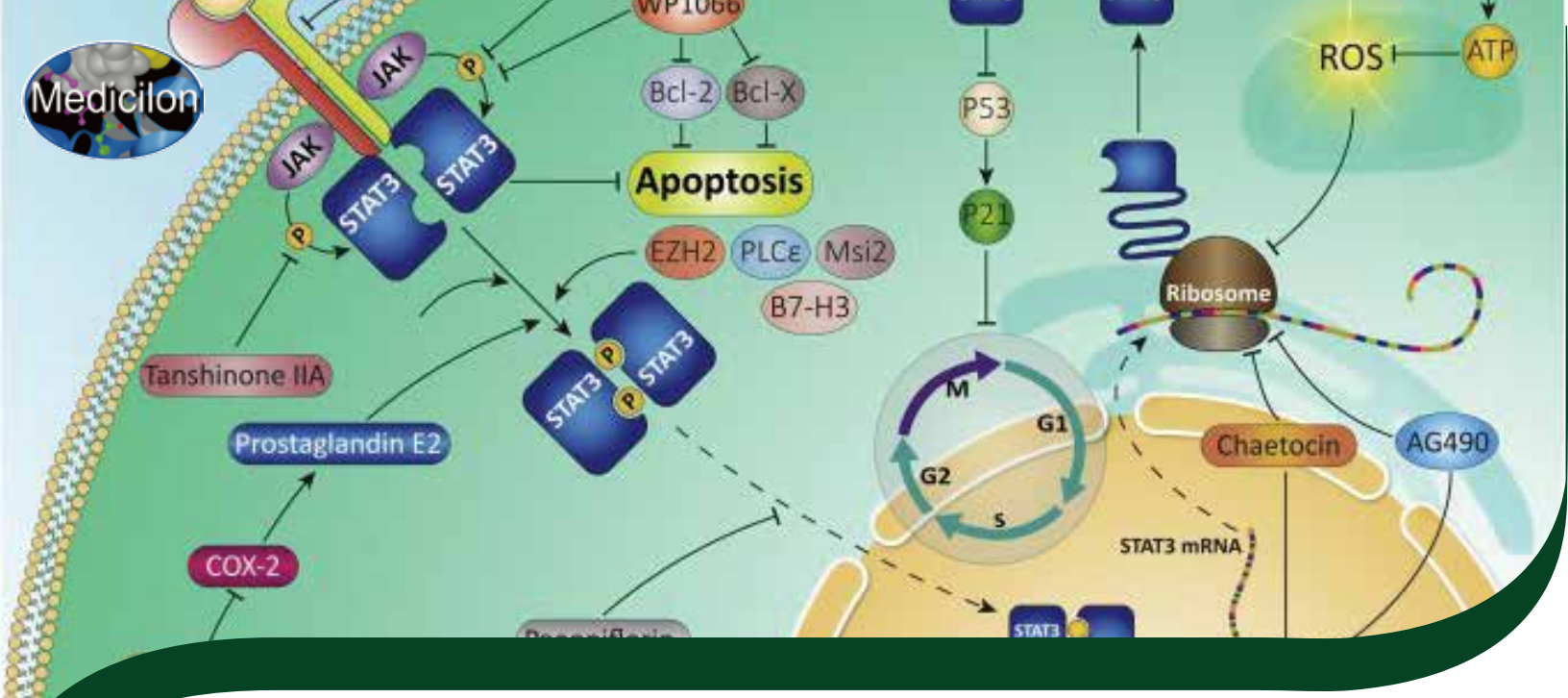


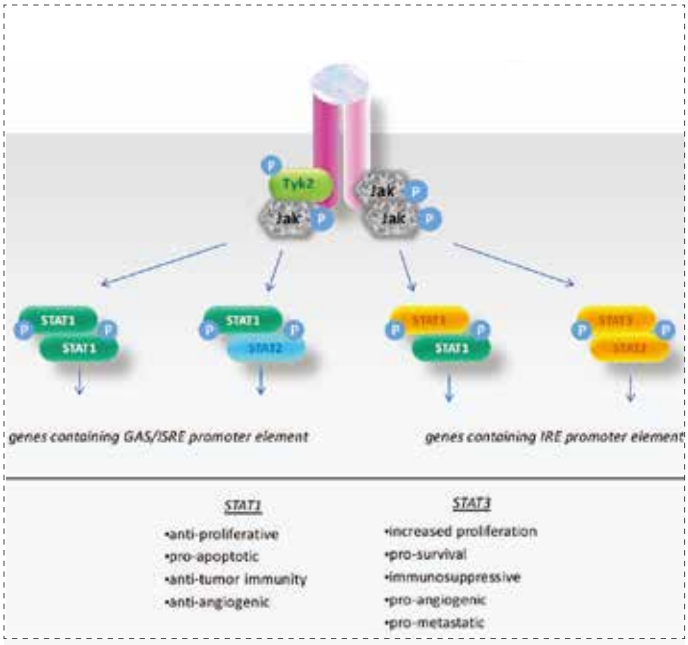
Medicilon STAT3-targeted Drugs R&D Service

STAT3, a member of the STAT family, is a latent transcription factor that is activated in response to various cytokines, growth factors, and oncogene signals. STAT3 is constitutively activated in various human cancers, and its activation is frequently associated with poor prognosis. As a transcription factor, STAT3 regulates a set of genes implicated in cancer cell survival, proliferation, angiogenesis, invasion, metastasis, drug resistance, and immune evasion.

Medicilon provides STAT3 drug discovery, CMC research (API + formulation), pharmacodynamics research, PK study, safety evaluation and other services.

Introduction of STAT3

The signal transducer and activator of transcription (STAT) is a family of intracellular cytoplasmic transcription factors involved in many biological functions in mammalian signal transduction. Among them, STAT3 is involved in cell proliferation, differentiation, apoptosis, and inflammatory responses, and also STAT3-related signaling pathways are aberrant over-activated in many types of cancer and are strongly associated with poor patient prognosis. STAT3 plays a pro-cancer role in a variety of cancers, and STAT3 inhibitors are used in pre-clinical and clinical studies. Excessive activation of STAT3 within tumor cells and other cells in the tumor microenvironment (TME) mediates a series of extracellular signals that enhances the immune inflammatory response in the TME, driving tumor cell proliferation, invasion, and metastasis, while strongly suppressing the anti-tumor immune response and creating an immunosuppressive microenvironment. Studies have indicated that persistently activated STAT3 is indispensable for various cancers including breast cancer and colorectal cancer, which makes it an ideal drug target.



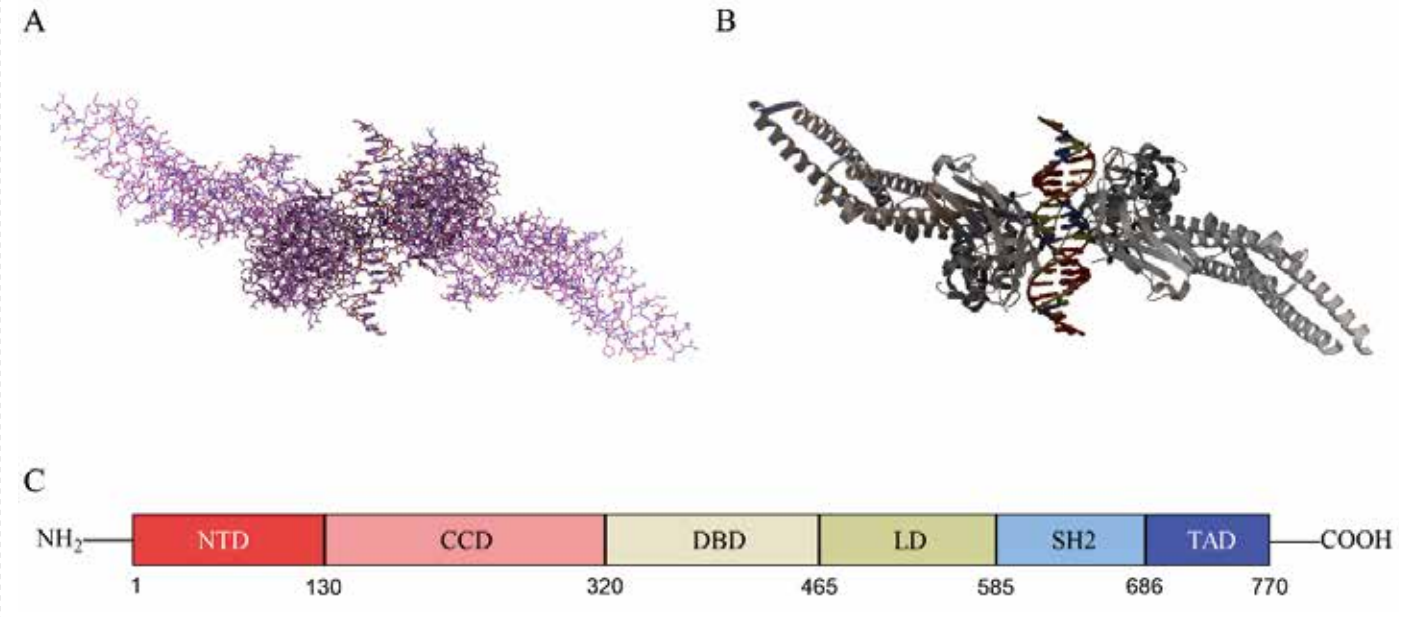
STAT3 signaling and effect in cancer[1]

Structure of STAT3

The STAT family consists of seven members, namely STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. STAT3 has two splicing isoforms with different functions, STAT3α and STAT3β. The domain of STAT3α is intact, but STAT3β lacks the 55 C-terminal amino acid residues. STAT3β has better specific DNA binding activity than STAT3α. The gene encoding STAT3 is located on human chromosome 17 and the protein consists of 770 amino acids.

STAT3 protein contains six structural domains:

- N-terminal domain (NTD): promotes the formation of STAT dimers, enabling their subsequent binding with transcription factors
- Coiled-coil domain (CCD): is essential for its SH2 domain-mediated receptor binding and subsequent activation induced by EGF and IL-6
- DNA-binding domain (DBD): recognizes and binds DNA sequences in regulatory regions of target genes
- Linking domain (LD): connects DBD to the SH2 domain
- SH2 domain: the most highly conserved STAT domain and plays a key role in signaling by binding to specific phosphorylated tyrosine motifs
- Transcriptional activation domain (TAD): undergoes serine phosphorylation and thereby recruits additional transcriptional activators, enhancing the transcriptional activity of STAT.



The three-dimensional structure and linear structure of STAT3[2]

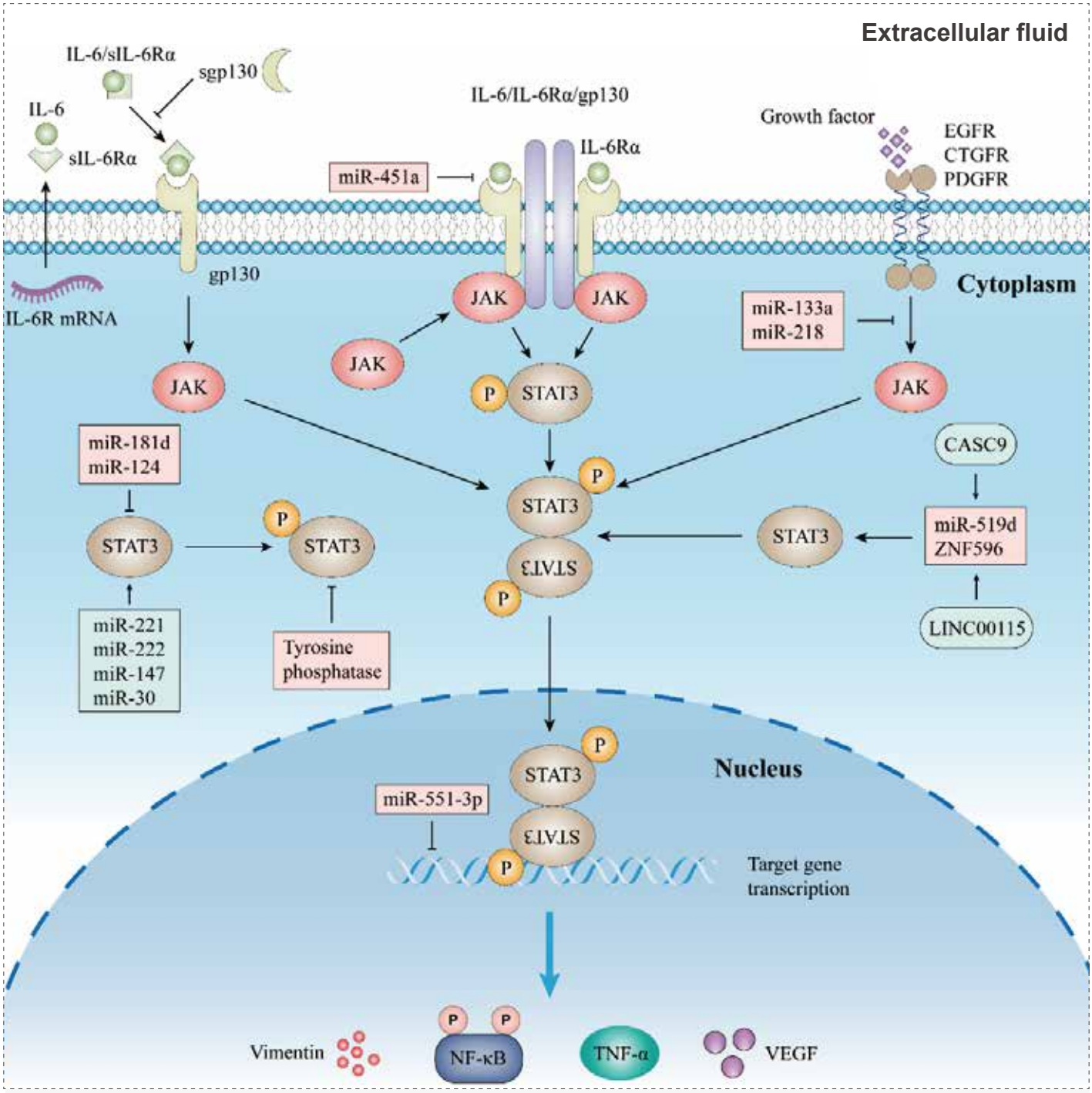
Signaling Pathway of STAT3

The JAK/STAT pathway is one of the most important signaling pathways regulating cellular function, and many cytokines and growth factors have been identified in this pathway, including interleukins, interferons, and angiogenic factors.

IL-6 is an inflammatory mediator. In TME, IL-6 can be produced by a variety of cells, including macrophages, neutrophils, fibroblasts, and tumor cells themselves. IL-6 can directly cause cancer cell proliferation, can also promote the production of other inflammatory factors in TME, and recruit large numbers of immune cells. Therefore, IL-6 is the key substance in chronic inflammation and tumor progression. In response to IL-6 stimulation, the JAK/STAT3 pathway is phosphorylated,

forming the critical IL-6/JAK/STAT3 pathway in the human body, which is involved in the processes of rheumatoid arthritis, inflammatory bowel disease, and many human cancers.

The classical IL-6 signaling pathway is mediated by IL-6 with the membrane-bound receptors IL-6 receptor α (IL-6Rα) and gp130, resulting in an IL-6/IL-6R/gp130 complex that leads to intracellular JAK activation. JAK protein binds to the intracellular structural domain of gp130, leading to phosphorylation of the tyrosine residue of gp130, forming a binding site for STAT3. After STAT3 recognition and binding to the phosphotyrosine docking site, the attached JAK enzyme with activity phosphorylates STAT3 at position 705 tyrosine, and the phosphorylated STAT3 forms a dimer that enters the nucleus and binds its downstream target genes.



IL-6/JAK/STAT3 signaling pathway[2]

STAT3 Inhibitors

Based on its important biological function in cancer, STAT3 has been investigated as a partial inhibitor for the next clinical therapeutic target in oncology. Up to now, most of the inhibitors applied to STAT3 targets have been designed to indirectly inhibit the biological function of STAT3 by blocking its upstream signaling mechanism. At present, many compounds have been reported to have STAT3-related pathway inhibitory activity, and many drugs have entered clinical practice. Current STAT3 inhibitors in clinical trials for cancer diseases are shown in the figure below. The indications include liver cancer, colorectal cancer, melanoma, leukemia and other diseases.

STAT3 Inhibitors	Cancer Types	Targets	Phase	NCT Number
IMX-110	Solid Tumor *	STAT3/NF-kB	I/II	NCT03382340
AZD9150	PC, NSCLC, CRC	STAT3	II	NCT02983578
Napabucasin	PC	STAT3	III	NCT02993731
Bazedoxifene	PC	IL-6 and gp130	NA	NCT04812808
Siltuximab	PC	IL-6	I/II	NCT04191421
CNTO 328	Solid Tumor	IL-6	II	NCT00841191
Ruxolitinib	PC	JAK1 and JAK2	I	NCT05440942
	PC	JAK1 and JAK2	II	NCT01423604
	PC, CRC	JAK1 and JAK2	I	NCT04303403
Itacitinib	Solid Tumor *	JAK1	I	NCT02646748
Ponatinib	CML	FGFR	II	NCT04043676
Sunitinib	RCCC	VEGFR, PDGFR	II	NCT03066427
	pNET	VEGFR, PDGFR	II	NCT02713763

STAT3 inhibitors in clinical trials[2]

Medicilon Case Study

STAT3 & HDAC Dual-Pathway Inhibitors

Simultaneous inhibition of multiple targets through drug combination is an important anticancer strategy owing to the complex mechanism behind tumorigenesis.

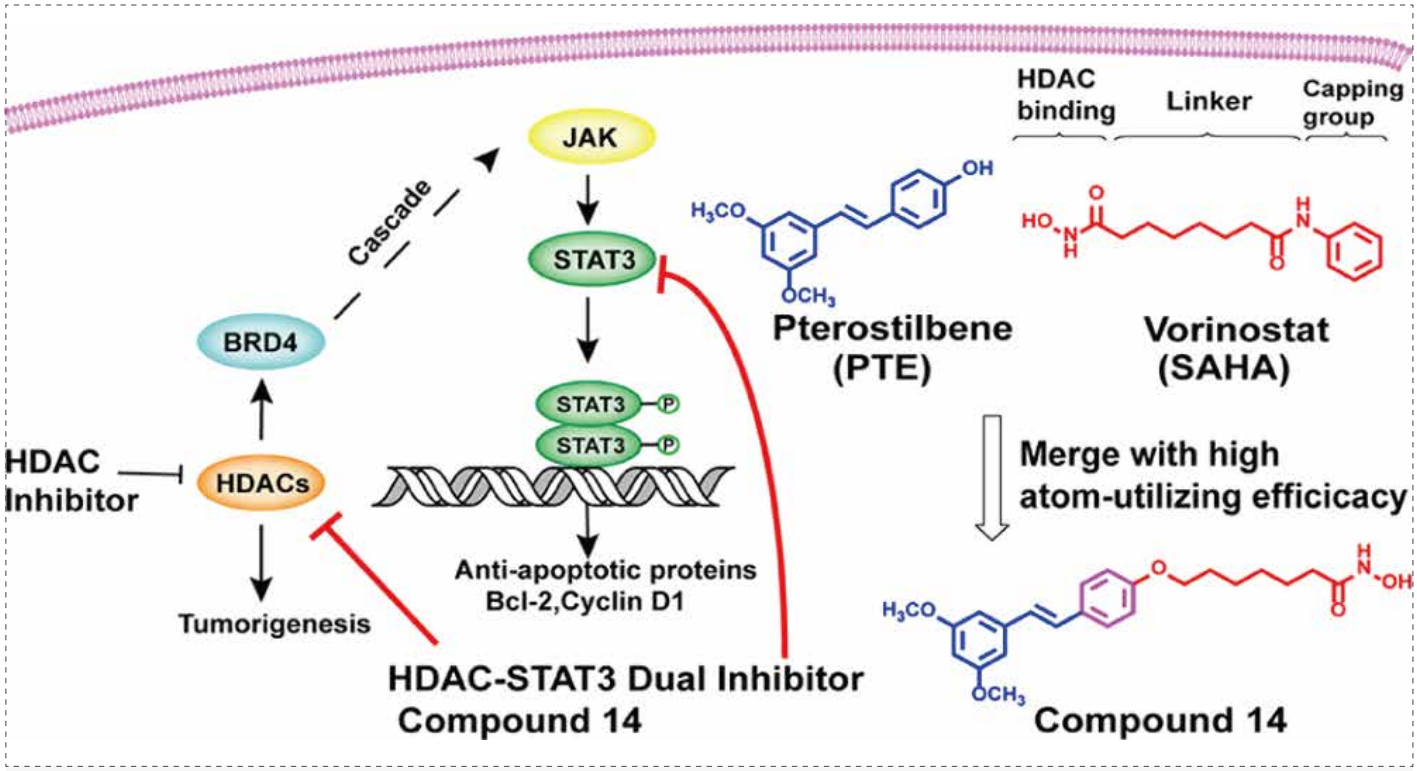
Traditional approaches represented by chemotherapy remain the primary choice to treat cancer. However, most chemotherapeutic agents merely act on one target, which may lead to drug resistance after a period of treatment and ultimately cause failure. An increasing number of therapies inhibiting two specific targets have achieved success in clinical research. Thus, a drug which regulates two or more relevant targets might result in a synergic therapeutic effect in cancer treatment. HDACs are a class of epigenetic enzymes closely related to tumorigenesis. HDACs play an important regulatory role in many vital cellular processes including cell growth, cell differentiation, and apoptosis.

Many HDAC inhibitors (HDACi) have shown significant anti-tumor effects in various types of cancer cells *in vitro*, including colon cancer, breast cancer, and liver cancer. The indications of the approved HDACi are concentrated on hematological malignancies such as lymphoma and myeloma. The inhibition of HDACs will lead to compensated activation of a notorious cancer-related drug target, STAT3, in breast cancer through a cascade, which probably limits the anti-proliferation effect of HDAC inhibitors in solid tumors.

Recent research studies have demonstrated that the inhibition of HDACs will lead to compensated activation of STAT3.

STAT3 activation by HDAC inhibition attenuates the intrinsic anticancer ability of HDACi. Inhibiting the factors in this cascade could sensitize breast cancer and colorectal cancer to HDACi. A compound selectively inhibiting both STAT3 protein and HDAC enzymes with high ligand efficiency might cure the resistant solid tumors.

Pterostilbene (PTE), a natural dimethylated analogue of Resveratrol extracted from blueberries, is chosen as the parent molecule. By incorporating the pharmacophore of the HDAC inhibitor SAHA (Vorinostat) into the STAT3 inhibitor Pterostilbene, a series of potent pterostilbene hydroxamic acid derivatives with dual-target inhibition activity are synthesized.



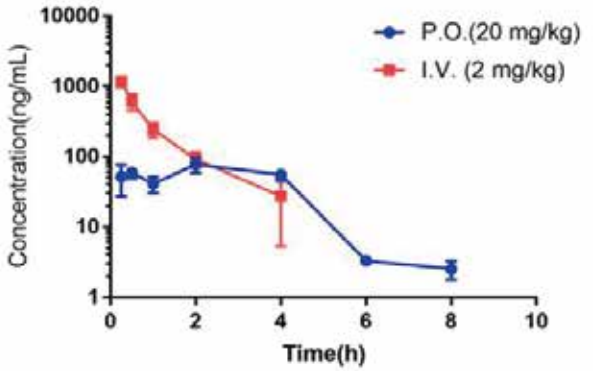
HDAC-BRD4-LIFR-JAK-STAT3 signal cascade[3]

An excellent hydroxamate derivate, Compound 14, directly binds to STAT3 with a robust affinity (K_D=33 nM). Compound 14 also inhibits HDACs with an IC_{50} of 23.15 nM *in vitro*. Compound 14 shows potent anti-proliferation ability *in vivo* and *in vitro*. Compound 14 is the most potent inhibitor in MDA-MB-231 (IC_{50}=0.78 μM) and HCT116 (IC_{50}=1.07 μM).

Compound 14 has excellent antitumor activity *in vitro* experiments, so a mouse 4T1 allograft tumor model is established to evaluate the *in vivo* antitumor activity of Compound 14. Compound 14 significantly reduces the tumor volume and weight. In the 15 mg/kg group of Compound 14 administration, the volume reduction reaches 54% and the weight reduction reaches 53% compared to the control group. In the 30 mg/kg group of Compound 14 administration, the volume reduction reaches 64%, and the weight reduction reaches 62%. In addition, Compound 14 does not cause apparent body weight change. The result indicated that Compound 14 is a promising anticancer compound with excellent safety performance.

The pharmacokinetics of Compound 14 is investigated in SD rats following a single intravenous injection (iv, 2 mg/kg) or oral administration (po, 20 mg/kg). This experiment is carried out by **Medicilon**. After intravenous injection of Compound 14 (2 mg/kg), the mean volume of distribution at the steady state (V_{ss}) is 1.65 L/kg. The mean blood clearance is 63.89 mL/min/kg. The mean half-life is 0.47 h. After oral administration of Compound 14 (20 mg/kg), the mean oral bioavailability is 5.81%, estimated based on AUC_{0-t}. The mean half-life is 0.88 h.

A Mean Plasma Concentration-Time Curve



B

Animal code	Route of Dosing	Dose Level mg/kg	HL_Lambda_ $T_{1/2}$ h	Tmax T_{max} h	Cmax C_{max} ng/mL	AUClast $AUC_{(0-t)}$ h*ng/mL	AUCINF_obs $AUC_{(0-\infty)}$ h*ng/mL	MRTlast $MRT_{(0-t)}$ h	MRTINF_obs $MRT_{(0-\infty)}$ h	C0 C_0 ng/mL	Vss_obs V_{ss} L/kg	Vz_obs V_z L/kg	Cl_obs Cl mL/min/kg
101	IV	2	0.59	0.083	1279.29	617.12	659.48	0.41	0.56	1660.96	1.71	2.59	50.54
102	IV	2	0.55	0.083	1303.09	514.48	536.51	0.32	0.43	1868.68	1.59	2.98	62.13
103	IV	2	0.27	0.083	945.00	419.95	422.04	0.34	0.35	1369.83	1.66	1.87	78.98
Mean			**0.47**	**0.08**	**1175.79**	**517.18**	**539.34**	**0.36**	**0.45**	**1633.16**	**1.65**	**2.48**	**63.89**
SD			**0.17**	**0.00**	**200.23**	**98.61**	**118.75**	**0.04**	**0.11**	**250.58**	**0.06**	**0.56**	**14.30**

C

Group	Route of Dosing	Dose Level mg/kg	$T_{1/2}$ h	T_{max} h	C_{max} ng/mL	$AUC_{(0-t)}$ h*ng/mL	$AUC_{(0-\infty)}$ h*ng/mL	$MRT_{(0-t)}$ h	$MRT_{(0-\infty)}$ h	F %
201	PO	20	0.89	0.3	78.64	273.63	277.45	2.34	2.40	5.29
202	PO	20	0.90	2.0	67.77	299.22	303.20	2.81	2.90	5.79
203	PO	20	0.85	2.0	98.98	329.23	331.68	2.51	2.56	6.37
Mean			**0.88**	**1.42**	**81.80**	**300.69**	**304.11**	**2.55**	**2.62**	**5.81**
SD			**0.03**	**1.01**	**15.84**	**27.83**	**27.12**	**0.24**	**0.25**	**0.54**

Pharmacokinetics of Compound 14 in SD rats[3]

Medicilon Assisted Projects

YY201

On July 20, 2023, the clinical trial application of YY201, a dual-function phosphorylation site-targeted inhibitor of STAT3 independently developed by Yuyao Biotech, was officially approved by NMPA. The indications are advanced solid tumors and relapsed and refractory hematologic malignancies.

Due to its relatively flat target interface, STAT3 cannot provide a "pocket" structure for specific binding of small molecules, and is recognized as a "undruggable target". The team of Yuyao biological scientists has been focusing on the basic and translational research of STAT3 targets, and with the help of AI technology, it has discovered and developed a nanomolar STAT3 dual phosphorylation site inhibitor for the first time, a world-leading First-in-class small molecule target drug "YY201". The drug directly binds to STAT3, inhibits the Tyr705 and Ser727 double-site phosphorylation of STAT3, inhibits STAT3 function and blocks downstream signal transmission, thereby exerting a tumor-suppressing effect. *In vitro* pharmacology and pharmacodynamics studies have shown that the affinity activity of YY201 and STAT3 is 1-10 nanomolar, and it is effective in pancreatic cancer, triple-negative breast cancer, lung cancer, acute myeloid leukemia, lymphoma and other solid tumors and blood tumors *in vitro* proliferation inhibitory activity at 1-10 nM. It exhibits significant anti-tumor effects in various *in vivo* models, and can cause complete regression of tumors at extremely low doses. Pharmacokinetic and toxicological studies have also confirmed the good druggability and safety of YY201.

Medicilon, as a partner of Yuyao Biotech, provided YY201 with pharmaceutical research services (including raw materials and preparations), preclinical research services (including drug efficacy, pharmacokinetics, safety evaluation) and IND declaration services, helping YY201 successfully pass IND approval and enter the clinical trial stage.

Summary and Outlook

STAT3 is an attractive cancer therapeutic target. In contrast to the antisense oligonucleotide approach of targeting STAT3, the PROTAC STAT3 degrader is capable of achieving complete tumor regression in multiple xenograft models, suggesting the potential superiority of STAT3 protein degradation over suppression of STAT3 mRNA expression as a therapeutic strategy. Degradation of STAT3 protein, therefore, is a promising cancer therapeutic strategy. A large body of cumulative evidence strongly supports STAT3 as an attractive therapeutic target in cancer and other human diseases.

References:

[1]Courtney Nicholas and Gregory B. Lesinski. The Jak-STAT Signal Transduction Pathway in Melanoma. Breakthroughs in Melanoma Research.

[2]Xin Li, et al. STAT3 Inhibitors: A Novel Insight for Anticancer Therapy of Pancreatic Cancer. Biomolecules. 2022 Oct 9;12(10):1450. doi: 10.3390/biom12101450.

[3]Yuhao Ren, et al. Discovery of STAT3 and Histone Deacetylase (HDAC) Dual-Pathway Inhibitors for the Treatment of Solid Cancer. J Med Chem. 2021 Jun 10;64(11):7468-7482. doi: 10.1021/acs.jmedchem.1c00136.

[4]Sailan Zou, et al. Targeting STAT3 in Cancer Immunotherapy. Mol Cancer. 2020 Sep 24;19(1):145.DOI: 10.1186/s12943-020-01258-7.

MEDICILON

Email: marketing@medicilon.com

Address: 50 Soldiers Field Place, Boston, MA 02135

Website: www.medicilon.com **Tel:** +1(626)986-9880