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POSTER ABSTRACTS

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

Genome-Wide CRISPR/Cas9 Knock-out Screen Identifies Apoptosis-Relevant Genes in Multiple Myeloma

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Background Bone morphogenetic proteins (BMPs) are members of the transforming growth factor (TGF)-β family and have several biological functions in different cells and tissues. In multiple myeloma, BMPs enable growth arrest and apoptosis of the cancer cells. BMPs act as ligands and signal by binding a tetrameric complex of type I and type II receptors, leading to activation of SMAD1/5/8 transcription factors which allocate into the cell nucleus. The main type I receptor in myeloma cells is ALK2, encoded by the *ACVR1* gene. Several BMP ligands can signal via ALK2, but the downstream signaling and mechanisms leading to apoptosis and growth arrest remain unknown. Mapping of this cascade could result in potential novel targets for treatment and therapies.
Methods A genome-wide CRISPR/Cas9 knock-out screen with the GeCKO v2 library was performed in INA-6 myeloma cells. To induce apoptosis and growth arrest two treatments were performed in parallel: BMP9 and a combination of Activin B and FK506 (tacrolimus), compared with medium control. Both BMP9 and Activin B signal via the type I receptor ALK2 combined with one or more of the type II receptors ACVR2A, ACVR2B or BMPR2. Top hits were defined as genes over- or underrepresented compared with control in both treatment conditions with a p-value lower than 0.05. Reactome pathway analysis of the top hits from the screen was performed. Validation of the top ranking hits was performed by generating single-gene specific knockouts, silencing with siRNA or with inhibitors. CellTiter Glo viability assay and annexin V/propidium iodide staining analyzed by flow cytometry were used to determine apoptosis. RT-PCR and western blots were used to determine specific mRNA and protein abundance.
Results The screen identified genes already known to be necessary in the BMP signaling pathway, such as *ACVR1* and *SMAD1* indicating technical robustness. Although the screen was designed to pick genes necessary for apoptosis and growth arrest, we were also able to identify genes that counteract BMP-induced apoptosis. Several novel genes/hits were found in the screen. Numerous well-established signaling cascades were identified from Reactome pathway analysis to be activated downstream of BMP mediated signaling. In particular, several genes belonging to the PI3K/AKT/mTOR cascade were significantly overrepresented in the screen, indicating they positively regulate ALK2-mediated apoptosis. The PI3K/AKT/mTOR pathway is known to be upregulated in several cancers, including multiple myeloma. Transcriptional regulators of *TP53* were found to be significantly underrepresented, indicating negative regulation in ALK2-mediated apoptosis. The protein encoded by the *TP53* gene is well known from several malignancies, including multiple myeloma. Additionally, 9 other genes with previously unknown apoptosis function were found to be relevant for its regulation.
Conclusion In conclusion, BMP-induced apoptosis in multiple myeloma cells rely on the canonical BMP signaling pathway, but also on a number of previously unknown genes. The screen confirmed the importance of the PI3K/AKT/mTOR pathway for positive apoptosis regulation and transcriptional factors affecting *TP53* for negative apoptosis regulation. Hits from this screen can be promising targets for future therapies and precision medicine targeting the apoptotic machinery in multiple myeloma cells.

Disclosures Aas: *Lybe Scientific:* Current holder of *stock options* in a privately-held company.

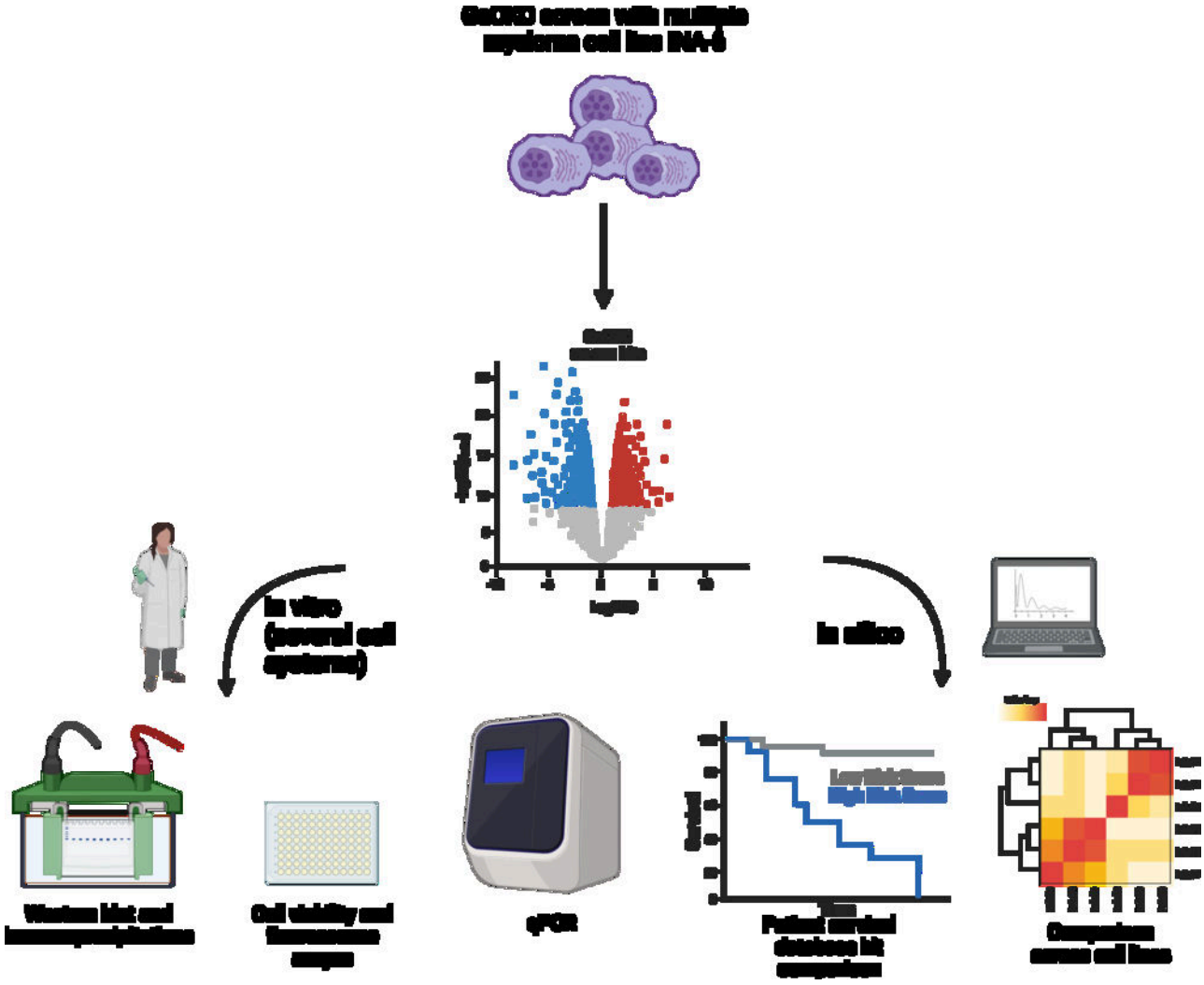

Figure 1

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