Selective gamma-secretase inhibition in combination with other targeted drugs effectively targets T-cell acute lymphoblastic leukemia cells in patient-derived xenograft models.

Inge Govaerts, Cristina-Prieto Fernandez, Olga Gielen, David Nittner, Johan Maertens, Nancy Boeckx, Heidi Segers, Jan Cools

Objectives

T-cell acute lymphoblastic leukemia (T-ALL) is a high-risk subtype that comprises 10-15% of childhood and 20-25% of adult ALL cases. Over 70% of T-ALL patients harbor activating mutations in the NOTCH1 signaling pathway. NOTCH1-driven T-ALL requires proteolytic cleavage of the NOTCH1 transmembrane receptor by the γ-secretase complex (GSC) and γ-secretase inhibitors (GSI) effectively inhibit proliferation of T-ALL in vitro and in vivo. However, clinical development is hampered by the modest efficacy of GSIs as well as by dose-limiting on-target gastro-intestinal toxicity due to goblet cell hyperplasia. We recently found that selective γ-secretase inhibition can overcome this toxicity and we identified MRK-560 as such a potent selective GSI. In this study we developed combination treatment strategies with MRK-560 and other targeted inhibitors, based on the genetic background of T-ALL.

Methods

T-ALL cell lines were treated *in vitro* with MRK-560 to confirm sensitivity to NOTCH1-inhibition. Based on the mutational background, cell lines were treated with a combination of MRK-560 and JAK-inhibitor ruxolitinib for DND-41 (IL7R L243_insSRCL) or Abl1-inhibitor imatinib for ALL-SIL (NUP214-ABL1 fusion) to study superiority of combination treatment over single-drug treatment and possible synergy between compounds. Additionally, all cell lines were treated with selective inhibitor of nuclear export KPT-8602 (eltanexor) alone and in combination with MRK-560. To confirm our findings *in vivo*, NOTCH1-driven patient-derived xenograft (PDX) samples with IL7-signaling mutations or the NUP214-ABL1 fusion were injected into immunodeficient NSG mice. After leukemia engraftment, mice were treated for 14 days with vehicle, single drug or a MRK-560 based combination with ruxolitinib, imatinib or KPT-8602. Disease progression was monitored by bioluminescence imaging and levels of human CD45-positive cells in the blood. A phase II-like trial including 9 different PDX samples was performed to study survival benefit of combination treatments.

Results

All NOTCH1-signaling dependent cell lines were sensitive to MRK-560. The combination of MRK-560 with ruxolitinib and MRK-560 with imatinib in DND-41 and ALL-SIL synergistically inhibited leukemia proliferation. These results were confirmed *in vivo* by slower disease progression and a significant reduction of tissue infiltration in combination-treated mice. We observed strong synergy between MRK-560 and KPT-8602 in all NOTCH1-dependent cell lines *in vitro*. Combination treatment significantly reduced leukemic infiltration *in vivo* when compared to both single treatment groups. This antileukemic effect resulted in a significant survival benefit for mice treated with a combination of MRK-560 and KPT-8602. We did not observe weight loss or goblet cell hyperplasia in single drug or combination treated mice when compared to control.

Conclusion

The antileukemic effect of PSEN1-selective γ-secretase inhibition can be synergistically enhanced by the addition of other targeted inhibitors *in vitro* and *in vivo*. We show that mutation-based combination treatment can effectively inhibit leukemia development. Moreover, the combination of MRK-560 with KPT-8602 is a highly effective treatment combination which circumvents the need for identification of additional mutations and provides a clear survival benefit *in vivo*. To conclude, we provide promising preclinical evidence for further development of combination treatment strategies based on a backbone of PSEN1-selective γ-secretase inhibition.