A novel class of Ribosome Modulating Agents (RMAs) targets ribosome heterogeneity in a subset of colorectal cancers

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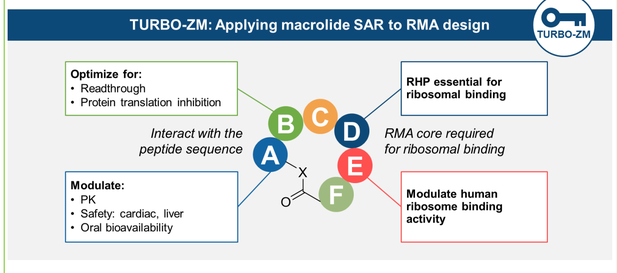
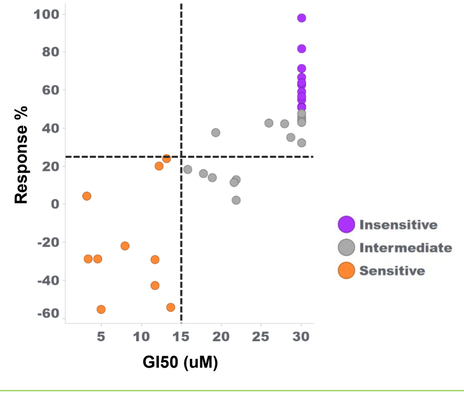
Introduction

Aberrant protein translation is a key driver in cancer, downstream of pro-oncogenic stimuli. This aberrant protein translation is frequently carried out by ribosomes that have cancer-specific alterations providing an opportunity to selectively target "oncoribosomes". Our unique synthetic chemistry generates novel macrolides that are Ribosome Modulating Agents (RMAs) capable of selectively targeting oncoribosomes. ZKN-157, a representative RMA, selectively inhibits protein translation in CMS2 subtype of CRC, which is characterized as Micro-satellite Stable (MSS) and typically shows high MYC and WNT pathway activity. The selectivity of protein translation inhibition is further characterized by preferential effect on positively charged proteins including those involved in ribogenesis and protein translation. This novel mechanism shows synergy with traditional cytotoxic therapy to generate potential new cancer therapeutics.

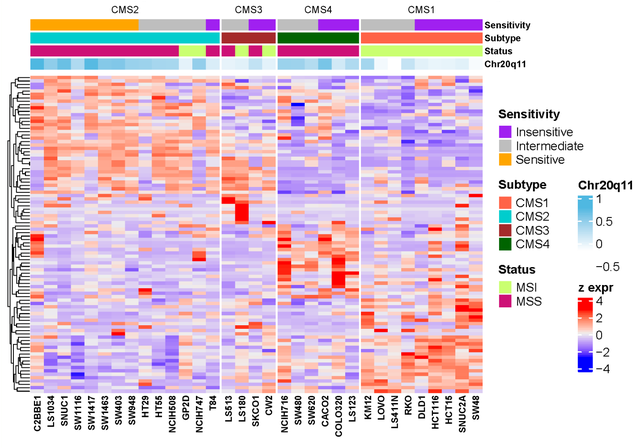
Novel synthetic macrolide-based allosteric modulators of the human ribosome



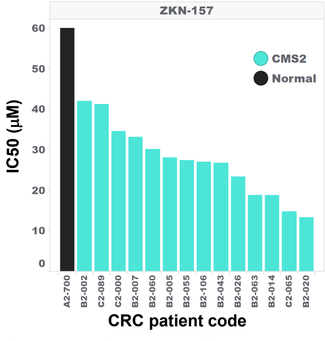
Subset of CRC cell lines are sensitive to ZKN-157



CMS2 subtype of CRC is enriched for sensitivity to ZKN-157



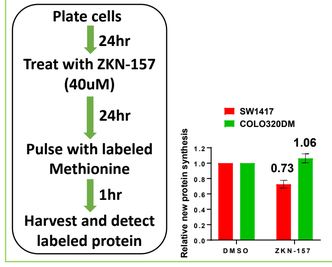
Sensitivity of CMS2 subtype was validated in CRC patient derived organoids (PDOs)

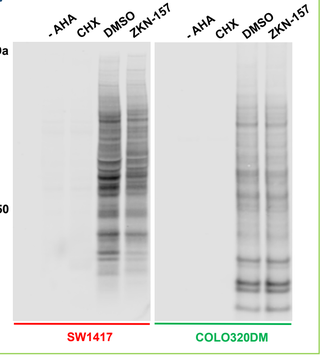


- CMS2 subtype account for **37%** of CRC cases
- CMS2 subtype distinguished by
  - High activity of Wnt and **Myc** pathways
  - High expression of genes involved in **ribogenesis** and protein translation

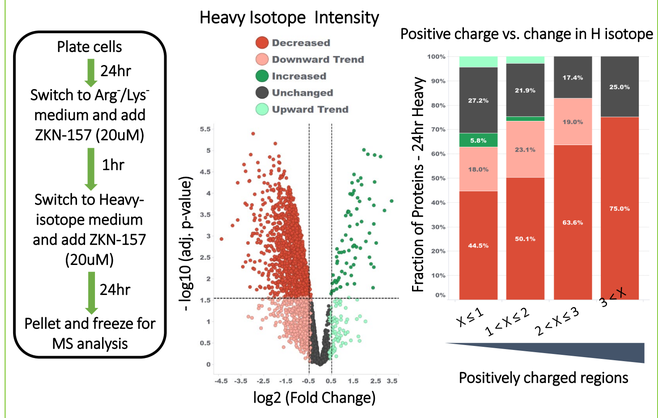
Anti-proliferative effect driven by selective inhibition of new protein synthesis

	ZKN-157	
Cell Line	GI50 (uM)	LD50 (uM)
SW1417	17.4	40.1
COLO320DM	60.0	60.0

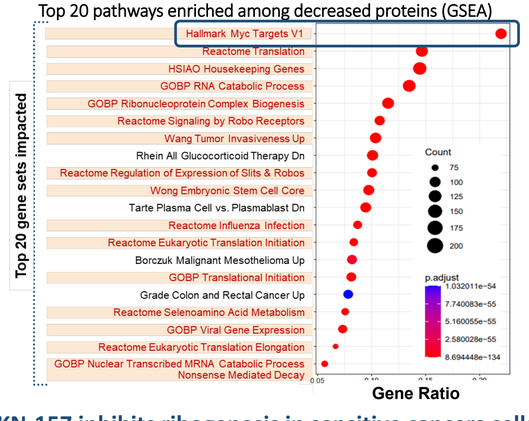




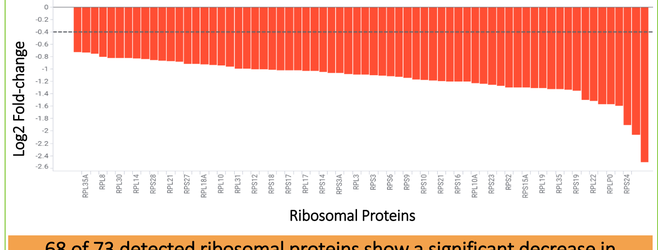
Proteins with higher positively charged regions are more sensitive to translation inhibition by ZKN-157



Decreased proteins show large impact on MYC pathway and protein translation machinery

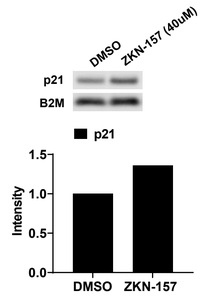


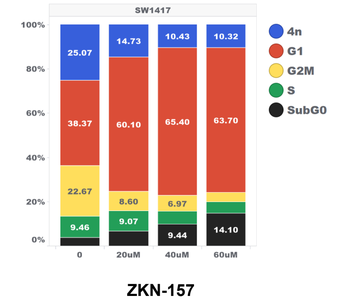
ZKN-157 inhibits ribogenesis in sensitive cancers cells



68 of 73 detected ribosomal proteins show a significant decrease in protein synthesis

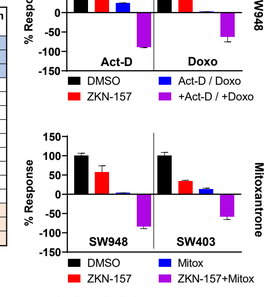
Inhibition of ribogenesis results in p21 induction leading to cell cycle arrest and apoptosis





DNA intercalating agents, known to inhibit rRNA synthesis, show synergy with ZKN-157

Inhibitor	Inhibitor's MoA	Combination Effect
Actinomycin D	DNA/Ribogenesis	Synergistic
Doxorubicin	Topoisomerase II/Ribogenesis	Synergistic
Mitoxantrone	DNA/Ribogenesis	Synergistic
SB525334	TGFβ Receptor	Additive
Oxaliplatin	DNA synthesis/Ribogenesis	Additive
Venetoclax (ABT-199)	Bcl-2	Additive
Camptothecin	Topoisomerase I/Ribogenesis	Additive
Sabutoclax	Bcl-2/xL, Mcl, Bfl-1	Additive
Irinotecan	Topoisomerase I	Additive
Regorafenib	Multi-kinase	Additive
Cetuximab	EGFR	Additive
5-FU	Anti-metabolite	Additive
Idelalisib	PI3K	Antagonistic
Dactolisib (BEZ-235)	PI3K/mTOR	Antagonistic
AZD8055	mTOR	Antagonistic



Multiple levels of selectivity of allosteric inhibition by RMAs

Novel Activity	Cause	Effect (of RMAs)
Ribosome selectivity	Ribosome heterogeneity	Target Ribosomes in a subset of cancers
Translation inhibition selectivity	Proteins containing regions with high density of positively charged amino acids	Inhibit translation of a subset of proteins

Conclusion

- Novel allosteric modulators of the human ribosome have activity on the CMS2 subtype of CRC
- Anti-proliferative effect driven by selective inhibition of ribogenesis and new protein synthesis
- Large impact on the MYC pathway may indicate other relevant cancer indications
- RMAs show additive and synergistic effects with major chemotherapy backbone agents