Novel Highly Selective Inhibitors of Cyclin Dependent Kinase 7 (CDK7)

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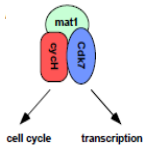
Cyclin-dependent kinase (CDK) family members that trigger passage through the cell cycle have been considered as attractive therapeutic targets for years, especially for cancer. Over time this enthusiasm vanished, since the 1st generation of CDK inhibitors resulted in disappointing clinical outcomes due to small therapeutic windows. CDK inhibitors affecting processes such as transcription and RNA processing have caught less attention so far, although experimental evidence for their involvement in different pathological processes is emerging. As a general regulator of cell cycle and transcription,

CDK7 is being discussed as a therapeutic target for cancer although it has also been considered a double edged sword because of its central role in transcription (TFIIH component) as well as in the cell cycle (CDK-activating kinase (CAK)). Extending the role of the molecular target, CDK7, to a CDK7 inhibitor, the double edged sword hypothesis argues either in favor of good oncology target properties or safety issues. To achieve clarity for this conundrum, we generated picomolar and mono-selective CDK7 inhibitors through rationale design efforts. Inhibitors from our proprietary lead series demonstrated *in vivo* efficacy in a breast cancer xenograft model without causing toxic or adverse effects. Even more surprisingly, such highly selective CDK7 inhibitors showed a distinct responder profile of sensitive cell lines from a panel of more than 120 different human cancer cell lines. Therefore, selective CDK7 inhibitors shall not be considered as non-specific cytotoxic agents. Rather they are novel starting points of personalized therapy for certain tumor types.

Dual role of CDK7 in cell cycle and transcription

- CDK7, in complex with cyclin H and Mat1 plays roles in both the cell-division cycle and transcription (CAK & TFIIH kinase) and as a component of the general transcription factor

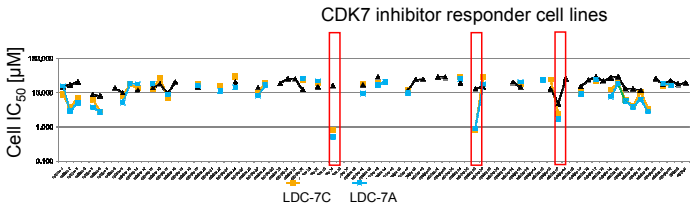


CDK7 controls several cancer relevant processes like cell cycle, activation of transcription factors, as well as general transcription

	Enzymatic IC50 LDC [µM]						Cellular IC50 [µM]			
Company code	CDK1	CDK2	CDK4	CDK6	CDK7	CDK9	cdc2 Thr161p	MDA-MB-468	HeLa	Company code
SNS-032	0.047	0.007	0.067	1.4	0.057	0.005		0.10	0.49	CDK Reference
Flavopiridol	0.037	0.053	0.21	0.79	0.25	0.02		0.18	0.69	CDK Reference
BS-181	21	3.2	>10	>10	0.057	1.9	18	18	30	CDK7 Reference
LDC-7A		>10			<0.005	>10		2.3	>30	CDK7 Inh. Generation 1
LDC-7B	4.8	0.29	>10	>10	<0.005	1.7	15	3.5	27	CDK7 Inh. Generation 1
LDC-7C	10	3.4	>10	>10	<0.005	4.7		4.0	>30	CDK7 Inh. Generation 1
LDC-7D	0.6	0.043	>10	>10	0.007	0.091	4.6	1.6	4.7	CDK7 Inh. Generation 1
LDC-7E	1.7	0.14	>10	>10	0.016	0.23	6.3	2.1	8.9	CDK7 Inh. Generation 1
LDC-7F	7.7	0.56	>10	>10	0.027	0.9	29	9.5	25	CDK7 Inh. Generation 1
LDC-7G	1.3	0.12	>10	>10	0.028	0.11		5.5	>10	CDK7 Inh. Generation 1
LDC-7H	17	1.7	>10	>10	0.087	6.4	19	18	27	CDK7 Inh. Generation 1

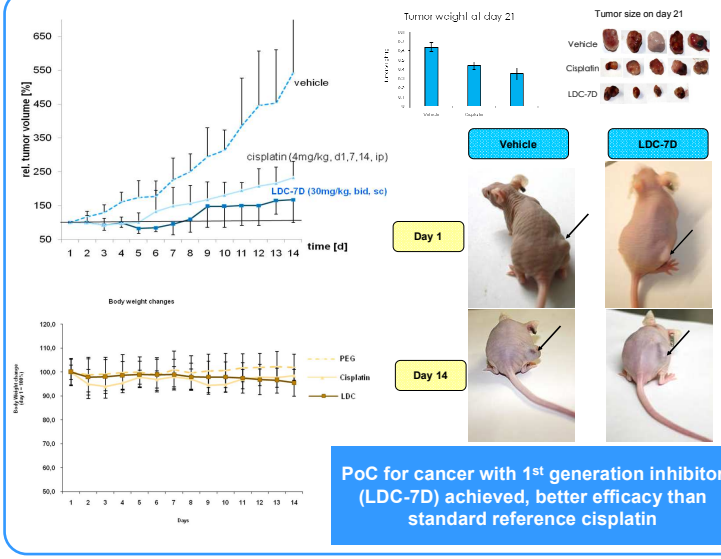
CDK7 inhibitors block phosphorylation of the cellular CDK7 substrate cdc2

Selective CDK7 inhibition is toxic for tumor cells, but non-toxic for HeLas & PBMCs



Screening of a panel of genetically defined tumor cells reveals CDK7-sensitive cell lines ➔ ***identification of biomarkers ongoing***

Inhibition of CDK7 is not generally toxic, therefore risk for target-related tox in development is reduced



PoC for cancer with 1st generation inhibitor (LDC-7D) achieved, better efficacy than standard reference cisplatin

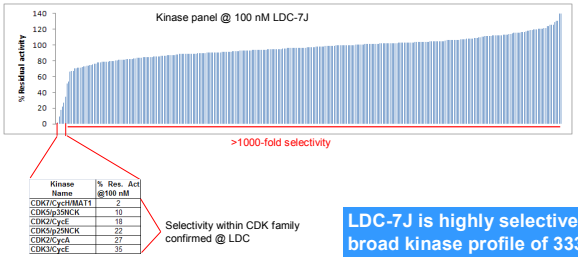
	Enzymatic IC50 [µM]						
Company code	CDK1	CDK2	CDK4	CDK6	CDK7	CDK9	Comment
SNS-032	0.047	0.007	0.067	1.4	0.058	0.005	CDK Reference
Flavo	0.037	0.053	0.21	0.8	0.25	0.016	CDK Reference
BS-181	21	3.2	>10	>10	0.057	1.9	CDK7 Reference
LDC-7C	>10	3.4	>10	>10	0.0043	4.7	1st Generation
LDC-7D	0.6	0.043	>10	>10	0.0059	0.091	1st Generation tested in Xenograft
LDC-7I	>1	1.1	>10	>10	0.00027	>10	2nd Generaton tested in PK cassette dosing
LDC-7J	>0.1	0.011	>10	>10	<0.000099	0.54	2nd Generaton tested in PK cassette dosing
LDC-7K	>10	6.9	>10	>10	0.0028	>10	2nd Generaton tested in PK cassette dosing
LDC-7L	>100	0.018	>10	>10	0.00011	2.7	2nd Generaton tested in PK cassette dosing
LDC-7M	>1	0.3	>10	>10	0.00039	3.1	2nd Generaton tested in PK cassette dosing
LDC-7N	>1	0.15	>10	>10	<0.000099	1.7	2nd Generaton tested in PK cassette dosing
LDC-7O	>1	0.03	>10	>10	0.0003	4.5	2nd Generaton tested in PK cassette dosing
LDC-7D	0.6	0.043	>10	>10	0.0059	0.091	1st Generation tested in Xenograft

			Biochemical IC50 [µM]						Cellular IC50 [µM]					
Company code	PAMPA	Sol	CDK1	CDK2	CDK4	CDK6	CDK7	CDK9	Cell line 1	Cell line 2	Cell line 3	HeLa	hPBMC	Cell line 4
LDC-7A	61,8	376,5		>10			0.005	>10	2,83	4,427	0,43	>30	>30	>30
LDC-7P	6,6	436,9		0,27			0.005	2,037	0,593	0,618	0,09	16,533	15,218	21,014
LDC-7Q	29,95	307		0,052			>1	2,426	4,694	23,282	12	>30	>30	>30
LDC-7R	53,6	289,8		0,022			<0.000099	0,32	0,055	0,28	0,029	3,697	8,672	6,028
LDC-7J	74,25	373,5	>10	0,0081	>10	>10	<0.000099	2,234	<0,014	0,109	<0,014	2,535	5,746	3,846
LDC-7L	18,35	395,2	>100	0,0179	>10	>10	0,00011	2,283	0,008	0,157	<0,007	10,217	14,062	13,208
LDC-7S	10,9	385,7	>0,1	0,0821	>10	>10	<0.000099	0,613	0,08	0,283	0,047	4,715	5,831	7,186
LDC-7N	29,9	333,3	>1	0,146	>10	>10	<0.000099	1,679	<0,014	0,157	<0,014	5,415	12,814	10,728

Two digit picomolar activity for 2nd generation of CDK7 inhibitors

The selectivity within the CDK-family is 1000-fold to more than 100 000-fold

Responder cell lines with cellular IC_{50}s <14 nM



Kinase Name	% Res. Act @100 nM
CDK7/CycH/MAT1	2
CDK5/p35NCK	10
CDK2/CycE	18
CDK5/p25NCK	22
CDK2/CycA	27
CDK3/CycE	35

LDC-7J is highly selective in a broad kinase profile of 333 kinases

Partnering Opportunity

Novel, highly selective inhibitors of CDK7

have anti-cancer activity *in vivo,* are well tolerated and might be suitable to treat inflammatory and viral diseases as well

Status:

- Lead nominated
- monoselective inhibitors with picomolar potency
- SAR clear
- no acute toxicity, good eADME properties
- Good oral bioavailability
- *in vivo* PoC in breast cancer xenograft model
- Responder breast and lung cancer cell lines
- Activity in cellular HCMV infection and inflammation models
- IP filed, recent analysis suggests FTO

Commercialization:

- In-licensing or joint lead optimization - approx. 1-2 years from IND
- known risks were addressed from early on