Singh Biotechnology
www.singhbiotechnology.com

Single domain antibodies—minimalist binders for maximal therapeutic efficacy

Singh Biotechnology is developing novel camelid-derived single domain antibodies that target key intracellular molecular switches in cancer. The technology has therapeutic applications beyond cancer, and the company is seeking partners to advance its assets from preclinical to commercialization.

Singh Biotechnology (SBT), a privately held start-up company established in 2014 and based in Tampa Bay, Florida, focuses on the discovery and development of proprietary single domain antibodies (sdAbs) for therapeutic application in a variety of cancers, autoimmune, ophthalmic, and infectious diseases. Single domain Abs, also called nanobodies, are small antigen-binding fragments (15 kDa) derived from VHH, the single N-terminal domain found in camelid heavy chain antibodies. Nanobodies represent the minimal antigen-targeting unit of a conventional antibody, and their advantages over conventional antibodies include high stability and solubility, and their ability, due to their small size, to interact with hard or impossible-to-target antigenic sites.

Recognizing the potential of sdAbs for targeting proteins traditionally considered to be undruggable, SBT has developed a platform for optimizing sdAbs that takes advantage of their three complementarity-determining regions (CDRs), which control the antigenic characteristics of the nanobodies. Using its technology platform, SBT has generated therapeutic sdAbs to specifically target intracellular molecules that are mutated, overexpressed, or significant in the pathogenesis of disease.

The company's lead asset is SBT-100, a bi-specific sdAb that binds both to KRAS and STAT3, two top cancer targets, and is capable of crossing the blood–brain barrier (BBB) and the cell membrane. SBT-100 exhibits a therapeutic effect against multiple human cancers both in vitro and in vivo.

"We believe that single domain antibodies represent a promising new approach for targeted cancer immuno-therapies due to their ability to specifically target intracellular proteins," said Sunanda Singh, SBT's founder and CEO. "We are extremely encouraged by the tremendous progress we have made, in a short time, to develop single domain antibody therapeutics with the potential to deliver highly targeted compounds that could help improve the quality of many cancer patients' lives."

SBT has started toxicology studies with SBT-100 for a planned oncology IND filing. It has already received Orphan Drug Designations from the U.S. Food and Drug Administration (FDA) for pancreatic cancer and osteosarcoma, and the pre-IND briefing packet for SBT-100, which included preclinical data in triple negative breast cancer (TNBC), details about the GMP manufacturing process for SBT-100, a proposal for toxicology studies in two species, and a roadmap for phase 1 clinical studies, also obtained a favorable FDA review.

SBT pipeline		Development stage			Comments
Therapeutic	Indication	Research	Preclinical	Phase 1	
SBT-100 (KRAS, P-STAT3)	TNBC breast cancer	→	→	2022	Pre-IND briefing packet reviewed by FDA
	Prostate cancer	→	→		Tested in vitro, in vivo in progress
	Glioblastoma	→	→		Tested in vitro
	Sarcoma*	→	→	2022	Orphan drug status granted by FDA
	Pancreatic cancer*	→	→	2022	Orphan drug status granted by FDA
SBT-101 (P-STAT3)	TNBC breast cancer and pancreatic cancer	→	→		Tested in vitro
SBT-102 (KRAS)	Pancreatic cancer	→	→		Tested in vitro
	Colorectal cancer	→			In progress
	Lung (NSCLC) cancer	→			In progress

*Indicates orphan cancers

Fig. 1 | Singh Biotechnology's pipeline. Over the past seven years, Singh Biotechnology has built a broad pipeline of proprietary single domain antibodies targeting STAT3 and KRAS—two key molecular switches in cancer—for therapeutic application across a wide range of cancers. FDA, Food and Drug Administration.

The company also has a broad pipeline of wholly owned and worldwide patented sdAbs in different stages of development, including SBT-101, a mono-specific anti-STAT3 sdAb, SBT-102, a mono-specific anti-KRAS sdAb, SBT-104, an anti-TNF-alpha sdAb, and SBT-106, an anti-Ebola virus VP24 sdAb (Fig. 1). SBT is planning to advance its sdAb pipeline in collaboration with partners interested in co-development and commercialization agreements, or in licensing the company's assets.

Two targets—many cancer applications

KRAS (Kirsten rat sarcoma 2 viral oncogene homolog), a small guanosine triphosphate (GTP) binding protein, and STAT3 (signal transducer and activator of transcription 3), a member of the STAT family of latent transcription factors, both play pivotal roles in the activation of signaling molecules and the transmission of signals from the cell surface to the nucleus where they modulate cellular processes such as cell differentiation, cell growth, and apoptosis (Fig. 2). KRAS is mutated in approximately 25% of all human cancers, and STAT3 is overexpressed in 50–90% of human cancers, most notably in pancreatic cancer, and has been linked to other conditions such as autoimmune diseases, blindness, fibrosis, endometriosis, and infectious diseases.

SBT's sdAbs have been optimized to jointly or individually target both KRAS and STAT3. Lead compound SBT-100, a bi-specific sdAb, binds tightly to KRAS with a K_D of 4.20 nM, to KRAS G12D, the most common KRAS mutant in humans, with a K_D of 15 nM, and to STAT3 with a K_D of 22.4 nM. The inhibition of KRAS GTPase activity of both SBT-100 and the mono-specific KRAS-targeting SBT-102, is further comparable to that of existing polyclonal KRAS antibodies.

In vitro, SBT-100 has shown beneficial effects against triple negative human breast cancer (MDA-MB-231) and human pancreatic cancer (PANC-1) at nanomolar concentrations. Treatment with SBT-100 results in a decrease of activated ERK (P-ERK), a downstream effector of the KRAS signaling pathway. PANC-1 contains the KRAS G12D mutation, while the breast cancer cell line MDA-MB-231 has a G13D mutation.

Similarly, the STAT3 binding activity of SBT-100 extends to both phosphorylated STAT3 (P-STAT3) and unphosphorylated STAT3

ADVERTISER RETAINS SOLE RESPONSIBILITY FOR CONTENT

(U-STAT3), which ensures that a broader range of genes turned on by these STAT variants can be modulated using SBT-100.

SBT has now tested the antiproliferative activity of SBT-100 in eleven different human cancer cell lines: PANC-1 and Bx-PC3 (pancreatic cancer), MDA-MB-231, MDA-MB-468, and MDA-MB-453 (TNBC), MCF-7 (estrogen receptor–positive (ER^+) and progesterone receptor–positive (PR^+) breast cancer), BT 474 (HER-2 amplified breast cancer), U87 (glioblastoma), SJSA-1 (osteosarcoma), HT-1080 (fibrosarcoma), and DU 145 (metastatic, chemo-resistant prostate cancer). Growth suppression ranged between 64% and 93% across all cell lines and with IC_{50}s in the nanomolar to single digit micromolar range.

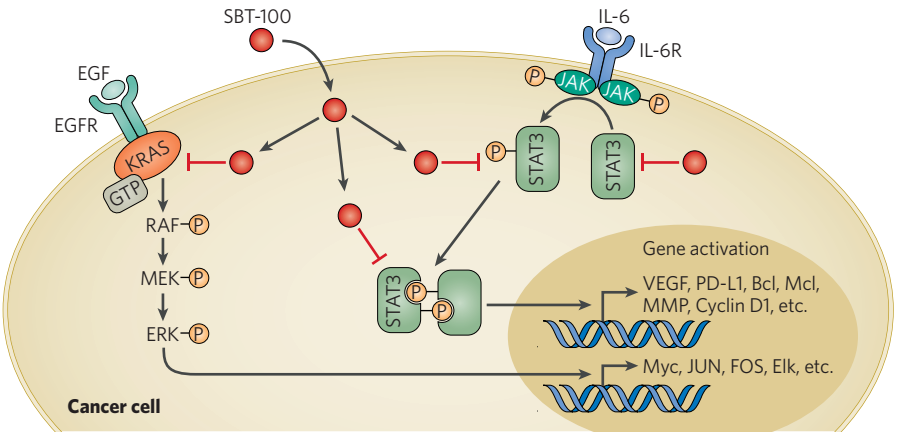

Fig. 2 | The multiple modes of action of SBT-100. SBT-100 is a bi-specific single domain antibody that inhibits both KRAS and STAT3, resulting in a multiplicity of signaling pathways being affected. These pathways include signaling cascades modulated by VEGF and IL-6. EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERKs, extracellular signal-regulated kinases; GTP, Guanosine-5'-triphosphate; IL-6, Interleukin 6; JAK, Janus kinases; MMP, matrix metalloproteinases; PD-L1 programmed death-ligand 1; VEGF, vascular endothelial growth factor.

Adding it up in vivo

Having documented the broad efficacy of SBT-100 against a range of tumor cell lines in vitro, SBT set out to confirm the efficacy of its lead compound in vivo. Following a 14-day course of treatment with SBT-100, well established and vascularized mouse xenografts of human TNBC (MDA-MB-231) measuring 50–100 mm^3 in size showed significant ($p<0.001$) growth suppression.

Similarly, a 14-day course of treatment with SBT-100, gemcitabine, or SBT-100 and gemcitabine, of well-established mouse xenografts of human pancreatic cancer (PANC-1) measuring 100–150 mm^3, resulted in growth suppression levels of 19.17%, 14.93%, and 31.52%, respectively, compared to controls. These data demonstrated the additive effect of SBT-100 on gemcitabine in suppressing pancreatic cancer growth.

In a third set of experiments, SBT demonstrated the effect of SBT-100 on doxorubicin in the treatment of human osteosarcoma. A 14-day course of treatment with doxorubicin or with SBT-100 and doxorubicin, of well-established mouse xenografts of human osteosarcoma (SJSA-1), resulted in a survival rate of 28% and 72%, respectively, suggesting SBT-100 reduces the toxicity for chemotherapeutic drugs such as doxorubicin and makes treatment more effective by improving safety and survival.

The additive effect observed in the above combination therapy settings suggests a potential role of SBT-100 as an agent for reducing chemotherapy-induced toxicity when treating human cancer patients. The overall preclinical safety profile of SBT-100 is further promising given that none of the mice used in SBT's oncology and non-oncology preclinical testing have died, lost weight, or developed signs of any toxicity.

Crossing barriers

A key advantage of the sdAbs is their ability to cross both the BBB and cell membranes. In vivo experiments with tumor-bearing mice containing established MDA-MB-231 tumors showed the presence of SBT-100 inside the MDA-MB-231 cancer cells and in the neurons and glial cells of the mouse brains. These findings suggest the possibility of targeting primary malignancies of the central nervous system (CNS), metastatic cancers that spread to the brain, cancer stem cells that hide in the CNS, and benign diseases where STAT3 plays a role in pathogenesis such as multiple sclerosis, Parkinson's disease, and Huntington's disease, with SBT-100.

Targeting STAT3 with SBT-100 has further advantages. At the molecular level, SBT's data suggest SBT-100 can be used with current checkpoint inhibitors to potentially augment their therapeutic effect. In MDA-MB-231 cells, SBT-100 causes both a decrease in STAT3 activation by reducing P-STAT3 levels, and also a decrease of total STAT3 levels in the cells. STAT3 is a transcription factor that turns on genes necessary for cancer growth, proliferation, survival, angiogenesis, host immunosuppression, and metastasis. One such target gene is PD-L1 (programmed death-ligand 1). Inhibition of STAT3 with SBT-100 resulted in a reduction of PD-L1 expression of 94% within 48 hours, suggesting the SBT-100 could potentially be used in the context of a broad spectrum of cancers since >50% of human cancers utilize the STAT3 pathway as a mechanism for malignant behavior. In addition, SBT-100 exhibits a potent anti-inflammatory effect, which may help reduce inflammatory complications typically associated with checkpoint inhibitor therapy.

Looking beyond cancer—other applications of sdAbs

Beyond cancer, SBT's sdAbs have shown potential for other therapeutic applications including ophthalmic and infectious diseases.

Through an ongoing collaboration with the National Eye Institute at the National Institutes of Health, SBT is investigating the potential of SBT-100 for treating ophthalmic conditions such as age-related macular degeneration (AMD) and uveitis, two of the most common forms of vision limitation or blindness in the U.S.

Aberrant growth of blood vessels in the retina due to dysregulation of vascular endothelial growth factor (VEGF) leads to limited vision and blindness in AMD, and STAT3 dysregulation leads to vision loss in uveitis. VEGF activity is modulated by STAT3 and thus inhibition of STAT3 is one of the therapeutic strategies being followed for these two conditions. SBT-100 has shown dramatic reductions in VEGF expression in an in vitro model of AMD ($p<0.0001$), and in an in vivo model of human uveitis preserves vision.

SBT is also collaborating with the United States Army Military Research Institute of Infectious Diseases to investigate the potential of using SBT-100 to treat viral infections including with Ebola virus, Zika virus, Venezuelan equine encephalitis virus, and Chikungunya virus.

STAT3 has been implicated in the pathogenesis of some viral diseases, and SBT has shown in vitro that SBT-100 can inhibit the viruses listed above by 90–95%.

SBT is now preparing to advance its SBT-100 program to clinical trials for several cancer indications. The human formulation of SBT-100 for intravenous administration has already been developed and shows high stability at both room temperature and 4°C for up to 12 months.

SBT's ability to move its various sdAb programs rapidly through the exploratory and preclinical phases was a result of its virtual approach. By working with top contract research organizations, SBT was able to be flexible and to streamline the work required to prepare for IND filings and clinical stage planning. For the next stages, SBT is looking to convert its 'virtual promise' into clinical realities on the ground by working with partners in pharma.

"As a virtual company, SBT has been able to rapidly advance its discovery and preclinical programs through a cost-effective and streamlined process that combines partnering with the optimal CROs to execute the different research and development components required, and to work with top GMP-certified manufacturers in the U.S.," said Singh. "We hope this same philosophy of applying an optimized and lean approach to the clinical and commercialization stages of our assets will guide future partnerships to bring our first-in-class sdAb therapeutics to patients worldwide."

CONTACT
Sunanda Singh, Founder and CEO
Singh Biotechnology, LLC
Tampa Bay, FL, USA
Tel: +1-813-892-6599
Email: ssingh@singhbiotechnology.com