CELL PENETRATING THERAPEUTIC ANTIBODY PLATFORM COMPANY

Single Domain Antibody for Cancers, Viral Infections, and Ophthalmic Diseases

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Singh Biotechnology Overview

Mission	Discover and develop proprietary drugs for the treatment of cancers, viral diseases, and ophthalmic diseases.
Technology	Novel sdAb cell penetrating antibody platform (**disruptive technology**): Advantages of biologics and small molecule drugs with applications in a number of diseases.
Pipeline	Developing therapeutics targeting intracellular proteins like STAT3 (Signal Transducer and Activator of Transcription 3) and KRAS.
Results	*In vitro* and *in vivo:* Significant reduction of inflammation induced cancer growth in multiple cancers, viral infections, and ophthalmic diseases
Stage	FDA has granted **two** Orphan Drug Designations: Pancreatic Cancer (August 2016) & Osteosarcoma (September 2017). Pre-IND stage completion by 3Q2021, IND filing by 4Q2021.
Funding	To date raised approximately $5 MM from Angel Investors.

SBT Therapeutic Pipeline: Oncology

SBT PIPELINE		Development Stage			
Therapeutic	**Indication**	**Research**	**Preclinical**	**Phase 1/2**	**Comments**
SBT-100 (KRAS, P-STAT3)	Breast Cancer (TNBC, ER+/PR+, HER2+)	→	→	2021	Tested In Vitro and In Vivo
	Prostate Cancer	→	→		Tested In Vitro, In Vivo in Progress
	Glioblastoma	→	→		Tested in vitro
	Sarcoma*	→	→	2021	Orphan Drug Status Granted by FDA
	Pancreatic Cancer*	→	→	2021	Orphan Drug Status Granted by FDA
SBT-101 (P-STAT3)	Breast Cancer (TNBC, ER+/PR+)	→	→		Tested In Vitro
SBT-102 (KRAS)	Pancreatic Cancer	→	→		Tested In Vitro
	Colorectal Cancer	→			In Progress
	Lung (NSCLC) Cancer	→			In Progress

Singh Biotechnology Patent Numbers:

- SBT-100: US 9,695,234, B2
- SBT-101: US 9,850,321, B2
- SBT102: US 9,663,570, B2

***Indicates Orphan Cancers**

- FDA Orphan Drug Designation Received by SBT.
- SBT owns full rights to all its programs & patents.
- SBT Technology & assets have **NOT** been licensed from any institutions or organization.
- PCT publication number: WO 2016/065323 A2

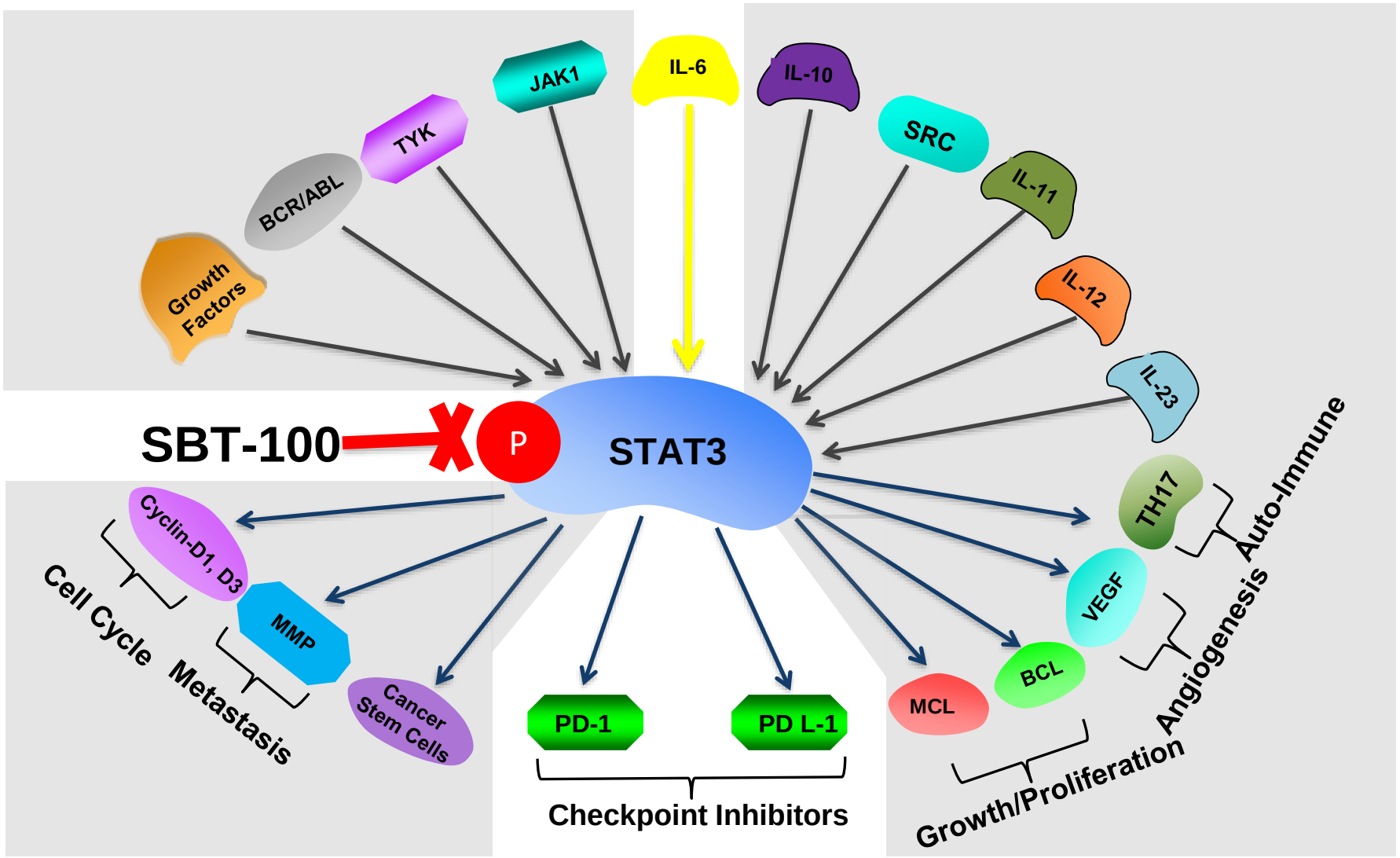
SBT Therapeutic Pipeline: Non-Oncology

SBT PIPELINE		Development Stage			
Therapeutic	**Indication**	**Research**	**Preclinical**	**Phase 1/2**	**Comments**
SBT-100	Infectious Disease (Ebola, Zika)	→	→		Tested In Vitro, In Vivo In Progress
	Infectious Disease (Viral Encephalitis)	→			In Progress
SBT-106 (Ebola VP24)	Infectious Disease (Ebola)	→	→		Tested In Vitro
SBT-107 (HIV 1 RT)	Infectious Disease (HIV)	→	→		Tested In Vitro

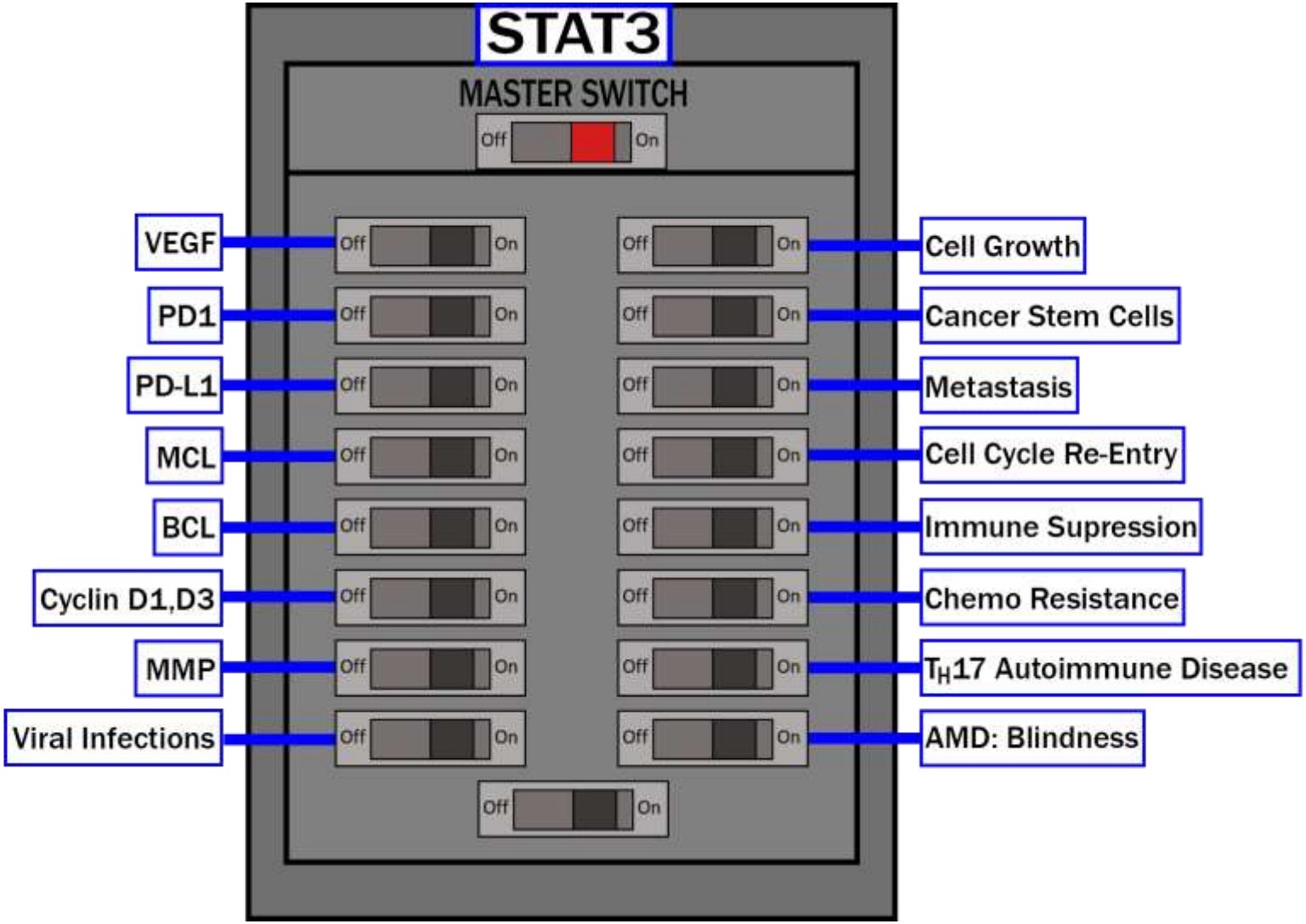
SBT-100 Currently in IND Enabling Studies

Preclinical	**Mechanism & Efficacy Demonstrated** - High affinity binding to targets - *In vivo* efficacy in animal models
Formulation	**Formulation Completed** - IV completed, other formulations in progress - Stable at room temperature
Manufacturing	**GMP Process: Established** - Cell line development complete - Gram level production established - Kilogram scale up underway
GLP toxicology	**Studies Planned for 1Q2021** - Design completed - CRO selected

STAT3 Induced Inflammation Drives Diseases

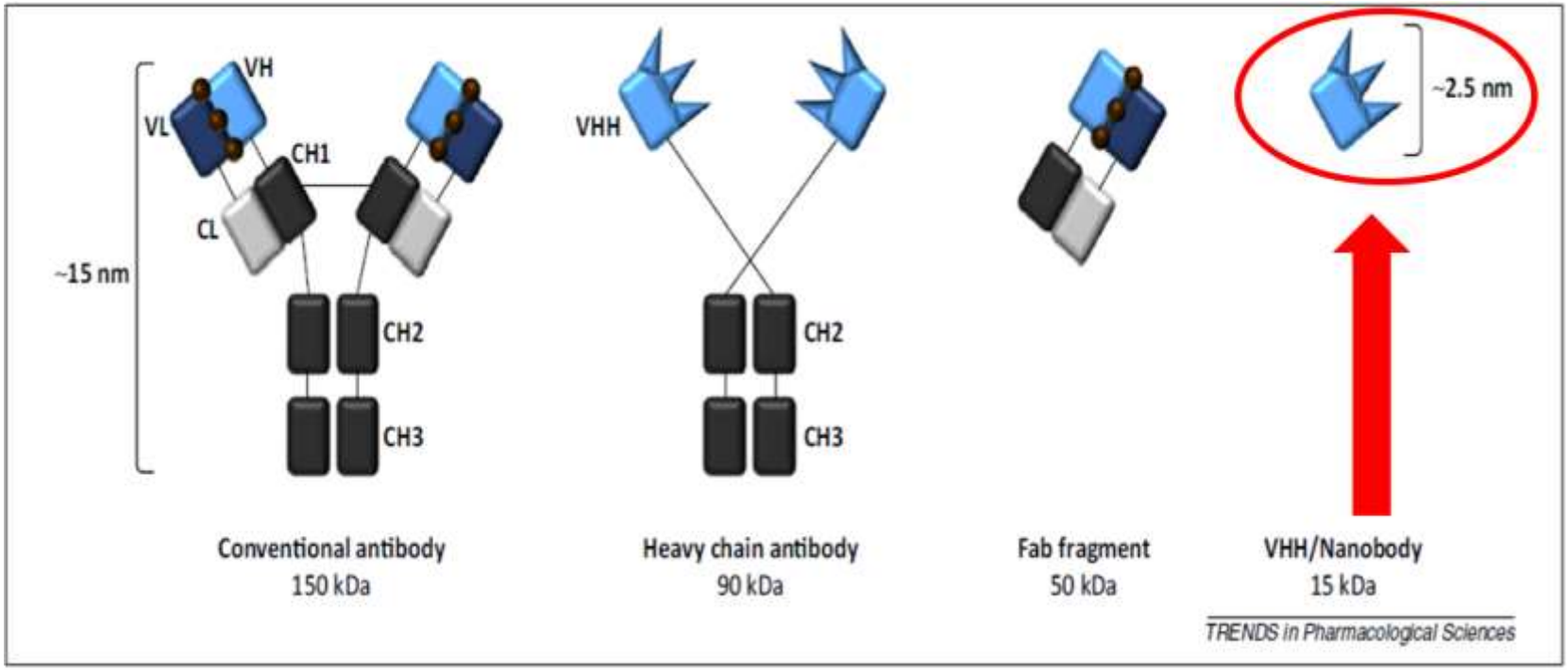


STAT3: Master Switch



SBT-100: Camelid-Derived, Single Domain Antibody (sdAb)

SBT-100 is a monomeric molecule but has bi-specific binding to KRAS and STAT3.



- VHH = Single Domain Antibody (sdAb)
- Most other sdAbs do not penetrate the cell
- Small size permits deep tissue penetration
- Renal Secretion, No toxicity

2014, 35:247-255

SBT-100 BINDS STAT3 & INHIBITS ITS FUNCTION

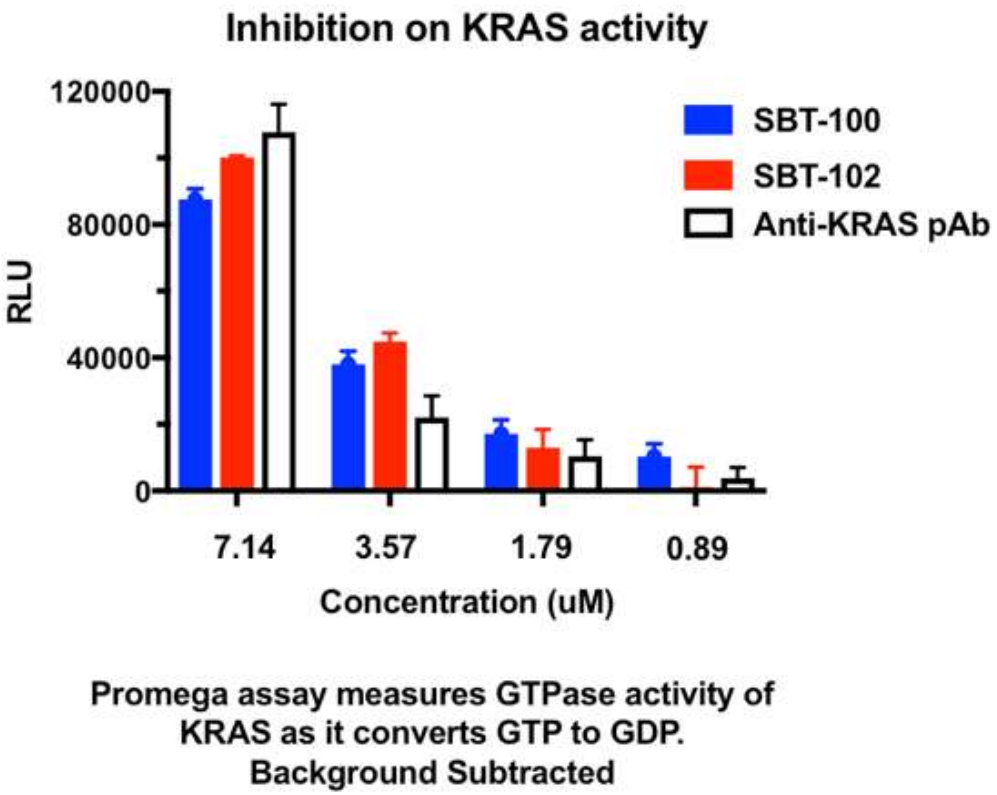
Affinity Binding of SBT sdAb in Biacore Assay

		Human STAT3 Protein	Human STAT5 Protein	Human KRAS Wildtype Protein	Human KRAS (G12D) Mutant	Ebola VP24	HIV 1 RT
1	Anti-KRAS / Anti-STAT3 VHH (SBT-100)	2.24×10^{-8}	7.50×10^{-8}	4.20×10^{-9}	1.50×10^{-8}		
2	Anti-KRAS VHH (SBT-102)	No Binding Observed	No Binding Observed	3.22×10^{-9}	1.48×10^{-7}		
3	Ebola VP24 VHH (SBT-106)					2.94×10^{-8}	
4	HIV 1 RT VHH (SBT-107)						3.85×10^{-9}

KD (M)

- SBT-100 binds & inhibits cancers with wild type KRAS, KRAS(G12D) (most common mutant), and KRAS(G13D) mutations.
- SBT-100 likely binds a common epitope near the KRAS GTPase active site, thereby making SBT-100 a pan-KRAS inhibitor.
- SBT-100's bi-specific property allows it to concentrate inside cells with high concentration of STAT3 which then brings SBT-100 into close proximity to KRAS.

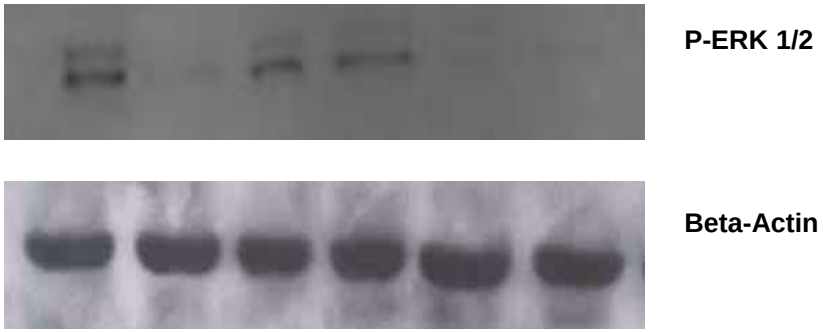
Assay: Inhibition of KRAS Activity



Promega assay measures GTPase activity of KRAS as it converts GTP to GDP. Background Subtracted

- Since there is no known inhibitor of KRAS commercially available, we used a polyclonal antibody to KRAS. This polyclonal antibody binds to multiple different epitopes on KRAS to inhibit function through steric hindrance.
- Inhibition of KRAS activity by SBT-100 and SBT-102 is comparable to the polyclonal antibody, which suggests that SBT-100 and SBT-102 bind to an epitope on KRAS that is close to the GTPase active site.

SBT-100 Reduces P-ERK in KRAS Mutant Cells



	Lane	P-ERK 1/2
1	MDA-MB-231 Control	0.747
2	MDA-MB-231 / SBT 100: 100 µg/ml	0.347
3	PANC-1 Control	0.502
4	PANC-1 / SBT 100: 100 µg/ml	0.465
5	BxPC3 Control	0.310
6	BxPC3 / SBT100: 100 µg/ml	0.292

- MDA-MB-231 (TNBC) & PANC-1 cells have KRAS activating mutations.
- BxPC3 cells **do not** have KRAS mutation.

- MDA-MB-231 has a larger amount of STAT3 than PANC-1, therefore SBT-100 can concentrate more in the MDA-MB-231 cells inhibiting its KRAS(G13D) mutation which leads to greater suppression of P-ERK 1/2.
- PANC-1 has much less intracellular STAT3 than MDA-MB-231 which then results in less SBT-100 concentrating inside PANC-1 cells.
  - Therefore SBT-100 cannot inhibit the KRAS(G12D) of PANC-1 as effectively and thus P-ERK 1/2 is reduced to a lesser amount.
  - In the literature PANC-1 has been described as a KRAS-independent cancer. It does not respond easily to KRAS inhibition. (https://www.cell.com/action/showPdf?pii=S1535-6108%2809%2900111-1)

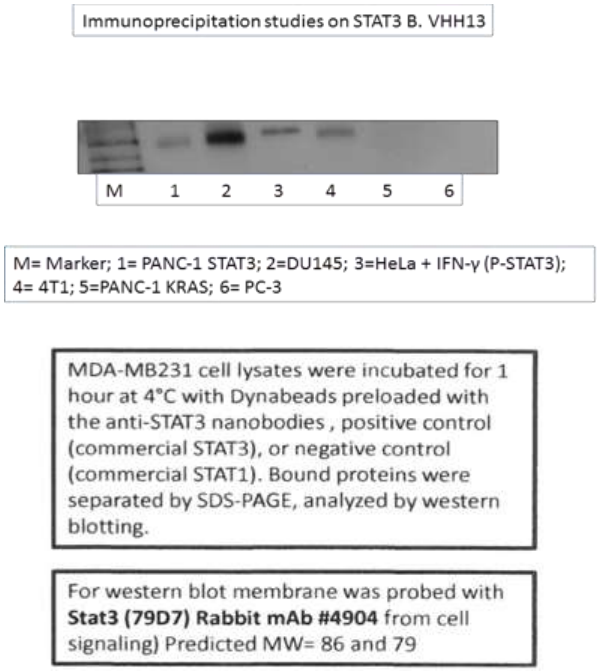
SBT-100 Binds STAT3 from Lysed Cell Lines

Unphosphorylated STAT3

Lanes:
M- Marker
1- STAT3 VHH13 (mammalian)
2- STAT3 VHH14 (mammalian)
3- STAT3 VHH13 (bacterial)
4- STAT3- VHH14 (bacterial)
5- Positive control (STAT-3)
6- Negative control (STAT-1)

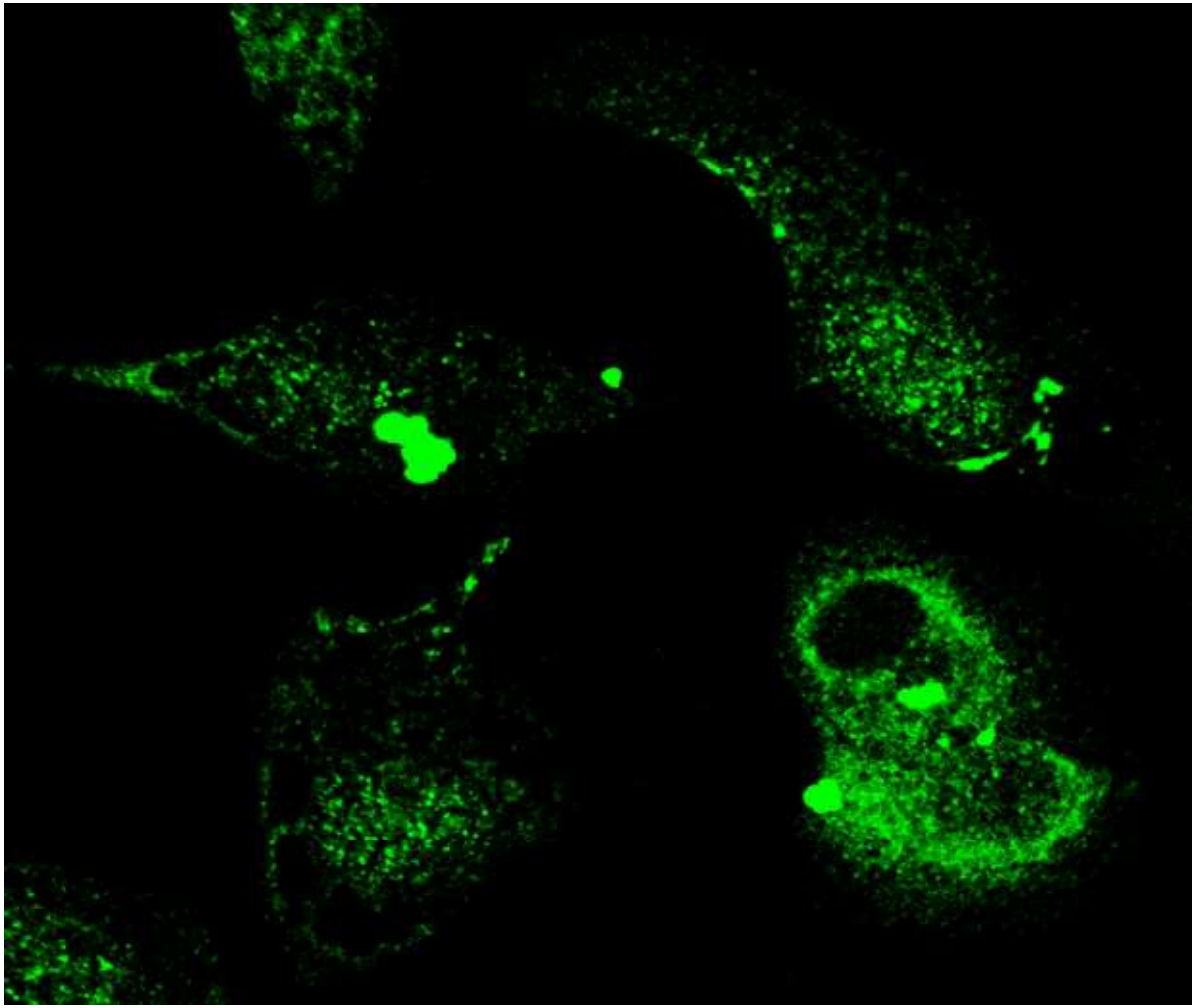
- The western blot on the left shows the amount of STAT3 in MDA-MB-231 cells & lane 1 of the western blot on the right shows the PANC-1 cells have much less intracellular STAT3.
- This difference may explain the difference in P-ERK inhibition in the previous slide (number 17).
- Lane 2, on the right, shows that DU145 prostate cancer cells have high concentration of STAT3. This may explain why it is more effectively suppressed compared to other cells with less STAT3.
- Lane 4 in the western blot on the right side shows that SBT-100 binds to mouse STAT3 which is 99% homologous to human STAT3.
- This is important for the FDA which wants to see that our in vivo data is done in animals where the target is affected in a comparable way as it would be in humans.

Phosphorylated STAT3



Positive control: SC-482 STAT3 Antibody (C-20) rabbit polyclonal; negative control: STAT1 #9172

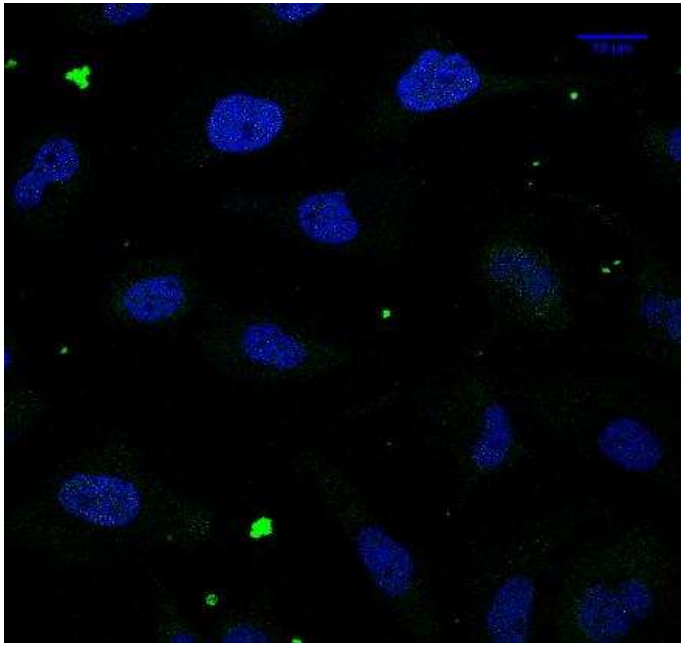
SBT-100 Binding in the Cytoplasm



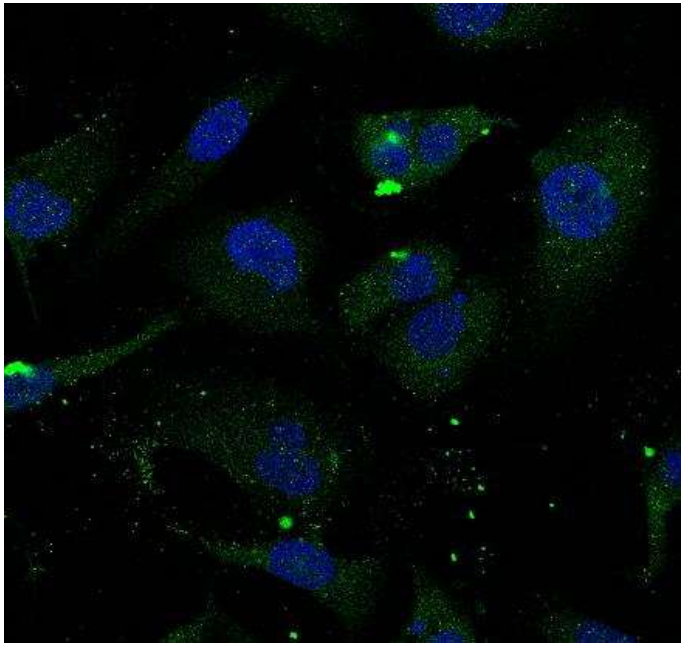
- Positive staining in cytoplasm. Confocal Image of anti-VHH IFA, SBT-100, 6hr in MDA-MB-231 (TNBC) cells.
- SBT-100 localizes inside cancer cells and the SBT-100 is bound by a secondary anti-llama antibody conjugated with a green fluorescent color.

SBT-100 Binding in the Nucleus & Cytoplasm

DAPI Treated & No VHH Antibody



DAPI & SBT-100: Positive Staining in Nucleus and Cytoplasm



- Confocal Images of DAPI detection via IFA (left) and following 6hr of SBT-100 anti-VHH antibody/DAPI treatment (right) in MDA-MB-231 (TNBC) cells *in vitro*.
- The left side is staining only with the vehicle, and the right side is staining with SBT-100.
- Both sides had the secondary anti-llama conjugated antibody added after six hours.

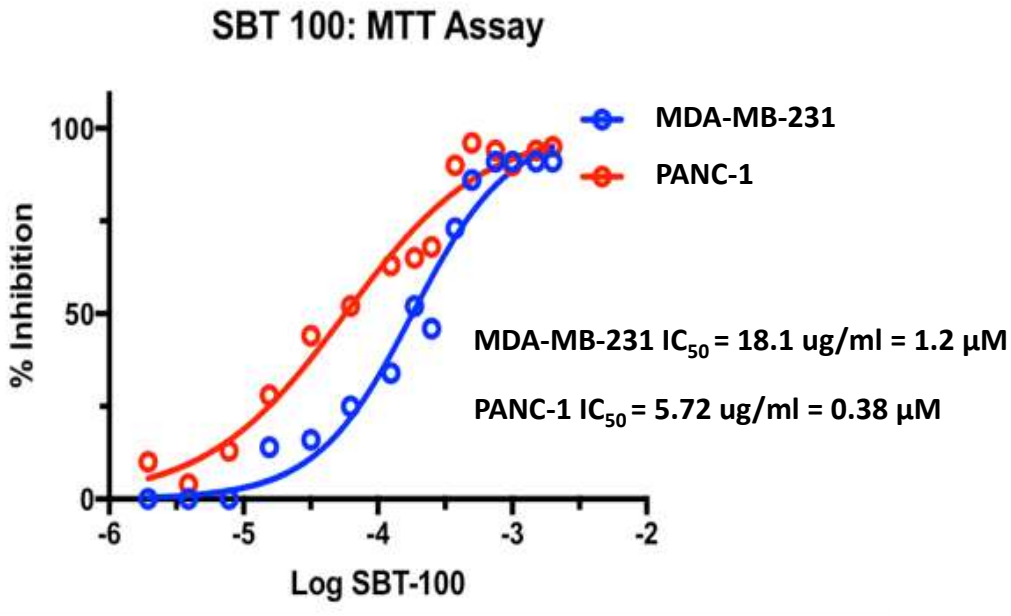
SBT-100: Inhibits Proliferation in Cancer Cell Lines

Cancer	Human Cancer Cell Line	KRAS Mutation/STAT3	% Inhibition in 3 Days with SBT-100 (100 ug/ml or 6.7 uM)	IC50 (µM)
Pancreatic	PANC-1	+ / +	85% (P < 0.001)	0.38
Pancreatic	Bx-PC3	- / +	90% (P < 0.001)	2.34
TNBC	MDA-MB-231	+ / +	89% (P < 0.001)	1.21
TNBC	MDA-MB-468	? / +	85% (P < 0.001)	0.83
TNBC	MDA-MB-453	- / +	64% (P < 0.001)	1.19
Breast	MCF-7	? / +	93% (P < 0.001)	0.99
Breast	BT474	? / +	93% (P < 0.001)	1.68
Glioblastoma	U87	- / +	62% (P < 0.001)	4.32
Osteosarcoma	SJSA	? / +	83% (P < 0.001)	3.39
Fibrosarcoma	HT-1080	- (+ NRAS)/ +	86% (P < 0.001)	2.13
Prostrate	DU-145	+ / +	92% (P < 0.001)	1.46

In Vitro Growth Inhibition Determined by MTT Assay.
Red indicates documented KRAS mutation.

- This table demonstrates SBT-100's broad application in multiple aggressive human malignancies.
- These include 2 pancreatic cancers, 3 TNBCs, ER+PR+ breast cancers (MCF-7), HER-2 amplified breast cancer (BT474), brain cancer, two types of sarcomas, and metastatic, chemo resistant prostate cancer (DU-145).
- The cells highlighted in red have a documented KRAS mutation in addition to over expression of P-STAT3.

SBT-100 *In Vitro* Dose Response Curves

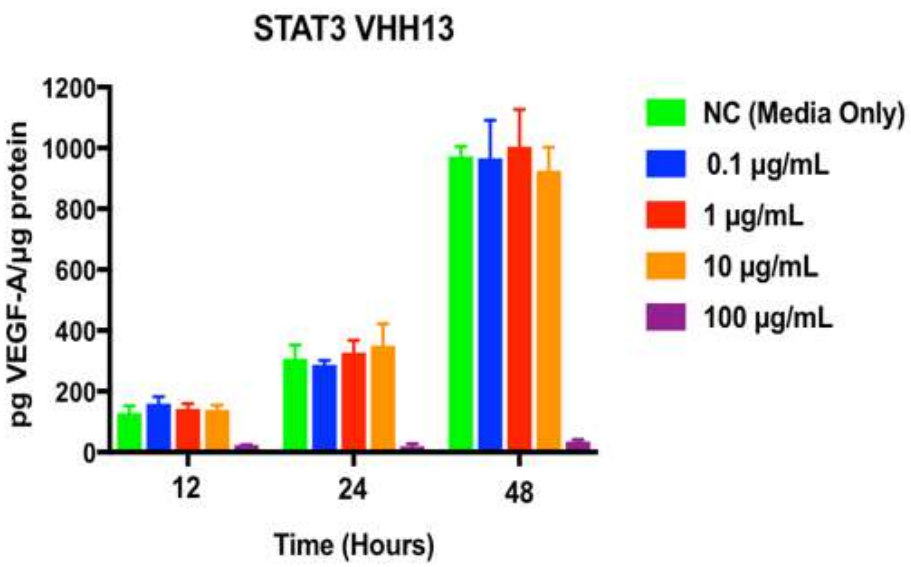


- MDA-MB-231 (TNBC) Cells [KRAS (G13D) Mutation]
- PANC-1 (Pancreatic) Cells [KRAS (G12D) Mutation]
- Doses response for SBT-100 in PANC-1 & MDA-MB-231 cancer cell lines *in vitro* MTT assay.

- This is an example of two cancers' dose response curves that generated the data for the table in the previous slide.
- Both of these two cancers have an activating KRAS mutation.

SBT-100 Rapidly Blocks VEGF Production *In Vitro*

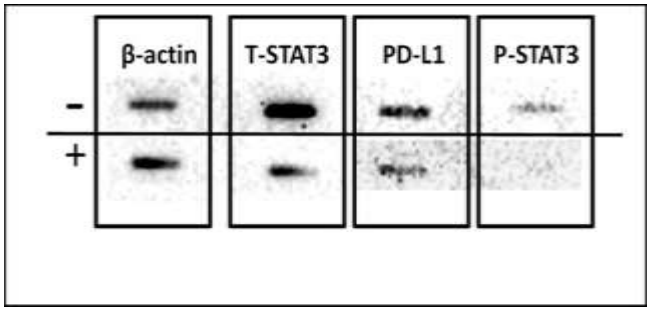
Macular Degeneration Model (AMD): Retinal Epithelial Cells



- SBT-100 significantly inhibits VEGF production within 12 hrs ($p<0.01$)
- Inhibition is maintained for 48 hrs ($p<0.01$)

- Retinal epithelial cells which continuously produce VEGF (vascular endothelial growth factor) are cultured with SBT-100 using log dosing.
- Within 12 hours the VEGF protein production drops to near zero and this is continued up to 48 hours with one dose of SBT-100.
- VEGF is critical for cancer tumor growth and for macular degeneration which is the most common form of blindness in the western world.

SBT-100 Protein Immunoblots



Representative slot blots of protein samples from untreated (-) and 24hr SBT-100 treated MDA-MB-231 cells.

Time (Hrs) of Treatment	200µM S3I-201*	50µg/ml (3.3 µM) SBT-100	100µg/ml (6.7 µM) SBT-100
0	1	1	1
3	2.57	2.69	2.01
6	0.72	0.71	0.65
24	0.35	0.12	0.06

Quantification of total STAT3 following treatment of MDA-MB-231 cells with S3I-201* or SBT-100 with increasing times.

The changes in the levels of total STAT3, P-STAT3 and PD-L1 induced by SBT-100 treatment in MDA-MB-231 cells were quantified by immunoblotting of protein extracts from untreated cells and treated MDA-MB-231 cells for 3hr, 6hr, 16hr and 24hr with SBT-100.

*S3I-201 is a cell-permeable Stat3 inhibitor that binds to the Stat3-SH2 domain, prevents Stat3 phosphorylation/activation, dimerization, and DNA-binding.

- To help appreciate the rapid changes induced by SBT-100 within 24 hours to total STAT3 (T-STAT3), activated STAT3 (P-STAT3), and PD-L1 immunoblots were done (upper left-hand corner).
- The data for T-STAT3 was quantified and is shown in the table in the upper right-hand corner, and in this table SBT-100's suppression of T-STAT3 is compared to S3I-201 (a small molecule inhibitor of STAT3).
- S3I-201 came from a chemical bank from the NCI and was patented by scientists at Moffitt Cancer Center in Tampa, Florida. SBT-100 gives significantly better suppression of T-STAT3 than S3I-201.

Quantification: Total STAT3, P-STAT3 & PD-L1

- Quantification of the levels of total STAT3, P-STAT3 and PD-L1 after treatment with SBT-100 for increasing times is shown in the graphs.
- SBT-100 treatment resulted in a decrease in the levels of total STAT3, P-STAT3, and PD-L1.
- The significant suppression of T-STAT3, P-STAT3, and PD-L1 by SBT-100 has been quantified and graphed to further appreciate the rapid change that occurs in 24 hours.

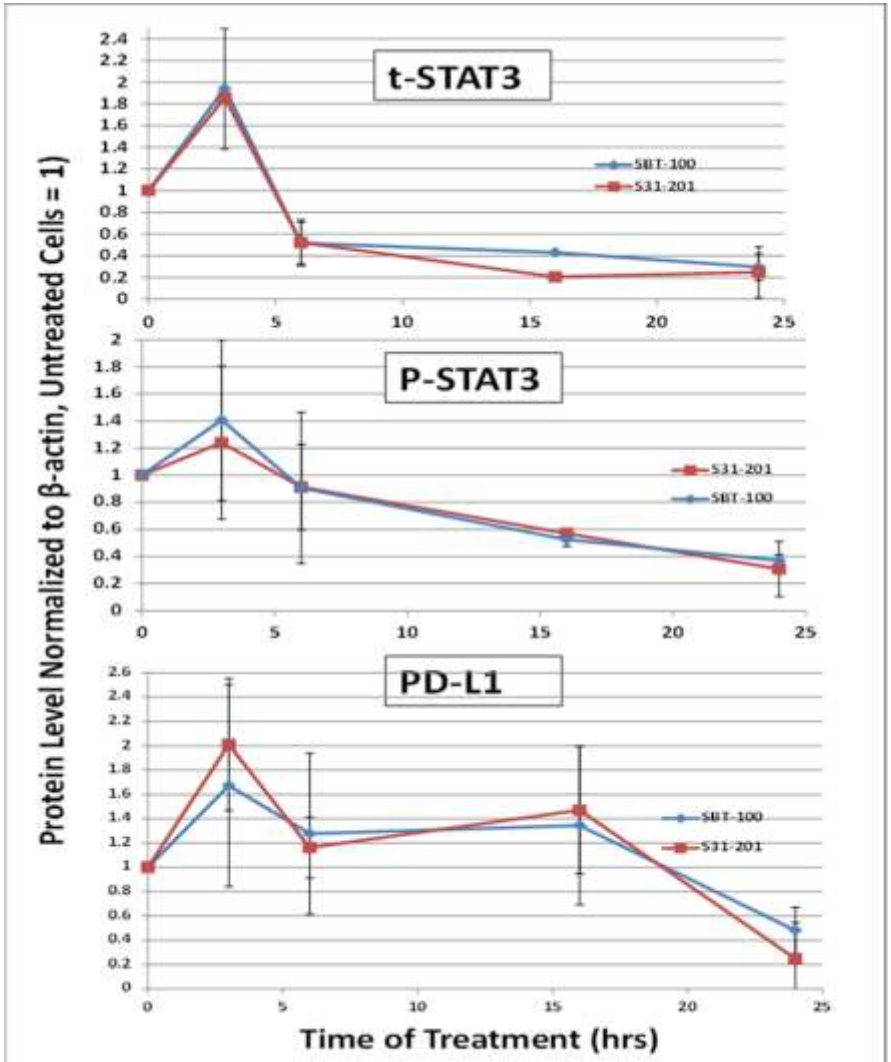
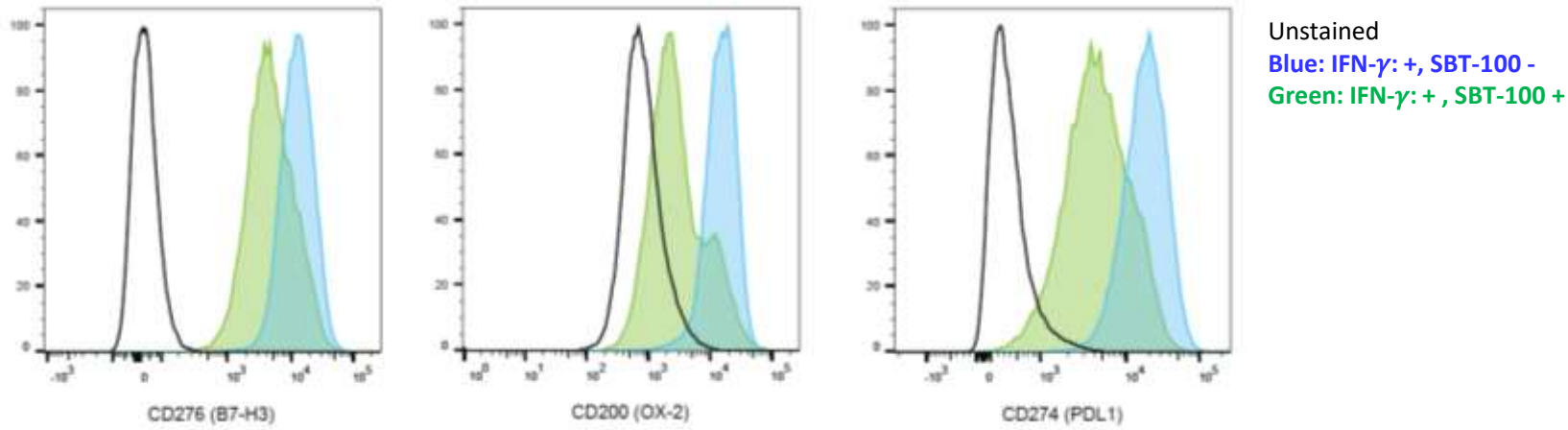


Fig. 11. The Effects of treatment with SBT-100 or S31-201 on t-STAT3, P-STAT3 and PD-L1 levels in MDA cells.

FACS Analysis: SJSA-1 Cells (Osteosarcoma)

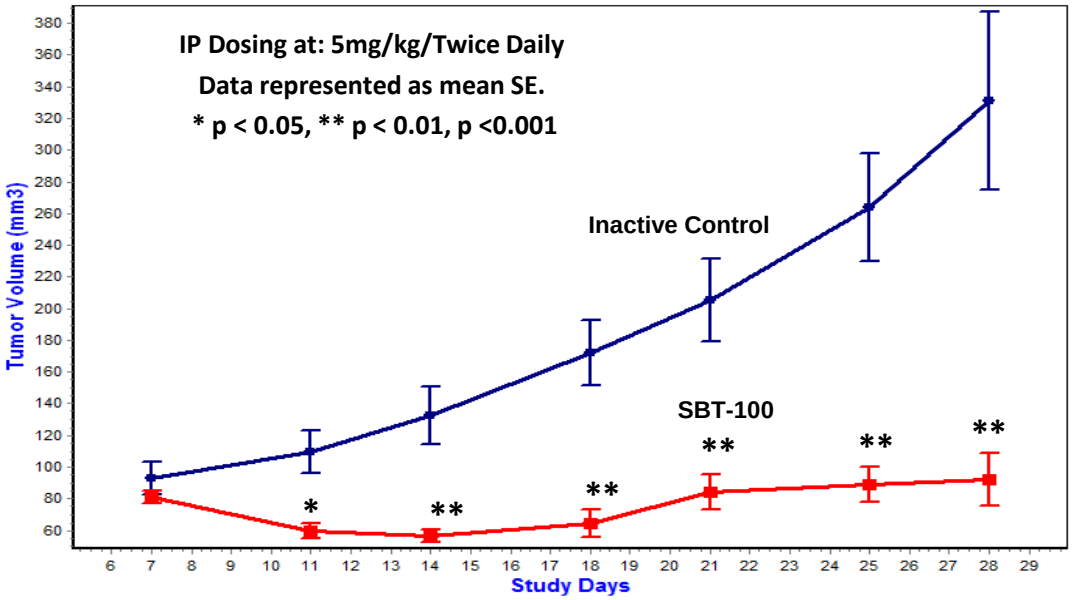
- Since the rapid suppression of PD-L1 by SBT-100 is so important, we had a second CRO test this finding with a different human cancer (osteosarcoma) using a different method (FACS analysis).
- The panel in the far right hand side shows the black & white curve as unstained cells, the blue curve are cells treated with IFN-γ to upregulate PD-L1 but had no SBT-100, and the green curve are the cells treated with IFN-gamma and SBT-100.
- Use of SBT-100 causes a left shift of the curve demonstrating a 10-fold decrease in PD-L1 expression of the surface of these human osteosarcoma cells.
- This finding suggests that SBT-100 can decrease cancer cell surface expression of PD-L1 thus potentially augmenting or synergizing with checkpoint inhibitor therapy.
- In addition SBT-100 has been shown to significantly decrease Th17 cells in an autoimmune disease model. This may help reduce or eliminate the development of autoimmune conditions that can develop with checkpoint inhibitor therapy. Thus reducing potential toxicity.



- Flow Cytometry study in SJSA-1 (Osteosarcoma) cells for checkpoint inhibitors.
  - PD-L1 inhibition is a key factor in cancer immunotherapy.
  - Orexin-2 receptor (OX-2) which is a GPCR (G-protein coupled receptor) expressed exclusively in the brain.
  - B7-H3, member of the B7 and CD28 families, plays an important role in the inhibition of T-cell function & autoimmunity.

SBT-100 *In Vivo* Efficacy: TNBC

Mean Tumor Volume in MDA-MB-231 Cells [KRAS (G13D) Mutation]

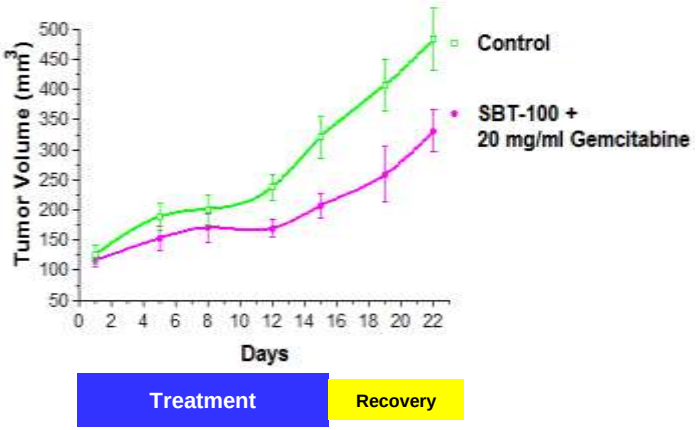


- No toxicity or weight loss observed through out the study
- Dosing for 14 days followed by 7 days of recovery and observation

- Using the NCI protocol, nude mice with human TNBC tumors between 50-100 mm^3 were randomized into control (blue line) and treatment (red lines) groups.
- This TNBC cells have a KRAS activating mutation.
- SBT-100 treated mice had significant suppression of tumor growth compared to control group which received vehicle only.
- After 14 days of treatment, the mice were in 7 days of recovery.
- None of the treated mice died, lost weight, or showed any clinical signs of toxicity.

SBT-100 *In Vivo* Efficacy in Pancreatic Cancer Cells: In Combination with Gemcitabine

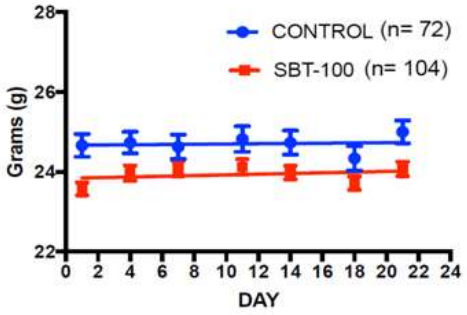
PANC1 [KRAS (G12D) Mutation]: SBT-100 & Gemcitabine



No Toxicity or Weight Loss Observed

% Inhibition of Control				
Group	Day 12	Day 15	Day 19	Day 22
20 mg/kg Gemcitabine	19.51	23.17	23.26	14.93
5 mg/kg SBT-100 BID	20.7	27.29	18.37	19.17
5 mg/kg SBT-100 + 20 mg/kg Gemcitabine	28.83	35.48	36.37	31.52

Body Weights



- This xenograft study also used the NCI protocol and the human pancreatic tumors were grown to between 100-150 cubic millimeters.
- This pancreatic cancer also has a KRAS activating mutation.
- Treatment with SBT-100 plus gemcitabine resulted in significant reduction in tumor growth (31.52% reduction versus control group).
- The graph in the bottom right shows the pooled data of mice weights from multiple studies. Mice treated with SBT-100 did NOT lose weight or show any signs of toxicity over the 3 weeks during these xenograft studies.

DOXORUBICIN STUDY

- Nude mice with human osteosarcoma tumors were treated with doxorubicin and had significant reduction in tumor growth compared to the control group which received vehicle only; however, this group had only 28% survival rate at the end of the 3-week study. They died due to doxorubicin toxicity.

- Nude mice with these tumors when treated with doxorubicin plus SBT-100 also had significant reduction in tumor growth compared to the control group, but this group had survival rate of 70% of the mice.

- **SBT-100 reduced doxorubicin induced toxicity.**

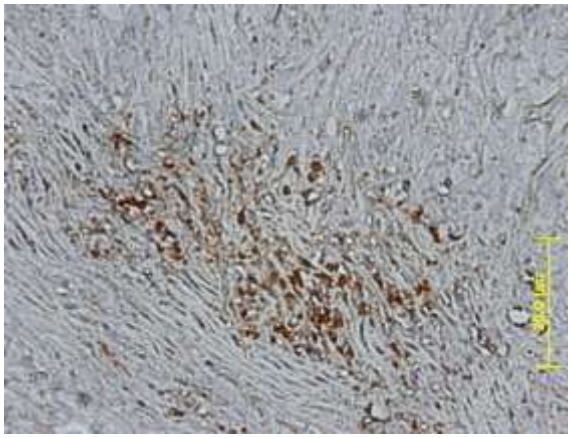
- This reduced toxicity associated with SBT-100 is the reason the FDA granted it Orphan Drug Designation for osteosarcoma.

SAFETY

- SBT-100 is a sdAb such as a camelid VHH.
- VHH as a class of antibodies have an excellent safety profile in humans.
- Ablynx has a VHH (caplacizumab) for aTTP which successfully completed Phase III and is now being used by patients. Three other VHHs are in Phase II, and multiple are in Phase I.
- Inhibiting STAT3 in adults is safe as demonstrated by Phase I studies by Dianippon/BBI (small molecule), Otsuka (2 small molecules), and AstraZenaca/ISIS (RNA inhibitor).
- With over 150 mice treated by USA CROs and NIH, no deaths, no toxicity, no weight loss has been noted with SBR-100.

SBT-100 Rapidly Penetrates the Cell and Crosses Blood Brain Barrier (BBB)

Tumor



Brain

- Intracellular localization of SBT-100 shown by IHC staining of tumor & brain cells done 15 minutes after mouse was injected with SBT-100 IP
- Evaluated by 2 independent clinical pathologists

- Xenograft mice with human TNBC tumors were injected intraperitoneally with SBT-100 and then 15 minutes later sacrificed.
- The tumors of these mice show that SBT-100 localizes in the cancer cells within the tumors.
- Staining of the brains show that SBT-100 localizes inside the neurons and glial cells.
- Thus demonstrating that SBT-100 rapidly crosses the BBB in vivo. Most chemotherapeutic drugs do NOT cross the BBB.
- The potential for targeting primary CNS cancers and metastatic cancers to the brain now exists. Also neurological conditions that are STAT3 mediated (multiple sclerosis, Parkinson's disease, Huntington's disease, etc.) can potentially be treated.

SBT-100 INHIBITS VIRAL REPLICATION

STAT3 in Viral Infections

- Ebola virus VP24 binds karyopherin alpha1 and blocks STAT1 nuclear accumulation

J. Virol. 2006, 80(11):5156

- The Ebola virus interferon antagonist VP24 directly binds STAT1

PLoS Pathogens, 2012, 8:2, e1002550

- In the absence of STAT1, no antiviral state is established

J. Virol. 1996, 70(1): 647

- STAT3 signaling is activated by DNA and RNA viruses, including EBV, MCMV, HCMV, HSV, VZV, HCV

- Activation or increased expression of STAT3 is required for the replication of a number of viruses by suppressing the type I IFN mediated antiviral response or regulating microtubule dynamics

Wang *et al*. STAT3 negatively regulates type I IFN-mediated antiviral response. J Immunol 187:257, 2011

- STAT3 inhibitors decreased viral replication significantly, e.g., SGIV

Huang Fish & Shellfish Immunology 41:308-16, 2014

STAT3 in Viral Infections

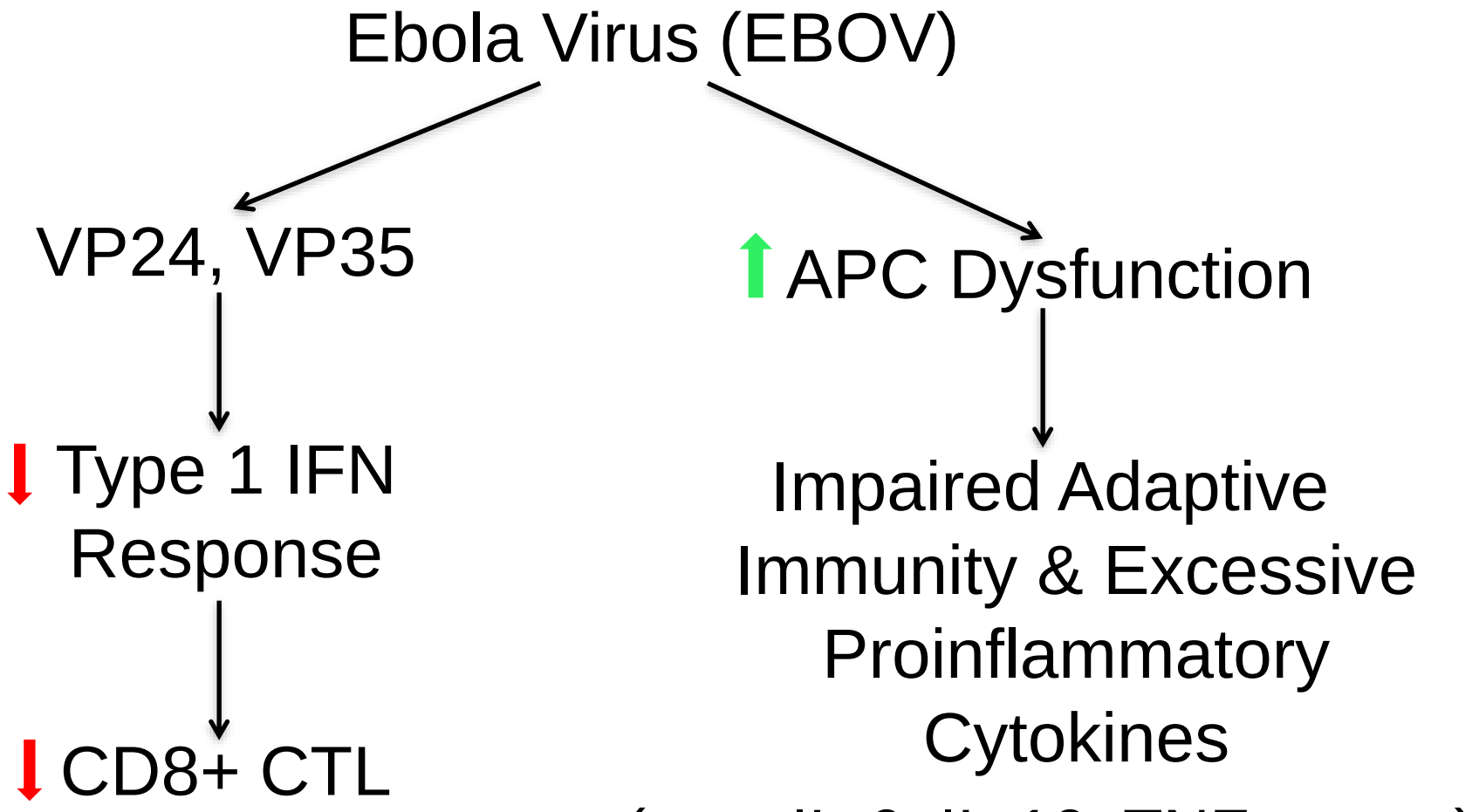
Viral manipulation of STAT3: Evade, exploit, and injure

Armando Andres Roca Suarez, Nicolaas Van Renne, Thomas F. Baumert, Joachim Lupberger

•Published: March 15, 2018
•https://doi.org/10.1371/journal.ppat.1006839

Abstract:
Signal transducer and activator of transcription 3 (STAT3) is a key regulator of numerous physiological functions, including the immune response. As pathogens elicit an acute phase response with concerted activation of STAT3, they are confronted with two evolutionary options: either curtail it or employ it. This has important consequences for the host, since abnormal STAT3 function is associated with cancer development and other diseases. This review provides a comprehensive outline of how human viruses cope with STAT3-mediated inflammation and how this affects the host. Finally, we discuss STAT3 as a potential target for antiviral therapy.

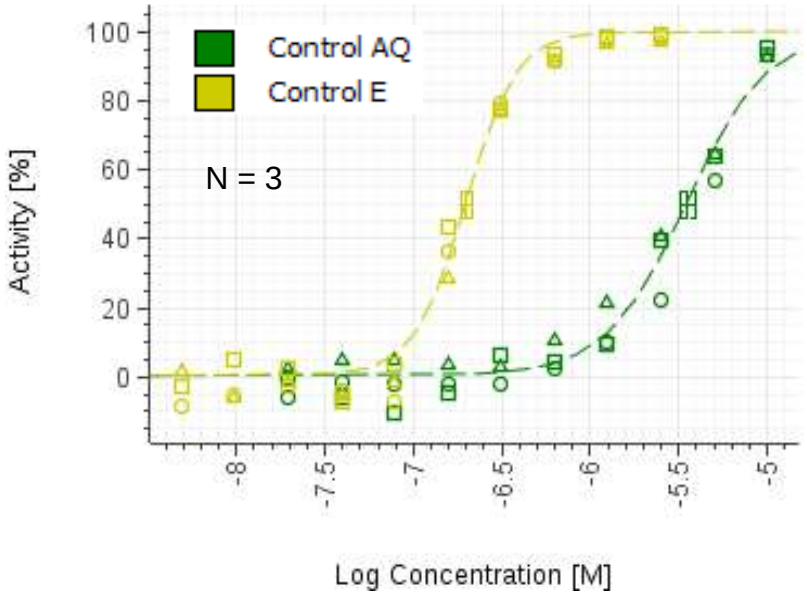
Ebola Virus Impaired Immune Response & Cytokine Storm



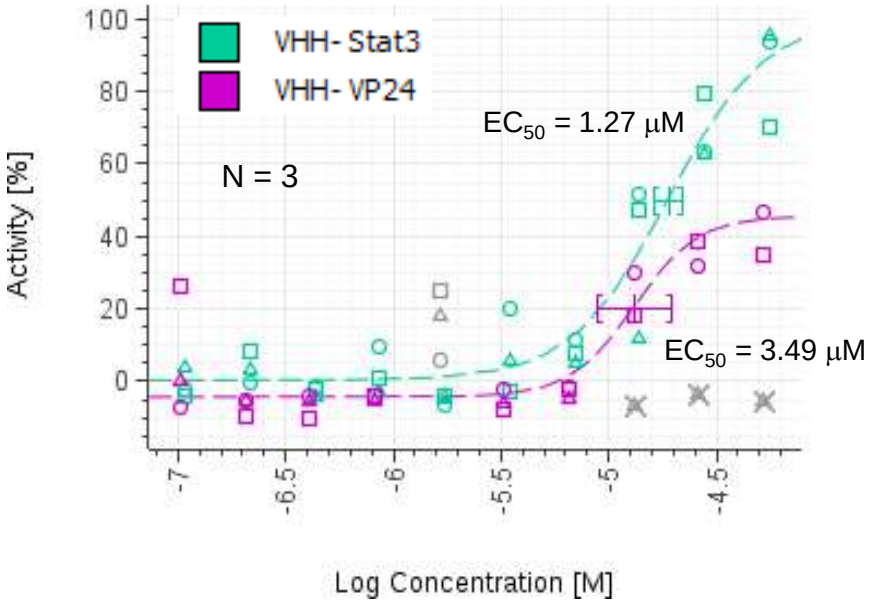
PLoS One. 2015 Feb 26;10(2):e0118345

SBT-100: Inhibits Ebola Proliferation

EBOV/Hela: % Inhibition



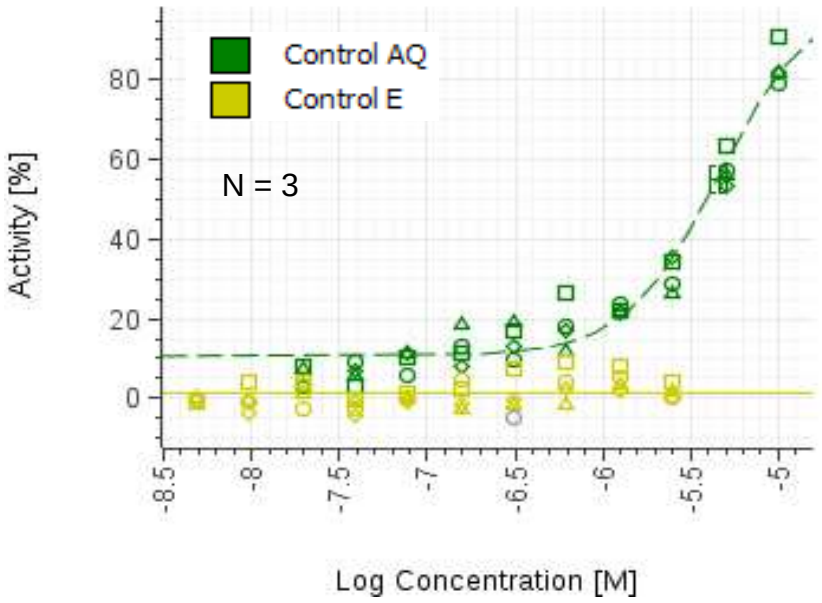
EBOV/Hela: % Inhibition



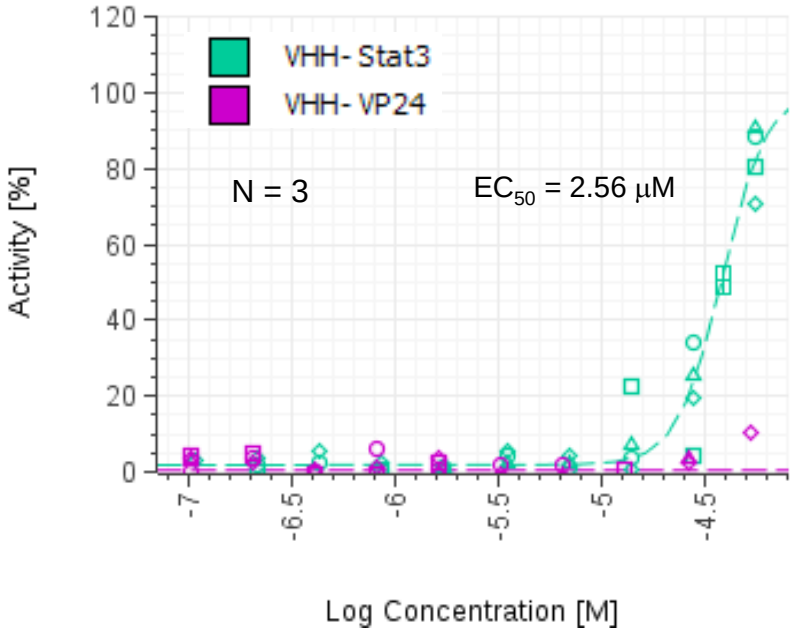
- Anti-STAT3 VHH (**SBT-100**) inhibits Ebola virus replication comparable to Control AQ
- Anti-Ebola VP24 VHH also inhibits Ebola replication

SBT-100: Inhibits Ebola Proliferation

EBOV/HFF: % Inhibition



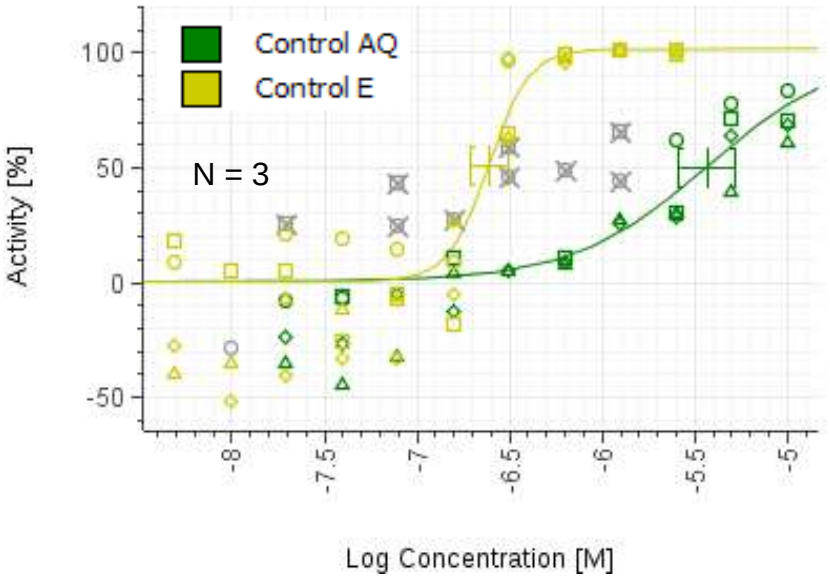
EBOV/HFF: % Inhibition



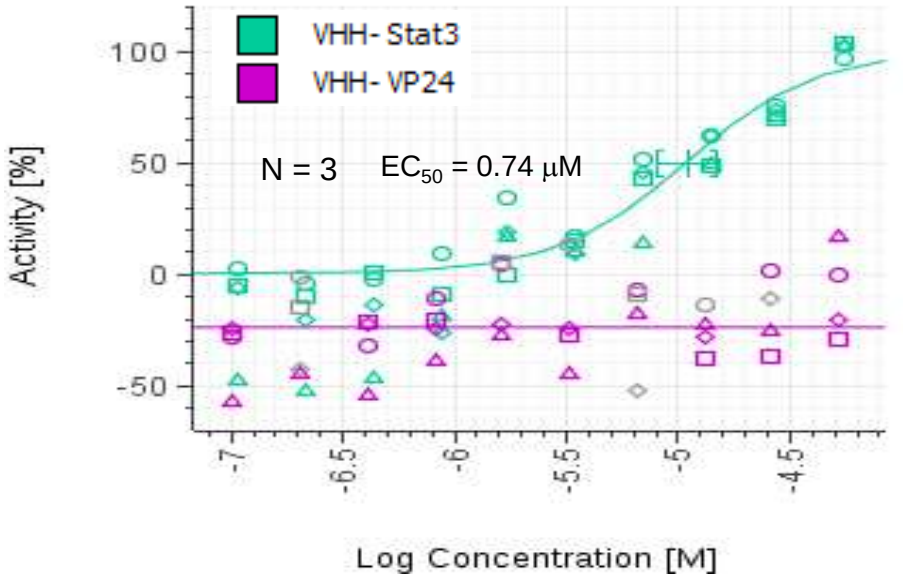
- Anti-STAT3 VHH (**SBT-100**) again inhibits Ebola virus replication comparable to Control AQ
- Anti-Ebola VP24 VHH does not inhibit Ebola replication in this model

SBT-100: Inhibits Zika Proliferation

Zika/Vero: % Inhibition



Zika/Vero: % Inhibition

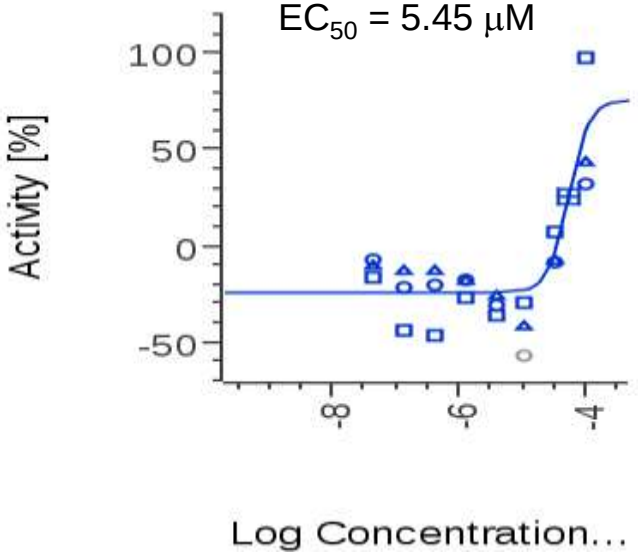


- Anti-STAT3 VHH (**SBT-100**) inhibits Zika virus replication comparable to Control AQ and Control E
- Anti-STAT3 VHH (**SBT-100**) inhibits both Ebola and Zika virus suggesting it maybe a therapeutic for inhibiting other viruses
- Anti-Ebola VP24 VHH does not inhibit Zika virus replication thus showing specificity for the Ebola virus

SBT-100: Inhibits Zika (DAKAR, Senegal) Virus Proliferation

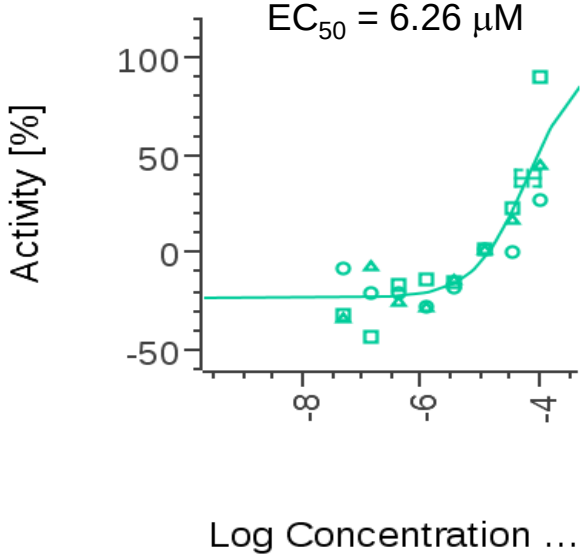
VERO E6 Cells

EC_{50} = 5.45 µM



HFF Cells

EC_{50} = 6.26 µM



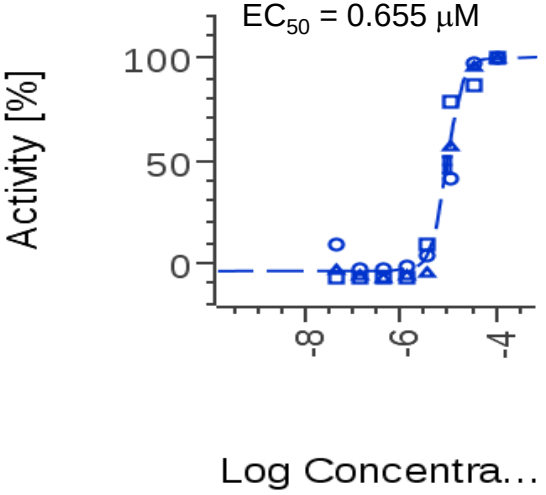
Cell line	Pathogen	% Infection	EC50 [uM]
Vero	Zika DAKAR , Senegal	58.68	5.457042
		54.69	
		41.47	
HFF	Zika DAKAR , Senegal	63.94	6.262912
		57.08	
		50.59	

- Anti-STAT3 VHH (**SBT-100**) inhibits Zika virus replication in different infected cell lines.

SBT-100: Inhibits Venezuelan Equine Encephalitis (TC83) Virus Proliferation

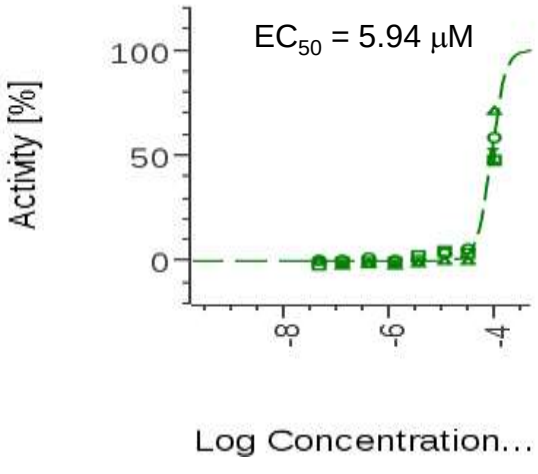
HeLa Cells

EC_{50} = 0.655 µM



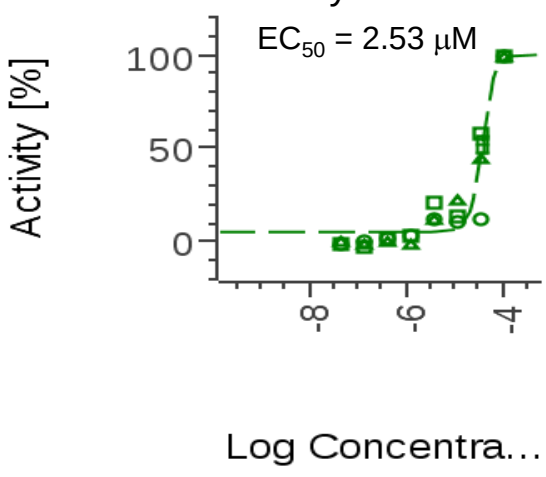
BE2M17: Glioblastoma Cells

EC_{50} = 5.94 µM



U87MG: Astrocytoma Cells

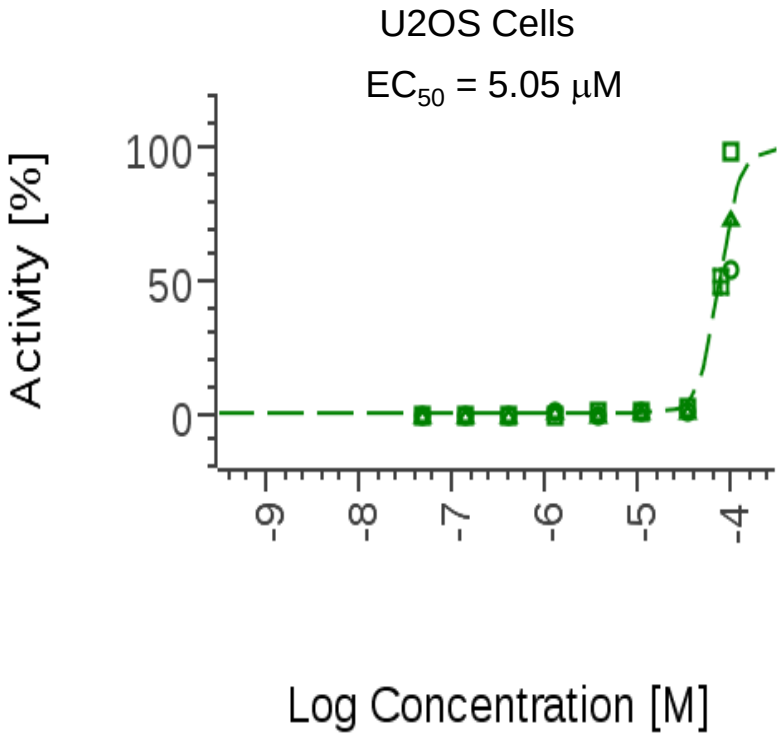
EC_{50} = 2.53 µM



Cell line	Pathogen	% Infection	EC50 [uM]
Hela	TC83	98.95	0.655027
		93.70	
		97.91	
BE2M17	TC83	96.89	5.944437
		96.97	
		96.29	
U87MG	TC83	97.24	2.534258
		95.73	
		96.28	

- Anti-STAT3 VHH (**SBT-100**) inhibits VEE virus replication in different infected cell lines.

SBT-100: Inhibits Chikungunya (CHIV 181/25) Virus Proliferation



Cell line	Pathogen	% Infection	EC50 [uM]
U2OS	CHIV (181/25)	99.94	5.051497
		99.96	
		99.96	

- Anti-STAT3 VHH (**SBT-100**) inhibits Chikungunya virus replication in U2OS infected cell line.

Viral Inhibition by SBT-100

SBT sdAb	Cell Line	Pathogen	Max % Inhibition	EC50 [µM]	Comment
SBT-100	HeLa	ENBOV (Ebola)	97%	1.27	Inhibts Ebola virus replication comparable to control compounds
SBT-106			45%	3.49	
SBT-100	HFF		95%	2.56	Inhibts Ebola virus replication comparable to control compounds
SBT-106			No Response	No Response	Does not inhibit Ebola virus replication in this model
SBT-100	Vero	Zika	96%	0.74	Inhibts Ebola virus replication comparable to control compounds
SBT-106			No Response	No Response	Does not inhibit Zika virus replication in this model
SBT-100	HeLa	TC83	96%	0.665	Inhibits Venezuelan Equine Encephalitis (VEE) virus proliferation in three different infected cell lines
SBT-100	BE2M17	TC83	97%	5.94	
SBT-100	U87MG	TC83	96%	2.53	
SBT-100	U2OS	CHIV (181/25)	99%	5.05	Inhibits Chickungunya (CHIV 181/25) virus proliferation
SBT-100	Vero	Zika DAKAR , Senegal	98%	5.45	Inhibits Zika virus replication
SBT-100	HFF	Zika DAKAR , Senegal	98%	6.26	Inhibits Zika virus replication

Development Pathway for Pathogen Targets: SBT-100 May Inhibit Replication of Other Viruses

- **Hemorrhagic Fever Viruses:**
  - Dengue
  - Marburg
  - Arenaviruses (Lassa & Junin Viruses)*
  - Bunyaviruses
- **Toga Viruses (Alpha Viruses): Mosquito-borne Encephalitis Viruses**
  - West Nile Virus (WNV)*
  - Venezuelan Equine Encephalitis Virus (VEE)*
  - Eastern Equine Encephalitis Virus (EEE)*
  - Western Equine Encephalitis Virus (WEE)*
  - Others
- **Chikungunya Virus**
- **Coronaviruses:**
  - Severe Acute Respiratory Syndrome (SARS)
  - Middle East Respiratory Syndrome (MERS)

*** Pathogens in the Queue to be tested against SBT-100**

*** Pathogens tested against SBT-100**

SBT-100 INHIBITS IL-6

- IL-6 is a key mediator of inflammation and uses the STAT3 pathway to do this.
- There is high levels of IL-6 in the blood of patients with severe infection with COVID-19.
- IL-6 plays a key role in the tumor microenvironment of many cancers.
- IL-6 plays promotes STAT3 mediate inflammation in the eye causing macular degeneration and uveitis.

Novel Coronavirus Testing

Regeneron and Sanofi reported today that they have launched a clinical program to test their IL-6 drug Kevzara on hospitalized Covid-19 patients. The companies report that they've already seen proof-of-concept data from a study of another IL-6 drug in China. The anti-inflammatory drug has the potential to lessen the damage the coronavirus can inflict on patients' lungs, which can be ravaged in the reaction to the new virus.

- Endpoints News March 16, 2020

China has listed Roche's rheumatoid arthritis drug, Actemra, in its latest provisional treatment guidelines for Covid-19, the disease caused by the novel coronavirus. The National Health Commission added the drug to treat patients who have "extensive lung damage or are in serious condition, with elevated levels of interleukin-6," updated guidelines state.

Roche's biologic therapy, generically known as tocilizumab, does not act on viruses, but it is known to inhibit IL-6, a type of protein that causes inflammation in the body, which, in this case, could be helpful in preventing a cytokine storm from occurring in Covid-19 patients.

- Endpoints News March 11, 2020

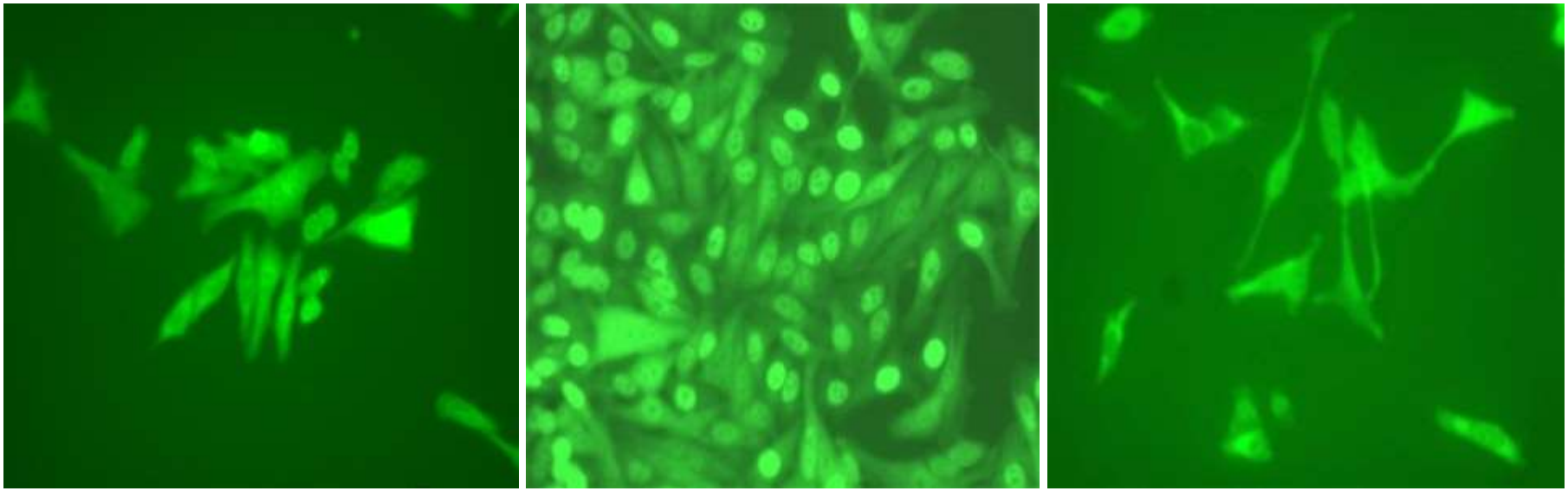
SBT-100 by blocking IL-6 and other pro-inflammatory cytokines effects may help to improve the patient's pulmonary compliance, and oxygenation in Covid-19 patients.

SBT-100 Blocks IL6 Induced Nuclear Translocation of STAT3 in HeLa Cells

No IL-6 stimulation:
STAT3 stains in cytoplasm

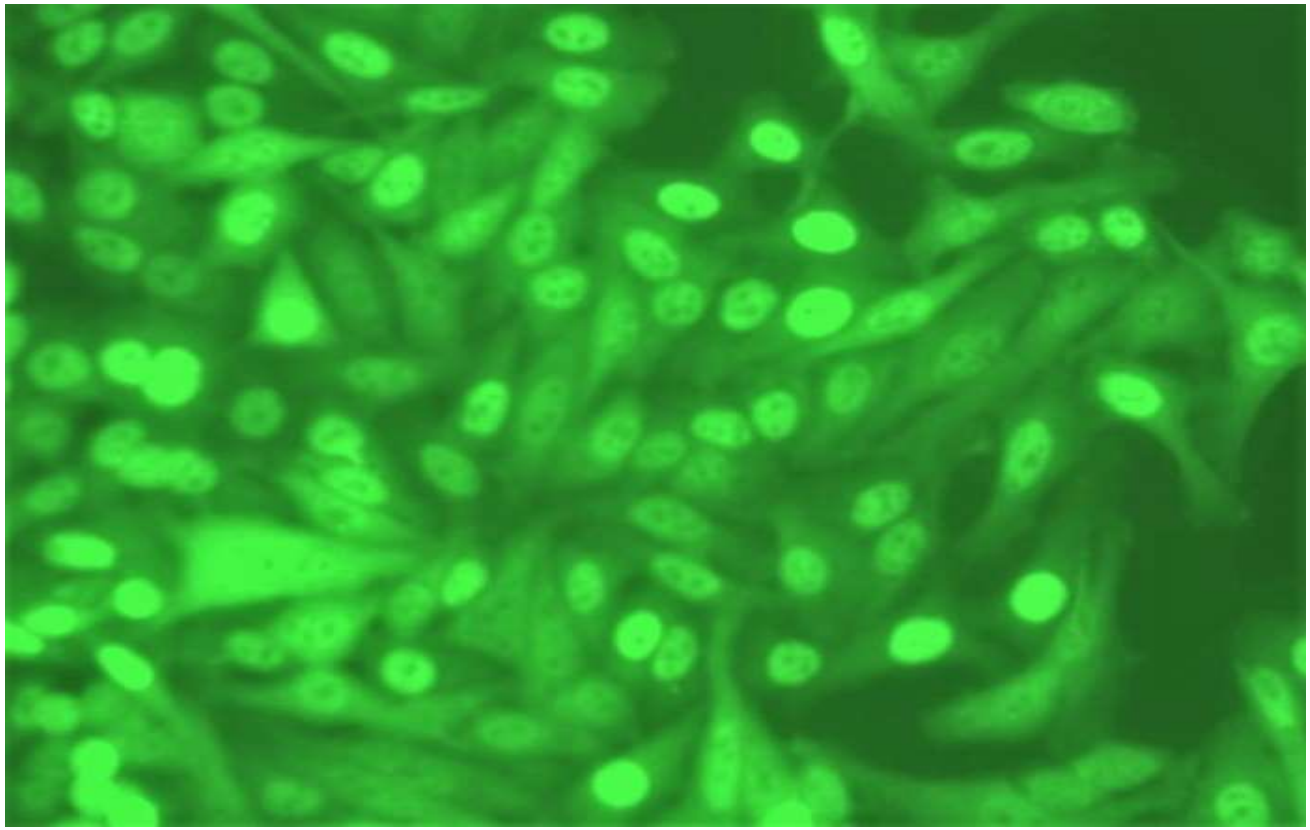
IL-6 stimulation:
STAT3 stains in nucleus

IL-6 Stimulation + SBT-100:
STAT3 stains in cytoplasm



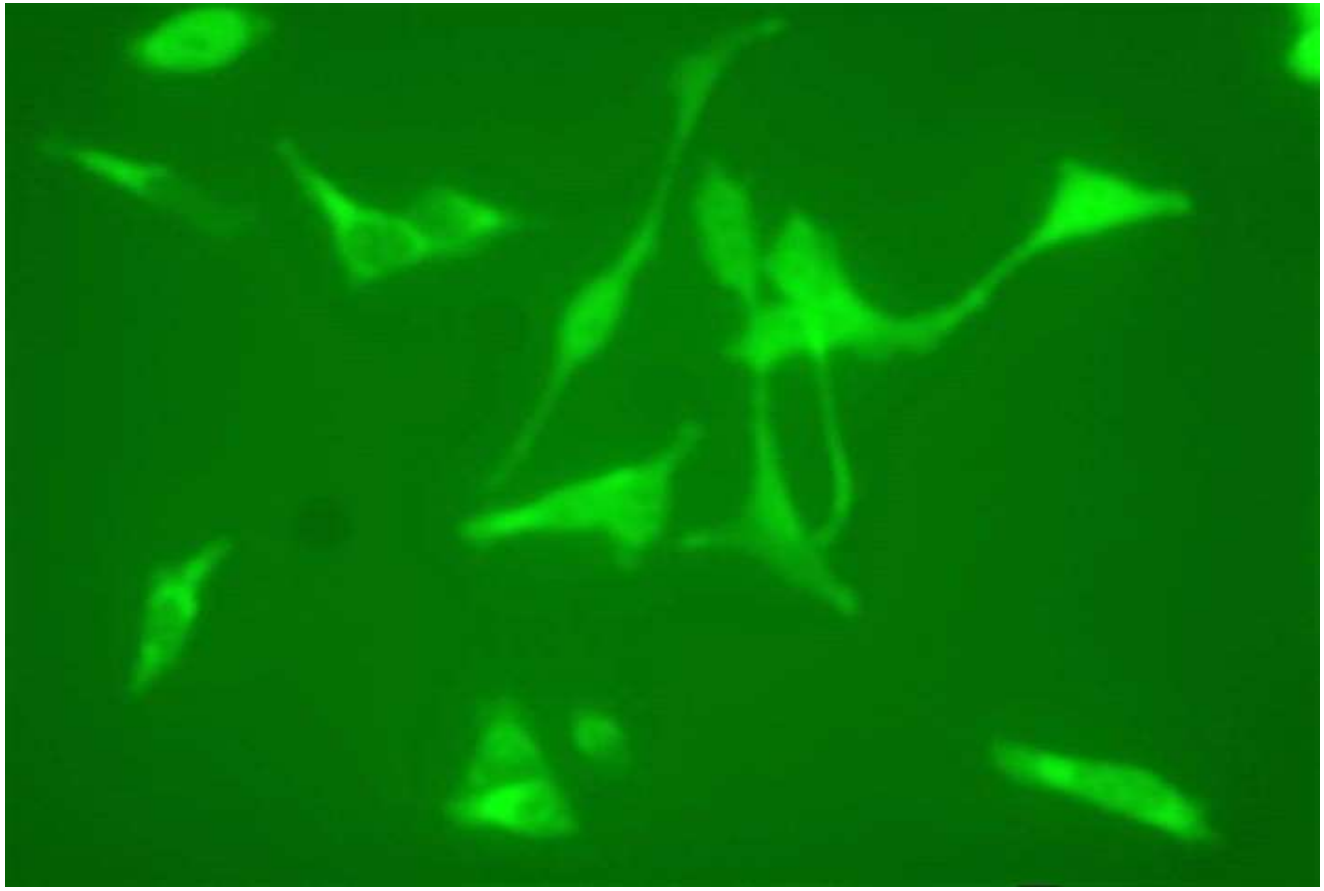
- IHC Staining: *In Vitro* IL-6 Stimulated HEp-2 (HeLa) Cancer Cells
- Similar results also observed in MDA-MB-231 (TNBC) & PANC-1 (pancreatic) cancer cells IHC staining.
- The left most picture show STAT3 staining in the cytoplasm only.

IL-6 Stimulation: STAT3 Stains in Nucleus of HeLa Cells



- Adding IL-6 to the cervical cancer cells (HeLa) activates STAT3 by phosphorylation into P-STAT3 which then forms P-STAT3 dimers.
- These dimers then translocate into the nuclei of the cancer cells which is where the dimers bind to STAT3 promotors next to the many genes that result in cancer cell growth, proliferation, immunosuppression, angiogenesis, metastasis, etc.
- The staining shows the STAT3 is localized primarily in the nuclei and the cancer cells are growing rapidly.

IL-6 Stimulation + SBT-100: STAT3 Stains in Cytoplasm of HeLa Cells



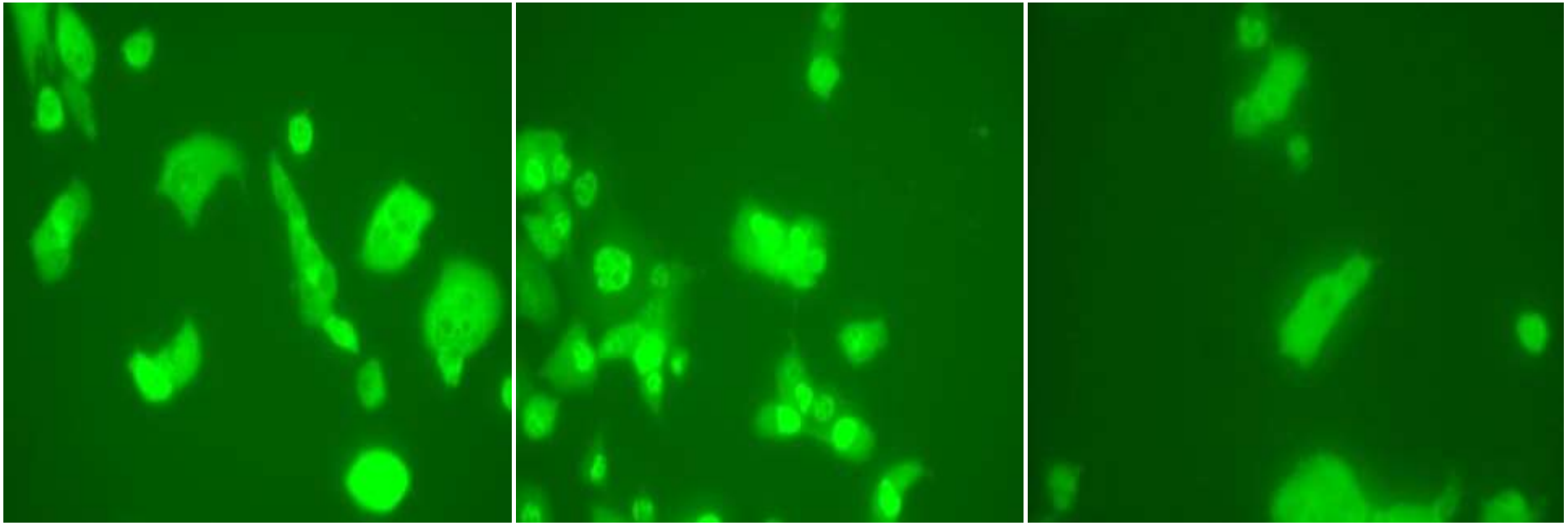
- When IL-6 is added in the presence of SBT-100, the STAT3 is bound in the cytoplasm and cannot translocate into the nuclei.
- Therefore the genes in the nuclei necessary for cancer proliferation and malignant growth cannot be turned on.
- In the upper left corner of the slide the three cells have gray hollow nuclei devoid of any STAT3.

SBT-100 Blocks IL-6 Induced Nuclear Translocation of STAT3 in PANC-1 Cells

No IL-6 stimulation: STAT3 stains in

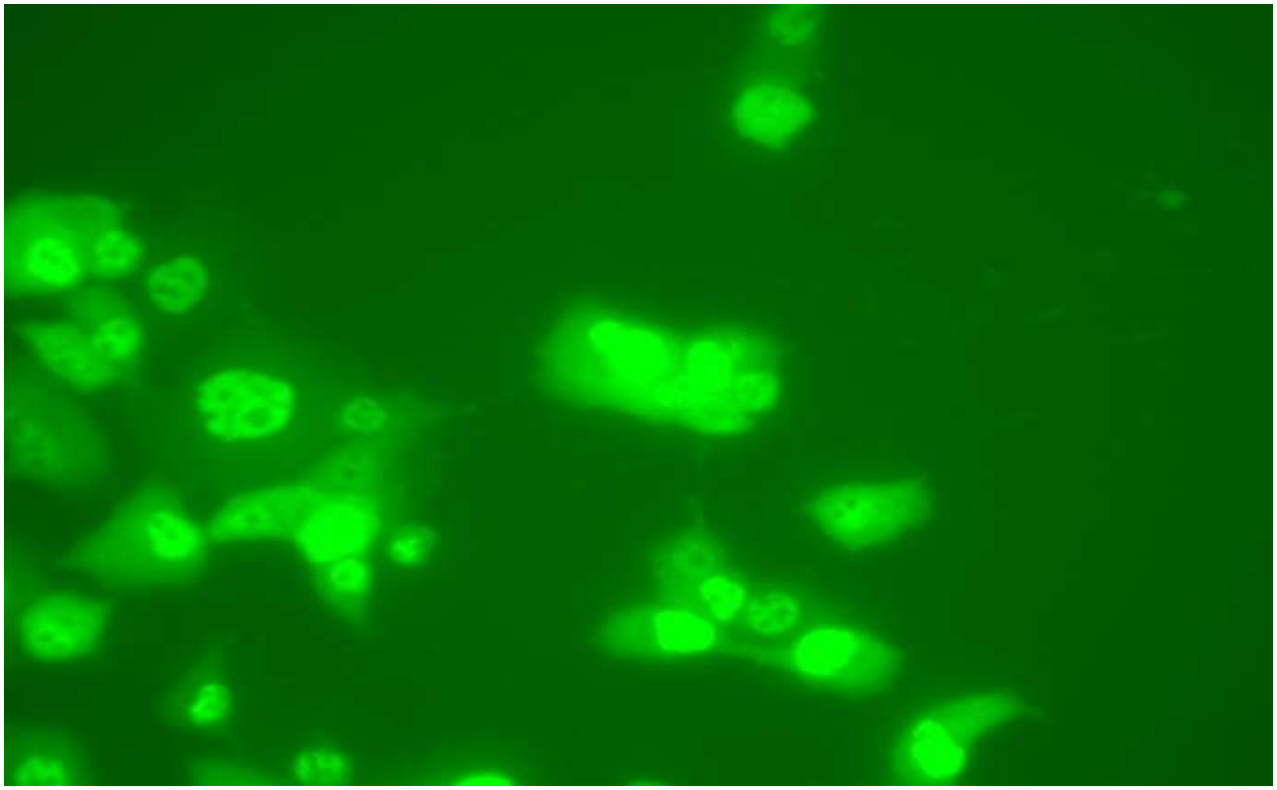
IL-6 stimulation: STAT3 stains in nucleus

IL-6 stimulation + SBT-100: STAT3 stains in



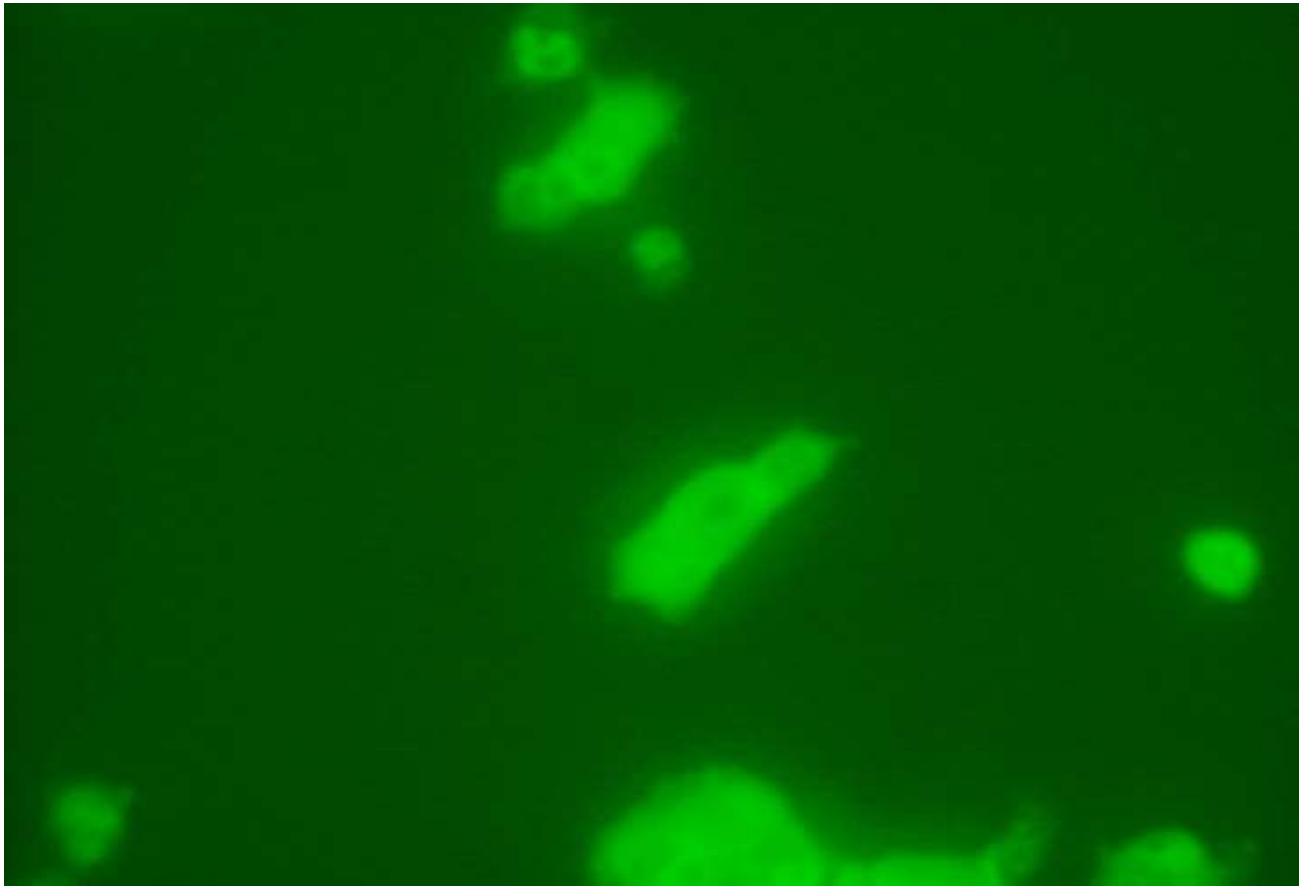
- IHC Staining: *In Vitro* IL-6 Stimulated PANC-1) Cancer Cells
- Similar results also observed in MDA-MB-231 (TNBC) & HeLa (cervical) cancer cells IHC staining.
- The left most picture show STAT3 staining in the cytoplasm only.

IL-6 Stimulation: STAT3 Stains in Nucleus of PANC-1 Cells



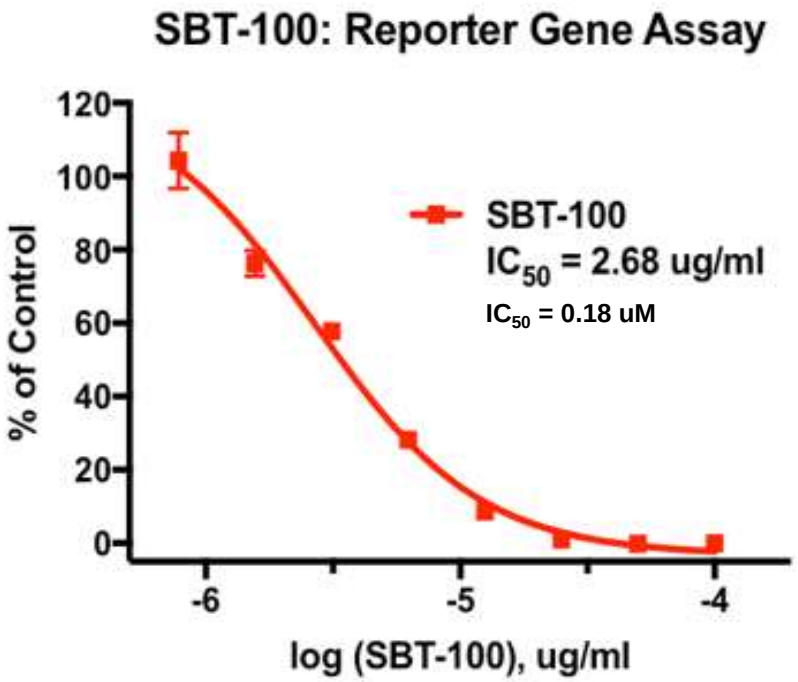
- Adding IL-6 to the pancreas cancer cells (PANC-1) activates STAT3 by phosphorylation into P-STAT3 which then forms P-STAT3 dimers.
- These dimers then translocate into the nuclei of the cancer cells which is where the dimers bind to STAT3 promotors next to the many genes that result in cancer cell growth, proliferation, immunosuppression, angiogenesis, metastasis, etc.
- The staining shows the STAT3 is localized primarily in the nuclei and the cancer cells are growing rapidly.

IL-6 Stimulation + SBT-100: STAT3 Stains in Cytoplasm of PANC-1 Cells



- When IL-6 is added in the presence of SBT-100, the STAT3 is bound in the cytoplasm and cannot translocate into the nuclei.
- Therefore the genes in the nuclei necessary for cancer proliferation and malignant growth cannot be turned on.

SBT-100 Rapidly Blocks IL-6 Production *In Vitro*



- Dual cell luciferase reporter assay using Promega's STAT3 HEK cell line to measure IL-6 stimulated STAT3 activity.

- The Reporter Gene Assay uses HEK cells transfected with a luciferase green protein gene linked to the STAT3 promotor.
- Adding IL-6 activates STAT3 in these HEK cells and then the P-STAT3 form dimers which translocate to the nuclei.
- There the P-STAT3 dimers bind to the promotor linked to the luciferase and this produces the green luciferase protein which is measured as the readout.
- When SBT-100 is titrated into the culture the IL-6 effect is inhibited with a classic dose response curve. The luciferase production drops down to almost zero.

Hypothesis

- SBT-100 may reduce SARS-CoV-2 replication in patients by binding and inhibiting STAT3.

- By blocking IL-6 effects, SBT-100 may help reduce pulmonary inflammation which may then improve the patient's pulmonary compliance, and oxygenation.

SBT-100 Treatment Approach

- All in vivo therapeutic results in oncology and ophthalmology using mice have been done by injecting SBT-100 intraperitoneally (IP).
- The human formulation for SBT-100 has already been developed and is PBS with 0.3% DSPE PEG 2000. It is highly stable at room temperature and refrigerator temperature (4°C).
- ICU COVID-19 patients who are critically ill and may be on the ventilator should received IP slow infusion of SBT-100 twice daily.
- Pretreatment and then daily measurements of pulmonary function and 30-day survival in the ICU recorded to help determine efficacy of SBT-100. Pretreatment and daily measurements of IL-6.

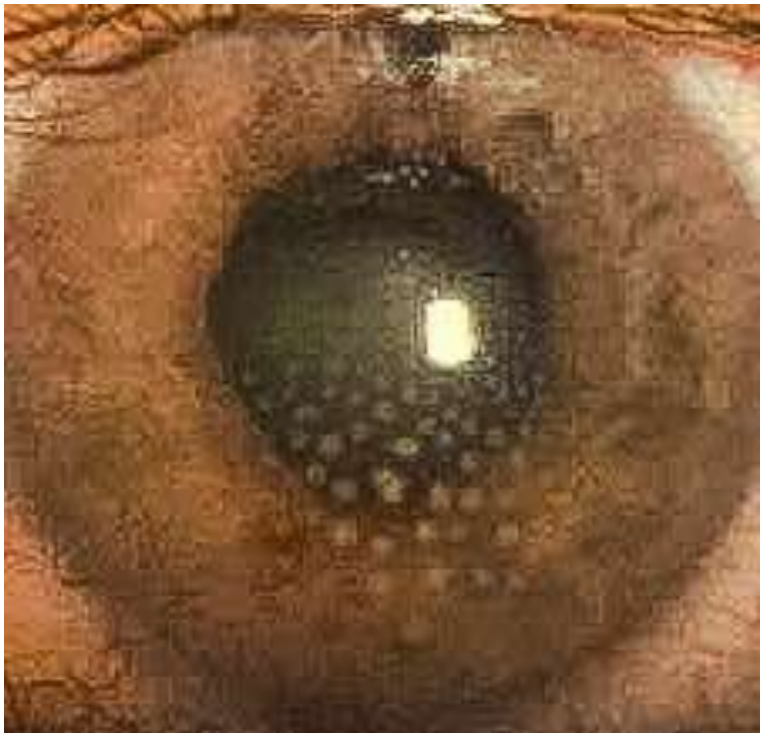
SBT-100 INHIBITS STAT3 MEDIATED EYE DISEASES

Inflammatory & Neovascular Diseases of the Eye are STAT3 Mediated

By blocking the inflammatory cascade and the vascular signaling pathways, inhibiting STAT3 may be effective in treating many ocular inflammatory and neovascular conditions like:

- Corneal Neovascularization
- Proliferative Diabetic Retinopathy
- Keratoconjunctivitis Sicca
- Macular Degeneration (AMD)
- Uveitis

Uveitis



- Ocular inflammation of the iris, ciliary body, or choroid that can occur from autoimmune conditions, trauma, and infections.
- A general term describing a group of inflammatory diseases that produces swelling and destroys the middle layer of tissues in the eye wall (uvea).
- Is not limited to the uvea but also affects the lens, retina, optic nerve, and vitreous, producing reduced vision or blindness.

Uveitis (Autoimmune)

- Uveitis is a group of intraocular inflammatory diseases responsible for 10 percent of vision loss in the United States.
- Th17 T-helper cell subset has been implicated in the etiology of uveitis in mice and humans.
- The Signal Transducer and activator of transcription 3 (STAT3) plays a critical role in the differentiation of Th17 cells and mice with targeted deletion of Th17 cells do not develop experimental autoimmune uveitis (EAU), the mouse model of human uveitis.
- Consequently, there is significant interest in developing drugs and Biologics that target STAT3 pathway as therapy for uveitis and other inflammatory diseases.
- We have used V_HH single-domain anti-STAT3 antibody (SBT-100) to investigate whether this Nanobody can be used to treat uveitis in mice.

Molecular Immunology Section, Laboratory Immunology, National Eye Institute, NIH, Bethesda, MD

Uveitis (Autoimmune): Conclusion

- “The single-domain anti-STAT3 antibody (SBT-100) attenuated severity of uveitis in mouse model of human uveitis, suggesting that the single domain anti-STAT3 antibody (V_HH Nanobody) can be exploited as therapy for Uveitis and other inflammatory diseases.”

- The number of TH17 cells is significantly reduced by SBT-100

Molecular Immunology Section, Laboratory Immunology, National Eye Institute, NIH, Bethesda, MD

SBT-100 PK and Tissue Distribution In Vivo

- SBT-100 gives significant efficacy in vivo in oncology xenograft mouse models with once daily dosing.
- In an autoimmune uveitis model study done at the National Eye Institute/NIH, SBT-100 dosed everyday or every other day gave significant benefit in protecting the retina and preserving vision.
- Within 15 minutes of in vivo injection, SBT-100 localizes inside cancer cells in a growing tumor in a xenograft mouse.
- SBT-100 serum half-life approximately 1 hour, while its biological half-life is over 24 hours.
- SBT-100 gets sequestered in cancer (abnormal) cells because it binds to intracellular STAT3/KRAS proteins, which are present in high concentrations compared to normal cells.

Expression Systems for SBT-100

- All preclinical development and data with SBT-100 has been produced in E. Coli.
- For preclinical work our CMO has been producing gram amounts of SBT-100 at a time.
  - The CMO can scale up to kilogram amounts of SBT-100 production.
- We have initiated production of SBT-100 in CHO cells and in Pichia.

COMMERCIALIZATION: SBT-100 Formulation & Stability

- Initial formulation for SBT-100 was done with it in PBS.
- Current formulation for SBT-100 is in 0.3% DSPE PEG 2000.
- The 0.3% DSPE PEG 2000 has greatly increased the solubility and stability of SBT-100.
- This lead formulation of SBT-100 is stable for 12 months at room temperature and at 4˚C.

Mode of Action

- Rationale for targeting KRAS and STAT3 signaling: about 30% of all human cancers have a KRAS mutation, and about 50%-90% of all human cancers have hyper-expression of P-STAT3. These will also reduce the risk of resistance developing in the cancer as occurs in mono-therapy.
- SBT-100 crosses the cell membrane without conjugates, linkers, or receptor mediated endocytosis.
- Biophysical Data: SBT-100 binds to KRAS & KRAS(G12D), which is the most common mutant of KRAS, in a Biacore assay with nanomolar affinity ($K_d = 10^{-8} – 10^{-9}$ M).
- Biochemical Data: SBT-100 inhibits KRAS GTPase activity equivalent to the polyclonal anti-KRAS antibody.
- Western Blot: SBT-100 down regulate P-ERK expression in cancer cells with activated KRAS mutations G12D and G13D.
- MTT Assay: SBT-100 significantly inhibits cell growth of human cancers with KRAS mutations (G12D and G13D) in vitro.
- In Vivo Xenograft Study: SBT-100 significantly inhibits the growth of human tumors with KRAS mutations (G12D and G13D).
- STAT3 sequesters SBT-100 inside cancer cells which increases its biological half-life despite the serum half-life being one hour.
- SBT-100 concentrates in cells with high STAT3 levels. This brings SBT-100 in close proximity to the KRAS protein which is then inhibited by hydrogen bonding with SBT-100.
- SBT-100 is a monomeric single domain antibody but has bi-specific binding to KRAS and STAT3.
- While normal healthy cells have little to no STAT3 present, cancer (unhealthy) cells have a very high concentration of STAT3. The high concentrations allows STAT3 to act as a “glue” to bind and sequester SBT-100 inside cancer cells where it is brought into contact with KRAS. This allows dual inhibition of KRAS and STAT3 pathways.

General Pharmacology

- SBT-100 gives significant efficacy in vivo in oncology xenograft mouse models with once daily dosing.
- In an autoimmune uveitis model study done at the National Eye Institute/NIH, SBT-100 dosed everyday or every other day gave significant benefit in protecting the retina and preserving vision.
- Within 15 minutes of in vivo injection, SBT-100 localizes inside cancer cells of a growing tumor in a xenograft mouse.
- SBT-100 serum half-life is approximately 1 hour, while its biological half-life is over 24 hours.
- SBT-100 gets sequestered in cancer (abnormal) cells because it binds to intracellular STAT3/KRAS proteins, which are present in high concentrations compared to normal cells.
- In vivo SBT-100 crosses the blood brain barrier (BBB) and enters the cancer cells within the tumor as soon as 15 minutes.
- The serum or tissue half-life does NOT always correlate with the biological half-life of antibodies. For example Lucentis has a vitreous half-life of 2.84 days but has a biological half-life of 4 weeks.
- SBT-100 does NOT have the traditional small molecule PK profile.

Executive Summary

- Singh Biotechnology, LLC (“SBT”) developed SBT-100, a first-in-class single domain antibody (sdAb) which can penetrate the cell membrane to bind intracellular KRAS and STAT3. This bi-specific nature gives SBT-100 broad application in oncology. The U.S. FDA has given SBT-100 Orphan Drug Designation for Pancreatic Cancer and Osteosarcoma. In addition, the FDA favorably reviewed SBT’s triple-negative breast cancer (TNBC) program, including all preclinical data on TNBC, GMP manufacturing of SBT-100, proposed IND-enabling toxicology studies, and proposed Phase I clinical trial.

- Collaboration with the NIH, using an autoimmune uveitis model for human uveitis, has demonstrated that SBT-100 gives significant protection of the retina in vivo with vision preservation. NIH scientists are preparing the results of these studies for publication. This work also demonstrated that SBT-100 via STAT3 down-regulation significantly reduces the pathogenic autoimmune Th17 cells. These same Th17 cell are involved in rheumatoid arthritis, inflammatory bowel disease, psoriasis, multiple sclerosis, and other diseases.

- SBT is a virtual company so all the R&D has been done by leading CROs in the USA, or in collaboration with the National Institutes of Health and US Army’s USAMRIID facility in Maryland. The data from these entities are all consistent with the mechanism that SBT-100 binds and inhibits KRAS and STAT3.

What makes SBT-100 unique

- SBT-100 is a demonstration of a highly disruptive technology and has created a new paradigm in drug development.
- SBT-100 is a First-in-Class therapeutic sdAb.
- SBT-100 is a 15 kD single domain antibody (i.e., camelid VHH), and it is the first demonstration of a cell penetrating antibody to give a therapeutic effective in oncology, ophthalmology, and virology models. The average human IgG is about 150 kD.
- SBT-100 is a monomeric molecule that crosses the cell membrane to bind to both KRAS and STAT3 with nanomolar affinity and inhibit their function.
- As in some other effective therapeutic antibodies, such as Lucentis, the biological half-life of SBT-100 is much longer (24 times as long) as its serum or tissue half-life.
- Based on our current data from CROs, NIH, USAMRIID, and the scientific literature SBT-100 has broad applications in oncology, ophthalmology, virology , fibrotic diseases (e.g., NASH) and autoimmune diseases. SBT-100 inhibits IL-6 in oncology & non-oncology models.
- SBT's composition of matter patents have been approved in the USA, & most of the world.
- SBT has at least 15 years left on these patents, which are solely owned by Singh Biotechnology.

Clinical Indication and Unmet Need

- At least 30% to 50% of human cancers with KRAS mutations and hyper-expression of P-STAT3.
- Ophthalmic diseases: wet AMD, uveitis, diabetic retinopathy, & neo-vascular diseases of the eye.
- Viral infections: HBV, HCV, VSV, EBV, & HCMV. Preclinical data for Ebola virus, Zika virus, VEEV, and Chikungunya virus.
- Autoimmune diseases: multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases, and psoriasis, etc.
- Fibrotic diseases: NASH, pulmonary fibrosis, kidney fibrosis, and scleroderma.
- SBT-100 can potentially be used for a very large number of patients with STAT3 mediated diseases.
- Since SBT-100 also binds KRAS and KRAS(G12D). SBT-100 inhibits the growth of cancers with KRAS(G13D) and KRAS(G12D) activating mutations. This suggests SBT-100 recognizes an epitope on KRAS and its mutants that is in close proximity to KRAS's GTPase active site. Thus SBT-100 is likely a pan-KRAS inhibitor.

Differentiation from the Competition

- Several pharmaceutical and biotechnology companies are focused on KRAS & STAT3 inhibition using small molecules, RNA inhibitors, and T-cell receptor targets. Toxicity and lack of efficacy in human clinical trials have limited the progress of some of these compounds.
- SBT-100 is First-in-Class based on US patent, world patent, protein database, and literature searches. Initial search done in 2015 found no other therapeutic single domain antibodies. This search was repeated in February of 2020 and reached the same conclusion.
- In 2019 Amgen and Mirati announced that they have small molecule drug(s) that are COVALENT binders to the thiol sidechain on the cysteine amino acid residue of KRAS(G12C) mutant. Thus, their KRAS inhibitors will bind the thiol residue on all cysteine residues on all proteins in the body.
- The potential toxicity these small molecules may create is yet to be determined, since the metabolic breakdown by these inhibitors in the liver is unknown.
- SBT-100 is secreted by the kidney due to its small size and has a short serum half-life. This contributes to its safety.
- In addition, an intrinsic property of sdAb is that it does not have a Fc portion which makes the rare antibody toxicity like cytokine storm not possible.

SBT Management Team

Sunanda Singh M.D., Ph.D., CEO/President & Founder
Immunology, Cancer Biology

- Ph.D. from University of Chicago in Cancer Immunology: NIH Funded Scholarship
- M.D. from University of Chicago: NIH Funded Scholarship
- Research Experience:
  - GLG Pharma (STAT3 inhibitors discovered at Moffitt Cancer Center)
  - Dr. Hans Schreiber's Lab (Cancer Immunology)
  - Dr. Frank Stuart's Lab (Organ Transplant Immunology)
  - Argonne National Laboratory (Antibody Drug Conjugate Development)

Anjali Singh M.D., COO & CMO, *Dermatology and Internal Medicine*

Felix Wong, Director of Business Development, *25+ years experience in Private Equity and Investment Banking*

Bruce Dobbs, MBA, CFM, Vice President of Corporate Communications. *Senior executive with experience in the investment banking, manufacturing, & advertising industries*

Ashutosh Parihar, Vice President Research & Development, *25+ years in experience Biotechnology / Pharmaceutical* sector including Abbott, *Wyeth, Pfizer*

SBT Scientific Advisory Committee

Nipun B. Merchant, M.D., Chairman of Scientific Advisor Committee

- Professor at The University of Miami Medical Center
- Alan S. Livingstone Professor of Surgery
- Vice Chair of Surgical Oncology Services
- Chief, Division of Surgical Oncology
- Chief Surgical Officer & Associate Director of Translational Research Sylvester Comprehensive Cancer Center

Hector Gomez, M.D., Ph.D., 35+ years in experience Biotechnology / Pharmaceutical industries including Merck, Ciba-Geigy, Vertex, Leader/Key Member of teams that brought 10 drugs to market

Charles E. Egwuagu, MPH, Ph.D., Chief, Molecular Immunology Section, Laboratory of Immunology National Eye Institute, National Institutes of Health

Rajendra Mehta, Ph.D., Cancer Biologist; Professor Emeritus at Illinois Institute of Technology, Professor at University of Illinois at Chicago (Formerly)

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