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Combined inhibition of two bad prognosis genes: Chk1 and Wee1 as a new therapeutic strategy for Multiple Myeloma

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Multiple myeloma (MM) is the second most common hematological malignancy characterized by an abnormal clonal proliferation of malignant plasma cells. Despite introduction of novels agents that have significantly improved clinical outcomes, most patients relapse and develop drug resistance. Therefore, novel therapeutic strategies to overcome chemotherapy resistance are needed in clinical settings.
MM is characterized by genomic instability and high level of replicative stress that arise during the disease's pathogenesis. In response to stress and DNA damage associated, MM cells activate various DNA damage signaling pathways that include the kinases Ataxia Telangiectasia Mutated-Related (ATR), CHK1 and WEE1. It is admitted that CHK1 is activated by ATR after DNA damage or replicative stress and promote cell cycle arrest *via* the regulation of S and G2 checkpoints. In parallel, the kinase WEE1 has a major cell cycle function on control of the G2/M transition. Interestingly, experimental studies have identified that the combination of an inhibitor of CHK1 (AZD7762) with an inhibitor of WEE1 (MK-1775) led to a therapeutic synthetic lethality in various solid and lymphoma tumors cells.
Here, we aimed to challenge the role of CHK1 and WEE1 in MM.

First we investigated the prognostic value of Chk1 and/or Wee1 gene expression in MM patients. Both Chk1 and Wee1 were significantly overexpressed in human myeloma cell lines (HMCLs) compared to BMPCs (*P<001 and P<001 respectively*). In addition, we identified Chk1 and Wee1 expression were significantly higher in the poor prognosis "proliferation" MM subgroup (*P<001 and P<001 respectively*).
Then, we showed in 4 independent cohorts of patients that a high Chk1 expression and a high Wee1 expression could predict for shorter overall survival (OS) (*P<0.001* and *P=0.007* in the HM cohort (N=206), *P<0.001* and *P=0.008* in UAMS-TT2 cohort (N=345), *P<0.001* and *P<0.001* in Hovon cohort (N=282) and *P=0.002* and *P<0.001* in TT3 cohort (N=186)). Moreover, Chk1 and Wee1 high expression were associated to bad prognosis in overall survival in a cohort of 242 patients treated by Bortezomib (Mulligan Cohort, *P<0.001* and *P=0.001 respectively*).
Interestingly, the high expression of both genes are associated to significantly shorter survival compared to expression of each gene alone in 4 independent cohorts investigated (*P=0.004* for EFS in the HM cohort, *P=0.005* for OS in UAMS-TT2 cohort, *P<0.001* for OS in Hovon

cohort, *P= 0.04* and *P=0.03* for OS and progression free survival respectively in Mulligan cohort).

According, to the association of Chk1 and Wee1 high expression with bad prognosis in MM, we tested the effect of both inhibitors AZD7762 and MK-1775 individually and in combination in MM cells.

AZD7762 induced a dose dependent inhibition of cell growth in 13 investigated HMCLs with a median IC50 of 179nM (range 69–366nM), and a correlation of HMCLs sensitivity with P53 mutation was observed.

MK-1775 induced also a dose dependent inhibition of cell growth in all investigated HMCLs (n=10) with a median of IC50 of 450nM (range 100–800nM).

Next, we performed combination treatment with the IC20 of MK-1775 in co-treatment with various doses of AZD7762 in 4 HMCLs and we showed a high synergy (combination index range 0.4-0.7) with a significant and remarkable reduction of AZD7762 IC50 (198nM to 20nM for XG6; 166nM to 25nM for AMO1; 386nM to 18nM for XG7 and 163nM to 21nM for OPM2). Investigation of the mechanisms involved showed a significant increase of apoptotic cells, a blockage of cell cycle and an increase in g-H2AX foci at 48h with the combination of both inhibitors in 3 HMCLs.

Finally, the combination of both inhibitors also significantly and specifically reduce the median number of viable primary myeloma cells of patients co-cultured with their bone marrow environment and this effect was significantly higher than each inhibitor alone (*P<0.0;* n=5). Of interest, the non-myeloma cells present in the culture were significantly less affected by the combination treatment.

Taken together, our results suggest a promising new combined targeted strategy for MM patients especially in patients characterized by high Chk1 or Wee1 expression and a poor prognosis.

Disclosures: no disclosures to declare.