Effect of IMiD Compounds on CD38 Expression on Multiple Myeloma Cells: MOR202, a Human CD38 Antibody in Combination with Pomalidomide

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Abstract

Background:
MOR202 (MOR03087), a human CD38 antibody currently under evaluation in a phase I/IIa trial, mediates antibody-dependent cell-mediated cytotoxicity/phagocytosis (ADCC/ADCP) of multiple myeloma (MM) patient-derived cells with high potency (EC50 ~200 pM). IMiD compounds such as lenalidomide (LEN) or pomalidomide (POM), both approved in MM, were evaluated *in vitro* for their ability to modulate CD38 expression and enhance the cytotoxicity of MOR202.

Method:
CD38 expression +/- LEN and POM on MM cell lines was analyzed by flow cytometry. The antitumor activity of POM combined with MOR202 was evaluated *in vitro*; analyses included the induction of direct cytotoxicity of MM cells and the activation of immune effector cells. On a functional level, the combinatorial effects of MOR202 with POM were assessed in ADCC assays. Different incubation schemes were used to separate the effect of POM on target and effector cells, as well as in the evaluation of the combined effects. The observed combination effects were analyzed for synergistic potential using the fractional product concept.

Results:
POM and LEN mediated a substantial CD38 upregulation on MM cell lines. POM as a single agent showed activation of effector cells and with high potency (EC50 ~150 nM), cytotoxic effects on MM cell lines. Additionally, POM dose-dependently induced an up to 3-fold CD38 upregulation (EC50 ~20 nM) on CD38-expressing MM cell lines. POM-mediated effects were time-dependent, with the most pronounced effects after 72 h incubation. Combining MOR202 with POM led to a synergistic enhancement of cytotoxic activity. The synergy benefit ranged between 1.2–3.1-fold above theoretical additivity, depending on the cell line used, and was most prominent in case of strong CD38 upregulation.

Conclusions:
Upregulation of CD38 was mediated by both LEN and POM and may represent a class effect of IMiD compounds. The cytotoxic activity of MOR202 on MM cells was enhanced by POM via multiple mechanisms; CD38 upregulation, activation of effector cells and direct cytotoxicity. These results provide a mechanistic rationale for the combination of MOR202 with IMiD compounds and warrant further clinical evaluation.

Modes of Action: MOR202 & IMiD Compounds

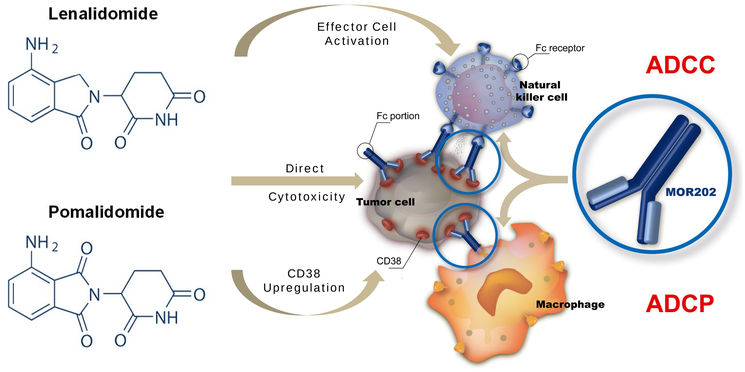

Figure 1: Relevant modes of action of MOR202 (Antibody dependent cellular cytotoxicity, antibody dependent cellular phagocytosis) and IMiD compounds (CD38 upregulation, effector cell activation, direct cytotoxicity) for *in vitro* combination therapy on CD38 positive MM cell lines

IMiD Compounds Mediate CD38 Upregulation

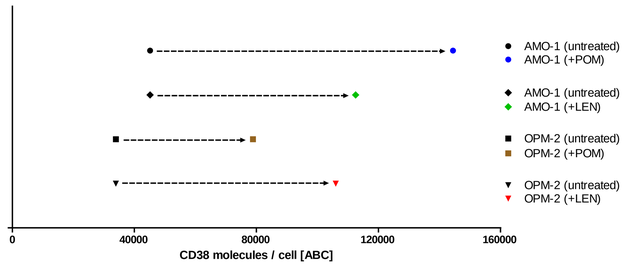

Figure 2: Potential of IMiD compounds to upregulate normal CD38 expression level on MM cells
Normal CD38 expression level on MM cell lines are substantially upregulated by pomalidomide and lenalidomide. The IMiD compounds converted these cells from CD38 low expressing cells into CD38 high expressing cells. Values represent the mean of at least three independent experiments. Antibodies bound per cell [ABC]

- IMiD compounds mediate CD38 upregulation on MM cell lines *in vitro*

Pomalidomide Shows CD38 Upregulation

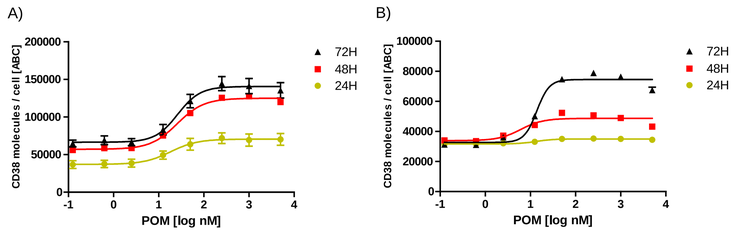

Figure 3: Time dependent dose response curves of pomalidomide mediated CD38 upregulation
Time dependent dose response curves of pomalidomide mediated CD38 upregulation on MM cell lines AMO-1 (A) and OPM-2 (B). Values represent the mean (±SEM) of three independent experiments.

- POM shows time and dose dependent CD38 upregulation on MM cell lines *in vitro*

Pomalidomide Shows Cytotoxic Effects

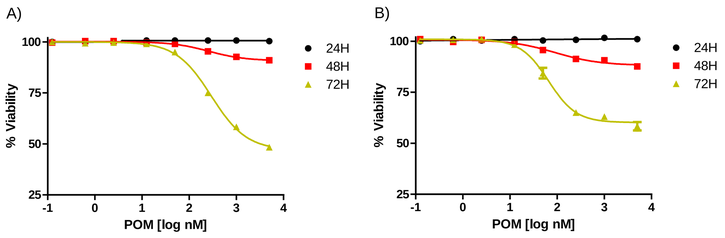

Figure 4: Time dependent dose response curves of pomalidomide mediated cytotoxic effect
Time dependent dose response curves of pomalidomide mediated cytotoxic effect on MM cell lines NCI-H929 (A) and OPM-2 (B). Values represent the mean (±SEM) of three independent experiments.

- POM shows time and dose dependent cytotoxic effects on MM cell lines *in vitro*

MOR202 & Pomalidomide Show Synergy

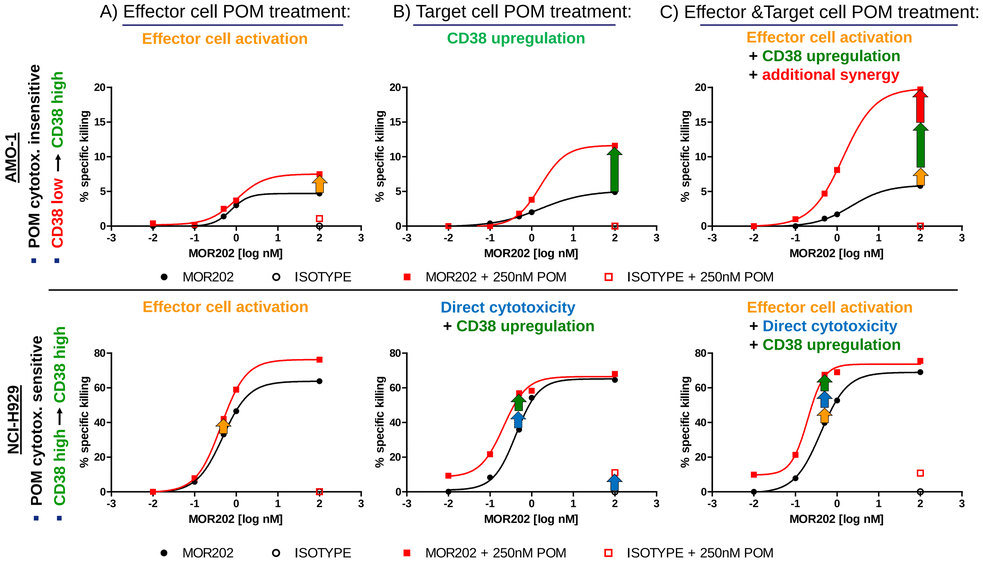

Figure 5: Influence of pomalidomide pretreated PBMC effector cells or target cells or effector and target cells on MOR202´s ADCC activity on MM cells *in vitro*.
Human peripheral blood mononuclear cells (PBMC) from healthy donors (A) or MM cell lines (B) or both (separately, C) were preincubated for 72 hours in the presence or absence of pomalidomide. Subsequently, the preincubated cells were utilized in a flow cytometry based ADCC assay in the presence of MOR202 to determine the influence of pomalidomide pretreatment on MOR202´s ADCC activity. In each individual setting the dose-response curves with and without pomalidomide preincubation are compared. As high mono therapy effects limit the therapeutic window for the combination treatment a sub-maximal MOR202 concentration close to the compounds EC_{50} was analysed for the NCI-H929 cell line. Cytotoxicity was determined by flow cytometry using PI staining. For the dose titrations, data of one representative experiment are shown including mechanistical contributions of pomalidomide effects. As indicated CD38 upregulation and effector cell activation are important factors for the enhanced efficacy of MOR202.

- Antibody dependent cellular cytotoxicity activity of MOR202 is enhanced via pomalidomide induced CD38 upregulation, effector cell activation and direct cytotoxicity on MM cells *in vitro*

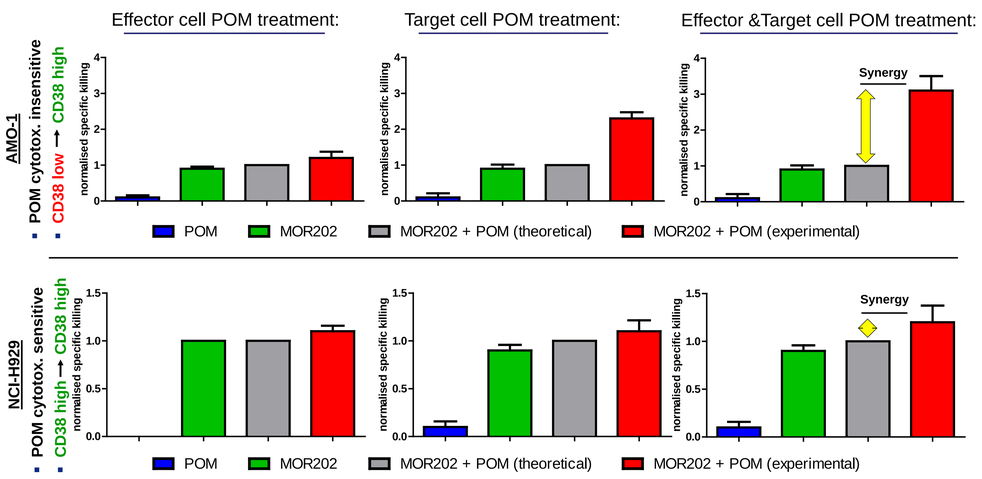

Figure 6: Effects of MOR202 and pomalidomide combination therapy on MM cells *in vitro*
Theoretical additivity and experimental results for MOR202 and pomalidomide as single agents and in combination are shown. Theoretical additivity was determined applying the fractional product concept *(Webb J.L.; 1963)*. Results were normalized for inter assay comparability. Data of three independent experiments are displayed as mean (+SEM). Experimental setup identical to Figure 5.

- In combination MOR202 and pomalidomide show synergistic effects on MM cell lysis *in vitro*

Conclusion

- CD38 upregulation on Multiple Myeloma cell lines may represent a class effect of IMiD compounds
- In combination therapy MOR202 activity on MM cell lines is significantly enhanced by pomalidomide via CD38 upregulation, effector cell activation and direct cytotoxicity