Abstract LB029

Degraders of TEAD Transcription Factors Based on Interface 3 Binders

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Abstract

TEAD transcription factors have emerged as clinically validated targets for Hippo-altered cancers, *e.g.*, mesothelioma driven by NF2 inactivation/deficiency. We have developed a series of novel small molecule targeted protein degraders of TEAD, based on binders to TEAD Interface 3. In cells, the compounds induce degradation of TEAD by formation of a ternary complex with Cereblon, leading to ubiquitination of TEAD and subsequent proteasomal degradation. In a cell-based luciferase reporter assay the degraders show low nanomolar activities. The downstream effects of TEAD degradation were further investigated by qPCR analyses of bona fide YAP–TEAD target genes such as CTGF, Cyr61 and AMOTL2. The effectiveness of the TEAD degraders were compared to other classes of TEAD modulators such as palmitoylation and YAP–TEAD protein–protein interaction inhibitors by means of cellular viability and proliferation assays using various mesothelioma cell lines. Finally, we performed an unbiased, quantitative high-throughput drug combination screening[1] by combining one selected TEAD degrader with a library of approximately 2,800 oncology-focused drugs.

From fragment to lead

The project was initiated by fragment screens against TEAD1 and TEAD3 resulting in three hits binding to Interface 3. Co-crystal structures enabled structure-based design.

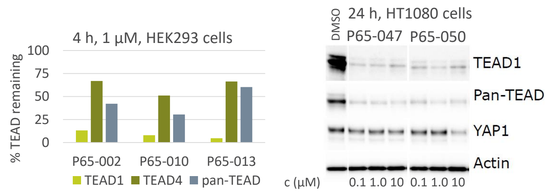
Fragment-based lead generation guided by SPR and crystallography resulted in a 10 nM lead starting from a 300 µM fragment hit.

Optimization of ternary complex formation

EC_{max} = The concentration of the ligand that results in the maximum concentration of the ternary complex at α = 1, *i.e.* no cooperativity.

Conclusions: Early compounds that gave limited ternary complex formation between TEAD and Cereblon were successfully optimised into potent inducers of ternary complexes. Both the extent of ternary complex formation and binary interaction data were found to be predictive of TEAD elimination by the degraders.

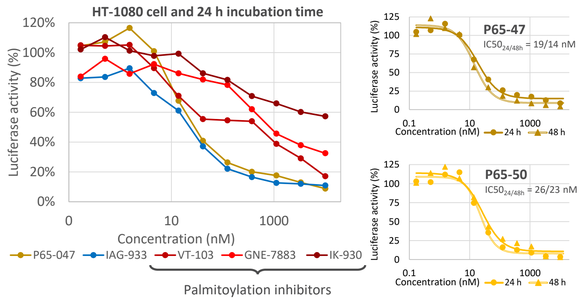
TEAD degradation and selectivity



Conclusions: Ternary complex-forming compounds induced substantial TEAD degradation with a preference for TEAD1 (left pane). With more advanced compounds, pan-TEAD degradation could also be achieved (right pane).

Luciferase reporter assay

Based on SPR affinity and propensity to induce a ternary complex, compounds were prioritized for degradation experiments and a TEAD luciferase reporter assay. Due to its robust TEAD luciferase signal, the HT-1080 cell-line was used.



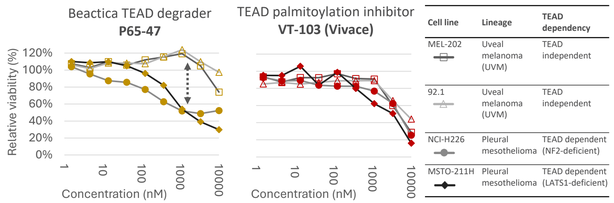
Conclusion: Interface 3-binding TEAD modulators/degraders outperform palmitoylation inhibitors in down-regulating TEAD-regulated luciferase expression in HT-1080 cells.

Expression of TEAD-regulated genes

Conclusion: Interface 3-binding TEAD modulators/degraders more strongly suppress the expression of TEAD-regulated genes compared to palmitoylation inhibitors.

End-point proliferation

TEAD modulators/degraders were evaluated against TEAD-dependent mesothelioma cell lines and TEAD-independent uveal melanoma cell lines in a CellTiter-Glo assay after 48 h incubation.

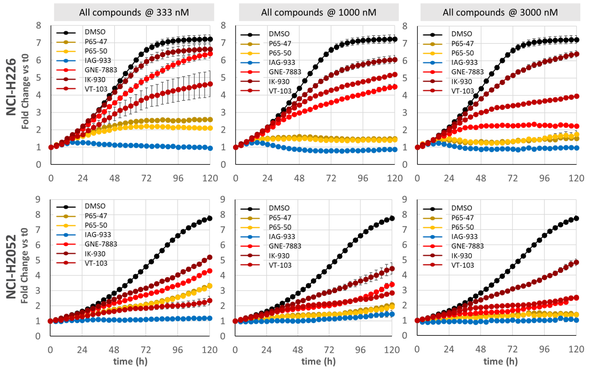


Cell line	Lineage	TEAD dependency
MEL-202	Uveal melanoma (UVM)	TEAD independent
92.1	Uveal melanoma (UVM)	TEAD independent
NCI-H226	Pleural mesothelioma	TEAD dependent (NF2-deficient)
MSTO-211H	Pleural mesothelioma	TEAD dependent (LATS1-deficient)

Conclusion: Beactica's TEAD degraders show a larger separation in cytostatic activity between TEAD-dependent (mesothelioma) vs. TEAD-independent (UVM) control cell lines, compared to palmitoylation inhibitors.

Live-cell proliferation

TEAD modulators/degraders from three mechanistically different compound classes were evaluated on two mesothelioma cell lines – NCI-H226 cells (NF2-deficient) and NCI-H2052 (NF-mutant) – for a period of 5 days in an Incucyte assay.



Conclusion: Interface 3-binding TEAD modulators/degraders outperform palmitoylation inhibitors in inhibiting the proliferation of mesothelioma cell lines.

Acknowledgements
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References
1) Evsen L, Morris PJ, Thomas CJ & Ceribelli M (2023) Comparative Assessment and High-Throughput Drug-Combination Profiling of TEAD-Palmitoylation Inhibitors in Hippo Pathway Deficient Mesothelioma. *Pharmaceuticals*, 16:1635.

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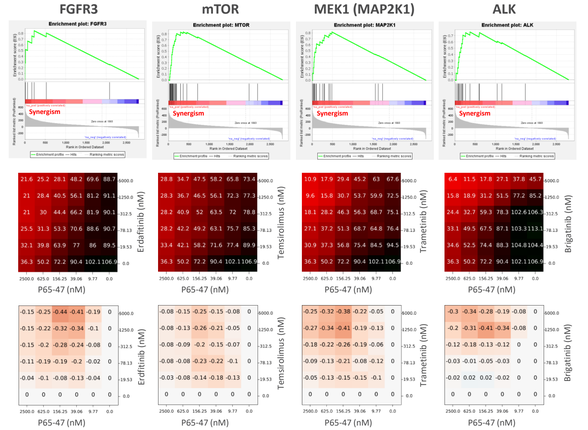
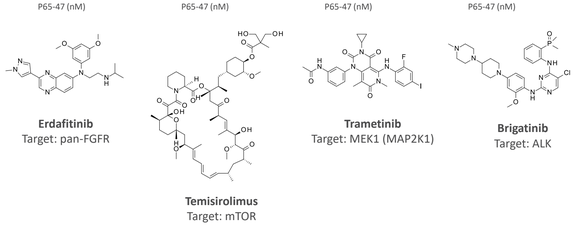
Drug combination profiling

An unbiased, quantitative high-throughput drug screen was performed by combining the TEAD degrader P65-047 with a library of approximately 2,800 oncology-focused drugs. By exploiting the mechanistic redundancy built into this library, several drug classes that synergized with P65-047 in inhibiting proliferation of NCI-H226 (NF2-deficient) cells could be identified (CellTiter-Glo assay after 72 h incubation). The enrichment analysis identified TEAD co-dependencies for several target classes, including FGFR, mTOR, MEK1, and ALK (see examples below), as well as HSP90, CDK8, PI3K-alpha, ABL1, and XPO1 (data not shown).





Summary

- Efficient proteolysis-targeting degraders of TEAD transcription factors were generated, based on TEAD Interface 3 binders and Cereblon ligands
- The degraders showed low nM potency in a TEAD luciferase assay and were found to efficiently down-regulate three bona-fide YAP–TEAD target genes: AMOTL2, CTGF, and CYR61
- In end-point proliferation assays, the TEAD degraders displayed selectivity towards TEAD-dependent cell lines in contrast to, e.g., the palmitoylation inhibitor VT-103
- The TEAD degraders outperformed the tested palmitoylation inhibitors in a live-cell proliferation assay using two mesothelioma cell lines
- A quantitative high-throughput drug combination screen enabled the identification of several druggable TEAD co-dependencies, including FGFR, mTOR, MEK1, ALK, HSP90, CDK8, PI3K-alpha, ABL1, and XPO1