### **PROTAC® Drugs: A Revolutionary Approach**



Karteek Kadimisetty Ph. D Director, R&D

LifeSensors Inc. 271 Great Valley Parkway Malvern PA 19355 Phone: 610-644-8845 bd@lifesensors.com www.lifesensors.com

LifeSensors

### LifeSensors Inc.

- Leading Biotech in PROTAC/UPS Drug Discovery and Diagnostic R & D
- ~500 Product lines, PROTAC assay plates, Ub ligases and DUBs, Affinity Reagents (TUBEs), Kits and SUMO Protein Expression Systems
- Drug Discovery, PROTAC/UPS in vitro and cell-based screening Services
- Profiling compounds against ubiquitin ligases and DUBs
- Custom Assay Development CRO Model
- Collaborative and Independent Research



# Proteolysis-targeting Chimeric Molecules PROTACs

- > Recruiting a Target Protein to an E3 ligase for ubiquitination and subsequent degradation
- > Heterobifunctional molecule that recognizes an E3 ubiquitin ligase and a Target Protein and joined by a linker domain



LifeSensors www.lifesensors.com

### **PROTAC®s are Essential Catalytic Activators**



LifeSensors

## **Challenges with PROTAC® Discovery**

- Lack of high-throughput assays to assess true efficacy of PROTACs
- Positive co-operativity (better K<sub>D</sub>) does not guarantee better degradation activity
- Ternary complex rigidity can be inhibitory to ubiquitination



### **Does All Ternary Binding Lead to Successful Degradation ?**





**TR-FRET- based dimerization assay** 





**Endogenous KRAS G12C Degradation** 

Zeng, M., Xiong, Y., Safaee, N., Nowak, R. P., Donovan, K. A., Yuan, C. J., ... & Gray, N. S. (2020). Exploring targeted degradation strategy for oncogenic KRASG12C. *Cell chemical biology*, 27(1), 19-31.



#### **Endogenous K-RAS vs GFP K-RAS Degradation**



**Invitro Ubiquitination Assay** 



Zeng, M., Xiong, Y., Safaee, N., Nowak, R. P., Donovan, K. A., Yuan, C. J., ... & Gray, N. S. (2020). Exploring targeted degradation strategy for oncogenic KRASG12C. *Cell chemical biology*, *27*(1), 19-31.

![](_page_6_Picture_5.jpeg)

## LifeSensors PROTAC Discovery Approach

#### **Traditional Approaches**

- **Reporter gene assays**: External tags with internal lysines lead to artifacts (Off target ubiquitination).
- **Proximity Ligand Assays:** Does not account for protein's intrinsic features crucial for ubiquitination.
- Western Blotting: Low throughput and Irreproducible.

#### LifeSensors Approach

- No reporter tags needed, suitable for all targets and applicable to targets limited to primary cell lines.
- Measures PROTAC mediated Ubiquitination and degradation simultaneously – dependent on intrinsic lysine exposure.
- High-throughput, reproducible and compatible for multiplexing.

![](_page_7_Picture_9.jpeg)

## **TANDEM UBIQUITIN BINDING ENTITIES (TUBEs)**

![](_page_8_Picture_1.jpeg)

- <u>PROTAC Screening</u>, PROTAC mediated of poly-ubiquitinated proteins from cells and tissue
- Superior to antibodies, detection by Western blot
- <u>E3 ligase</u> and <u>DUB</u> assays
- *In situ* detection with fluorescence
- <u>Ubiquitin mass spec proteomics</u> bypassing SILAC

![](_page_8_Picture_7.jpeg)

### **HTS-in vitro PROTAC Screening Platform**

Monitor PROTAC mediated ternary complex formation and ubiquitination

![](_page_9_Figure_2.jpeg)

**TUBE Capture and Substrate Detection** 

![](_page_9_Figure_4.jpeg)

![](_page_9_Picture_5.jpeg)

### **VHL K-RAS PROTAC®**

HTS - In vitro Ubiquitination Assays with KRAS G12C vs G12D vs WT

![](_page_10_Figure_2.jpeg)

In vitro ubiquitination assay with KRAS G12C VHL based degraders: (A & B) VHL-based PROTAC LC2 in a dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C, G12D and wildtype. CL intensities plotted in response to ½ log dose response demonstrates PROTAC mediated ubiquitination. (C) Western blot analysis to confirm PROTAC mediated ubiquitination via characteristic poly-ubiquitination smears with anti-KRAS antibody.

Fesensors www.lifesensors.com

### **Cerebion MRTX - K-RAS PROTAC®**

HTS – In vitro Ubiquitination Assays with KRAS G12C

![](_page_11_Figure_2.jpeg)

In vitro ubiquitination assay with KRAS G12C CRBN based degraders: ((A) CRBN-based PROTAC degrader 1 (compound 518) in a dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C. CL intensities plotted in response to ½ log dose response demonstrates PROTAC mediated ubiquitination. (B) Western blot analysis to confirm PROTAC mediated ubiquitination via characteristic poly-ubiquitination smears with anti-KRAS antibody.

![](_page_11_Picture_4.jpeg)

### **Cerebion ARS-1620 K-RAS PROTAC®**

HTS - In vitro Ubiquitination Assays with KRAS G12C vs Cell based assay

![](_page_12_Figure_2.jpeg)

(A) In vitro ubiquitination assay with ARS 1620 KRAS G12C CRBN based degrader in a dose response study to monitor PROTAC mediated ubiquitination of KRAS G12C. CL intensities plotted in response to ½ log dose response demonstrates PROTAC mediated ubiquitination. (B) Cell based assay to monitor degradation of KRAS G12C in H358 cells - PROTAC mediated degradation in a dose response study.

![](_page_12_Picture_4.jpeg)

### **CRBN Bromodomain PROTAC®**

HTS – In vitro Ubiquitination Assays

![](_page_13_Figure_2.jpeg)

fesensors

### **VHL Bromodomain PROTAC®**

HTS – In vitro Ubiquitination Assays

![](_page_14_Figure_2.jpeg)

PROTAC	In vitro Ubiquitination vs Binding Affinity		
	Ub <sub>Max</sub> ( <i>In vitro</i> ), μΜ	Kd, μM	Hook effect ( <i>In vitro</i> ), μM
AT1	0.1-0.03 (3-fold Peak)	0.04	0.1
MZP54	0.03 (10-fold Peak)	0.004	0.3
NE987	0.03 (8-fold Peak)	0.004	0.1

ONSORS www.lifesensors.com

### **PROTAC®** Assay Plate – Cell Based Assays

![](_page_15_Figure_1.jpeg)

\_ifeSensors

### **CRBN & VHL – K-RAS G12C degraders**

Cellular ubiquitination and degradation profiles – Dose Response Study

![](_page_16_Figure_2.jpeg)

Monitoring Ubiquitination – Dose Response : changes in ubiquitination profiles of endogenous KRAS and subsequent degradation in H358 cells with changes in dose of both VHL and CRBN KRAS degraders. VHL and CRBN PROTACs designed with covalent ligands to engage KRAS G12C and successfully ubiquitinate and degrade with 3hrs of treatment between 30-100 nm.

### LifeSensors www.lifesensors.com

### **CRBN & VHL – K-RAS G12C degraders**

Cellular ubiquitination and degradation profiles – Time Course Study

![](_page_17_Figure_2.jpeg)

**Monitoring Ubiquitination Kinetics –** changes in PROTAC mediated ubiquitination profiles of endogenous KRAS and subsequent degradation in H358 cells. VHL and CRBN PROTACs designed with covalent ligands to engage KRAS G12C and successfully ubiquitinate and degrade within 2-4hrs of treatment.

### LifeSensors

### **TUBE-based Proteomics**

![](_page_18_Figure_1.jpeg)

- Rapid, Inexpensive and Simple method for identifying total Ubiquitome changes
- Quantitative method for examining drug effect of PROTACs in cells

![](_page_18_Picture_4.jpeg)

### **Novel E3 Ligase K-RAS PROTAC®**

![](_page_19_Figure_1.jpeg)

![](_page_19_Picture_2.jpeg)

### **MS Evaluation of K-RAS PROTAC®**

Comparative E3 Ligase Study – In vitro Ubiquitination Mass Spectrometry Analysis

![](_page_20_Figure_2.jpeg)

### **TUBE-based Proteomics-PROTAC**<sup>®</sup>

Multi-kinase CRBN-PROTAC ubiquitinated ~15 kinases that were rescued by proteasome inhibitor

![](_page_21_Figure_2.jpeg)

\_ifeSensors www.lifesensors.com

### **CRBN Multi-Kinase degraders**

HTS PROTAC mediated Ubiquitination of AURKA

![](_page_22_Figure_2.jpeg)

**AURKA** *invitro* ubiquitination assays: recombinant AURKA ubiquitination was monitored as function of cereblon PROTAC dose response. Excess kinase inhibitor competes with PROTAC interaction compromising ubiquitination of the target. A complementary gel-based assay to represent changes in poly-ubiquitination profiles mediated by PROTACs.

www.lifesensors.com

### **CRBN Multi-Kinase degraders**

Cellular ubiquitination HTS In vitro Screening

![](_page_23_Figure_2.jpeg)

- A time course study to evaluate intracellular ubiquitination and degradation to establish the cooperative binding between CRBN and AURKA.
- Monitor degradation kinetics to compare Ub<sub>max</sub> with degradation establish the ubiquitination as precursor step of degradation.
- Evaluate DC<sub>50</sub> of the promiscuous kinase PROTAC in K562 cells using Ub<sub>max</sub>

www.lifesensors.com

### **TUBE based PROTAC® Assays**

- Establish rank-order potencies of PROTAC variants in a HT fashion
- Rapid ubiquitination and degradation kinetics of native targets
- Guiding Med Chem to establish rapid SAR

#### **"Ub<sub>Max</sub>" A better way to measure potency of PROTACs**

### **TUBEs based PROTAC® Assays provides a link between ubiquitination and degradation**

![](_page_24_Picture_6.jpeg)

![](_page_25_Figure_0.jpeg)

ifeSensors

### **Thank You**

#### We are your partner for PROTAC Drug Discovery

#### **Contact Us!**

#### **Contact Information**

<b>Research &amp; Product Inquiries</b>	R&D	<u>info@lifesensors.com</u>	610-644-8845 (ext 339)
<b>Custom Service &amp; Assays</b>	BD	bd@lifesensors.com	610-644-8845 (ext 310)

LifeSensors