Targeting of BMI-1 in mantle cell lymphoma

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Background

MCL is an incurable disease characterized by balanced t(11:14) translocation and cyclin D1 overexpression.

BMI-1 implicated in self-renewal and maintenance of tumor-initiating cancer stem cells.

BMI-1 is upregulated in MCL cells, most prominently in side population (SP) cells that have high in vivo tumorigenicity and self-renewal capability.

BMI-1 levels are increased in R/R patients.

PTC-209 and PTC596 are BMI-1 inhibitors with different modes of action. PTC-209 inhibits BMI-1 production while PTC596 accelerates BMI-1 degradation.

Methods

Reagents: The BMI-1 inhibitors PTC-209 and PTC596 from PTC Therapeutics (South Plainfield, NJ, USA) and ibrutinib from Cayman Chemical Company (Ann Arbor, MI). Cells: 8 MCL cell lines and patient samples that were analyzed under the protocol approved by the institutional review board at Saga University (2014-10-05). Samples were obtained after informed consent, according to institutional guidelines per the Declaration of Helsinki.

Apoptosis analysis: Annexin V binding assay. Protein expression: Western blotting.

Statistical analyses were performed using a two-sided Student's t-test, the non-parametric Mann-Whitney U-test, and the Pearson correlation coefficient as appropriate. A P value < 0.05 was considered statistically significant. Average values were expressed as means $\pm$ standard deviations.

Results

Basal BMI-1 levels determine the sensitivity to the BMI-1 inhibitor PTC596

PTC596 induces mitochondrial apoptosis

PTC596 decreases MCL-1 levels

PTC596-mediated decrease in MCL-1 is NOT associated with caspase activation

PTC596 eradicates side population cells

PTC596 showed additive anti-MCL effects with doxorubicin, bortezomib and cytarabine

Cell line	IC ₅₀	CI for anti-proliferative effect				CI for apoptotic effect			
		IC ₅₀	IC ₅₀	IC ₅₀	Averaged CI from IC ₅₀ , IC ₅₀ and IC ₅₀	ED ₅₀	ED ₅₀	ED ₅₀	Averaged CI from ED ₅₀ , ED ₅₀ and ED ₅₀
PTC596	Z-138	1.3	1.1	1.0	1.1	1.1	1.0	1.0	1.1
+ doxorubicin	JPM-2	0.7	0.9	1.4	1.0	1.1	1.0	1.0	1.0
MAVER-1	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PTC596	Z-138	1.2	1.1	1.1	1.1	1.2	1.2	1.1	1.2
+ bortezomib	JPM-2	0.8	0.9	1.2	1.0	1.1	1.2	1.2	1.2
MAVER-1	1.3	1.2	1.1	1.2	1.2	1.1	1.2	1.2	1.2
PTC596	Z-138	1.7	1.4	1.3	1.5	1.5	1.5	1.4	1.5
+ cytarabine	JPM-2	1.0	1.2	1.0	1.0	1.0	1.0	1.0	1.0
MAVER-1	2.2	1.3	1.0	1.5	1.5	1.6	1.5	1.4	1.5

PTC596 synergizes with ibrutinib to induce apoptosis

PTC596-induced apoptosis is enhanced by combination with ibrutinib in primary cells

Conclusion

- PTC596 induced apoptosis in MCL cells at nanomolar concentrations (= clinically relevant conc.; Cmax 3.7 μ M @ RP2D).
- High BMI-1 expression predicted high susceptibility to PTC596.
- PTC596 efficiently killed lymphoma-initiating side population cells.
- The non-chemotherapeutic combination of PTC596/ibrutinib synergistically induced apoptosis in MCL.
- There are currently no drugs that specifically target cancer stem cell fractions and targeting of BMI-1 may offer a novel therapeutic strategy for MCL.

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Conflict of Interest

Kensuke Kojima received research support as an unrestricted gift from PTC Therapeutics. Marla Weetall, Liangxian Cao and Arthur Branstrom are employees of PTC Therapeutics Inc. (South Plainfield, NJ).