

Combination Therapy with IMGN901 and Lenalidomide Plus Low-Dose Dexamethasone is Highly Effective in Multiple Myeloma Xenograft Models

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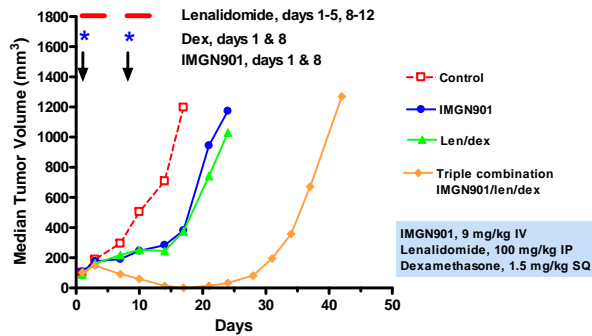


INTRODUCTION

- IMGN901 (huN901-DM1) is an antibody-maytansinoid conjugate which targets CD56. It is being evaluated as a single agent in patients with CD56-positive multiple myeloma (MM) in a Phase I clinical trial and also for the treatment of CD56-positive solid tumors.
- Lenalidomide, used in combination with dexamethasone, is approved for the treatment of MM in patients who have received at least one prior therapy. More recent studies demonstrated that lenalidomide in combination with low-dose dexamethasone was highly active in newly-diagnosed MM patients.
- The activity of IMGN901 used in combination with lenalidomide plus low-dose dexamethasone (len/dex) was examined using the MOLP-8 human CD56-positive MM xenograft model. Tumors were collected from satellite groups of animals after 48 hours of treatment to investigate mechanisms of action for the combination therapy using immunohistochemical (IHC) analysis of markers of cell proliferation, apoptosis, and angiogenesis.
- Combination activity of IMGN901 and lenalidomide/dexamethasone against MOLP-8 cells in an *in vitro* cytotoxicity assay was also evaluated using median effect analysis¹.

Triple Combination of IMGN901/Lenalidomide/Dexamethasone is Highly Active against MOLP-8 Multiple Myeloma Xenografts

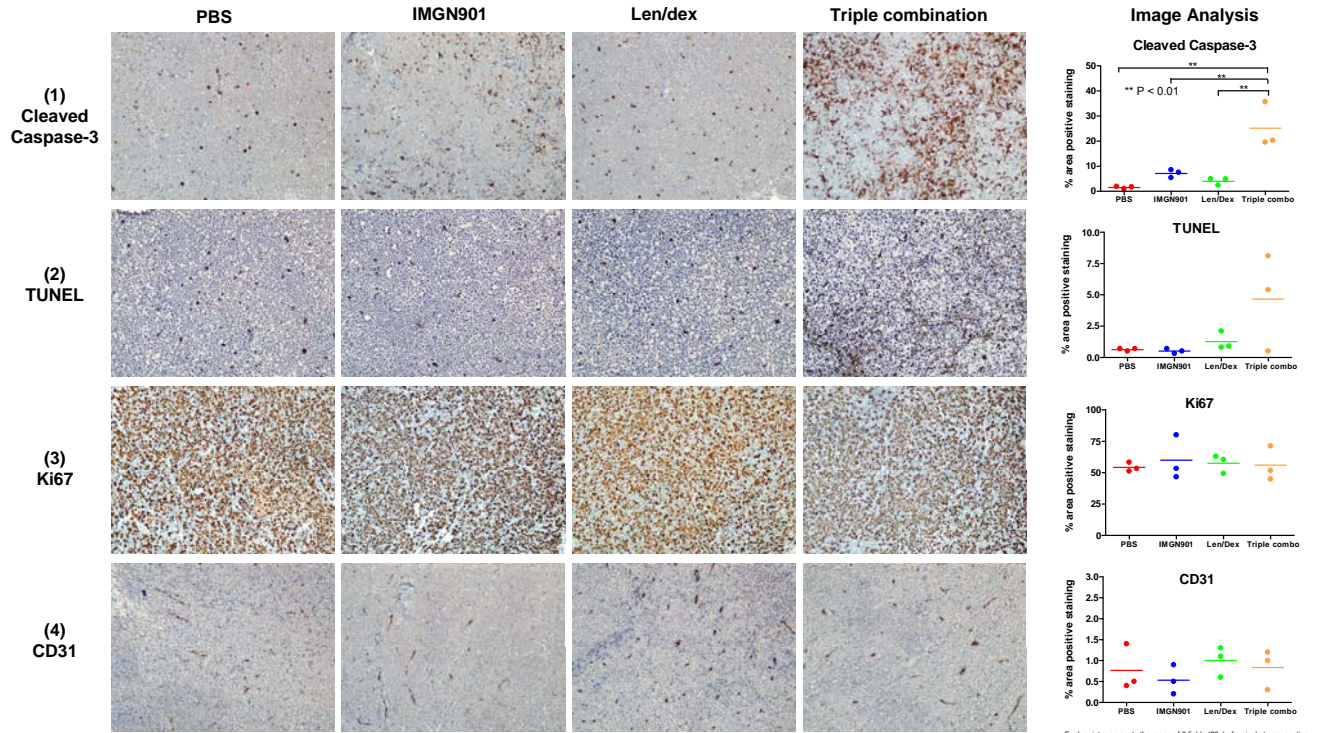
Efficacy in established xenografts (~100 mm³) in CB.17 SCID mice



Treatment	Agent	Dosage per injection	Dose schedule	T/C (%)	log cell kill	Regressions PR	CR	Conclusions
IMGN901	IMGN901	9 mg/kg	1qw x 2	33	0.5	0/6	0/6	active
len/dex	lenalidomide dexamethasone	100 mg/kg 1.5 mg/kg	qd x 5 x 2w 1qw x 2	33	0.5	0/6	0/6	active
IMGN901/len/dex	IMGN901 lenalidomide dexamethasone	9 mg/kg 100 mg/kg 1.5 mg/kg	1qw x 2 qd x 5 x 2w 1qw x 2	0	1.4	6/6	4/6	HIGHLY ACTIVE

- Female CB.17 SCID mice bearing subcutaneous MOLP-8 xenograft tumors were treated with IMGN901 (9 mg/kg, iv) once weekly for 2 weeks. Lenalidomide (100 mg/kg, ip) was administered 5 days per week for 2 weeks as a suspension in 1% carboxymethylcellulose in PBS. Dexamethasone (1.5 mg/kg, sq) was administered once weekly for 2 weeks. All treatments were started on Day 1.
- Log₁₀ cell kill (LCK), was calculated from the formula: LCK = (T - C) / T₀ x 3.32, where tumor growth delay (T - C), is the median time (in days) for treatment (T) and control (C) tumors to reach 1000 mm³. T₀ is the tumor doubling time, and 3.32 is the number of cell doublings per log of cell growth. A mouse was considered to have a partial regression (PR) when tumor volume was reduced by 50% or greater and to have a complete tumor regression (CR) when no palpable tumor could be detected.
- Tumor growth inhibition (T/C %) was calculated as the ratio of median tumor volumes at the time when control tumors reached 1000 mm³. By NCI standards, T/C ≤ 42% are considered active and T/C < 10% highly active.²
- Satellite groups (n=3) of MOLP-8 tumor-bearing SCID mice were treated in parallel, sacrificed on Day 3 (48 hours post treatment initiation) and tumors were formalin-fixed and paraffin-embedded for immunohistochemical analysis

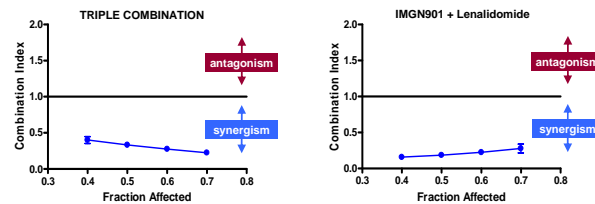
Significant Increase in Apoptosis in Tumors is Observed After 48 hours of Treatment with the IMGN901/len/dex Triple Combination Relative to Groups Treated with either the Single-Agent IMGN901 or Len/dex Combination



At 48 hours, increased apoptosis was observed in the triple combination group, with no changes in cell proliferation or endothelial cell markers.

- Tumors were stained with (1) Rabbit Anti-Caspase-3 (Cell Signaling Technology) and (2) TUNEL (APOPTAG Peroxidase in situ Apoptosis Detection Kit, CHEMICON) to assess apoptosis; (3) Horse Anti-Ki67 (Dako) to determine the tumor cell proliferative index; and (4) Goat Anti-CD31 (Santa Cruz Biotechnology), an endothelial cell marker. The percent area positive staining was determined using ImageJ image analysis software and one-way ANOVA statistical analysis was performed using GraphPad Prism software package.

Combination of IMGN901 plus Len/dex is Synergistic against MOLP-8 Multiple Myeloma Cells *in Vitro*



Addition of dexamethasone to treatment maintains strong *in vitro* synergy.

- Combinations of IMGN901 with lenalidomide only or lenalidomide/dexamethasone were analyzed by median effect analysis¹. Briefly, drugs were combined at their equipotential ratio, based on the IC₅₀ molar concentrations for each agent. Cells are marginally sensitive to dexamethasone as a single agent *in vitro*, and the dose used in the triple combination was based on the ratio used in *in vivo* studies. Cells were incubated for 5 days with various concentrations of IMGN901, lenalidomide, len/dex or their combinations.
- Surviving fraction of cells relative to control was determined using Alamar Blue (Invitrogen) cell viability assay. The Combination Index (CI) was determined using the median effect analysis software CompuSyn (ComboSyn, Inc.) for combination concentrations where 40-70% of the cells were affected (i.e., killed). A Combination Index greater than 1, equal to 1 or less than 1 indicates antagonism, additivity and synergism, respectively.

CONCLUSIONS

- Our preclinical results provide strong rationale to support the clinical evaluation of IMGN901 in combination with lenalidomide/low dose dexamethasone for the treatment of patients with CD56-positive multiple myeloma.
- The combination of IMGN901 and len/dex demonstrated greater-than-additive anti-tumor activity in subcutaneous MOLP-8 MM tumor xenografts as compared to treatment with either agent alone. All of the mice treated with the triple-agent combination experienced tumor regressions, versus none of the mice in either single-therapy group at the doses used in this study.
- The combination of IMGN901 with len/dex was strongly synergistic in an *in vitro* cytotoxicity assay as determined by median effect analysis.
- IHC analysis of treated xenograft tumors demonstrated a significant increase in apoptotic activity in the IMGN901/len/dex treated mice as early as 48 hours post treatment, well before any indications of tumor regressions were detected in the efficacy portion of the study. Extended time points post treatment will be investigated to study effects of the triple combination on cell proliferation and tumor vasculature.

REFERENCES

1. Chou, T.C. *Adv. Enzyme Regul.* 22, p.27-55, 1984
2. Bissery, M. et al. *Cancer Res.* 51, 4845-4852, Sept. 1991.