CD45^{neg} but Not CD45^{pos} Human Myeloma Cells Are Sensitive to the Inhibition of IGF-1 Signaling by a Murine Anti-IGF-1R Monoclonal Antibody, mAVE1642

Géraldine Descamps, Soraya Wuillème-Toumi, Valérie Trichet, Corinna Venot, Laurent Debussche, Thierry Hercend, Madeleine Collette, Nelly Robillard, Régis Bataille and Martine Amiot

*J Immunol* 2006; 177:4218-4223; doi: 10.4049/jimmunol.177.6.4218

http://www.jimmunol.org/content/177/6/4218

---

**References**  This article cites 24 articles, 13 of which you can access for free at: http://www.jimmunol.org/content/177/6/4218.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
CD45\textsuperscript{neg} but Not CD45\textsuperscript{pos} Human Myeloma Cells Are Sensitive to the Inhibition of IGF-1 Signaling by a Murine Anti-IGF-1R Monoclonal Antibody, mAVE1642\textsuperscript{1}

Géraldine Descamps,\textsuperscript{2*} Soraya Wuillème-Toumi,\textsuperscript{2*} Valérie Trichet,\textsuperscript{‡} Corinne Venot,\textsuperscript{§} Laurent Debussche,\textsuperscript{§} Thierry Hercend,\textsuperscript{§} Madeleine Collette,* Nelly Robillard,† Régis Bataille,* and Martine Amiot\textsuperscript{3∗}

Insulin-like growth factor 1 (IGF-1) is a well-known growth factor for myeloma cells. Thus, therapeutic strategies targeting IGF-1R have been proposed for multiple myeloma treatment. In this study, we investigated the effect of the antagonistic anti-IGF-1R murine AVE1642 Ab (mAVE1642). We show that mAVE1642 selectively inhibits IGF-1R but not insulin signaling in human myeloma cell lines. Since we have previously shown the functional relevance of CD45 expression in the growth of myeloma cells and the association of CD45-negative (CD45\textsuperscript{neg}) status with a less favorable clinical outcome, both CD45-positive (CD45\textsuperscript{pos}) and CD45\textsuperscript{neg} myeloma cell lines were selected for our study. We found that mAVE1642 strongly inhibits the growth of CD45\textsuperscript{neg} myeloma cell lines, leading to a G\textsubscript{1} growth arrest, whereas it has almost no effect on the growth of CD45\textsuperscript{pos} myeloma cell lines. Furthermore, mAVE1642 binding induced a significant reduction of IGF-1R expression. We next demonstrated that the overexpression of IGF-1R in the CD45\textsuperscript{neg} myeloma cell line increased Akt phosphorylation but was not sufficient to sensitize these cells to mAVE1642. In contrast, we generated a stable CD45-silencing XG-1 cell line and showed that it became sensitive to mAVE1642. Thus, for the first time, we provided direct evidence that the expression of CD45 renders cells resistant to mAVE1642. Taken together, these results support that therapy directed against IGF-1R can be beneficial in treating CD45\textsuperscript{neg} patients. The Journal of Immunology, 2006, 177: 4218 – 4223.

\textsuperscript{1} Institut National de la Santé et de la Recherche Médicale, Unité 601, Université de Nantes, Unité de Formation et de Recherche Médicine et Techniques Médicales, LNC Label; \textsuperscript{2} Laboratoire d’Hématologie; \textsuperscript{3} EA 3822 Institut National de la Santé et de la Recherche Médicale ER17, Nantes, Cedex, France; and \textsuperscript{4} Sanofi-Aventis, Oncology Therapeutic Department, Vitry sur Seine, France

Received for publication March 15, 2006. Accepted for publication July 4, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{*} This work was supported by the Ligue Nationale Contre le Cancer.

\textsuperscript{†} G.D. and S.W.-T. contributed equally to this work.

\textsuperscript{‡} Address correspondence and reprint requests to Dr. Martine Amiot, Institut National de la Santé et de la Recherche Médicale, Unité 601, Département de Recherche en Cancérologie, 9, quai Moncoussu 44093 Nantes, Cedex 01, France. E-mail address: mamiot@nantes.inserm.fr

\textsuperscript{§} Abbreviations used in this paper: MM, multiple myeloma; HMCL, human myeloma cell line; IGF-1, insulin-like growth factor 1; IRS, insulin receptor substrate; IR, insulin receptor; shRNA, short hairpin RNA; mAVE1642, murine AVE1642.

Copyright © 2006 by The American Association of Immunologists, Inc.

0022-1767/06/$02.00

Materials and Methods

\textbf{mAbs and reagents}

The anti-IGF-1R mAb EM164 (murine IgG1), the murine version of the mAb AVE1642 (named mAVE1642) was provided by Sanofi-Aventis (17). Anti-actin was purchased from Chemicon International. Anti-IGF-1R\textsubscript{β} Ab was obtained from Santa Cruz Biotechnology Anti-phospho-Akt (Ser\textsuperscript{473}) and anti-phospho-p44/42 MAPK Abs were purchased from Cell Signaling. Human recombinant IGF-1 and anti-phospho-IRS-1(Tyr\textsuperscript{612}) Ab were purchased from Sigma-Aldrich.
**Human myeloma cell lines (HMCL) and culture conditions**

The XG-1, XG-2, XG-6, and BCN HMCL have been previously established in our laboratory and were cultured in the presence of 3 ng/ml IL-6 (Novartis). LP-1, L.363, and NCI-H929 HMCL were purchased from DSMZ. U266 was purchased from American Type Culture Collection. JIM-3 was provided by Prof. L. Bergsagel (Cornell University, New York, NY). Cell lines were maintained in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, antibiotics, and 5 × 10⁻⁵ M 2-ME.

**Immunofluorescence analysis**

Cells (0.5 × 10⁷) were sampled in each condition and washed with PBS before incubation with either anti-CD45-FITC, CD90-PC-5 (Beckman Coulter), or anti-IGF-1R-PE (BD, Biosciences) for 20 min. After two washes, cells were fixed in 1% formaldehyde. Flow cytometry analysis was performed on a FACSCalibur using the CellQuest program (BD Biosciences). The ratio of fluorescence was determined by dividing the mean fluorescence intensity by the mean fluorescence intensity of the respective control.

**Transfection**

Plasmid encoding IGF-1R (pcVN 7346) was provided by Dr. Baserga (Thomas Jefferson University, Philadelphia, PA). Five micrograms of plasmid DNA was mixed with 5 × 10⁶ 293FT cells in suspension in buffer T transferred to a 2-mm electroporation cuvette and electroporated using the electrical protocol A3 of the Amaxa Nucleofector apparatus (Amaxa). After selection in geneticin, cells were labeled with anti-IGF-1R-mAb and bright positive cells were selected by FACS.

**Lentiviral construction for RNA interference**

Two lentiviral vectors, pLenti-CD90-sh-CD45 and pLenti-CD90-sh-luci, were generated to produce short hairpin RNA (shRNA) directed against human CD45 (GenBank accession number NM002838) or firefly luciferase mRNA, respectively. Briefly, they were derived from the pLentSV5-GW/LacZ plasmid (Invitrogen Life Technologies), whose LacZ and Blasticidin genes have been replaced by a marker gene coding for CD90 membrane protein. In addition, they contain a genomic cassette composed of H1 promoter plus sequence for shRNA synthesis. CD45 shRNA were designed to target the following CD45 gene sequence: GAGTACACCTCTACTCATT.

**Virus production and cell transduction**

Viral productions were performed following the guidelines of the Viral Power Lentiviral Expression System (Invitrogen Life Technologies). Briefly, 3 × 10⁶ 293FT cells were transfected using the calcium phosphate method with 9 μg of the optimized packaging mix and 3 μg of pLenti-CD90-shRNA. Virus containing supernatants were harvested 48 h post-transfection. For titration, serial dilutions of viral production were tested on CD90-shRNA. Virus-containing supernatants were harvested 48 h post-infection. We usually obtained titers ranging from 5 × 10⁶ to 5 × 10⁷ viral units/ml.

To generate stably modified XG-1 cell lines, 10⁶ XG-1 cells were plated in 200 μl of medium within a well of a 24-well plate and infected at a multiplicity of 8 with either Lenti-CD90-sh-CD45 or Lenti-CD90-sh-luci viral units. Ten days after infection, 20% of transduced XG-1 cells were identified by their overexpression of CD90 and sorted out using FACS analysis. By this way, we obtained a control population, named shLuci-XG-1, and another one, named shCD45neg-XG-1. Both express CD90 and were generated to produce short hairpin RNA (shRNA) directed against CD45 mRNA, respectively.

**Cell viability and detection of apoptotic cells**

Cell viability was determined by vital dye (0.4% eosin) exclusion and assessed by visual inspection in a hemocytometer. Cell death was assessed by Annexin V(FITC) (Beckman Coulter) staining according to the manufacturer’s recommendation. Flow cytometry analysis was performed on a FACSCalibur using the CellQuest software (BD Biosciences).

**DNA synthesis**

Myeloma cells (10⁶ cells/well) were cultured in triplicate in 96-well plates in the presence or absence of mAVE1642 for 72 h. Cells were pulsed with 1 μCi of [³H]thymidine during the last 8 h of culture, harvested onto glass filters with an automatic cell harvester (PerkinElmer), and the uptake of [³H]thymidine was monitored using a 1450 Microbeta Jet beta counter (PerkinElmer).

**Immunoblot analysis**

Cells (4 × 10⁶) were resuspended in lysis buffer (10 mM Tris-HCl (pH 7.6), 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, 2 mM Na₃VO₄, 1 mM NaF, 2 μM aprotinin, 1 μM leupeptin, and 0.5% Nonidet P-40#). After 40 min on ice, lysates were cleared by centrifugation at 12,000 × g for 30 min at 4°C. Protein concentration was measured using bicinchoninic acid (BCA protein assay; Pierce). One hundred micrograms of proteins was loaded for each lane. The proteins were separated by 10% SDS-PAGE and then electrotransferred to polyvinylidene fluoride membranes. Western blot analysis was performed by standard techniques with ECL detection (Roche).

**Cell cycle distribution**

For cell cycle analysis, cells were fixed in 70% cold ethanol for 30 min at 4°C, washed twice in PBS, and stained with propidium iodide (20 μg/ml) for 10 min at room temperature. Flow cytometry analysis was performed on a FACSCalibur using CellQuest Pro software (BD Biosciences). Data were gated on the FL2-area and FL2-Width cytogram to exclude doublets and aggregates, and a minimum of 2 × 10⁶ gated cells was collected per sample. Analysis of the cell cycle was performed using the ModFit LT.

**Statistical analysis**

For statistical analysis, we used the nonparametric Wilcoxon rank sum test or the Student t test.

**Results**

mAVE1642 selectively inhibits IGF-1R but not insulin signaling in LP-1 HMCL

To confirm the selectivity of mAVE1642 for IGF-1R and not for IR, we performed Western blotting analysis of cell lysates prepared from LP-1 cells treated with either IGF-1 or insulin in the presence or the absence of mAVE1642 preincubation. We demonstrated that the level of phosphorylated IRS-1 due to IGF1 was significantly reduced by mAVE1642. Those of phosphorylated Akt and ERK1/2 were totally inhibited by mAVE1642, whereas none of them were affected in response to insulin (Fig. 1). These results demonstrated that mAVE1642 selectively inhibits IGF-1R signaling but not insulin signaling in LP-1 HMCL.

mAVE1642 significantly inhibits the growth of CD45neg HMCL but has only a weak inhibitory effect on CD45pos HMCL

We have previously demonstrated that the growth of CD45neg LP-1 HMCL was highly dependent on the PI3K pathway. Thus, we used this HMCL to determine the effect of concentrations of mAVE1642 ranging from 1.2 to 12.2 μg/ml on its growth.

**FIGURE 1.** Specific inhibition by mAVE1642 of IGF-1 but not insulin signaling in LP-1 HMCL.

Viral productions were performed following the guidelines of the Viral Power Lentiviral Expression System (Invitrogen Life Technologies). Briefly, 3 × 10⁶ 293FT cells were transfected using the calcium phosphate method with 9 μg of the optimized packaging mix and 3 μg of pLenti-CD90-shRNA. Virus-containing supernatants were harvested 48 h post-transfection. For titration, serial dilutions of viral production were tested on CD90-shRNA. Virus containing supernatants were harvested 48 h post-infection. We usually obtained titers ranging from 5 × 10⁶ to 5 × 10⁷ viral units/ml.

To generate stably modified XG-1 cell lines, 10⁶ XG-1 cells were plated in 200 μl of medium within a well of a 24-well plate and infected at a multiplicity of 8 with either Lenti-CD90-sh-CD45 or Lenti-CD90-sh-luci viral units. Ten days after infection, 20% of transduced XG-1 cells were identified by their overexpression of CD90 and sorted out using FACS analysis. By this way, we obtained a control population, named shLuci-XG-1, and another one, named shCD45neg-XG-1. Both express CD90 and shRNA against either luciferase or CD45 mRNA, respectively.

**Cell viability and detection of apoptotic cells**

Cell viability was determined by vital dye (0.4% eosin) exclusion and assessed by visual inspection in a hemocytometer. Cell death was assessed by Annexin V(FITC) (Beckman Coulter) staining according to the manufacturer’s recommendation. Flow cytometry analysis was performed on a FACSCalibur using the CellQuest software (BD Biosciences).

**DNA synthesis**

Myeloma cells (10⁶ cells/well) were cultured in triplicate in 96-well plates in the presence or absence of mAVE1642 for 72 h. Cells were pulsed with 1 μCi of [³H]thymidine during the last 8 h of culture, harvested onto glass filters with an automatic cell harvester (PerkinElmer), and the uptake of [³H]thymidine was monitored using a 1450 Microbeta Jet beta counter (PerkinElmer).**
concentrations induced a maximum plateau inhibition and the concentration of 6 μg/ml was chosen for all of the following experiments (Fig. 2A). By cell counting over 4 days in standard culture conditions, mAVE1642 was shown to induce a significant growth inhibition of the CD45neg HMCL of 92 and 75% for LP-1 and NCI-H929, respectively (Fig. 2B). In contrast, mAVE1642 induced a weak growth inhibition of CD45pos HMCL XG-1 and XG-6 of 16 and 24%, respectively. In another experiment, the antiproliferative effect of mAVE1642 on a panel of four CD45pos HMCL and five CD45neg HMCL was measured by thymidine incorporation at 3 days. Results presented in Fig. 2C showed that the growth inhibition induced by mAVE1642 is significantly different between CD45pos and CD45neg HMCL (p < 0.05). All HMCLs expressed significant levels of IGF-1R and there was no correlation between IGF-1R expression and the CD45 phenotype of HMCL (Table I). We noted that both the highest IGF-1R-expressing cell lines LP-1 and JIM-3 are within the CD45neg subgroup and that both are the most mAVE1642-sensitive cell lines for proliferation inhibition.

mAVE1642 induces G1 growth arrest in LP-1 CD45neg HMCL but not in XG-1 CD45pos HMCL

The effect of mAVE1642 on the cell cycle phase distribution was investigated in both CD45pos HMCL (LP-1) and CD45neg HMCL (XG-1). After 40 h of mAVE1642 treatment, the cell cycle was unchanged in XG-1 but impaired in LP-1 (Fig. 3A). Indeed, in LP-1, the S phase was reduced (40% vs 13%; p < 0.001), whereas the G1 phase was significantly increased (55% vs 81%; p < 0.001). Of note, no significant hypodiploid DNA peak was observed. Finally, we demonstrated that mAVE1642 did not induce any apoptosis as shown by annexin staining analysis (Fig 3B). Altogether these results indicated that mAVE1642 induces a significant growth arrest in only the CD45neg HMCL.

mAVE1642 down-regulates the IGF-1R expression on LP-1 CD45neg HMCL

To evaluate whether treatment with mAVE1642 affected the expression of IGF-1R, we assessed the level of IGF-1R by flow

Table I. CD45 and IGF-1R expressions on myeloma cell lines

<table>
<thead>
<tr>
<th></th>
<th>LP-1</th>
<th>NCI-H929</th>
<th>L363</th>
<th>BCN</th>
<th>JIM-3</th>
<th>U266</th>
<th>XG-1</th>
<th>XG-2</th>
<th>XG-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean fluorescence ratio CD45</td>
<td>1</td>
<td>1.6</td>
<td>1</td>
<td>1.7</td>
<td>1</td>
<td>4.6</td>
<td>19.6</td>
<td>5</td>
<td>109</td>
</tr>
<tr>
<td>Mean fluorescence ratio IGF-1R</td>
<td>11</td>
<td>3.2</td>
<td>3.8</td>
<td>1.8</td>
<td>21</td>
<td>3.8</td>
<td>3.3</td>
<td>2.7</td>
<td>4</td>
</tr>
</tbody>
</table>

a Myeloma cells were stained with isotype-matched control mAb or with anti-IGF-1R PE or with CD45 FITC. Results are shown as mean fluorescence ratio.
cytometry after 1- to 3-day treatment of mAVE1642 on LP-1 HMCL. As shown in Fig. 4A, exposure to mAVE1642 induced a significant reduction of the mean fluorescence intensity ratio of IGF-1R from 13 to 7. This decrease of the IGF-1R expression was also confirmed by Western blot analysis, an important down-regulation of IGF-1R expression was observed after 24 h of exposure to mAVE1642 (Fig. 4B).

Overexpression of IGF-1R in XG-6 CD45<sup>pos</sup> HMCL is not sufficient to sensitize it to mAVE1642

Based on the observation that LP-1 and JIM-3, which are the highest IGF-1R-expressing cell lines, appeared to be the most mAVE1642-sensitive cell lines, we attempted to determine whether ectopic overexpression of IGF-1R could alter sensitivity to mAVE1642. XG-6 HMCL was stably transfected with the cDNA of IGF-1R. Overexpression of IGF-1R was assessed by flow cytometry. The mean fluorescence ratio of IGF-1R intensity was of 30 for XG-6-IGF-1R compared with 3 for XG-6-WT (Fig. 5A). We next demonstrated that the phosphorylation of Akt remained activated for a longer time after IGF-1 stimulation in XG-6-IGF-1R compared with the one observed in XG-6-WT (Fig. 5B). However, mAVE1642 had no effect on the growth of either XG-6-WT or XG-6-IGF-1R, indicating that high levels of IGF-1R were not able to sensitize these cells to mAVE1642 (Fig. 5C). We have also observed that addition of IGF-1 similarly increased DNA synthesis in XG-6-IGF-1R compared with XG-6-WT (Fig. 5D).

FIGURE 4. Kinetics of down-regulation of IGF-1R expression on LP-1 cells. A, LP-1 cells were treated with (filled histogram) or not treated (open histogram) with 6 μg/ml mAVE1642 for 3 days. Then cells were stained with anti-IGF-1R PE. B) LP-1 cells were treated with 6 μg/ml mAVE1642 for 6 and 24 h. Equivalent amounts of cell lysates were separated by SDS-PAGE, then immunoblotted with anti-IGF-1Rβ. Protein loading was controlled with an anti-actin.

FIGURE 3. A, mAVE1642 induced a G<sub>1</sub> growth arrest in LP-1. Myeloma cells (2 × 10<sup>5</sup> cells/ml) were cultured in RPMI 1640 with 5% FCS in the presence or absence of mAVE1642 (6 μg/ml) for 40 h. Three nanograms of IL-6 per milliliter is added for XG-1 HMCL. Then cells were labeled with propidium iodide and cell cycle was analyzed by flow cytometry. B, mAVE1642 did not induce any apoptosis in HMCL. LP-1 and XG-1 cells were treated with different concentrations of mAVE1642 for 48 h. Then cells were labeled with Annexin V<sup>FITC</sup> and the percentage of dead cells was analyzed by flow cytometry.
synthesis of both XG-6-WT and XG-6-IGF-1R serum-starved cells (data not shown).

Stable CD45-silencing XG-1 HMCL are sensitive to mAVE1642

To evaluate the role of CD45 down-regulation in the sensitivity of myeloma cells to mAVE1642, we made use of RNA interference technology. We infected XG-1 cells with lentivirus for the delivery of the shRNA sequence designed for CD45. shLuci-XG-1 cells were generated to evaluate the potential effects caused by small interfering RNA and distinguished them from specific effects due to silencing CD45. Fig. 6A illustrated that the shCD45 neg XG-1 cells are characterized by a strongly attenuated expression of surface CD45, corresponding to about 10-fold reduction of the mean fluorescence intensity ratio compared with wild-type or shLuci-XG-1 cell populations. mAVE1642 inhibits the growth of shCD45neg XG-1 by 56%, whereas it has almost no effect on the growth of shLuci-XG-1 (Fig. 6B). A similar inhibitory effect was observed using the PI3K inhibitor wortmannin (59% of inhibition), indicating that the growth of shCD45neg XG-1 cells is dependent on the PI3K pathway (data not shown). Consistent with this observation, we found that the Akt phosphorylation in response to IGF-1 is stronger in shCD45neg XG-1 than in shLuci-XG-1 (Fig. 6C).

Discussion

In this study, we used the antagonist mAb mAVE1642, which binds to the IGF-1R, to assess its effect on the proliferation and survival of HMCL. The specificity of this Ab and its high affinity for the IGF-1R has been already described (17). We demonstrated that treatment with mAVE1642 inhibits the IGF-1-activated signaling in these cells, resulting in a reduction of the phosphorylation levels of IRS-1 and a total inhibition of the activated downstream substrates (Akt, Erk). Importantly, mAVE1642 did not block the IR-activated signaling in these myeloma cells. We have shown that mAVE1642 induced a dramatic growth inhibition of CD45neg HMCL, whereas it induced a weaker or no effect on CD45pos HMCL. Since mAVE1642 treatment triggers a G1 growth arrest, the inhibition of CD45neg HMCL growth induced by mAVE1642 can be mainly attributed to a cytostatic effect. This cytostatic effect is due to both the blockade of IGF-1 binding to IGF-1R and to the
down-regulation of IGF-1R induced after mAVE1642 exposure. The down-regulation of IGF-1R induced by treatment with different Abs against IGF-1R has been already reported and seems to occur by an internalization and degradation of the IGF-1R (17, 18). Thus, the ability of mAVE1642 to trigger receptor surface down-regulation can contribute to its effectiveness to inhibit long-term growth of CD45neg HMCL. We found that treatment by mAVE1642 does not induce apoptosis in myeloma cells, indicating that the apoptotic effect described on HMCL by small tyrosine kinase inhibitor molecules as AEW541 or cyclolignan (14, 15) are probably due to some off-target effect.

The fact that the inhibitory effect of mAVE1642 is restricted to CD45neg HMCL is in agreement with our previous study showing that myeloma cell proliferation due to the PI3K pathway was clearly associated with the CD45neg phenotype. We also addressed the role of IGF-1R expression level by overexpressing it in a CD45pos HMCL. In this cell line, which is dependent on IL-6 for its growth, high levels of IGF-1R are not sufficient to induce sensitivity to mAVE1642. Even though the PI3K/Akt pathway can be strongly activated in response to IGF-1, this pathway does not seem to be responsible for the growth of this HMCL in serum, indicating that IGF-1R expression level has not significantly modified the balance between tyrosine kinase and CD45 phosphatase. By overexpressing CD45, it was shown that CD45 could inhibit the signaling and cellular response to the IGF-1 and platelet-derived growth factor (19, 20). To explore the function of CD45 phosphatase, we generated a stable CD45neg XG-1 HMCL using shCD45RNA. This CD45-silencing cell line became equally sensitive to both mAVE1642 and wortmannin, indicating that the PI3K/Akt pathway is now responsible for the growth of this cell line. Consistent with the role of CD45 in IL-6 signaling (11), the CD45neg XG-1 HMCL does no longer proliferate in response to IL-6 (data not shown). Taken together, these results provided direct evidence that CD45 is a negative modulator of IGF-1 signaling and positive regulator of IL-6 signaling.

Therapeutic strategies using mAb have emerged as very potent clinical therapeutics (21, 22). The best example is provided by the use of herceptin a humanized mAb against the epidermal growth factor receptor 2, which is a tyrosine kinase receptor critical in some cancers. Herceptin has been shown to be effective for the treatment of patients with breast cancer (23, 24). Our results support the concept that the humanized anti-IGF1R mAb mAVE1642 may be beneficial in treating the subgroup of MM lacking CD45, which is the most aggressive one with a poor clinical outcome (10). Finally, given the very specific effect of AVE1642 on IGF-1R expression and signaling without any off-target effect, we provided the rational for using it safely in combination regimens.

Acknowledgments

We thank Dr. R. Baserga and Dr. R. Breathnach for the gift of the cDNA of the IGF-1R and the lentiviral vector (pLenti-CD90), respectively.

Disclosures

The authors have no financial conflict of interest.

References