Combination Therapy with Interleukin-6 Receptor Superantagonist Sant7 and Dexamethasone Induces Antitumor Effects in a Novel SCID-hu In vivo Model of Human Multiple Myeloma

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Abstract

Interleukin-6 (IL-6) plays an important role in mediating growth, survival, and drug resistance in multiple myeloma (1–4). Multiple myeloma cells express functional IL-6 receptors (IL-6R; refs. 4, 5) and are dependent on IL-6 for growth. Their proliferation is inhibited by anti–IL-6 antibodies (4). In vivo administration of anti–IL-6 monoclonal antibodies results in cytostatic effects on tumor cells (6). Importantly, IL-6 protects against apoptotic cell death induced in multiple myeloma by a variety of agents, including dexamethasone, suggesting that inhibition of IL-6 may be of potential value for therapy of multiple myeloma (7, 8).

Glucocorticoids have been widely used in the treatment of multiple myeloma (9, 10), both as single agents and combined with conventional and novel agents. Blockade of IL-6 signaling seems to be essential for dexamethasone-induced cell death (7) because apoptotic pathways activated by dexamethasone can be abrogated by IL-6 (11, 12). Furthermore, dexamethasone only partially suppresses the paracrine production of IL-6 by bone marrow stromal cells, which in turn counteracts dexamethasone-induced cell death (13). Therefore, continued IL-6 production in the bone marrow microenvironment may account, at least in part, for clinical drug resistance. In the past, molecular variants of IL-6 have been generated (14–16), which bind to the IL-6Rα chain with high affinity and prevent binding and/or dimerization of the signaling chain gp130. The most potent of these molecules, the superantagonist Sant7, inhibits cell proliferation and is an effective proapoptotic factor for IL-6–dependent multiple myeloma cells (16). We have previously shown that Sant7 overcomes autocrine IL-6–mediated cell...
resistance to dexamethasone (17) and may also enhance the anti–multiple myeloma activity of dexamethasone and/or zoledronic acid (18) in vitro, suggesting that inhibition of the IL-6 survival pathway may represent a promising anti–multiple myeloma strategy.

In this study, we evaluated for the first time whether an IL-6R antagonism can enhance the efficiency of glucocorticoids in vivo. For this purpose, we first showed the in vitro cytotoxicity induced by the combination of Sant7 with dexamethasone against an IL-6–dependent multiple myeloma cell line, in the presence or absence of bone marrow stromal cells. Gene profiling was utilized to delineate potential molecular targets mediating antiproliferative effects and apoptosis. We then used a novel SCID-hu model of human multiple myeloma, which allows for the evaluation of in vivo effects of single agents or combination therapies against IL-6–dependent multiple myeloma cells in a human bone marrow milieu. To assess its clinical safety, we examined the effect of Sant7 and/or dexamethasone on CD34+ hematopoietic progenitor cells. Our findings show that the combination of Sant7 and dexamethasone produces significant and specific anti–multiple myeloma effects in vivo and provide the framework for derived clinical trials to improve patient outcome in multiple myeloma.

Materials and Methods

Reagents. The IL-6 receptor superantagonist Sant7 was in part kindly provided by Sigma-Tau (Pomezia, Italy) and in part produced as previously described (14). Dexamethasone (Soldsam) was obtained from American Pharmaceutical Partners, Inc. (Schaumburg, IL). IL-6, IL-3, stem cell factor (SCF), and Flt3 ligand were purchased from PeproTech EC, Ltd. (London, England, United Kingdom). Granulocyte colony-stimulating factor (G-CSF) and erythropoietin were purchased from Doppe-Biotec (Milan, Italy). Granulocyte-macrophage colony-stimulating factor (GM-CSF) was obtained from Schering-Plough (Milan, Italy). Anti-CD34 (HPCA-2) monoclonal antibody was purchased from Becton Dickinson (San Jose, CA).

Cells. The establishment, characterization, and in vitro culture of the IL-6–dependent human multiple myeloma cell line INA-6 has been previously described (19). Cells were maintained in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS (HyClone, Logan, UT), 2 mmol/l L-glutamine (Life Technologies), 100 μg/mL streptomycin (Life Technologies), and 100 units/mL penicillin (Life Technologies) in the presence of 2.5 ng/mL of IL-6 at 37°C in a 5% CO2 atmosphere. Bone marrow cells were isolated using Ficoll-Hypaque density gradient sedimentation from bone marrow aspirates obtained from multiple myeloma patients following informed consent. Bone marrow stromal cells were obtained by long-term cultures of bone marrow cells (4-8 weeks) in RPMI 1640 supplemented with 20% fetal bovine serum. Mobilized peripheral blood CD34+ hematopoietic progenitor cells were isolated from leukapheresis products of patients affected by hematopoietic and nonhematopoietic malignancies treated with high-dose chemotherapy and G-CSF or GM-CSF. Peripheral blood mononuclear cells were obtained by centrifugation through a Ficoll-Hypaque density gradient sedimentation, washed, and subjected to positive selection of CD34+ progenitors using MiniMACS (Miltenyi Biotech, Bergish Gladbach, Germany). Purity of isolated CD34+ hematopoietic progenitor cell was >85% as assessed by flow cytometry (Coulter, Birmingham, United Kingdom); viability, evaluated by propidium iodide staining of cells and trypan blue exclusion, was >90%.

Cell proliferation assay. Cell proliferation was measured by [3H]thymidine (NEN Life Science Products, Boston, MA) incorporation as previously described (20). Cells (2 × 10^4 cells/well) were incubated in triplicate in 96-well culture plates in the presence or absence of human IL-6 or confluent bone marrow stromal cells at 37°C, with or without drug for 72 hours. [3H]thymidine (0.5 μCi) was then added to each well for the last 8 hours. Cells were harvested onto glass filters with an automatic cell harvester (Cambridge Technology, Cambridge, MA) and counted using a Micro-Beta Trilux counter (Wallac, Gaithersburg, MD).

Detection of apoptosis. Dual staining with FITC-labeled Annexin V and propidium iodide was carried out to detect induction of apoptotic cell death. After treatment of 1 × 10^6 tumor cells for 48 hours, cells were washed with PBS and resuspended in 100 μL of HEPES buffer containing Annexin V-FITC and propidium iodide (Annexin V-FLILOS staining kit; Roche Diagnostic, Indianapolis, IN). Following 15-minute incubation at room temperature, cells were analyzed using a Coulter flow cytometer for the presence of an Annexin V-FITC–positive/propidium iodide–negative apoptotic cell population.

Gene expression and data analysis. INA-6 cells (2 × 10^6) were exposed to Sant7 and/or dexamethasone for 24 hours to avoid gene profiling on dying cells at later time points. Total RNA was isolated utilizing an RNeasy kit (Qiagen, Inc., Valencia, CA) and gene expression profile was evaluated using the HG-U133 array chip (Affymetrix, Santa Clara, CA) representing ~33,000 human genes. GeneChip arrays were scanned on a GeneArray Scanner (Affymetrix). Array normalization, expression value calculation, and clustering analysis were done using the dChip Analyzer (21). The Invariant Set Normalization method was used to normalize arrays at probe level to make them comparable, and the model-based method was used for probe selection and to compute expression values (21). These expression levels were assigned SE based on replicates, which were subsequently used to compute 90% confidence intervals of fold changes in intergroup comparisons. The lower confidence bounds of “fold change” were conservative estimates of the actual changes. Expression of key genes involved in proliferation/maintenance, cell cycle control, and apoptosis was analyzed.

SCID-hu INA-6 mouse model and in vivo treatments. Six- to 8-week-old male CB-17 severe combined immunodeficient (SCID) mice (Taconic, Germantown, NY) were housed and monitored in our Animal Research Facility. All experimental procedures and protocols had been approved by the Institutional Animal Care and Use Committee (VA Boston Healthcare System). Human fetal long bone grafts were s.c. implanted into SCID mice (SCID-hu), as previously described (22). Four weeks following bone implantation, 2.5 × 10^6 INA-6 multiple myeloma cells in 30 μL PBS were injected directly into the human bone implant. Because INA-6 cells release soluble human IL-6 receptor (shu-IL6R), we used this marker to monitor tumor growth in SCID-hu mice. In these mice, shu-IL6R is a sensitive indicator of tumor burden as shown by injection of fluorescent INA-6 cells (INA-6^shu^), which allows an external visualization of multiple myeloma cell growth (23). Mouse sera were serially monitored for shu-IL6R levels by ELISA (R&D Systems, Inc., Minneapolis, MN). Mice developed detectable serum shu-IL6R − 4 weeks following INA-6 cell injection and then were treated daily with Sant7 (3.3 mg/kg) and/or dexamethasone (1 mg/kg) s.c. for 6 consecutive days. Three days after the last injection, blood samples were collected and analyzed (R&D Systems). Kinetics of Sant7 was determined in SCID-hu mouse serum from peripheral blood collection using human IL-6 ELISA (R&D Systems) using recombinant Sant7 protein as standard.

Liquid culture of purified CD34+ human hematopoietic progenitor cells. Purified CD34+ hematopoietic progenitor cells were cultured at a density of 1 × 10^5 cells/well in 24-well plates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) in 1 mL of Iscove's modified Dulbecco's culture medium (Life Technologies) supplemented with 10% FCS (HyClone) and 1% deionized bovine serum albumin (Sigma, St. Louis, MO). To induce granulomonocytic or erythroid differentiation, cells were stimulated with IL-3 (50 ng/mL), GM-CSF (100 ng/mL), G-CSF (100 ng/mL) or IL-3 (50 ng/mL), GM-CSF (100 ng/mL), SCF (50 ng/mL), and erythropoietin (3 units/mL).
respectively. Cells were also cultured in the presence of IL-6 (0.2 ng/mL) with Sant7 (200 ng/mL) and/or dexamethasone (10^{-5} mol/L) to study their effect on cell cycle and differentiation. The cultures were maintained in a humidified atmosphere with 5% CO_{2} at 37°C and were harvested at day 6. Viable cells were enumerated by trypan blue exclusion.

**Clonogenic progenitor assays.** Clonogenic progenitor assays were done in methylcellulose as previously described with minor modifications (24). Briefly, 1 x 10^{4} freshly isolated CD34^{+} hematopoietic progenitor cells were seeded in Iscove’s modified Dulbecco’s culture medium (Life Technologies) containing 1% methylcellulose, 30% FCS (Hyclone), 1% bovine serum albumin (Sigma), 2 mmol/L L-glutamine (Life Technologies), and 10^{-4} mol/L 2-mercaptoethanol (StemCell Technologies, Inc., Vancouver, Canada). To induce granulomonocytic and erythroid differentiation, cells were stimulated by IL-3 (50 ng/mL), GM-CSF (100 ng/mL) or IL-3 (50 ng/mL); and GM-CSF (100 ng/mL), SCF (50 ng/mL) and erythropoietin (3 units/mL) respectively. When indicated, IL-6 (0.2 ng/mL), Sant7 (200 ng/mL), and/or dexamethasone (10^{-5} mol/L) were added to the cultures. Aliquots of 1 mL were plated in triplicate in 35-mm culture dishes (Falcon) at 37°C in a humidified atmosphere with 5% CO_{2}. Granulomonocytic colonies (granulocyte-macrophage colony-forming cells) and erythroid bursts (blast-forming unit-erythroid) were counted at 14 days by using an inverted microscope.

**Data analysis.** The statistical significance of differences between the individual and the combined treatments was analyzed using the t test; differences were considered significant when P ≤ 0.05. Synergistic effects were quantified by synergism quotient (SQ; refs. 25, 26). SQ was defined as the net effect induced by the combination (Sant7 + dexamethasone) divided by the sum of the net individual effect (Sant7) + (dexamethasone). A quotient >1 indicates a synergistic effect, whereas a quotient of <1 indicates an antagonistic effect.

**Results**

**Combination of Sant7 and dexamethasone induces synergistic anti–multiple myeloma effects in vitro.** INA-6 cells require exogenous IL-6 for in vitro growth (Fig. 1A). We first used this cell line to evaluate the effect induced by the receptor superantagonist Sant7, either alone or in combination with dexamethasone, on the in vitro growth of multiple myeloma cells. INA-6 cells were cultured in the presence of human exogenous IL-6 (2.5 ng/mL) and then cell proliferation and apoptosis were assessed after 3-day exposure to drugs. Abrogation of IL-6 signaling by a saturating concentration of Sant7 (1 μg/mL) induced growth inhibition and apoptosis (Fig. 1B and C), whereas dexamethasone (1 μmol/L) alone did not. Importantly, the combination of Sant7 (1 μg/mL) and dexamethasone (1 μmol/L) resulted in significant (P < 0.05) and synergistic antiproliferative and apoptotic effects (SQ = 1.5 and 2, respectively).

![Fig. 1. In vitro effects of Sant7 and/or dexamethasone (Dex) on the IL-6–dependent human multiple myeloma cell line INA-6 after day 3 of culture. A, cell proliferation in the presence or absence of exogenous IL-6 (1 ng/mL) as determined by [3H]-Tdr incorporation. B, growth inhibitory effect of Sant7 (1 μg/mL) and/or dexamethasone (1 μmol/L) in cell cultures in the presence of exogenous IL-6 (2.5 ng/mL); data are expressed as the percentage of control value. C, apoptosis induced in cell cultures in the presence of exogenous IL-6. Apoptotic cell death was determined by flow cytometric analysis of Annexin V and propidium iodide staining. D, growth inhibitory effect on INA-6 cells adherent to bone marrow stromal cells in the absence of exogenous IL-6. Growth inhibition effect was calculated as percentage of control value. The P value of the combination was calculated versus the effect induced by both single drug alone. \*P < 0.05. SQ > 1 indicates a synergistic effect.\]**

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Because paracrine production of IL-6 is induced in bone marrow stromal cells when multiple myeloma cells adhere to bone marrow stromal cells (27), we next evaluated the in vitro growth of INA-6 cells adherent to bone marrow stromal cells in the presence or absence of drugs. INA-6 cells were seeded on 70% to 80% confluent bone marrow stromal cells in the absence of exogenous IL-6, and cell proliferation was assessed 3 days after treatment. As shown in Fig. 1D, adherence of INA-6 cells to bone marrow stromal cells prevented growth inhibition and partially abrogated the inhibitory effect of Sant7. Cytotoxicity of dexamethasone was completely inhibited. However, the combination of these agents exerted synergistic (SQ = 1.9) and significant (P < 0.05) growth inhibition.

Gene expression profiling. We next examined whether gene transcription profile was modulated in INA-6 cells following drug treatment. Cells cultured in the presence of exogenous IL-6 (2.5 ng/mL) were exposed to Sant7 (1 μg/mL) and/or dexamethasone (1 μmol/L) for 24 hours followed by gene microarray analysis. In Fig. 2, representative changes in
proliferation/maintenance, cell cycle control, and apoptotic gene transcripts between untreated and treated cells with combination of Sant7 and dexamethasone are shown. A total of 149 genes showed altered expression pattern (>3-fold changes), including genes that are suppressed (Fig. 2A) or induced (Fig. 2B) in response to combination treatment. In particular, we observed significant down-regulation of CDC14, Rad52, Ras, IGFR, fibroblast growth factor, and vascular endothelial growth factor triggered by Sant7 and dexamethasone combination, but not by either drug alone. As shown in Figs. 2B and 3, a variety of proapoptotic gene transcripts were significantly induced in response to the combination, including myeloperoxidase, p21cip, chemokine, caspase family members, granzyme A, and tumor necrosis factor family members, compared with the effect induced by single drugs.

Sant7 and dexamethasone induce antitumor effects in a novel SCID-hu model of human multiple myeloma in vivo. To evaluate the in vivo effect of Sant7 and dexamethasone combination therapy on multiple myeloma cells in a human bone marrow milieu, we have used a novel murine model of human multiple myeloma in which INA-6 cells are directly injected and engrafted into a human fetal bone chip implanted s.c. in a SCID mouse (SCID-hu mouse). This new model allows for the growth of human IL-6-dependent multiple myeloma cells in a human bone marrow microenvironment. We first determined the pharmacokinetics of Sant7 after its s.c. injection in SCID-hu mice. As shown in Fig. 4A, after a single injection of Sant7 (3.3 mg/kg), peak serum Sant7 levels were rapidly reached after 30 minutes, with the drug remaining in the circulation for 4 hours after the injection. We, therefore, treated a cohort of 19 SCID-hu mice previously engrafted with INA-6 cells s.c. with Sant7 and/or dexamethasone for 6 consecutive days, and serum shuIL-6R levels were serially determined as a marker of tumor growth. As shown in Fig. 4B, treatment of SCID-hu mice with Sant7 (3.3 mg/kg; n = 4) or dexamethasone alone (1 mg/kg; n = 4) did not induce any significant reduction of shuIL-6R (P = 0.5 and P = 0.3, respectively) compared with control (PBS; n = 7). In contrast, despite the relatively fast clearance of the recombinant protein, the combination of Sant7 (3.3 mg/kg) and dexamethasone (1 mg/kg; n = 4) synergistically (SQ = 2.1) and significantly (P = 0.04) reduced shuIL-6R levels up to 70% versus control. At these experimental conditions, the activity of this combination was timely linked to continued drug exposure.

**Effect of Sant7 and/or dexamethasone on human hematopoietic progenitor cell.** To evaluate the safety of Sant7 and dexamethasone combination for clinical use, in particular in a posttransplantation setting, we next evaluated the effects induced by these drugs on human hematopoietic progenitor cell. CD34+ cells were purified from leukapheresis products harvested, after informed consent, from cancer patients. These CD34+ cells were treated with recombinant hematopoietins and proliferation/maintenance, cell cycle control, and apoptotic gene transcripts between untreated and treated cells with combination of Sant7 and dexamethasone are shown. A total of 149 genes showed altered expression pattern (>3-fold changes), including genes that are suppressed (Fig. 2A) or induced (Fig. 2B) in response to combination treatment. In particular, we observed significant down-regulation of CDC14, Rad52, Ras, IGFR, fibroblast growth factor, and vascular endothelial growth factor triggered by Sant7 and dexamethasone combination, but not by either drug alone. As shown in Figs. 2B and 3, a variety of proapoptotic gene transcripts were significantly induced in response to the combination, including myeloperoxidase, p21cip, chemokine, caspase family members, granzyme A, and tumor necrosis factor family members, compared with the effect induced by single drugs.

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then exposed to Sant7, dexamethasone, or the combination, followed by clonogenic assays and flow cytometric analysis. The results of colony assays (Table 1) indicate that Sant7 does not inhibit generation of granulocyte-macrophage colony-forming cell and blast-forming unit-erythroid in response to hemopoietins. In contrast, dexamethasone resulted in a decrease in the number of both types of colonies. Importantly, Sant7 did not potentiate the inhibitory effect of dexamethasone.

Flow cytometric analysis of the DNA content was done on liquid cultures of CD34+ cells stimulated for 6 days with hemopoietins (IL-3 + G-CSF + GM-CSF + IL-6 or IL-3 + GM-CSF + erythropoietin + IL-6) plus Sant7, dexamethasone, or the combination (Fig. 5). Although Sant7 did not affect cell proliferation, the addition of dexamethasone reduced by 20% the number of cells in the S phase. The combination of Sant7 and dexamethasone showed an effect similar to dexamethasone alone. No significant apoptosis was detected.

Discussion

This study provides evidence that in vivo treatment with the combination of the IL-6 superantagonist Sant7 and dexamethasone induces anti–multiple myeloma effects in a novel SCID-hu murine model of human multiple myeloma. To our knowledge, this is the first report of an in vivo synergistic effect between an IL-6–neutralizing agent and a glucocorticoid with potential important implications for the treatment of human multiple myeloma.

At present, dexamethasone remains one of the most active drugs in the treatment of multiple myeloma (9, 10). Prior studies have shown that its therapeutic efficacy may be significantly counteracted by paracrine IL-6 secretion in the bone marrow microenvironment, which is highly enhanced by the adhesion of multiple myeloma cells to bone marrow stromal cells (7, 27–29). Therefore, using dexamethasone in combination with agents capable of neutralizing the IL-6 may improve its efficacy. In the past, a variety of biological agents have been used for this purpose (6, 30–33). We have previously reported that treatment of IL-6–autocrine multiple myeloma cells with Sant7 may overcome in vitro IL-6–mediated cell resistance to dexamethasone (17), suggesting a combined approach to enhance anti–multiple myeloma activity.

In the present report, we show that Sant7 treatment of a human IL-6–dependent multiple myeloma cell line, cultured alone or in the presence of bone marrow stromal cells, enhances the antiproliferative effects of dexamethasone. These data confirm that Sant7 may overcome, at least in part, the combination between an IL-6–neutralizing agent and a glucocorticoid with potential important implications for the treatment of human multiple myeloma.

Table 1. Clonogenic assays of purified CD34+ hemopoietic progenitor cells were done in semisolid culture medium

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>CFC-GM</th>
<th>BFU-E</th>
<th>Total</th>
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<tbody>
<tr>
<td>IL3 + GM + G</td>
<td>90 ± 6</td>
<td>90 ± 6</td>
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</tr>
<tr>
<td>+ IL6</td>
<td>76 ± 1</td>
<td>76 ± 1</td>
<td></td>
</tr>
<tr>
<td>+ IL6 + Sant7</td>
<td>73 ± 2</td>
<td>73 ± 2</td>
<td></td>
</tr>
<tr>
<td>+ IL6 + Dex</td>
<td>54 ± 5</td>
<td>54 ± 5</td>
<td></td>
</tr>
<tr>
<td>+ IL6 + Sant7 + Dex</td>
<td>55 ± 1</td>
<td>55 ± 1</td>
<td></td>
</tr>
<tr>
<td>IL3 + GM + SCF + Epo</td>
<td>21 ± 3</td>
<td>75 ± 2</td>
<td>96 ± 2</td>
</tr>
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<td>+ IL6</td>
<td>30 ± 2</td>
<td>45 ± 1</td>
<td>75 ± 1</td>
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<td>+ IL6 + Sant7</td>
<td>21 ± 2</td>
<td>50 ± 2</td>
<td>71 ± 1</td>
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<td>+ IL6 + Dex</td>
<td>11 ± 2</td>
<td>29 ± 3</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>+ IL6 + Sant7 + Dex</td>
<td>13 ± 2</td>
<td>37 ± 1</td>
<td>50 ± 1</td>
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NOTE: Cells (1 × 10⁶/plate) were seeded in the presence of hemopoietins to induce granulomonocytic (IL-3 + GM-CSF + G-CSF) or erythroid (IL-3 + GM-CSF + SCF + erythropoietin) differentiation. The cytokine concentrations used were as follows: IL-3, 50 ng/mL; GM-CSF, 100 ng/mL; G-CSF, 100 ng/mL; SCF, 50 ng/mL; and erythropoietin, 3 units/mL. Cultures were scored on day 14. The data represent the mean ± SD of triplicates from a representative experiment.

Abbreviations: G, granulocyte; GM, granulocyte-macrophage; Dex, dexamethasone; Epo, erythropoietin; CFC-GM, granulocyte-macrophage colony-forming cell; BFU-E, blast-forming unit-erythroid.
protective effect of paracrine IL-6 against dexamethasone. Most important, we used a novel murine model of human multiple myeloma to evaluate the in vivo activity of Sant7 or dexamethasone alone or in combination. In this model, IL-6–dependent multiple myeloma cells were directly injected into a human fetal bone chip previously implanted into SCID mice, providing a biologically relevant in vivo system for evaluating the impact of novel drugs targeting multiple myeloma cells in their bone marrow milieu. Using this model, we observed that in vivo blockade of IL-6R by Sant7 alone did not induce significant antitumor activity. This observation is consistent with previous studies showing that IL-6/gp130/STAT3 pathway is not essential for survival of human multiple myeloma cells grown in the presence of bone marrow stromal cells in vitro, suggesting that other factors in the human bone marrow microenvironment may substitute for a requirement of IL-6 (34). Nevertheless, Sant7 might be of potential therapeutic benefit, because blocking of IL-6R overcomes bone marrow stromal cell–mediated drug resistance of multiple myeloma cells in vitro (35). Furthermore, in our in vivo model, we failed to show activity of relatively low doses of dexamethasone because adherence of multiple myeloma cells to bone marrow stromal cells induced IL-6 and related drug resistance. In fact, we showed human IL-6 in the serum of mice injected with INA-6 cells, especially in aspirates from implanted bone marrow (data not shown). Importantly, in our study, blocking IL-6R in vivo with Sant7 in combination with dexamethasone resulted in synergistic anti–multiple myeloma activity, suggesting, therefore, a potential clinical utility of this combination. An important issue is the route of Sant7 administration: Although s.c. injections of Sant7 significantly prolonged half-life compared with i.v. injections (data not shown), this route of administration may limit clinical application. This limitation may be overcome either by continued infusion or alternative approaches for controlled release of Sant7 to maintain saturating Sant7 concentrations during dexamethasone treatment. Interestingly, we already showed in vitro and ex vivo IL-6 inhibitory activity by an IL-6 superantagonist (Sant1) expressed after gene transfer mediated by a first-generation adenovirus containing its cDNA (36). More recently, we showed that a new adenoviral vector, known as helper-dependent adenoviral vector, induces in immunocompetent mice high level and long-lasting expression of erythropoietin, a cytokine sharing the same structural features of IL-6 (37) with negligible toxicity (38, 39). Therefore, construction of an helper-dependent adenoviral vector driving the expression of the IL-6–derived superantagonist Sant7 may be a suitable option to maintain saturating Sant7 concentrations during dexamethasone treatment.

These preclinical studies have important implications for the therapy of multiple myeloma. Specifically, the combination of an IL-6R antagonist and glucocorticoid could represent an effective approach to overcome multiple myeloma drug resistance and enhance activity of conventional anti–multiple myeloma treatments. Glucocorticoids are in fact usually included in chemotherapeutic regimens containing both novel biological as well as conventional drugs; therefore, addition of Sant7 might significantly potentiate the therapeutic activity of these drugs. Here, we also showed that Sant7, alone or in combination with dexamethasone, did not significantly affect CD34+ hematopoietic progenitor cell growth and survival, further supporting its clinical promise. An additional novel and important finding is that the combination of Sant7 and dexamethasone triggered changes in the gene expression profile in multiple myeloma cells. A variety of cell proliferation/maintenance and cell cycle control genes were significantly down-regulated, whereas apoptotic genes were up-regulated, including p21cip, myeloperoxidase, caspase family members, and granzyme A. p21 is tightly regulated by p53, which induces apoptosis by down-regulation of Bcl2 and up-regulation of Bax. Interestingly, we found that

![Cell cycle analysis of hematopoietic progenitor cell exposed to Sant7 and/or dexamethasone. Flow cytometric profile of a representative experiment in which hematopoietic progenitor cell cycle was analyzed by propidium iodide staining. Analysis was carried out using the CellQuest program (Becton Dickinson). Treatments and percentages of cells in S-phase (S) are indicated.](www.aacrjournals.org/clinicanimage)
up-regulation of Bcl-2L1, which has been associated to a prosurvival effect (40), was induced by dexamethasone or Sant7 alone but not by the combined treatment. Myeloperoxidase mediates H$_2$O$_2$-induced apoptosis in many cell types, including lymphocytes (41) and cancer cells, triggered by chemotherapeutic treatments (42). Also of interest is the induction of caspase family members, including caspases 4, 9, and 10, suggesting a caspase-dependent apoptosis triggered by the combination as well as up-regulation of granulys A, which, in contrast, induces caspase-independent apoptosis (43).

Taken together, these transcript modulations induced by combined Sant7 and dexamethasone treatment are consistent with induction of a death program by several distinct pathways.

Therefore, our findings suggest that Sant7 and dexamethasone can be combined to enhance multiple myeloma cell cytotoxicity without adverse effects on hematopoietic progenitor cells. Combined treatment with Sant7 and glucocorticoids is, therefore, a promising approach to overcome drug resistance and improve patient outcome in multiple myeloma.

References

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