficacy, namely patients in the lower dose cohorts having longer stability of c-peptide and hemoglobin A1c (Bluestone et al., 2015).

Tregs in this study were tagged by metabolic labeling of cells with deuterium [6,6-2H2] glucose, enabling them to be tracked. Labeled cells were transferred into the patients and the Tregs analyzed for deuterium enrichment in the DNA. At Day 1, a significant percentage of deuterium label was present in the DNA of circulating Tregs. Treg cell numbers peaked at 7 to 14 days; approximately 25% of the peak labeling was still observed by Day 90. In a majority of patients, the labeled Tregs remained present in the circulation at greater than 10% of the total Treg subset at least 1 year after transfer (Bluestone et al., 2015).

It remains unclear whether the decrease of Tregs in the circulation was due to cell death or migration into the tissues, however, the remaining adoptively transferred Tregs remained phenotypically stable as virtually all of the deuterium label remained confined to cells within the $CD4^+CD127^{lo/-}CD25^+$ Treg cell population. Importantly, the data suggest that the infused Tregs remained stable, and did not transdifferentiate into detectable Teff cells over time, showing that the cell phenotype was maintained and continued to be safe and well tolerated (Bluestone et al., 2015).

Polyclonal Treg therapy has been further investigated as a possible treatment for other autoimmune conditions, including systemic lupus erythematosus (SLE) and pemphigus. A case report describes the effects of autologous adoptive Treg therapy to a patient with SLE with active skin disease (Dall' Era et al, 2019). In this case study, Treg cells tagged with a deuterium tracer illustrated the transient presence of cells in peripheral blood. These nuclear scans also identified increased percentages of highly activated Treg cells in diseased skin. Results from both flow cytometry and whole transcriptome RNA sequencing also showed that Treg cell accumulation in the skin was related to attenuation of the interferon-γ pathway as well as amplification of the IL-17 pathway. These relationships were more pronounced in skin when compared to peripheral blood.

A pilot study was conducted (n=10), which aimed to induce tolerance and minimize the need for immunosuppression (currently needed to prevent acute cellular rejection of the transplanted organ) using a novel Treg therapy in living donor liver transplant (Todo et al., 2016). Ex vivo cells were generated using recipient lymphocytes with irradiated donor cells to generate alloantigen-specific Tregs. The cells generated displayed cell-number-dependent donor-specific inhibition in the mixed lymphocyte reaction. Infusions were well tolerated, and seven of ten patients were able to taper fully off their immunosuppressive agents and remained drug free at the time of publication (16-33 months). This study demonstrates an example for the safety of antigen-specific Treg therapy, as well as showing that this approach can minimize the need for immunosuppressive drugs, which come with significant risks and known side effects.

In conclusion, these clinical studies and case reports demonstrate the Tregs are well tolerated in patients with autoimmune diseases as well as being phenotypically stable and persistent out to one year post administration. Taken together, these data support the pursuit of CAR Treg therapy in patients with chronic diseases including RA and HS.

2.3.3. Rationale for SBT777101 in RA and HS

Citrullinated vimentin (CV) is one example of an antigenic protein that is found in the extracellular matrix of inflamed synovial tissue of RA patients and in the dermis of HS subjects (Van Steendam et al., 2010; Byrd et al., 2019) and data on file, Sonoma Biotherapeutics). Given that Cit-Prot are present at sites of chronic inflammation in the joints of patients with RA and in the skin of patients with HS, they constitute a potential localized antigen source that can be targeted by a Cit-Prot specific CAR on a Treg (Orvain et al., 2021). Importantly, the presence of Cit-Prot in the extracellular matrix and synovial fluid (or NETosis in the dermis for HS) allows for SBT777101 CAR Tregs to effectively recognize and target the modified proteins in the inflammatory milieu, which when combined with the ability of Treg to function through bystander suppression (i.e., the suppression of localized inflammatory activities in the near vicinity of Treg activation) provides the appropriate conditions for Treg-mediated suppression of local inflammation across patient MHC haplotypes (Raffin et al., 2020).

Multiple studies have demonstrated that patients with autoimmune diseases have Treg populations that are insufficient in their ability to control disease, especially in the inflammatory sites of disease (Brusko et al., 2008). This was reported both in HS (Kim et al., 2023) and RA (Ehrenstein et al., 2004) patients. Chronic inflammation and an overabundance of Teff cells contribute to a shift in the immune balance, resulting in existing Tregs being ineffective in controlling the inflammatory consequences of autoimmunity.

One approach to augmenting Treg cells has been the use of low dose IL-2 to selectively promote Treg expansion (Wu et al., 2020). This therapy has shown promising therapeutic effects in a number of preclinical autoimmunity models. Early-stage clinical data in the RA setting using low dose IL-2 treatment showed that the cytokine could activate and expand the functionality of Tregs in vivo (Zhang et al., 2021). Clinical data from a Phase 2 study in patients with active RA showed moderately reduced disease activity in patients treated with low dose IL-2 plus methotrexate (MTX) as compared to MTX alone (Zhang et al., 2022). While these data support the therapeutic use of IL-2 treatment, several studies have questioned the adequacy of Treg selectivity over Teff cells (particularly activated Teff cells) with low-dose IL-2, the requirement for continuous treatment to maintain increased Treg numbers and an unclear activity at the site of inflammation. The small therapeutic window of IL-2 (Treg activity versus Teff activity), has prompted development of multiple IL-2 muteins with greater Treg selectivity (Ghelani et al., 2020), but clinical data for this approach is not available for RA. Finally, IL-2 treatment does not have selective activity or specificity for the inflammatory sites and acts systemically, potentially leading to immunosuppression of immune responses more generally.

That said, the combination of preclinical evidence and early clinical data with IL-2 provide a clear rationale for Treg therapy in RA and HS. Moreover, inclusion of the CAR in the Treg drug product, SBT777101, will promote activation of the Tregs product directly at the sites of inflammation, specifically the joints and tissues of RA and HS patients where Cit-Prot are enriched. In principle, the CAR Tregs will increase the local activity of the treatment and lead to a specific and effective suppression of disease activity at the inflamed site with reduced risk of generalized immunosuppression.

2.4. Background of Rheumatoid Arthritis

2.4.1. RA Disease Background

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that primarily affects diarthrodial joints but frequently involves other organs. A major portion of the pathogenesis of RA is initiated by antigen-specific effector T cells (Teff) in addition to B cells that produce pathogenic autoantibodies. The pathogenesis of RA is built upon the concept that self-reactive $CD4^+$ T cells become activated by antigen-presenting cells (APCs) through interactions between the T cell receptor and class II MHC-peptide antigen with co-stimulation through the CD28-CD80/86 pathway (Yap et al., 2018). Synovial $CD4^+$ T cells differentiate into T_H1 and T_H17 cells, each with distinctive cytokine profiles. $CD4^+$ T follicular helper (FH) cells in turn activate B cells, some of which differentiate into autoantibody-producing plasma cells. In addition, the presence of $CD8^+$ T cells producing inflammatory cytokines in the synovial fluid is associated with disease severity (Carvalheiro et al., 2015). Teff cell activation promotes macrophages and fibroblasts to produce pro-inflammatory cytokines, further amplifying the chronic inflammatory response. This process contributes to osteoclast activation and proliferation of synoviocytes surrounding the joint that can ultimately expand, resorb cartilage and bone, and present radiographically as erosions. This conceptual model continues to be refined with the molecular deconstruction and reconstruction of immune cells and stromal cells in the synovial microenvironment using single cell mRNA sequencing and reinforces the concept that RA is a heterogeneous disease.

Approximately 1% of the general population is affected worldwide, with females being two to three times more likely affected than males (Cross et al., 2014). In the United States and northern Europe, estimates of RA prevalence are between 0.5 to 1 percent and the annual incidence rate is estimated to be 40 per 100,000 persons (Myasoedova et al., 2010; Eriksson et al., 2013; Hunter et al., 2017). While the etiology of RA is unknown, there is evidence that the disease is caused by a combination of both genetic and environmental risk factors. Among the genetic risk factors, the HLA locus is the most significant, with HLA-DRB1 alleles that encode an HLA-DRβ chain containing an amino acid sequence motif called the 'shared epitope' increasing RA risk and severity (Gregersen et al., 1987). Cigarette smoking is the strongest known lifestyle or environmental risk factor for RA (Sugiyama et al., 2010). Smoking is known to cause lung injury, which can lead to PAD enzyme induction and the generation of Cit-Prot.

Citrullination and the formation of ACPA have been reported to be hallmarks of disease during the development of RA (Darrah and Andrade, 2018; Fox, 2015; Holers, 2013). Citrulline is generated via a post-translational conversion of protein associated arginine to citrulline by PADs. It has been established that citrullination leads to the generation of autoantigens during inflammatory responses (Muller and Radic, 2014). These autoantigens lead to the development of pathogenic autoantibodies. Nearly 70% of cases of established RA are characterized by the presence of autoantibodies, rheumatoid factor (RF) and/or antibodies directed against ACPA, of which antibodies reactive to cyclic citrullinated peptides (anti-CCP) are the most specific clinical test currently available (Nielen et al., 2004; Rantapaa-Dahlqvist et al., 2003; Majka et al., 2008). Recent studies provide evidence for a preclinical phase characterized by the presence of circulating RF and anti-CCP antibodies as long as 10 years prior to the clinical onset of disease (de Brito Rocha et al., 2019). In the context of this autoimmune diathesis, a "second hit" is postulated to initiate a chronic inflammatory response, with a strong predilection for the joints.

2.4.2. RA Therapeutic Options and Unmet Need

Conventional synthetic disease modifying drugs (csDMARDs) are the current treatment standard of care (Fraenkel et al., 2021). In general, csDMARDs are effective for patients with mild to moderate disease who are at low risk of developing erosions. These drugs are typically well tolerated and have a favorable benefit-risk profile (Smolen et al., 2018). However, a significant proportion of patients either don't respond to therapy, have a partial response, are inadequate responders, or relapse after an initial response to therapy. Thus, based on current treatment guidelines, these patients are treated with biologic or targeted synthetic therapies (bDMARDs and tsDMARDs) as monotherapy or in combination with standard DMARDs.

While many patients with early disease can achieve low disease activity with csDMARDs, over time response rates decline and b/tsDMARDs are introduced as second line therapies. These include most prominently inhibitors of tumor necrosis factor (TNFα), inhibitors of the IL-6/IL-6 receptor pathway, CTLA4-Ig (which binds CD80/86 and downregulates T cell activation), Janus kinase (JAK) inhibitors (which suppress multiple cytokine and growth factor receptor pathways), and rituximab (anti-CD20 B cell depleting antibody). Despite these options, about 40% to 50% of patients treated with b/tsDMARDs fail to achieve ACR50, a clinically meaningful response, and these therapies lose efficacy in many patients later during therapy. In fact, patients become inadequate responders to treatment with TNFα blockers more rapidly than those who go on to fail earlier lines of treatment with standard DMARDs such as MTX (Aletaha and Smolen, 2018). Overall, long term response rates with b/tsDMARDs are poor, with clinical study data showing that only 10%–17% of patients that have failed prior treatment TNF inhibitors are able to achieve an ACR70 response (Smolen et al., 2018). Many patients fail to achieve low disease activity with DMARD therapy and only 10%–15% of patients can achieve DMARD-free remission (Ajeganova and Huizinga, 2017). Even with aggressive goals of treating to remission and guidelines recommending switching rapidly to therapies with alternative mechanisms of action (Fraenkel et al., 2021), response rates continue to decrease with increasing disease duration and multiple drug exposures.

While a number of bDMARDs and tsDMARDs with differing mechanisms of action are available, treatment may not be well tolerated by patients and these medications are associated with serious side effects. A systematic literature review (Köhler et al., 2019) reported the safety of biological DMARDs. Risks associated with treatment with these therapies include increased rates of serious infection, opportunistic infections, malignancy, and hematologic changes. JAK inhibitors, the only approved tsDMARDs to date, are associated with increased risk of infection, lipid and liver enzyme elevations and reactivation of herpes zoster (Clarke et al., 2021), as well as thrombosis, cardiovascular events and malignancy.

Thus, despite there being multiple therapeutic options for patients with RA, existing therapies are inadequate for long term disease management in most cases. A recent study reported that almost three quarters of patients are dissatisfied with their treatments (Radawski et al., 2019). Patients reported symptoms with a moderate to severe impact on patient quality of life including fatigue (82%), pain (76%) and physical wellbeing (75%) (Radawski et al., 2019). Many commonly used approved therapies require chronic administration and are given via frequent infusion or injection, causing an ongoing burden to the patient. Thus, there is still a significant unmet need for treatment options that are safe, effective, and durable.

2.4.3. New Therapeutic Options for RA

As a result of the continued unmet medical need, the development landscape for RA includes a broad range of targets being interrogated. A systematic review (Blaess et al., 2020) conducted in 17 clinical study databases (search date June 1, 2019) identified a total of 242 therapeutic studies, involving 243 molecules having been or currently being evaluated in RA. Of these, 141 (58%) molecules had already been withdrawn from development.

Another systematic review (Huang et al., 2021) identified 58 compounds being evaluated in Phase 1 and 2 clinical studies, including those targeting cytokines, chemokines, and proteins involved in inflammatory cellular pathways. Yet very few novel therapies were being investigated in Phase 3 clinical studies. In fact, many new therapies currently under development for RA are biosimilars of existing therapies (Smolen et al., 2019). While these therapies will offer patients easier access to medications against proven targets in RA, they will not target alternative mechanisms for treatment of disease. Otilimab, an antibody against granulocyte-macrophage colony stimulating factor (GM-CSF), was the only novel disease modifying agent in development that had reached Phase 3 studies for the treatment of RA. Other Phase 3 programs were investigating agents with similar or identical mechanisms of action as existing approved therapies, including olokizumab, an antibody targeting IL-6, ofatumumab, a fully human anti-CD20 monoclonal antibody, and peficitinib, a JAK inhibitor.

One newer approach is the use of mesenchymal stem cells (MSC). A small study (n=9) evaluated the effect of bone marrow derived MSCs on immunological biomarkers and clinical parameters in patients with RA (Ghoryani et al., 2019). The results from this study suggested a trend towards improvements in clinical signs and symptoms. The utility of MSC transplant in RA is being further evaluated in early phase clinical studies (NCT04170426, 2022; NCT03186417, 2022; NCT03618784, 2022). However, studies have shown that most patients who achieve a treatment response ultimately relapse.

Overall, this landscape analysis supports the conclusion that there remains a significant unmet medical need in this disease indication.

2.5. Background of Hidradenitis Suppurativa

2.5.1. HS Disease Background

Hidradenitis suppurativa (HS) is a chronic inflammatory skin condition characterized by recurrent painful lesions, including inflammatory nodules (N), abscesses (A), and purulent draining tunnels or fistulae (dT), in the axillae, buttocks, groin, and anogenital region (Sellheyer and Krahl, 2005; Alikhan et al., 2019a; Sabat et al., 2020)While the underlying mechanisms of the disease are not completely understood, it is hypothesized that skin lesions are formed from occlusion and rupture of hair follicles, possibly due to the formation of epithelial tendrils in the follicle, which initiates a chronic cutaneous feed-forward inflammatory response mediated by dysregulated innate and adaptive immune pathways, ultimately leading to more follicular occlusion and subsequent inflammation (Fletcher et al., 2020; Moran et al., 2017; Sabat et al., 2020; Frew et al., 2020b; Dunstan et al., 2020; Navrazhina et al., 2021). Recent data suggest that there is a significant role of immunopathogenesis in the disease manifested by dysfunction of Type 2 conventional dendritic cells, relatively reduced Treg cell function, an influx of memory T cells and a plasma cell/plasmablast infiltrate in the skin of HS patients (Lowe et al., 2022). In addition, data suggest that in HS there are tertiary lymphoid structures in which B and T cells are primed and actively undergo maturation which suggests that the disease has a significant underlying adaptive immune mechanism (Lowe et al., 2023). While HS is not considered an infectious disease, there may also be an infectious component because occluded hair follicles can act as a site for bacterial colonization, leading to additional immune activation (Wolk et al., 2020).

2.5.2. HS Therapeutic Options and Unmet Need

Currently, there are limited therapeutic options. Treatment for HS includes both local and systemic interventions. Local management of disease includes topical or intralesional corticosteroids, topical antibiotics or retinoids, laser or light therapies, and in severe and extensive refractory cases, surgery (Gracia Cazaña et al., 2020; Ravi et al., 2022; Alikhan et al., 2019b). Systemic treatments include oral medications, such as corticosteroids, antibiotics, retinoids, and agents with antiandrogenic effects, as well as biologics, including but not limited to subcutaneous adalimumab and IV infliximab (Orenstein et al., 2020; Alikhan et al., 2019a;). Of these interventions, adalimumab, an anti-TNFα biologic, and secukinumab, and bimekizumab which are anti-IL17 biologics, are currently the only FDA-approved systemic medications for moderate to severe HS. A subset of patients still do not respond to these biologics (Midgette et al., 2022). For example, only 41.8% to 58.9% of patients receiving weekly adalimumab reached HS Clinical Response (HiSCR50) at 12 weeks after administration, compared to 26%–28% on placebo treatment. HiSCR50 is a dichotomous endpoint, defining a responder as achieving at least a 50% reduction in the abscess and inflammatory nodule (AN) count, without any increases in counts of abscesses or draining tunnels (dTs) (Kimball et al., 2016). Anti-IL17 therapies have shown efficacy similar to adalimumab (Kimball et al., 2023; Kimball et al., 2024).

Overall, these therapies are inadequate for long-term disease management in most moderate to severe cases, and many patients experience a high disease burden, which has a negative impact on their quality of life (QOL), including physical and mental health (Ingram et al., 2022; Kouris et al., 2016; Marvel et al., 2019; Matusiak, 2020).

A recent global survey evaluating patients' unmet needs in HS found that many patients are dissatisfied with available medical or procedural treatments (Garg et al., 2020). Patients experience flares often (23.0% daily, 29.8% weekly, and 31.1% monthly), and 12.5% of patients had visited the hospital at least 5 times due to their HS, incurring significant financial burden. Greater than 60% of patients rated their recent HS-related pain as moderate or higher, while 4.5% described it as the worst possible. Extreme impact on QOL was reported by 43.3%, and 14.5% were classed as disabled, as measured by the HS Quality of Life instrument (HiSQOL, (Zouboulis et al., 2021). Current treatments have limited QOL improvement, low treatment effectiveness, and inadequate pain control (Willems et al., 2022).

2.5.3. New Therapeutic Options for HS

In patients with HS presenting with inflammatory lesions, skin tunnels, and scarring do not typically achieve satisfactory, sustained disease control. Other medical treatment options under investigation include other biologics targeting IL-1, TNF and alternative IL-17 inhibitors.

3. PHYSICAL, CHEMICAL, AND PHARMACEUTICAL PROPERTIES AND FORMULATIONS

3.1. Product Description

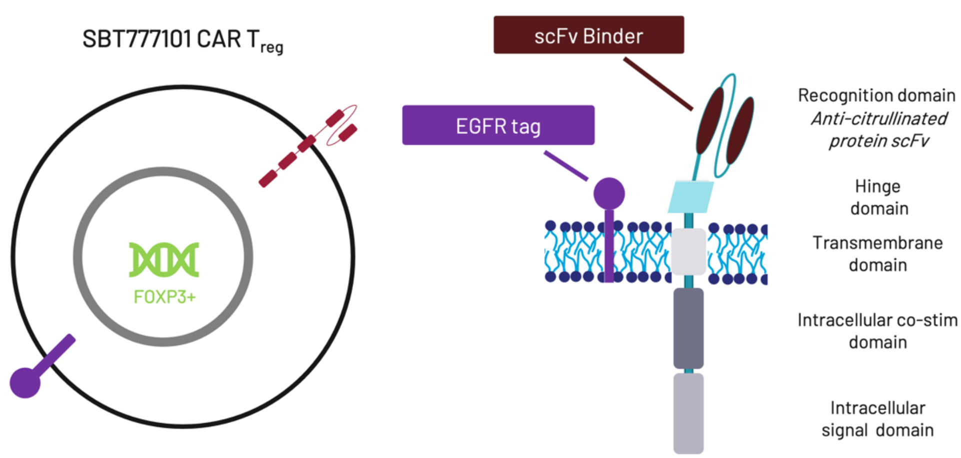
3.1.1. Nomenclature

SBT777101 is a cryopreserved autologous human Treg cell therapy product, expressing a chimeric antigen receptor (CAR) transmembrane protein targeting Cit-Prot in the extracellular matrix of patients with inflammatory diseases.

3.1.2. Product Overview

SBT777101 is a cryopreserved ex vivo expanded autologous Treg cell preparation that has been transduced with a lentiviral vector encoding both a CAR specific for Cit-Prot and an inert truncated epidermal growth factor receptor (EGFR) tag for identification. The SBT777101 CAR contains an intracellular signaling domain, an intracellular co-stimulatory domain, a transmembrane domain, and an extracellular recognition domain (anti-Cit-Prot single-chain variable fragment [scFv]) to enable specific binding and Treg activation at the site of target protein expression. (Figure 1).

Figure 1: SBT777101 Schematic



3.1.3. SBT777101 Manufacturing Overview

Starting cells are collected from the patient through nonmobilized leukapheresis at qualified facilities associated with each clinical study site. Once the Leukapheresis Product is received at the drug product manufacturing site, it is enriched for $CD25^{+}$ cells via a cell sorting process. The resulting Treg cells ($CD4^{+}CD127^{lo/-}CD25^{+}$) are activated using anti-CD3/anti-CD28-coated beads (CD3/CD28 beads) and cultured in vitro for expansion. Following activation, the cells are transduced with a lentiviral vector carrying a CAR construct specific for Cit-Prot and further expanded. The cells are restimulated again with CD3/CD28 beads on Day 9 and harvested on

Day 14. Upon harvest, cells are formulated using a cryopreservation solution and aliquoted into cryobags.

3.1.4. Biological Characterization and Drug Product Attributes

SBT777101 lot release is performed assessing product quality attributes that includes safety, identity, strength/potency, and purity. Testing includes measures of viability, viable cell concentration, markers of identity, vector copy number, and transgene expression (see Investigational Product Manual).

In addition to lot release, additional product characterization assessing the biological activity of the product will be evaluated throughout the Phase 1 studies.

3.1.5. Formulation

SBT777101 is formulated in a cryopreservative buffer with a targeted cell density of 30 million cells/mL. Upon formulation, cells are aliquoted into cryobags and cryopreserved using a controlled-rate freezer.

3.1.6. Storage and Handling of SBT777101

3.1.6.1. Storage and Shipping Conditions

Cryopreserved SBT777101 is stored in a continuously monitored vapor -phase liquid nitrogen freezer and transported in liquid nitrogen dry vapor shippers, which are validated to maintain temperature for a minimum of 15 days. The product will be shipped via a qualified courier with continuous data loggers and chain of custody documentation. At the time of receipt at the clinical site, the bags are removed from the shipper and prepared for infusion.

3.1.6.2. Handling and Use of SBT777101

SBT777101 is supplied in cryopreserved bags. The product must remain frozen and should only be thawed when required for administration. As soon as the product is completely thawed, SBT777101 will be administered to the patient intravenously.

Please refer to the clinical protocols and the Investigational Product Manual for specific instructions on handling, thawing and administration of SBT777101.

3.1.7. Stability Data

In-use stability studies of thawed SBT777101 have been performed and data demonstrated that SBT777101 remained viable and suited for infusion for 3 hours after thaw. Long-term stability studies of cryopreserved SBT777101 are being performed.

4. NONCLINICAL STUDIES

SBT777101 is a CAR Treg cell therapy product that was designed to recognize antigens produced in the context of certain autoimmune diseases and exhibit anti-inflammatory activity. Nonclinical studies documented the specificity of SBT777101 for Cit-Prot and the Treg cell phenotype of SBT777101 via flow cytometric analysis of FOXP3 expression, FOXP3 Treg-specific demethylated region (TSDR) analysis, cytokine profiling, and evaluation of the suppressive activity of SBT777101. Studies demonstrated that the SBT777101 CAR specifically recognizes Cit-Prot and does not exhibit off-target binding. When SBT777101 was manufactured using whole blood samples from patients with RA and from patients with HS, cells were transduced efficiently and exhibited the same phenotype post transduction as cells from healthy donors. SBT777101 exhibits regulatory/immunomodulatory functions in vitro. An immunohistochemistry-based tissue cross reactivity study showed that the SBT777101 CAR stains mostly cytoplasmic and nuclear elements in multiple cell types and that it is associated with membranous staining only in rare epithelial and mononuclear leukocytes in various tissues. Staining of extracellular material was also observed in various tissues, as further detailed below. The nonclinical assessment of SBT777101 has demonstrated in vivo that SBT777101 does not cause adverse events towards normal tissues including under proinflammatory conditions, that SBT777101 is not activated in vivo within normal tissues, that SBT777101 exhibits a stable Treg phenotype under pro-inflammatory conditions, and that SBT777101 exhibits an immunoregulatory activity in vivo that is similar to the activity of untransduced polyclonal Tregs. An evaluation of the risk of lentiviral vector mediated insertional mutagenesis showed a multi-site integration profile with no dominant integration site which is consistent with numerous published studies of lentiviral vectors similar to that used in the SBT777101 vector, and no abnormal impact of the transduction of Tregs on cell growth activity.

4.1. Nonclinical Pharmacology

4.1.1. In Vitro Pharmacology

In Vitro Activation of the SBT777101 CAR in a Jurkat Reporter Cell Line

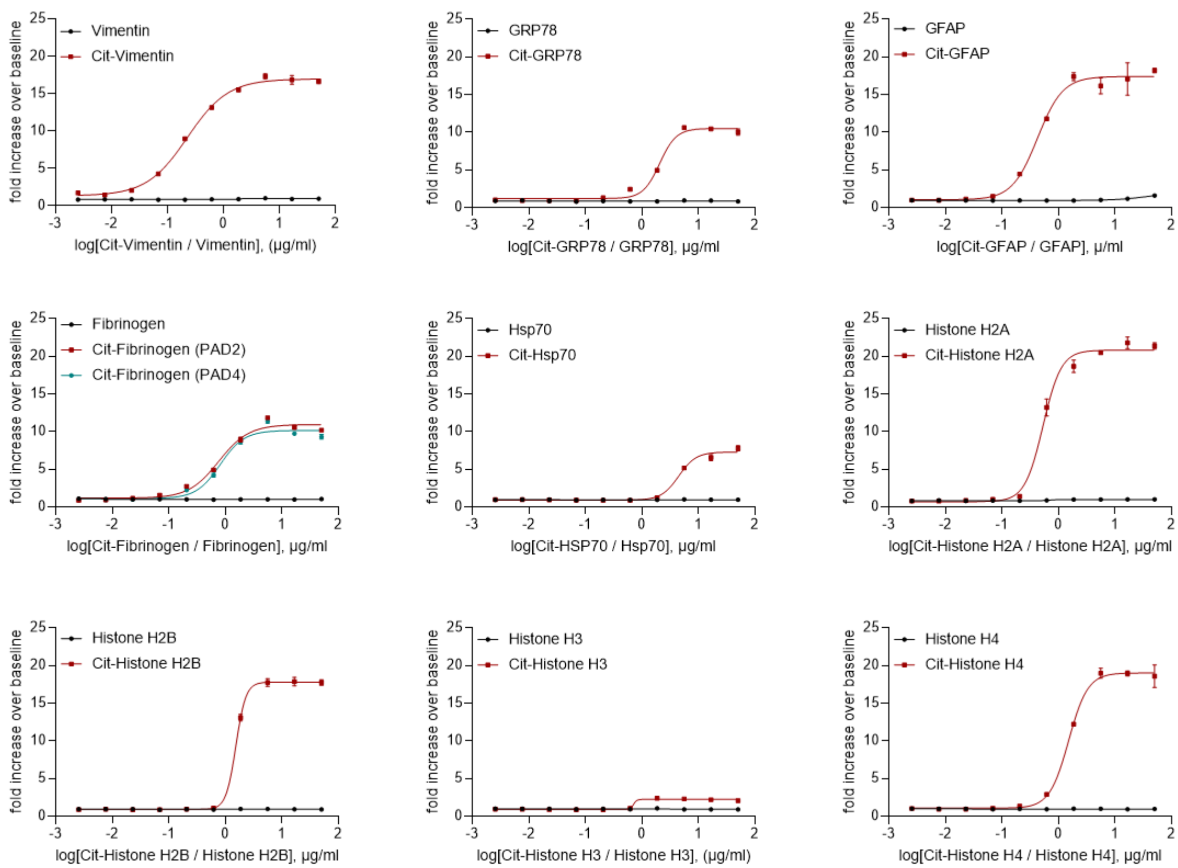
The objective of these experiments was to demonstrate that the SBT777101 CAR can be activated and transduce signaling upon binding to target citrullinated but not control, non-citrullinated version of the same proteins. Evaluation of the activation of the SBT777101 CAR was conducted using a Jurkat cell line containing a nuclear factor of activated T-cells (NFAT)-luciferase reporter system (BPS Bioscience, Inc., San Diego, USA). The Jurkat reporter cell line was transduced with the SBT777101 vector to express the SBT777101 CAR. Activation of the CAR upon binding to target proteins was evaluated by measuring the bioluminescence signal resulting from NFAT activation and increased luciferase activity in the presence of D-luciferin.

A panel of proteins known to be citrullinated in inflammatory disease were purchased from Cayman Chemicals and evaluated in this system to demonstrate antigen-specific functional activity of the SBT777101 CAR (Aggarwal et al., 2009).

Plate coated Cit-Prot (citrullinated by either peptidyl arginine deiminase type 2, PAD2, or PAD4), including vimentin, glial fibrillary acidic protein (GFAP), glucose-regulated protein, 78-kDa (GRP78), fibrinogen, heat shock protein 70 (Hsp70), Histone H2A, Histone H2B, Histone

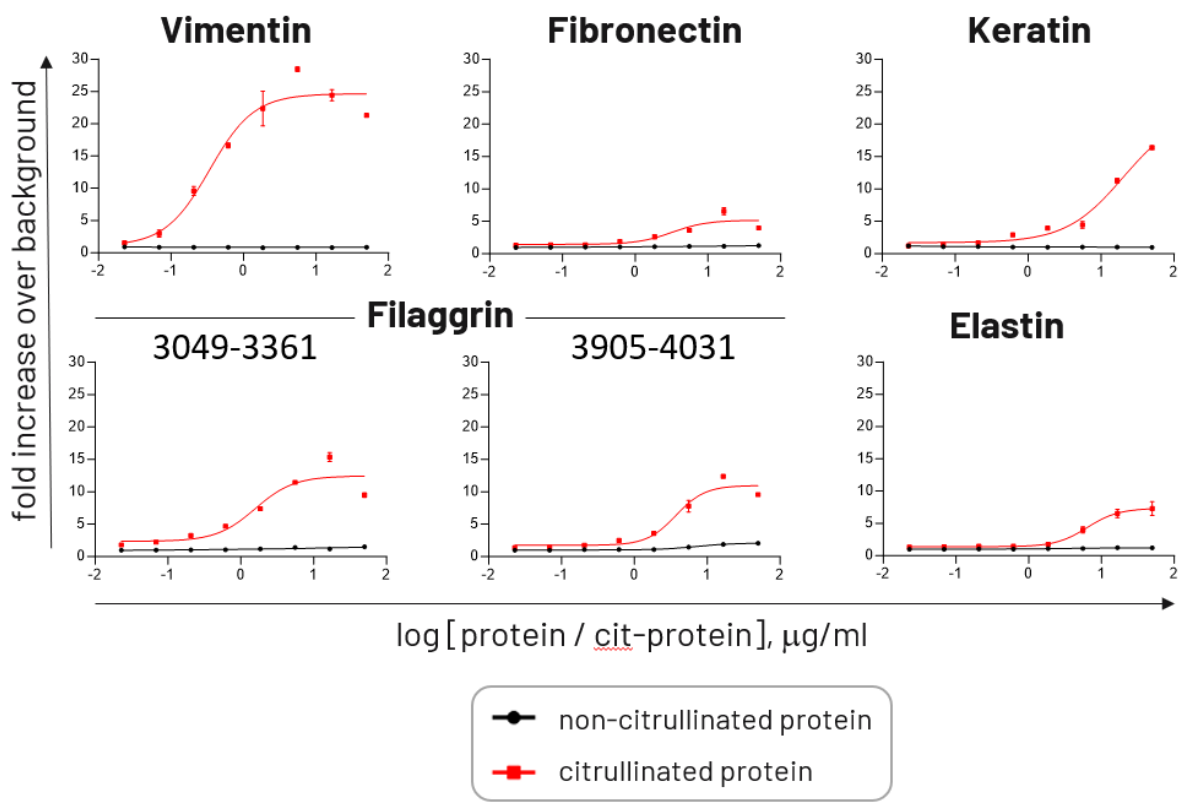
H3 and Histone H4 were able to activate SBT777101 CAR expressing Jurkat cells while the non-citrullinated form of each of these proteins did not activate the Jurkat reporter cells (Figure 2). In another set of experiments, a different panel of Cit-Prot (skin and/or HS related Cit-Prot) was evaluated in the same reporter assay. Expression of the SBT777101 CAR resulted in specific and selective activation upon exposure to Cit-CV, Cit-Keratin, Cit-Filaggrin (two Filaggrin fragments tested), Cit- Fibronectin, and Cit-Elastin, while no activation occurred in the presence of corresponding non-Cit-Prot (Figure 3).

Figure 2: Activation of the SBT777101 CAR by Citrullinated Proteins



Level of luciferase activity (y axis) with increasing protein concentrations. Red lines represent Cit-Prot and black lines represent native proteins.

Figure 3: Activation of the SBT777101 CAR by Skin and/or HS Related Citrullinated Proteins

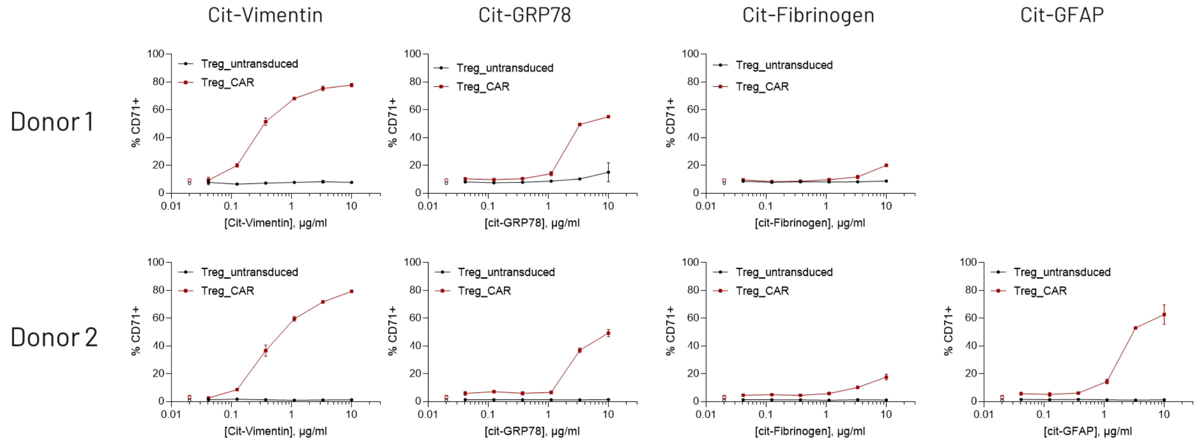


Under the conditions of these experiments, citrullinated vimentin (CV) was the most potent activator of the CAR, with a low half maximal effective concentration (EC_{50}) value and a high NFAT activation signal, both parameters contributing to the sensitivity to a given antigen. The level of activation of the SBT777101 CAR in the Jurkat reporter cell line was significantly lower when stimulation was provided by soluble CV instead of plate-bound CV, likely reflecting a lower potential for circulating soluble Cit-Prot to stimulate SBT777101 in comparison to tissue localized Cit-Prot.

Citrullinated Protein-Mediated Activation of SBT777101

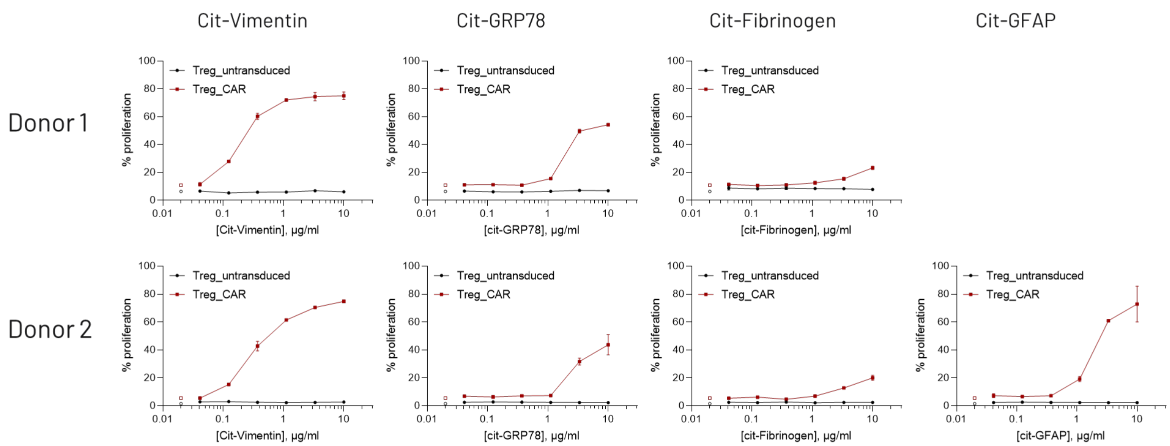
Some select SBT777101 specific CAR ligands were used to demonstrate antigen-specific activation and proliferation of SBT777101. SBT777101 was incubated with increasing concentrations of plate-bound Cit-Prot for 72 hours and upregulation of CD71 was measured as a marker of activation of the CAR T cells while proliferation was assessed by carboxyfluorescein succinimidyl ester (CFSE) fluorescence dilution. There was a dose-dependent increase in CD71 expression, a marker of activation, (Figure 4) and proliferative response (Figure 5) of SBT777101in response to CV, citrullinated GRP78 (glucose-regulated protein 78), citrullinated fibrinogen, and citrullinated GFAP (glial fibrillary acidic protein). In contrast, untransduced Tregs were not activated and did not proliferate in the presence of the same Cit-Prot.

Figure 4: SBT777101 Activation in Response to Citrullinated Proteins



Percentage CD71 expression according to concentration of Cit-Prot. Red lines represent SBT777101 (referred to as Treg_CAR) and black lines represent untransduced Treg controls (referred to as Treg_untransduced).

Figure 5: In Vitro Proliferation of SBT777101 in Response to Citrullinated Proteins

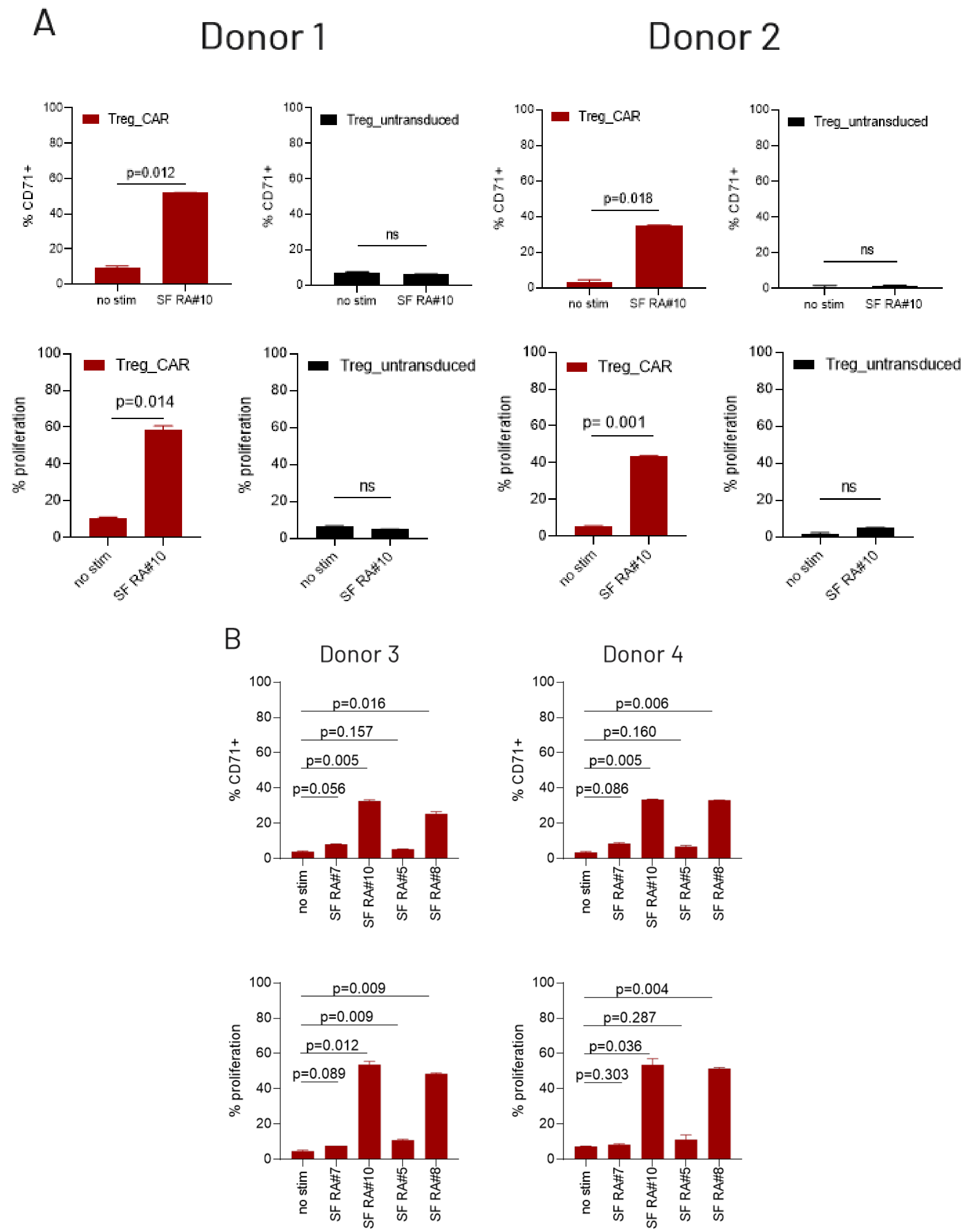


Percentage proliferation of Tregs according to concentration of Cit-Prot. Red lines represent SBT777101 (referred to as Treg_CAR) and black lines represent untransduced Treg controls (referred to as Treg_untransduced).

Similarly, activation and proliferation of SBT777101 was observed in response to RA patient--derived synovial fluid (Figure 6). SBT777101 was prepared from four different Treg RA donors and incubated with synovial fluid from patients. Activation demonstrated by CD71 expression and proliferation based on cell tracing dye were used as readouts to detect SBT777101 responses. There were no observed responses in untransduced control cells noted by lack of CD71 expression and proliferation dye dilution (donor#1, donor#2). Activation and proliferative responses of transduced cells (identified as Treg_CAR in the figure) were observed with the synovial fluids tested (donors #1 to #4) showing increased CD71 and loss of proliferation dye. The different synovial fluids tested (SF RA#7, 10, 5, 8) were associated with different degrees of activation and proliferation of SBT777101. These data demonstrate that

SBT777101 made from four different donors responds in a similar fashion to synovial fluids from RA patients.

Figure 6: Activation and Proliferation of SBT777101 Induced by Patient-Derived Synovial Fluid



Percentage CD71 expression (top row) and proliferation (bottom row) of Tregs derived from donors with RA (Donor 1-4). **A)** SBT777101 and untransduced cells from the same donor were stimulated with synovial fluid from an RA patient (referred to as SF RA#) or media controls (referred to as no stim); **B**). SBT777101 from two additional donors were incubated with a larger panel of synovial fluids. P values are a result of a paired T test.

SBT777101 Flow Cytometric Analysis

The flow cytometric analysis of SBT777101 evaluated the phenotypic characteristics of the product based on cluster of differentiation (CD)4, CD8, EGFRt, and FOXP3 expression. This analysis demonstrated that the manufacturing process yielded $CD4^{+}CD8^{-}FOXP3^{+}$ Treg cells, in addition to measuring the percentage of CAR transduced cells. This analysis showed that SBT777101 derived from RA and HS patients exhibit a consistent and similar FOXP3 Treg phenotype as SBT777101 derived from healthy volunteers.

SBT777101 Cytokine Profiling

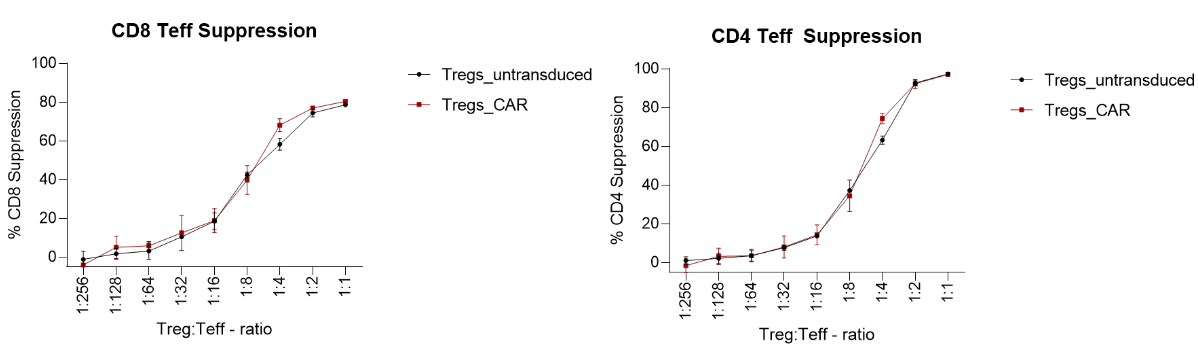
Characterization of the cytokine-based regulatory phenotype of SBT777101 showed that the cytokine profile of SBT777101 derived from RA and HS patients was consistent with SBT777101 derived from healthy volunteers. The cytokine profiles showed high IL-10 levels upon CAR stimulation as compared to relatively lower GM-CSF, IFNγ, TNFα, and IL-17A levels.

SBT777101 derived from HS-patients had slightly lower GM-CSF, IL-10 and IFNγ production, however, ratios of donor level IL-10/IFNγ were similar to what was observed with SBT777101 derived from healthy donors. A similar trend was also observed for IL-10/GM-CSF ratios.

SBT777101 Regulatory Function

It has been demonstrated that the inherent function of Tregs isolated from peripheral blood is maintained in Tregs expressing the SBT777101 CAR. Autologous purified T cells (Teff) were labeled with the CellTrace™ Violet (CTV) tracer dye while Tregs were labeled with the CFSE tracer dye. Cells were incubated at various ratios and evaluated for proliferation after incubation with anti-CD3/anti-CD28 coated beads used as stimulus. In the presence of increasing amounts of Treg cells, a reduction in proliferation in response to the CD3/CD28-mediated stimulation was observed (Figure 7). SBT777101 inhibited Teff cell proliferation in a similar manner to untransduced Tregs, demonstrating that the expression of the CAR and the truncated EGFR tag did not impact the inherent Treg function of transduced cells.

Figure 7: Inhibition of T Cell Proliferation in the Presence of Tregs

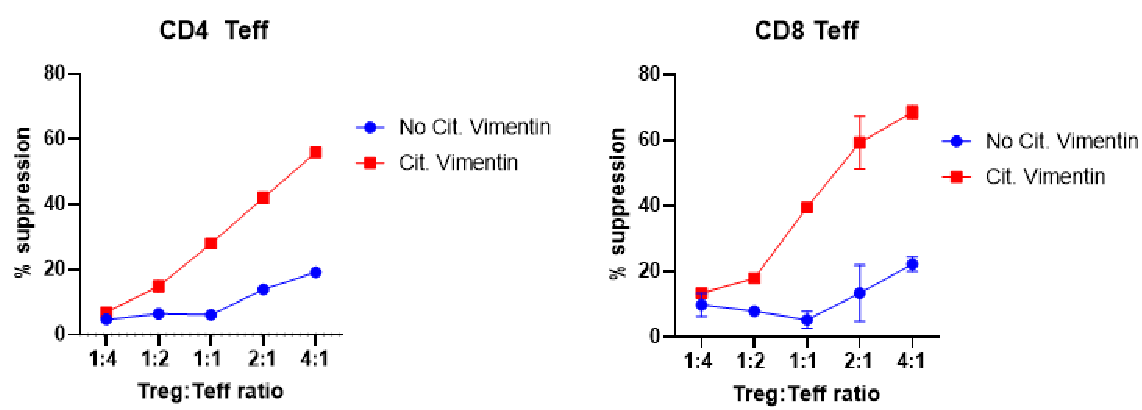


Percentage CD4 proliferation (left) and CD8 (right) of Teff according to Treg:Teff ratio. Red lines represent SBT777101 (referred to as Treg_CAR) and black lines represent untransduced Treg controls (referred to as Treg_untransduced).

This experiment demonstrated that SBT777101 exhibits regulatory T cell function as defined by inhibition of proliferation of co-cultured activated T cells and that this activity is similar to that observed with untransduced Tregs. It is therefore not altered by the transduction process.

SBT777101 is designed to activate the multifaceted activity of Tregs upon engagement of the CAR on the surface. When activated via the neoantigen, citrullinated vimentin, SBT777101 exhibited regulatory activity of activated T cells. Similar to above, autologous purified T cells (Teff) were labeled with CTV tracer dye while Tregs were labeled with the CFSE tracer dye. In this assay, Teffs were incubated with anti-CD3/anti-CD28-coated beads used as stimulus to promote robust polyclonal activity. After overnight activation, activated Teff cells were incubated at various ratios with SBT777101 in the presence of citrullinated vimentin and evaluated for proliferation. In the presence of increasing amounts of SBT777101 Treg cells stimulated via the CAR with citrullinated vimentin, a reduction in proliferation in response to the CD3/CD28-mediated stimulation was observed demonstrating that activation of SBT777101 via the CAR can promote Treg regulatory activity (Figure 8).

Figure 8: SBT777101 Suppression of Effector T cells

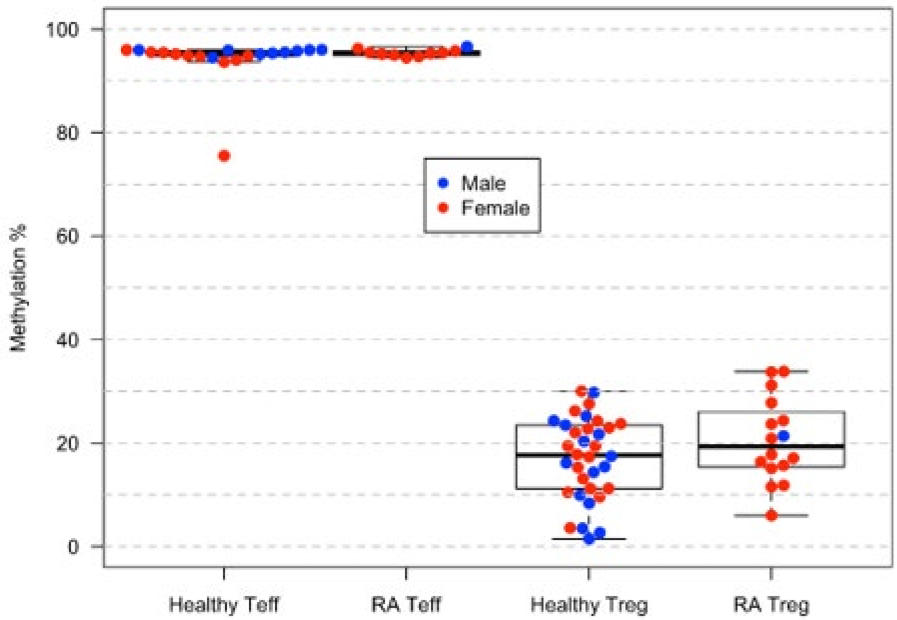


Percentage CD4 proliferation (left) and CD8 (right) of Teff according to Treg:Teff ratio. Red lines represent platebound citrullinated vimentin with SBT777101 and Teff (referred to as Cit. Vimentin Stim). Blue lines represent control plates with no citrullinated vimentin (referred to as No Cit. Vimentin) with SBT777101 and Teff.

SBT777101 Treg Specific Demethylated Region (TSDR)

SBT777101 has an epigenetic profile of the TSDR region consistent with stable FOXP3 expression (Rossetti et al., 2015) (Figure 9). Tregs from whole blood and leukopacs from RA patients and healthy donors were isolated using a scaled-down version of the manufacturing process of SBT777101. In some donors, $CD4^+$ T cells (non-Tregs) were also isolated. SBT777101 generated from RA patient blood had a similar TSDR profile, with high level of demethylation, to that of healthy donors Tregs. This profile was consistent with published reports of stable FOXP3 expression. $CD4^+$ non-Tregs from healthy donors and RA patients had high methylation of the TSDR region. SBT777101 prepared from Tregs isolated from HS patients showed the same TSDR profile.

Figure 9: TSDR Profile of SBT777101 is Consistent with Stable FOXP3 Expression



Box and whisker plot shows percent methylation (y-axis) research scale from 34 healthy (H) volunteers and 16 RA patient derived Treg cells. In addition, percent methylation for paired expanded Teff cells are shown. Males are indicated in blue, and females are indicated in red.

Truncated EGFR tag

SBT777101 cells express a truncated version of the EGFR on their cell surface which can be leveraged to identify and select transduced cells. This tag can be recognized by an anti-EGFR antibody.

4.1.2. In Vivo Pharmacology

Exploratory studies in the mouse indicated that there is no animal model properly reflecting the increased amount or constitution of Cit-Prot observed in RA patients and triggering activation of SBT777101 in vivo. However, an alternative model was used to show pharmacological activity in vivo. SBT777101 was administered intravenously (IV) to NCG mice at a dose of 5.0 x 10^6 cells per animal, in the presence of autologous peripheral blood mononuclear cells (PBMCs) causing graft-versus-host disease (GvHD) and associated systemic production of pro-inflammatory human cytokines, with or without the administration of LPS. Treatment with SBT77101 was associated with a reduction of the production of GvHD-related pro-inflammatory cytokines, which was similar to the reduction caused by CAR-negative Tregs. The administration of SBT777101 also appeared to be associated with a slight reduction in the incidence/severity of GvHD-related microscopic splenic changes, which was also similar to the reduction caused by CAR-negative Tregs.

4.1.3. Secondary Pharmacology – Tissue Cross Reactivity Study

A tissue cross-reactivity study was conducted to determine the potential cross reactivity of a fusion protein containing the SBT777101 CAR scFv, in cryosections from a full panel of normal human tissues. The fusion protein stained the membrane, cytoplasm, and/or cytoplasmic granules and occasionally nucleus of some epithelial cell types as well as mononuclear leukocytes in various tissues. Nucleus, cytoplasmic, and/or cytoplasmic granular staining was observed in glial cells and processes of the cerebellum, cerebral cortex, and spinal cord. Cytoplasmic and/or cytoplasmic granular staining was observed in spindle cells, retinal cells and lens fibers in the eye, cells and processes associated with peripheral nerve, pituicytes, smooth myocytes, striated skeletal myocytes, and adipocytes.

Staining of extracellular material was also observed in various tissues including adrenal gland, bone marrow, cerebral cortex, kidney, large intestine, liver, lymph node, lung, mammary glands, striated (skeletal) muscle, peripheral nerve, placenta, pituitary, salivary gland, spinal cord, thymus, tonsil, and ureter. Extracellular staining was mostly observed over stromal or interstitial elements or within the lumens of glands, kidney tubules, or vessels.

The majority of observed test article staining was cytoplasmic or nuclear in nature. Membrane staining was rare as compared to cytoplasmic staining in the tissue elements where membrane staining was observed. The cytoplasmic and nuclear staining in this study was considered expected based on the reported expression of PAD2 and PAD4 (citrullinating enzymes) in various tissues. The observed membrane staining, due to the general rarity and restricted pattern within the epithelial cells and rare mononuclear leukocytes is considered of minimal toxicologic relevance.

Given the rarity of cells with membranous staining, it is anticipated that SBT777101 would have minimal CAR-mediated interactions with cells from normal tissues.

4.1.4. Safety Pharmacology

No in vivo Pharmacology studies have been conducted with SBT777101.

4.2. Pharmacokinetics and Drug Metabolism in Animals

In the absence of a proper animal model, and consistent with ICH S12 (draft version, 2021) (ICH S12, 2021) indicating that in general, biodistribution assessment of ex vivo genetically modified cells of hematopoietic origin is not critical based on expected widespread distribution following systemic administration, formal pharmacokinetic and biodistribution studies with SBT777101 were not conducted.

4.3. Toxicology

4.3.1. In Vivo Toxicity

As stated in Section 4.1.2, it is challenging to develop animal models that properly reflect the increased amount or constitution of Cit-Prot observed in RA patients and that would trigger activation of SBT777101 in vivo. However, because SBT777101 scFv binds extracellularly in various healthy tissues as well as to the membrane of rare epithelial cells in select tissues and mononuclear leukocytes in several tissues, in vivo studies were conducted in triple immunodeficient NOD-Prkdc^{em26CD52}Il2rgem^{26Cd22}/NjuCrl (NCG) mice whose unique phenotype decreases the immune-mediated rejection of human transplanted cells.

Intravenous administration of SBT777101 to NCG mice at a dose of 5.0 x 10^6 cells per animal was well tolerated with no adverse effects observed either 7 or 14 days post-dose based on clinical observations, body weight, gross necropsy, and light microscopic examination of tissues.

Intravenous administration of SBT777101 to NCG mice at a dose of 5.0 x 10^6 cells per animal, in the presence or absence of administration of lipopolysaccharide (LPS, used as a pro-inflammatory agent) was well tolerated with no adverse effects observed 14 days post-dose and no evidence of activation of SBT777101 cells in various tissues. The cells surviving after 14 days maintained a Treg phenotype as defined by FOXP3 expression. Similar findings were observed following the administration of CAR$^-$/EGFRt$^+$ Tregs.

As described in Section 4.1.2, an in vivo study was conducted with intravenous administration of SBT777101 to NCG mice at a dose of 5.0 x 10^6 cells per animal along with autologous peripheral blood mononuclear cells (PBMCs) to induce graft-versus-host disease (GvHD) and associated systemic production of pro-inflammatory human cytokines, with and without the administration of LPS. SBT777101 was well tolerated with no adverse effects observed up to 14 days post-dose. The administration of SBT777101 was associated with a reduction of the production of GvHD-related pro-inflammatory cytokines, which was similar to the reduction caused by CAR-negative Tregs. The administration of SBT777101 also appeared to be associated with a slight reduction in the incidence/severity of GvHD-related microscopic splenic changes, which was similar to the reduction caused by CAR-negative Tregs. SBT777101 and CAR-negative Tregs were similarly stable (as defined by FOXP3 expression) in these GvHD-associated pro-inflammatory conditions.

4.3.2. Genotoxicity

The manufacture of SBT777101 involves the transduction of T lymphocytes with a third-generation self-inactivating lentiviral vector. Viral vectors derived from the *Retroviridae* family are of special interest for introducing modifications to human cells because they can convert their RNA genome into DNA and integrate this DNA into the chromosomes of target cells through reverse transcriptase and integrases enzymes. Lentiviral vectors are attractive technologies for this gene transfer because of the efficient transfer and stable integration of the transgene in the host genome. There are many active clinical studies involving CAR T cell immunotherapies with gene transfer being performed with lentivirus transduction (Holzinger et al., 2016). A potential risk associated with lentiviral transduction is the insertional mutagenesis caused by the integration of the proviral DNA and viral promotor within or in close proximity to active genes. To address this risk, an insertion site analysis was conducted for SBT777101. This analysis showed a polyclonal integration site profile with no dominant integration site of concern observed.

4.3.3. Carcinogenicity Studies

No in vivo carcinogenicity study has been conducted with SBT777101.

4.3.4. Reproductive and Development Toxicity

No in vivo reproductive and development toxicity study has been conducted with SBT777101.

4.3.5. Special Studies

Assessment of the Off-Target Binding Potential of SBT777101

A cell microarray technology was used to screen for potential off-target binding interactions of a human Fc fusion protein which contains the scFv contained in the SBT777101 CAR fused to a human Immunoglobulin G1 (IgG1) Fc. The fusion protein was used as a tool molecule to evaluate the potential for the SBT777101 CAR to bind to off-target proteins. This study demonstrated that the CAR scFv did not bind to off-target proteins. It can therefore be concluded that the SBT777101 CAR is not associated with off-target binding activity.

IL-2 Independent Growth

A theoretical concern associated with engineering T cells is the disruption of normal cell growth control mechanisms. The ability of SBT777101 CAR Treg cells to grow in the absence of IL-2 was therefore evaluated in vitro. SBT777101 cells were plated with or without 300 IU of human IL-2. Untransduced cells were used as a control. Cell counts and viability were followed for 14 days after IL-2 withdrawal. A steady decrease in cell counts was observed over 14 days in the absence of IL-2, indicating a lack of unexpected cell growth after transduction.

4.3.6. Other Toxicity Studies

The potential for unwanted immunosuppression has been evaluated using several data sets, including the comparison of the immunomodulatory activity of SBT777101 with the immunomodulatory activity of untransduced polyclonal Tregs, since in more than 40 completed human Treg studies reported to date, Treg therapies have been shown to be well tolerated and safe. Similar to untransduced polyclonal Tregs, SBT777101 showed no impact on NK cell activation and cytotoxicity in response to an erythroleukemic cell line. In addition, inhibition of antiviral $CD8^+$ T cell response was only observed if SBT777101 cells were fully activated prior to encountering $CD8^+$ T cells and similar to what was observed with untransduced polyclonal Tregs. Lastly, it was demonstrated in vivo that the low levels of expression of SBT777101 target in normal tissues did not lead to SBT777101 activation (as measured by cytokine production) and that the level of immunomodulatory activity of SBT777101 in a GvHD model was similar to that observed with untransduced polyclonal Tregs. Altogether, these data indicate a low risk for unwanted immunosuppression and a safety profile likely similar to what has been reported for untransduced polyclonal Tregs.

5. EFFECTS IN HUMANS

5.1. Designs of Clinical Studies

The clinical development program for SBT777101 includes two ongoing Phase 1 studies, each evaluating single ascending doses (SAD) of SBT777101 administered IV as summarized in Table 3.

REGULATE-RA (study SBT777101-01) is an open-label, multicenter, dose escalation and expansion study to assess the safety, PK, and preliminary efficacy of a single IV infusion of SBT777101 in participants with active RA who have failed at least three prior biologic or targeted synthetic disease modifying anti-rheumatic drug (b/tsDMARD) therapies with different mechanisms of action.

REGULATE-HS (study SBT777101-02) is an open label, multicenter, dose escalation study to assess the safety, PK, and preliminary efficacy of a single IV infusion of SBT777101 in participants with active hidradenitis suppurativa (HS) who have failed at least one biologic medication for HS.

Table 3: Ongoing Studies in Support of SBT777101 by Indication

Study Name and number	Study Design and Purpose	Dosage and Dosage Regimen	Planned Sample Size	Target Population	Duration of Treatment	Status
Rheumatoid Arthritis (RA)						
REGULATE-RA; SBT777101-01	Open-label, single dose escalation and expansion study designed to investigate safety, pharmacokinetics, and e**xploratory** efficacy	Dose Levels: Single dose escalation: 100 million cells 300 million cells 900 million cells Route: IV	Up to 28 participants (up to 18 in dose escalation and up to 10 in dose expansion)	Moderate to severe RA who have failed at least 3 prior b/tsDMARDs	1 day	Ongoing
Hidradenitis Suppurative (HS)						
REGULATE-HS; SBT777101-02	Open-label, single dose escalation study designed to investigate safety, pharmacokinetics, and e**xploratory** efficacy	Dose Levels: Single dose escalation: 100 million cells 300 million cells 900 million cells Route: IV	Up to 24 participants	Moderate to severe HS who have failed at least 1 biologic therapy for HS	1 day	Ongoing

Abbreviations: b/tsDMARD, logic or targeted synthetic disease modifying anti-rheumatic drug; IV, intravenous

5.2. Pharmacokinetics and Drug Metabolism in Humans

Pharmacokinetics and drug metabolism data in humans have not been established with SBT777101.

5.3. Safety and Efficacy

Efficacy data in humans have not been established with SBT77101.

REGULATE-RA and REGULATE-HS are ongoing. As of February 1, 2025, 4 participants (3 participants in the RA study and 1 participant in the HS study) were each exposed to a single dose of 100 million cells of SBT77101. To date, there have been no reports of serious adverse events (SAEs), dose limiting toxicities (DLTs), neurotoxicity, or cytokine release syndrome (CRS). No additional data is available.

5.4. Marketing Experience

SBT777101 has not been approved for use and is not marketed in any region.

6. SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATORS

6.1. Approved Indications

There are no current approved indications for SBT777101.

6.2. Contraindications

There are no known contraindications.

6.3. Warnings and Precautions

There is currently no clinical experience with SBT777101.

Similar to protein-based biotherapeutics and specifically monoclonal antibody-based biologics, administration of CAR Treg therapeutics may lead to the formation of anti-drug antibodies (ADAs) against the scFv expressed at the surface of the engineered cell. These may have an impact on persistence or efficacy of the CAR Treg cells. Participants receiving SBT777101 will be monitored at regular intervals for the development of ADAs.

See Section 6.4.1 for potential risks and Section 6.4.2 for theoretical risks for SBT777101.

6.4. Potential Risks and Adverse Effects

No risks have been identified yet for SBT777101.

6.4.1. Potential Risks

Two initial clinical studies evaluating SBT777101 in participants with RA (study SBT777101-01) and HS (study SBT777101-02) are ongoing and, to date, no safety signals have been identified. Potential risks described below are primarily informed by nonclinical and clinical literature concerning the safety profile of polyclonal autologous Treg therapy in autoimmune diseases (see Section 2.3.2).

6.4.1.1. Infusion Related Reactions

There was no evidence of infusion related reactions in studies with polyclonal Tregs in autoimmune indications (see Section 2.3.2). However, there remains a potential risk of immediate or delayed infusion related reactions with an autologous CAR Treg cell therapy product such as SBT777101.

Symptoms could include fever, chills, pain, nausea, vomiting, generalized rash, angioedema, hypotension, bronchospasm, wheezing, or hypoxia. Any such reactions may require treatment and could be fatal. Investigators should carefully monitor participants for signs or symptoms of infusion related reactions during and after the infusion of SBT777101.

6.4.1.2. Infections

As with prior polyclonal Treg based treatments, it is not anticipated that treatment with SBT777101 will increase infection rates above baseline levels. However, it is unknown whether the antigen specificity conveyed by the SBT777101 CAR will concentrate Tregs not only at sites of inflammation but also at sites of low-level indolent infections. If that is the case, then there may be an increased risk of infections. These infection risks may include viral reactivation, reduction in modulation of commensal organisms or increased susceptibility to commonplace infections. In the absence of specific human data, the extent of risk is unknown at this time. However, clinical studies of polyclonal autologous Treg therapy in autoimmune diseases have not reported increased frequency or severity of infections.

Physicians should exercise caution when considering the use of SBT777101 in participants with a history of opportunistic and/or recurrent infections or those with underlying conditions that may predispose them to infections (e.g., diabetes). Since Cit-Prot may be expressed in tissues other than joints (e.g., lungs, lymph nodes, and the periodontal cavity) (Musaelyan et al., 2018) eligibility criteria will also exclude participants with active infection.

Participants should be monitored closely for signs and symptoms of infection. See the study protocols for detailed eligibility criteria related to inclusion and management of patients at increased risk of infection.

6.4.1.3. Viral Reactivation

While reactivation of viral (e.g., Epstein-Barr virus (EBV)) or other serious infections has been observed with biologic therapies for RA, the potential for this to occur with SBT777101 is unknown. Reactivation of latent viral infections is considered a potential risk for SBT777101 (Brunstein et al., 2013; Zhang et al., 2018). However, it should be noted that there has been no evidence of reactivation of viral or other serious infections observed in clinical studies in other autoimmune diseases using polyclonal Treg adoptive immunotherapy. One participant in the Type 1 diabetes study developed Grade 2 pharyngitis and had low-copy number cytomegalovirus (CMV), but this was presumed to be due to a new infection with CMV occurring before receiving cells (Bluestone et al., 2015).

Participants should be monitored closely for signs and symptoms suggesting potential reactivation of viruses and treated according to standard of care.

6.4.2. Theoretical Risks

Theoretical risks described here are based on assessment of CAR Teff therapy in oncology. However, it should be noted that the cell population used in the SBT777101 product is phenotypically and functionally distinct from that used in CAR Teff therapies, with no evidence of the cytokine-release-related toxicities that have been associated with CAR Teff cell therapies.

6.4.2.1. Cytokine Release Syndrome

Cytokine Release Syndrome (CRS) is thought to result from a high level of immune activation of lymphocytes, macrophages and/or myeloid cells with subsequent massive release of proinflammatory cytokines. CRS is associated with markedly increased levels of IL-6, IL-10, TNFα and INFγ, and the sequelae may be severe or life-threatening. Administration of CAR Teff therapy is associated with CRS, with symptoms typically appearing within 14 days of CAR Teff administration (Chou and Turtle, 2020).

While instances of CRS have been well documented in T effectors expressing CARs, CRS has not been reported in studies of polyclonal Tregs in patients with autoimmune indications (see

Section 2.3.2), and thus while the risk of CRS with SBT777101 is unknown, it is not expected. It should be noted that the cell population used in the SBT777101 product is distinct from that used in CAR Teff therapies, with no evidence of the cytokine-release-related toxicities that have been associated with CAR Teff cell therapies. Nonetheless, it is important to closely monitor participants for signs and symptoms of onset of CRS.

The diagnosis of CRS requires a fever (temperature of ≥38°C or 100.4°F). It is critical to exclude potential infections during the initial evaluation of any participant presenting with a fever.

Laboratory studies for the evaluation of possible CRS should include markers of inflammation, especially IL-6 and IFNγ when available. Although C-reactive protein (CRP) is frequently elevated in CRS, it is often elevated in patients with autoimmune diseases in general. Therefore, a diagnosis of CRS should not rely solely on a finding of abnormal CRP.

Participants receiving SBT777101 should be monitored closely for signs and symptoms of CRS. Management of CRS should occur according to site protocols or standard clinical practice.

6.4.2.2. Neurotoxicity

CAR Teff cell therapy has been shown to be associated with neurological toxicities, also known as CAR T-Cell Related Encephalopathy (CRES) or Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) that may correlate with high cytokine levels. In general, onset of neurologic symptoms has been seen to begin five to seven days after CAR Teff therapy administration.

The risk of neurotoxicity with SBT777101 treatment is unknown but, it should be noted that there is a distinct difference in mechanism of action of SBT777101 from CAR Teff therapies used in the oncology setting.

Participants receiving SBT777101 should be monitored closely for signs and symptoms of neurotoxicity. Management of neurologic toxicity should occur according to site protocols or standard clinical practice.

6.4.2.3. Malignancies

The overall risk of malignancy for SBT777101 is unknown but considered low based on data from prior Treg clinical studies. Participants with malignancy within the last five years will be excluded from the study. Enrolled participants will be followed for at least one year as part of protocols SBT777101-01 and SBT777101-02 and will be required to participate in a 15 year follow up study for longer term safety surveillance in accordance with current FDA guidance.

SBT777101 contains a CAR that is encoded using a lentiviral vector. One concern of using a lentivirus is the potential for insertional mutagenesis caused by the integration of vector DNA near an oncogene. The risk for this believed to be low since lentiviral integration patterns favors sites away from cellular promoters and the CAR copy number itself will be low (Scholler et al., 2012). In vitro studies conducted with SBT777101 showed a polyclonal integration site profile with no dominant integration site of concern observed (see Section 4.3.2).

The polyclonal integration site profile of the SBT777101 lentiviral vector and the lack abnormal growth of SBT777101 in the absence of IL-2 are consistent with the demonstrated safety profile of lentiviral vectors broadly used in therapeutic settings and indicate a low likelihood of transformation of the transduced Tregs (Milone and O'Doherty, 2018).

The lentiviral vector used to manufacture SBT777101 is a third-generation vector designed to minimize risks of formation of replication-competent lentivirus (RCL). Nevertheless, RCL testing will be performed on participant samples at timepoints defined in the study protocols.

6.4.2.4. Thrombosis/Thromboembolism

Thromboembolic events (arterial thrombosis and septic thrombophlebitis) have been described in patients with Crohn's disease following administration of autologous ovalbumin-specific Tregs (Desreumaux et al., 2012). The risk of thrombosis/thromboembolism with SBT777101 is unknown.

To minimize the risk for thrombosis or thromboembolism, use of estrogen replacement therapy and estrogen-containing contraception is prohibited. In addition, participants should be followed for evidence of coagulopathy and any participant presenting with limb edema or dyspnea should be evaluated for thromboembolism.

6.5. Special Patient Populations

There are no data available on the use of SBT777101 in special patient populations.

6.5.1. Pregnancy

No studies have been conducted to assess the reproductive and development toxicity of SBT777101 in pregnant patients. It is not known whether SBT777101 can cross the placenta and cause harm to the fetus if administered to pregnant women.

As such, precautions have been implemented in the inclusion/exclusion criteria of the protocols regarding dosing of SBT777101 in women of childbearing potential and women who are pregnant are excluded from study participation.

6.5.2. Nursing Mothers

It is not known whether SBT777101 is excreted in human milk. Clinical recommendations for women who become pregnant and choose to breast feed will be made by the investigator in referral with other physicians, if needed, at the research center.

6.5.3. Children

There is no information on the use of SBT777101 in patients under 18 years of age. Therefore, children are not eligible for treatment with SBT700101.

6.5.4. Geriatric Patients

There is no safety information regarding the use of SBT777101 in geriatric patients. Investigators should consider the benefits and risks of SBT777101 in patients aged 65 years and older. Patients ≥70 years of age will not be allowed to enroll in studies with this product. Any use of SBT777101 in geriatric populations will consider FDA Guidelines for the Study of Drugs Likely to be Used in the Elderly and ICH E-7 Guideline for Industry Studies in Support of Special Populations: Geriatrics.

6.6. Concomitant Use with Other Medications

No clinical studies assessing the interaction of SBT777101 with other concomitant medications have been performed. Details on the potential interaction of SBT777101 with permitted and prohibited concomitant medications are included in the study protocols.

6.7. Overdose

No data on overdose has been generated to date. Guidelines on monitoring and reporting overdose are provided in the study protocols.

6.8. Starting Dose, Maximum Dose and Dose Escalation

Given the lack of direct translatability to in vivo models, it is not possible to use a no observed adverse effect level (NOAEL) or minimal observed biologic effect level (MABEL) approach from nonclinical studies to determine the starting dose or an expected maximum dose level. The starting dose for the initial cohort in the Phase 1 study is 100 $x10^6$ CAR^+ T cells (based on EGFR expression) administered by IV infusion. This low dose level is similar to the starting dose level administered to participants in multiple clinical studies and case reports using polyclonal autologous and alloantigen-specific Treg cellular therapy in disease indications including Type 1 diabetes, graft versus host disease, kidney and liver transplantation, lupus and Crohn's disease (Bluestone et al., 2015; Chandran et al., 2017; Dall' Era et al, 2019; Desreumaux et al., 2012; Marek-Trzonkowska et al., 2014; Mathew et al., 2018; Roemhild et al., 2020). The dose selected for the next cohort will be no more than 3-fold higher than that administered in the cleared cohort. This aligns with the dose escalation regimen utilized in other Treg studies (Bluestone et al., 2015; Brunstein et al., 2011; Mathew et al., 2018; Roemhild et al., 2020).

Unlike most other Treg products, SBT777101 includes the addition of a CAR. While this introduces a modification to the cell, addition of the CAR to the Tregs is not expected to negatively alter the benefit-risk ratio compared to untransduced Treg cells. This is supported by data from studies with allo-antigen specific Tregs, where the potency of the allo-antigen Tregs is increased over that for polyclonal Tregs (Jiang et al., 2006; Golshayan et al., 2007). The increase in specificity of the Treg via the T cell receptor did not lead to a change in safety profile compared to polyclonal Treg therapies in clinical studies and the doses administered were within range of the proposed starting dose and were well tolerated. The starting dose level is also consistent with the doses of allo-antigen Treg therapy previously given to transplant patients (Brunstein et al., 2011; Todo et al., 2016; Trzonkowski et al., 2009; NCT02474199, 2022).

The proposed maximum dose is up to 900 x 10^6 CAR^+ cells (based on EGFR expression) administered by IV infusion. A small fraction (approximately 2%) of Tregs can be found in the bloodstream, with some cells being sequestered in the lymph nodes, intestine, and bone marrow. It is therefore appropriate to dose up to the proposed level to ensure that sufficient study drug is present in the circulation to be able to traffic to the site of inflammation and reach the CAR target antigen within the synovial tissue.

7. REFERENCE SAFETY INFORMATION FOR ASSESSMENT OF EXPECTEDNESS OF SERIOUS ADVERSE REACTIONS

There are currently no expected serious adverse reactions (SARs) associated with SBT777101. All SARs will be reported as suspected unexpected serious adverse reactions (SUSARs) in accordance with current global regulatory guidelines.

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