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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Menin Inhibitor Induced Menin Protein Degradation Contributes to Menin Inhibitor Efficacy

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The chromatin adaptor protein Menin (*MEN1*) is necessary for sustained proliferation in acute leukemias driven either by *Lysine Methyltransferase 2A* (MLL1/ *KMT2A*) gene rearrangements. Small molecule Menin inhibitors (MIs) disrupt the MENIN-KMT2A protein interaction by competing with KMT2A for a binding pocket in Menin. This disruption leads to a downregulation of Menin-KMT2A transcriptional programs, induces leukemia differentiation and decreases leukemic proliferation. Multiple MIs have progressed to clinical trials with promising early-phase results. However, a subset of leukemias that initially responded developed mutations in the *MEN1* gene that result in mutant Menin proteins with decreased affinity to MI binding and thus drug resistance. This prompts the possibility that redesigned MIs might prevent development of this type of resistance. One potential novel feature of MI re-design considerations includes targeting Menin protein stability. Menin protein degradation has been observed following treatment with several MIs though this mechanism and its clinical utility is not well understood. Here, we characterize the mechanism by which Menin protein is degraded upon MI treatment as well as characterize the clinical implications of MI treatment induced Menin protein degradation.

Using protein degradation CRISPR-based screening technology we have identified HECT ubiquitin ligase UBR5 as a regulator of Menin protein stability. Furthermore, depletion of UBR5 partially rescues the anti-proliferative effects of MI treatments through blunting transcriptional inhibition of canonical Menin/KMT2A target genes such as *MEIS1/HOXA*. UBR5 colocalizes with Menin and KMT2A proteins on chromatin and contributes to transcriptional inhibition during MI treatments.

We further characterize Menin degradation by using CRISPR base editor screens coupled to FACS in an endogenously edited *MEN1*-mScarlet human leukemia cell line to identify key Menin residues responsible for Menin degradation after MI treatment. We found and validated, using Menin overexpression systems, residues near the KMT2A interaction interface that prevented degradation and are outside of the MI binding pocket. Our data suggests that Menin degradation necessitates MI induced KMT2A displacement and that UBR5/KMT2A possibly compete for Menin association at a putative degron.

In our recent study we identified mutations that induce resistance to Menin inhibitors via base-editor screens and these mutations were also identified in AMLs from patients (Perner et al. 2023). In that screen we also observed a mutation (D136N) that is outside the Menin MI-binding pocket and conferred a selective advantage on AML cells during MI treatment; this mutation has not been observed in patients but suggests a yet uncharacterized resistance mechanism. We generated *MEN1*-D136N mutated human leukemia cell lines and observed both protein degradation rescue as well as MI resistance, albeit less

so than binding pocket mutations. Menin/KMT2A-FITC-peptide fluorescence polarization assays suggest that MI binding is not affected by D136N. This suggests an alternative resistance mechanism mediated by evasion of Menin degradation. We next applied our base editing technology in *MEN1*-M327I mutated human leukemia cell lines to ask if degradation evasive compound mutations could deepen resistance phenotypes. We observed that the D136N substitution was the top compound mutation hit conferring a selective advantage in M327I *MEN1* cells during a MI treatment course in our screen.
Finally, we compared the Menin degradation potential of several MIs currently under clinical investigation and discovered variability among MIs for induced Menin degradation suggesting this is a designable feature. Additionally, we found that Menin degradation is not tightly linked to drug binding affinities opening the potential to overcome MI drug binding mutations through degradation mechanisms. Taken together our data suggests that MI induce degradation is an essential and designable feature of MI design/efficacy. Our data also suggests that degradation evasive mutations in *MEN1* could be an alternative resistance mechanism to contend with going forward in clinical trials.

Disclosures Fischer: *Civetta Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees, Other: Founder; *Lighthorse Therapeutics:* Current equity holder in private company; *Proximity Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees, Other: Founder; *Neomorph, Inc.:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees, Other: Founder; *Avilar Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *Photys Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *Novartis:* Consultancy; *Sanofi:* Consultancy; *EcoR1 Capital:* Consultancy; *Deerfield:* Consultancy; *Deerfield:* Research Funding; *Novartis:* Research Funding; *Ajax:* Research Funding; *Interline:* Research Funding; *Astellas:* Research Funding. **Ebert:** *Exo Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *Abbvie:* Consultancy; *Calico:* Research Funding; *Neomorph Inc.:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *Skyhawk Therapeutics:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *TenSixteen Bio:* Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; *Novartis:* Research Funding. **Armstrong:** *Syndax:* Research Funding; *Neomorph Inc:* Consultancy, Current holder of *stock options* in a privately-held company; *Imago Biosciences:* Consultancy, Current holder of *stock options* in a privately-held company; *Cyteir Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company; *C4 Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company; *Nimbus Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company; *Accent Therapeutics:* Consultancy, Current holder of *stock options* in a privately-held company; *Janssen:* Research Funding; *MENIN inhibition:* Patents & Royalties: WO/2017/132398A1.

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