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POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Identification of Novel Targets Based on Splicing Alterations for Undruggable RAS/CDK Signaling Cascade in Multiple Myeloma

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Abstract Background: RAS/CDK-dependent pathways play essential roles in multiple myeloma (MM) pathogenesis. Targeting these pathways represents a novel therapeutic strategy in MM. Our ongoing studies (*>420 patients)* demonstrate that aberrantly spliced transcript expressions can predict MM patient survival outcomes better than gene expression alone, indicating a significant role of splicing mechanism in MM pathophysiology. These studies also identified intron retentions as the predominant recurrent alterations (~32% of spliced genes were retained introns) in MM. We evaluated splicing alterations associated with pathway-level responses after RAS/CDK inhibition in order to identify and validate novel molecular targets.
Methods/results: MM cells were treated with selected Erk1/2 and CDK4/6 inhibitors (Ei, Ci) to inhibit RAS and CDK pathways. Our studies demonstrated strong synergistic (IC<0.5) MM cytotoxicity triggered by this combination treatment, which triggered dose-dependent manner G0/G1 phase growth arrest. We assessed early death cascade in MM cells after Ei+Ci treatment, and demonstrated significant priming to selective peptides BIM, BAD, and MS1 or HRK, suggesting dependency on BCL2 and MCL1 or on BCL-XL proteins. Our studies showed that Ei+Ci treatment induced inhibition of key target molecules in Erk1/2 and CDK4/6 signaling including c-myc, p-RSK, p-S6, p-RB, and E2F1, suggesting on-target activity of Ei and Ci. Patient MM cells co-cultured with or without autologous BM stromal cells remain equally sensitive to Ei+Ci, suggesting that this combination can overcome the protective effects of the MM BM milieu. Moreover, our *in vivo* study demonstrated a significant *(P=0.0004)* MM burden decrease in Ei+Ci-treated mice. We evaluated the effect of Ei+Ci treatment on target gene expression in BM cells isolated from flushed femurs of treated animals with Ei, Ci or Ei+Ci, and observed downregulation of Erk1/2-CDK4/6-dependent gene signature. Therefore, we suggest that these inhibitors selectively target Erk1/2, CDK4/6 and their downstream substrates both *in vitro/vivo*.
We next evaluated aberrantly spliced transcript expression in MM cells, with/without Erk1/2 knockdown (KD) or with Ei+Ci treatment. Unsupervised clustering of deregulated genes showed dose-dependent treatment effects. This observation was

further supported by principal component analyses: upregulation in response to Erk1/2 KD and downregulation due to treatment with Ei+Ci were considered spliced gene-signatures linked to RAS/CDK modulation. Gene/pathway enrichment analyses of these genes showed their involvement in cell proliferation and regulation of epigenetic networks in MM. Importantly, these analyses suggest that overexpression of *RAVER1*/*SNRPB* core splicing regulator genes are associated with RAS/CDK pathway regulation. These genes encode subunits of U1/2/4/5 spliceosome complex and are involved in intron retention processes, a marker of malignant transformation. We compared signature-gene expressions from 558 MM patient samples to the signature-genes in plasma cells from normal donors and observed significant ($p<2e-11$) upregulation of genes with progression from MGUS to sMM, and, also to overt MM **.** *SNRPB* overexpression is associated with shorter overall patient survival ($p<0.01$), while *RAVER1* is linked with poor outcomes. SNRPB proteins are also overexpressed in MM cells. Our studies evaluating SNRPB effects on RNA splicing showed both upregulation of transcripts with full intron retention and transcripts with cryptic stop codons utilizing intronic sequences causing their partial retention. We evaluated RAVER1 and SNRPB expression in BM cells from animals treated with Ei and Ci alone or in combination. We observed significant downregulation of *RAVER1*/*SNRPB (p=0.001) in BM samples obtained from animals treated with Ei+Ci. We observed decreased intron retention events in genes in treated samples,* consistent with our *in vitro* analyses in MM cell lines and patient samples. Thus, *RAVER1*/*SNRPB* overexpression contributes to the aberrant transcriptome splicing associated with RAS/CDK cascade in MM. **Conclusions:** Our studies 1) show an association between RNA processing and RAS-CDK pathways in MM, 2) identify a core splicing protein, SNRPB/RAVER1, as a novel target for modulating this cascade, and 3) suggest that targeting spliceosome complexes represents a promising therapy in MM.

Disclosures Letai: *Zentalis Pharmaceuticals:* Other: equity holding member of the scientific advisory board; *Dialectic Therapeutics:* Other: equity holding member of the scientific advisory board; *Flash Therapeutics:* Other: equity holding member of the scientific advisory board. **Anderson:** *Bristol Myers Squibb:* Membership on an entity's Board of Directors or advisory committees; *Millenium-Takeda:* Membership on an entity's Board of Directors or advisory committees; *Gilead:* Membership on an entity's Board of Directors or advisory committees; *Janssen:* Membership on an entity's Board of Directors or advisory committees; *Celgene:* Membership on an entity's Board of Directors or advisory committees; *Sanofi-Aventis:* Membership on an entity's Board of Directors or advisory committees; *Pfizer:* Membership on an entity's Board of Directors or advisory committees; *Scientific Founder of Oncopep and C4 Therapeutics:* Current equity holder in publicly-traded company, Current holder of *individual stocks* in a privately-held company; *AstraZeneca:* Membership on an entity's Board of Directors or advisory committees; *Mana Therapeutics:* Membership on an entity's Board of Directors or advisory committees.

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