Single Domain Antibody (sdAb) Localizes Inside Cancer Cells to Inhibit Signal Transducer and Activator of Transcription 3 (STAT3) Resulting in Therapeutic Inhibition of Multiple Cancers

Abstract # 4699 / 15

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ABSTRACT

STAT3 is involved in the pathogenesis of many malignancies, so we developed an anti-STAT3 VHH (variable region of the heavy chain), SBT-100, that internalizes in cancer cells and binds unphosphorylated STAT3 (U-STAT3) and phosphorylated STAT3 (P-STAT3) and results in significant inhibition of multiple cancers.

ATCC cell lines for triple negative breast cancers (MDA-MB-231, MDA-MB-468, MDA-MB-453), ER+/PR+ breast cancer (MCF-7), HER2+ breast cancer (BT474), pancreatic cancers (PANC-1, BX-PC3), murine mammary cancer (4T1), STAT3 null cells (PC-3), and castrate-resistant prostate cancer (DU145) were tested. Athymic nude mice were obtained from Envigo. The STAT3 Reporter Cell Assay was obtained from Promega. VEGF inhibition ELISA was done using retinal epithelial cells (ATCC). In vitro growth inhibition was done using a MTT assay.

Immunoprecipitation (IP) and western blot studies in MDA-MB-231, PANC-1, HeLa, DU145, 4T1 and PC-3 showed that SBT-100 binds U-STAT3 and P-STAT3. No STAT3 binding was seen in PC-3. Binding to P-STAT3 was seen in lysates from the 4T1 cells, which have constitutively activated STAT3. Since rodent and human STAT3 have a 99% homology, rodents are excellent models for extrapolating to human disease for over production of P-STAT3. Within 24 hrs STAT3 assay showed that SBT-100 blocked the production of IL-6 (p< 0.0001) compared to control. The degree of IL-6 suppression was comparable to the negative control, BB160B (STAT3 inhibitor). VEGF ELISA showed significant (p<0.0001) inhibition of VEGF production within 12 hrs and was maintained for up to 48 hrs. After 3 days with SBT-100 the MTT assay showed growth inhibition (p<0.001) in BT474 (93%), MCF-7 (93%), MDA-MB-231 (77%), MDA-MB-468 (85%), MDA-MB-453 (64%), PANC-1 (79%), BX-PC3 (90%), and DU145 (92%). MDA-MB-231 tumors grown in xenograft athymic mice showed suppression (p<0.001), IHC staining in the cytoplasm, and nucleus after 7 days of SBT-100 treatment.

U-STAT3 and P-STAT3 activate genes that promote growth, proliferation, angiogenesis, immune suppression, cancer stem cells, metastasis, and apoptosis inhibition. SBT-100 enters the cancers cells, binds STAT3 and P-STAT3 causing growth inhibition (p<0.001) in MCF-7, BT474, MDA-MB-231, MDA-MB-468, MDA-MB-453, PANC-1, BX-PC3, and DU145. These results suggest that SBT-100 can be developed as a therapeutic for cancers expressing either STAT3 or P-STAT3.

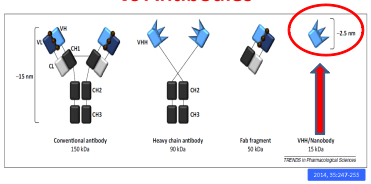
MATERIALS & METHODS

- •Cell lines (MDA-MB-231, MDA-MB-468, MDA-MB-453, MCF-7, BT474, PANC-1, BX-PC3, 4T1, PC-3 and DU145) were obtained from ATCC and grown according to ATCC guidelines.
- •Athymic nude mice for xenograft studies were obtained from Envigo (Indianapolis, IN).
- •Immunoprecipitation and western blot analysis was used to demonstrate binding of SBT-100 to STAT3 and P-STAT3.
- •The STAT3 Reporter Cell Assay was obtained from Promega and performed according to manufactures guidelines with the exception that the cells were pre-treated for 24 hours with the test article (SBT-100).
- •VEGF inhibition ELISA was done using retinal epithelial cells from ATCC.
- •In vitro growth inhibition was determined using a MTT assay.

BACKGROUND

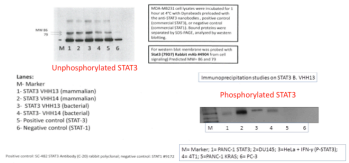
- STAT3 (signal transducer and activator of transcription 3) is an intracellular transcription factor that is over-expressed and is present in its activated form (phosphorylated-STAT3 or P-STAT3) in many human malignancies.
- P-STAT3 activates many genes (Bcl-xL, Cyclins D1 & D3, c-Myc, Mcl-1, VEGF, survivin, MMP-2, HIF-1 alpha) that promotes proliferation, growth, survival, angiogenesis, immune system evasion, and metastasis.
- RAS oncogene increases IL-6 production in the tumor microenvironment and subsequent STAT3 activation.
- We have produced a sdAb (SBT-100) that binds and inhibits STAT3 & P-STAT3 function.
- Mouse and rat STAT3 have 99% protein homology with human STAT3, thus making them an excellent model for study in cancer.
- STAT & KRAS are both involved in a high percentage (90%) of glioblastomas and pancreatic cancers.

Nanobodies (sdAb or VHH) vs Antibodies

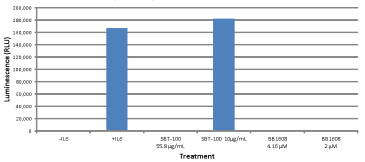


VHH = Nanobody = Single Domain Antibody (sdAb)

SBT-100: Intracellular STAT3 binding in human triple negative breast cancer (TNBC) MDA-MB-231 cell line



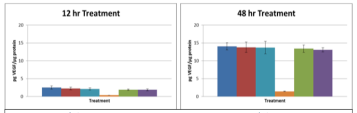
SBT-100 Blocks IL-6 Release in STAT3 Reporter Cell Assay



At 24 & 48 hrs in the STAT3 assay SBT-100 blocked the production of IL-6 (p< 0.0001)

SBT-100 Blocks VEGF Production

Retinal Epithelial Cells



- SBT-100 significantly inhibits VEGF production in 12 hrs (p<0.0001).
- This inhibition is maintained for 48 hrs (p<0.0001).

SBT-100 ANTI-CANCER EFFICACY *IN VITRO*

	HUMAN CANCER CELL LINE	IC50 (ug/ml)	% Inhibition in 3 days at 100 ug/ml
1	PANC-1 (Pancreatic Cancer)	41.15	79% (p<0.001)
2	Bx-PC3 (Pancreatic Cancer)	35.07	90% (p<0.001)
3	MDA-MB-231 (TNBC)	10.05	77% (p<0.001)
4	MDA-MB-468 (TNBC)	12.36	85% (p<0.001)
5	MDA-MB-453 (TNBC)	17.96	64% (p<0.001)
6	MCF-7 (ER+/PR+ Breast Cancer)	14.8	93% (p<0.001)
7	BT474 (HER2+ Breast Cancer)	25.24	93% (p<0.001)
8	U87 (Gilablastoma)	65	62% (p<0.001)
9	SJSA (Osteosarcoma)	51	83% (p<0.001)
10	HT-1080 (Fibrosarcoma)	33	86% (p<0.001)
11	DU-145 (Metsatatic Androgen Resistant Prostate Cancer)	21.87	92% (p<0.001)

In Vitro Growth Inhibition Determined by MTT Assay

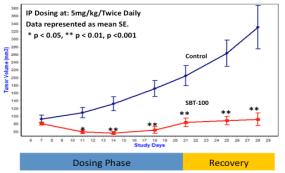
XENOGRAFT STUDIES

- Athymic nude-Foxn1nu female mice aged 5 to 6 weeks were purchased from Envigo Laboratories (Indianapolis, IN).
- All animals were housed under pathogen-free conditions and experiments were performed in accordance with the IIT Research Institute Animal Use and Care Committee.
- MDA-MB-231 cells at a density of 5×10^7 were injected subcutaneously into the right flank.
- Animals are randomized when tumors reach a range size of 55 - 150 mm^3 using the stratified random sampling algorithm.
- Treatment (SBT-100) or Vehicle (PBS) was initiated the day following randomization.
- Dosing schedules were as follows:

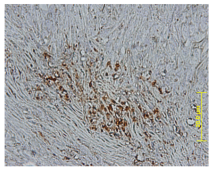
Group	TA	Dose	Schedule	Route
1	PBS	0	BIDx14	IP
2	SBT-100	5 mg/Kg	BIDx14	IP

SBT-100 Inhibits TNBC Growth *In Vivo*

Mean Tumor Volume in MDA-MB-231

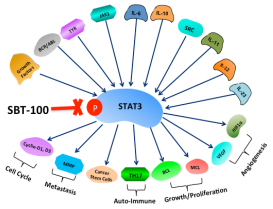


IMMUNOHISTOCHEMICAL (IHC) STAINING: TUMOR XENOGRAFT CELLS



Intracellular localization of SBT-100 shown by IHC staining of tumor was done 15 minutes after mouse xenograft was injected with SBT-100 IP.

Proposed Actions of SBT-100



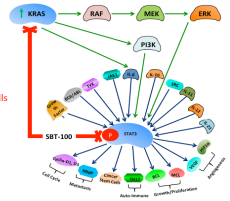
CONCLUSION

- •SBT-100 is a single domain antibody that binds both STAT3 and P-STAT3.
- •Unphosphorylated STAT3 and P-STAT3 both have been shown to activate genes the promote growth, proliferation, angiogenesis, immune suppression, cancer stem cells, metastasis, and inhibit apoptosis.
- •SBT-100 enters the cancers cells and inhibits STAT3 and P-STAT3 resulting in highly statistical significant (p<0.001) growth suppression of ER+/PR+ breast cancer (MCF-7), HER2+ breast cancer (BT474), triple negative breast cancers (MDA-MB-231, MDA-MB-468, MDA-MB-453), pancreatic cancer (PANC-1, BX-PC3), and castrate-resistant prostate cancer (DU145).
- •In vivo xenograft model using MDA-MB-231 treated with SBT-100 underwent highly statistical significant (p<0.001) growth suppression.

***These results suggest that SBT-100 can be developed as possible targeted chemotherapeutic agent for several cancers expressing either STAT3 or P-STAT3.**

*KRAS & STAT3 is Inhibited by SBT-100: Bi-Specific sdAb

- •Upregulates CTLA-4, PD-1, and PD-L1
- •Immune Suppression: DC Inhibition, Treg Promotion
- •Promotes Myeloid Derived Suppressor Cells (MDSC)
- •Cell Growth and Proliferation
- •Inhibits apoptosis
- •Promotes Metastasis
- •Angiogenesis



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