Syngene

Discovery on TARGET

OCTOBER 17-20, 2022 BOSTON, MA

P071

Targeted Protein Modulators

Cellular-to-ADME-to-PK/PD Models and Correlations

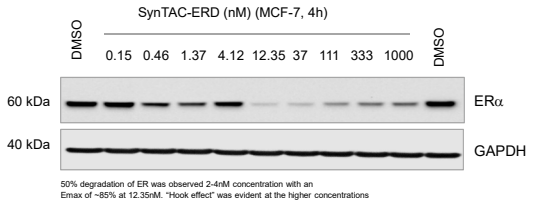
Debasish SinghaRoy, Rashika Dhar, Sujatha Subbanna, Krushali Powale, Inba Kumar V, Sakshi Anilkumarbhatele, Prafull Kumarsingh, Sripathi Obulesu, Kruti Upadhyay, Kanchanmala Singh, Pankaj Paliwal, Neha Kamran, Ravi Kumar N, Amol A Raje, Rajesh Vyas, and Atul Tiwari[#] *[#]Corresponding Author (atul.tiwari@syngeneintl.com)*

Abstract

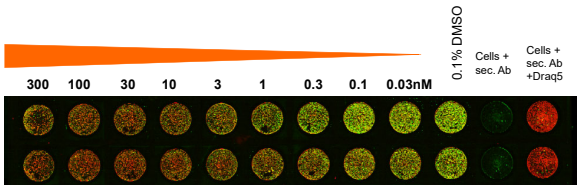
The proteolysis targeting chimeras (PROTACs) and other similar targeted protein modulators (TPMs) have revolutionized the field towards the discovery of new drugs. Different assay platforms with differentiation on conventional approaches, relevant to targeted protein degraders for their activity, efficacy and PK-PD correlation are presented in the form of a case study. Further perspective has been drawn on key ADME properties and plasma exposure to understand such correlations.

We hereby attempted to develop a correlation between the cellular activities in mechanistic and functional models, ADME, Pharmacokinetic (PK) and Pharmacodynamic (PD) properties using a tool PROTAC called as SynTAC-ERD, an Estrogen Receptor (ER) degrader. Studies confirmed a good correlation between cellular activity- degradation and decrease in cell proliferation in different models further translating to good PD response and efficacy in the animal model. The compound exhibited good PK to PD correlation, however a poor correlation between the *in vitro* ADME properties to the mouse PK was observed.

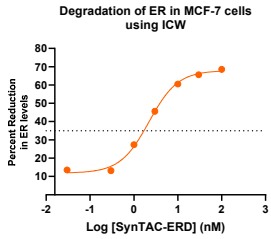
SynTAC-ERD is a potent degrader of ER in MCF-7 cells



50% degradation of ER was observed 2-4nM concentration with an Emax of ~85% at 12.35nM. "Hook effect" was evident at the higher concentrations



Dose-dependent ER degradation was observed in MCF-7 cells treated with SynTAC-ERD with a DC50 of 2.2nM



Target Degradation & Engagement to functional response in MCF-7 cells through Live cell Imaging using Incucyte

Decrease in cell viability in 2D and 3D spheroid models with SynTAC-ERD in a time-course study

Fig. A) MCF-7 cells 2D cultures + compound treatment; 120hrs

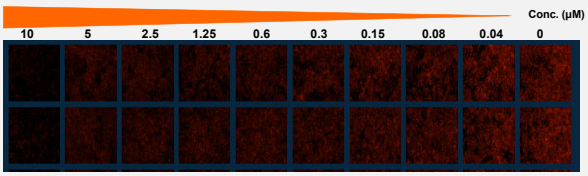


Fig. B) MCF-7 cells 3D spheroids + compound treatment; 6 days

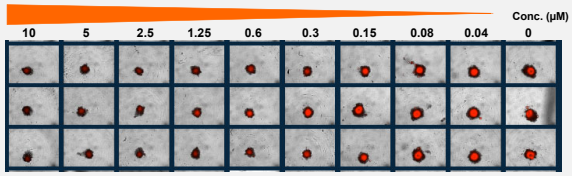


Fig. C) IC50 comparison- 2D vs. 3D

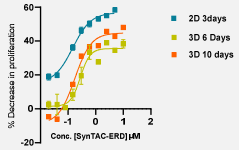
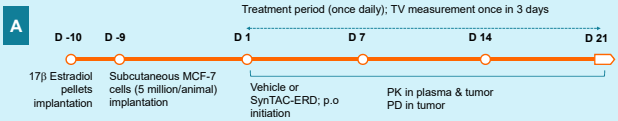


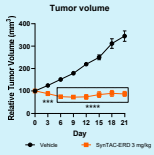
Fig. A & B) MCF-7 cells were treated with syn-TAC-ERD. Decrease in red fluorescence intensity indicates reduced number of cells in wells (cytotoxicity of target cells and/or decreased proliferation). The IC50 values were in the range of 1-4nM (Fig. C) under different conditions within DC50 range, though lower Emax was observed under 3D spheroid conditions compared to 2D cultures.

SynTAC-ERD significantly reduces tumor growth *in vivo* and improves survivability in MCF-7 Xenograft nude mice

A

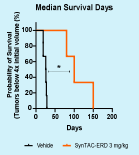


B



****p<0.001 & ****p<0.0001 vs Vehicle (Unpaired t-test)*

C



**p<0.05 vs Vehicle; (Log-Rank (Mantel-Cox) test)*

A. Schematic outline of in vivo MCF-7 xenograft study in female Athymic Nude-Foxn1^{nu} mice.
B. Relative tumor growth inhibition by SynTAC-ERD dosed at 3 mg/kg PO daily for 21 days.
C. Kaplan-Meier survival plot showing the median time to reach 4x initial tumor size. Data is represented as Mean +/-SEM (n=8/group). P<0.05 considered as significant Vs Vehicle

SynTAC-ERD exhibited

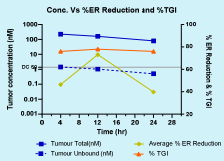
- 76% reduction in tumor growth
- 100 days of median survivability vs 26 days in vehicle group

D Efficacy corelates with ER Degradation and unbound tumor concentration

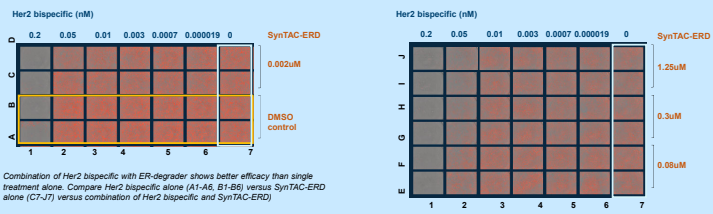
Western blot PD samples	% ER Reduction
3mpk	80%

21-day post 12hr treatment

PK Parameters	Day 21; 3 mpk	
	Plasma	Tumor
Cmax (nM)	96	226
Tmax (h)	4.0	4.0
AUClast (hr*nM)	922	3438



Combination of ER degrader with Her2 bispecific antibody in MCF-7 cells demonstrates improved response for tumor cell killing compared to Her2 bispecific and ER degrader alone



Combination of Her2 bispecific with ER-degrader shows better efficacy than single treatment alone. Compare Her2 bispecific alone (A1-A6, B1-B6) versus SynTAC-ERD alone (C7-J7) versus combination of Her2 bispecific and SynTAC-ERD)

Summary & Conclusions

- Using a tool compound SynTAC-ERD we hereby demonstrate the cellular translation to degradation and functional responses using different platforms.
- Live cell imaging using Incucyte offers an advantage for kinetic read-outs for target degradation and further studying the functional response with or without combination with other drugs. Preliminary studies suggest an additive/synergistic effect in combination with HER2 bispecific.
- In MCF-7 xenograft model, SynTAC-ERD as a single agent treatment led to marked ER degradation that further resulted into 76% relative tumor growth inhibition with an increase in Tumor Volume Quadrupling Time (TVQT) to ~100 days vs 26 days in Vehicle group.
- Efficacy of SynTAC-ERD corelates well with ER Degradation and unbound tumor concentration, however key ADME properties like Caco-2 permeability and solubility do not correlate well with plasma exposure.
- The physicochemical properties and mechanism of action for protein degraders can lead to unique pharmacokinetic (PK) and pharmacodynamic (PD) properties relative to traditional small molecule drugs, requiring a shift in perspective for translational pharmacology.

Affiliations: Discovery Biology, Syngene International Ltd., Bangalore, INDIA