



Oncalis cell-based High-Throughput Screening (HTS) for the early selection of “drug-like” kinase inhibitors

Background

In many human cancers, the processes of tumor progression and metastasis are initiated by activation of Receptor Tyrosine Kinases (RTKs) and the consequent signaling cascades. Therefore, RTKs and proteins involved in their downstream signaling have become front-line therapeutic targets for the treatment of cancer¹.

Oncalis has developed a unique and proprietary cell-based High-Throughput Screening (HTS) for intracellular kinase inhibitors (small chemical entities). This HTS platform utilizes a yeast *Saccharomyces cerevisiae* cell-based growth selection system that allows the rapid identification of specific and cell-active inhibitors of human RTKs.

In Oncalis yeast cell-based assay, active RTKs reduce cell proliferation. Inhibition of kinase activity by small molecule compounds restores the proliferation in a concentration-dependent manner, thus enabling a “positive read-out” for the identification of kinase-specific inhibitors by their virtue of stimulating cell growth².

Being a cellular assay and thanks to the “positive read-out”, Oncalis’ innovative cell-based assay has significant benefits over other assays, enabling the selection in a single system of compounds that have “drug-like” properties:

- a) penetrate the cell membrane
- b) bind to RTKs in active or inactive states (at equilibrium in living and growing cells)
- c) do not show generalized cytotoxic properties and have appropriate solubility, metabolic stability and other physicochemical properties for activity *in vivo*.

Hence, Oncalis cell-based High-Throughput Screening (HTS) for kinase inhibitors reduces the time and investment usually spent on *in vitro* assays of molecules that must be abandoned at the “final” stage of cellular proof-of-concept.

Oncalis is offering its cell-based High-Throughput Screening (HTS) for kinase inhibitors for a fee for service. This fee is on a per-well basis.

The HTS can be pan-hits (i.e. screening of numerous hits for selected kinases) or pan-kinases (i.e. screening of limited number of hits for all or a large panel of kinases).

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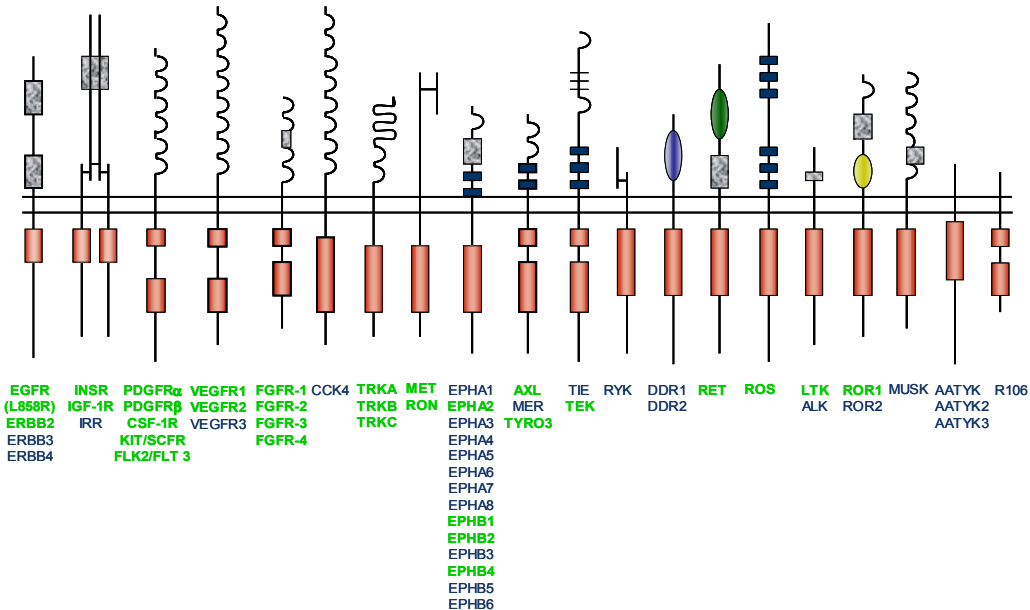


RTKs in Oncalis HTS platform as of March 2008

As of March 2008, Oncalis functional assays in yeast cover 31 human RTKs out of the 58 known human RTK genes (Fig 1). These 31 genetically engineered yeast strains are the basis for Oncalis' proprietary and patented screening platform technology. These strains lack key ABC membrane transporters, thus increasing drug sensitivity by preventing rapid compound efflux, an otherwise common problem with yeast systems. Small molecule compound libraries are currently screened in a 96-well format in an automated HTS system. The read-out is optical density (OD) at 600 nm.

AXL	FGFR3	LTK	TEK
CSF1R	FGFR4	MET	TRKA
EGFR (L858R)	FLT3	IGF1R	TRKB
ERBB2	KIT	INSR	TRKC
EphA2	KIT (D816F)	PDGFR α	TYRO3
EphB1	KIT (D816V)	PDGFR β	VEGFR1
EphB2	KIT (D816Y)	RET	VEGFR2
EphB4	KIT (K642E)	RON	
FGFR1	KIT (N822H)	ROR1	
FGFR2	KIT (V560G)	ROS	

Figure 1: Receptor Tyrosine Kinase (RTK) Families. Kinases highlighted in green are currently available as part of Oncalis' RTK assay platform





Compound Logistics

Compounds are delivered to Oncalis as follows:

- ❑ 2 mM stock solutions in 100% DMSO
- ❑ 40 μ L per well in 96-well polypropylene V-shaped plates (e.g. from Greiner # 651201 or Matrix # 4919) sealed with cap mats (see layout below)
- ❑ Isis Database containing plate/well ID of compounds
- ❑ Layout: Lane 12 contains our general control compound (growth stimulator). If available, please provide a known inhibitor of your selected RTK target; we will test it in the yeast cell-based assay and add it in an appropriate concentrations in wells F-H of lane 12 as additional positive control.

Fig. 2 Layout of shipped compounds in 96-well plates

Row/Col	1	2	3	4	5	6	7	8	9	10	11	12
A	DMSO	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	EMPTY
B	DMSO	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	EMPTY
C	DMSO	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	EMPTY
D	DMSO	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	EMPTY
E	DMSO	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	EMPTY
F	DMSO	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	EMPTY
G	DMSO	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	EMPTY
H	DMSO	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	CMPD	EMPTY

- ❑ Shipment on dry ice



Screening Procedure

1. HTS at 20 μ M

Throughput: 8400 compounds/week

2. "Hit" identification and confirmation in yeast

A "hit" is a compound that stimulates yeast cell proliferation compared to negative (DMSO) and positive control over a defined threshold (fold growth stimulation). Primary "hits" are picked and tested for confirmation in a concentration-dependent experiment (8 concentrations, n=1) in the same yeast strain.

Deliverables:

- Lists of primary "hits" exceeding defined threshold levels
- "Hit" confirmation: yes/no answer
- Concentration-dependent curves of confirmed "hits"

3. Selectivity profile in yeast

Selectivity profile of selected compounds with our panel of currently 31 RTKs is measured at one concentration (generally 10 μ M)

Example of an HTS

Fig. 3 HTS at one compound concentration. Typical 96-well assay plate with negative control (DMSO) in column 1, positive control compounds in column 12. Numbers indicate fold stimulation of cell proliferation. Green background: ≥ 1.3 fold stimulation (defined threshold for "hit" selection).

80 different compounds at 20 μ M

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.94	1.35	1.28	1.30	1.04	0.96	1.08	1.01	0.90	0.99	0.89	6.16
B	1.00	1.01	1.11	0.94	1.06	0.96	0.98	0.98	1.00	1.09	1.14	6.22
C	0.98	0.95	1.55	1.06	1.06	0.93	1.01	1.12	1.01	1.01	1.13	6.12
D	1.00	1.07	1.55	0.99	1.12	0.96	0.98	1.16	1.09	1.08	1.09	6.09
E	0.93	1.03	1.24	1.10	0.98	1.03	1.04	0.54	1.00	1.09	0.99	6.09
F	0.99	1.13	0.90	1.03	1.05	1.01	0.85	1.21	0.94	1.12	1.14	1.55
G	1.01	1.25	1.15	1.02	1.01	1.03	1.04	1.03	1.08	1.15	1.05	1.59
H	1.06	1.16	1.20	1.13	1.18	0.99	1.17	1.05	1.05	1.16	1.06	1.54

DMSO

Positive control ("protepx")

Validated control inhibitor at 2 μ M

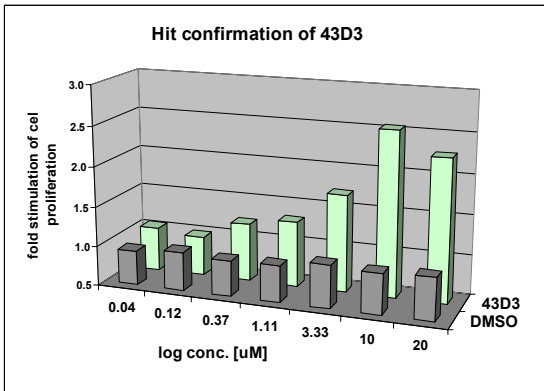


Fig. 4 Concentration-dependent test. Compound 43D3 (plate # 43, lane D, column # 3), among others, was picked and tested for growth stimulation in a concentration series in the same strain.

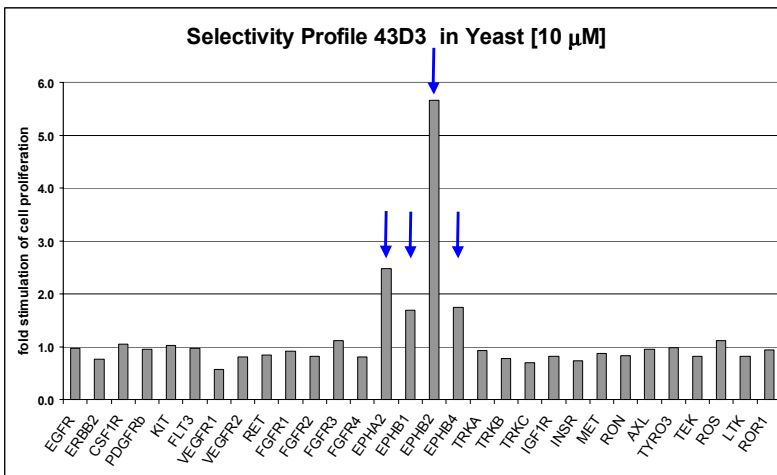


Fig. 5 Selectivity profile against RTK panel. Compound 43D3 was tested at 10 μ M against the RTK panel of Oncalis. An effect is seen only with the EphA and EphB family kinases.



Proof-of-principle experiment with Gleevec®

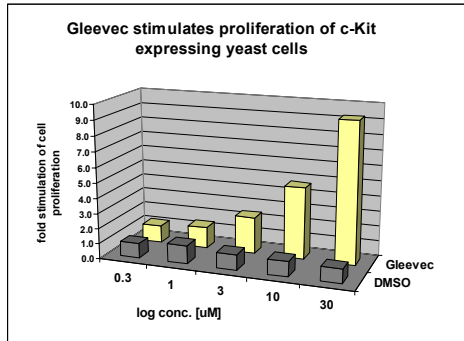


Fig. 6 The marketed drug Gleevec®, an inhibitor of Bcr-Abl, c-Kit and other kinases³, stimulates proliferation of c-Kit expressing yeast cells in a concentration-dependent manner.

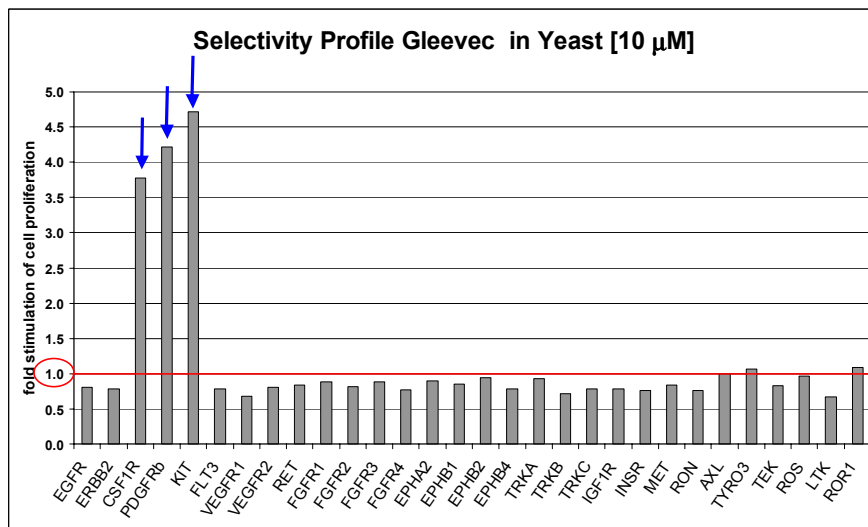


Fig 7 The drug Gleevec® was measured at 10 µM against Oncalis' RTK panel. In this panel, Gleevec® inhibits CSF1R, PDGFRβ and c-Kit, thus resulting in stimulation of cell proliferation of the respective yeast strains. These results that faithfully correlate with published data on Gleevec® selectivity profile.



References

1. Insight Pharma Reports. Kinase therapeutic pipelines: An assessment of targets and agents in development, by Norman P., December 2007
2. Barberis A. et al., Yeast as a screening tool. Drug Discov Today Tech, Vol 2, Issue 2, 2005, 187-192
3. Wisniewski D. et al., Characterization of potent inhibitors of the Bcr-Abl and the c-kit receptor tyrosine kinases. Cancer Res 2002 Aug 1;62(15): 4244-55.