Lack of Methylthioadenosine Phosphorylase Expression in Mantle Cell Lymphoma Is Associated with Shorter Survival: Implications for a Potential Targeted Therapy

Silvia Marce,1 Olga Balagué,1 Luis Colomo,1 Antonio Martinez,1 Sylvia Höller,3 Neus Villamor,1 Francesc Bosch,2 German Ott,2 Andreas Rosenwald,3 Lorenzo Leoni,4 Manel Esteller,5 Mario F. Fraga,5 Emili Montserrat,2 Dolors Colomer,1 and Elias Campo1

Abstract

Purpose: To determine the methylthioadenosine phosphorylase (MTAP) gene alterations in mantle cell lymphoma (MCL) and to investigate whether the targeted inactivation of the alternative de novo AMP synthesis pathway may be a useful therapeutic strategy in tumors with inactivation of this enzyme.

Experimental Design: MTAP gene deletion and protein expression were studied in 64 and 52 primary MCL, respectively, and the results were correlated with clinical behavior. Five MCL cell lines were analyzed for MTAP expression and for the in vitro sensitivity to L-alanosine, an inhibitor of adenylosuccinate synthetase, and hence de novo AMP synthesis.

Results: No protein expression was detected in 8 of 52 (15%) tumors and one cell line (Granta 519). Six of these MTAP negative tumors and Granta 519 cell line had a codeletion of MTAP and p16 genes; one case showed a deletion of MTAP, but not p16, and one tumor had no deletions in neither of these genes. Patients with MTAP deletions had a significant shorter overall survival (mean, 16.1 months) than patients with wild-type MTAP (mean, 63.6 months; P < 0.0001). L-Alanosine induced cytotoxicity and activation of the intrinsic mitochondrial-dependent apoptotic pathway in MCL cells. 9-β-D-Erythrofuranosyladenine, an analogue of 5′-methylthioadenosine, selectively rescued MTAP-positive cells from L-alanosine toxicity.

Conclusions: MTAP gene deletion and lack of protein expression are associated with poor prognosis in MCL and might identify patients who might benefit from treatment with de novo AMP synthesis pathway – targeted therapies.
lost in different human tumors usually due to gene deletions associated with the coincident loss of the INK4a-ARF locus (12, 13, 15). Malignant cells lacking MTAP, and consequently having an impaired AMP and methionine salvage pathway, are completely dependent on de novo AMP synthesis and exogenous methionine supply and thus are expected to be more sensitive to chemotherapies with antimetabolites blocking this pathway, such as l-alanosine, an amino acid analogue obtained from Streptomyces alanosinus that blocks de novo AMP synthesis from IMP via the inhibition of the adenylosuccinate synthetase activity (16, 17). Because MTAP-deficient cells cannot salvage adenosine, and l-alanosine interferes with the de novo AMP synthesis, this compound is an ideal candidate therapy for MTAP-deleted tumors (18, 19).

The aims of this study were to investigate whether the frequent losses of the INK4a-ARF locus in MCL also implicate MTAP gene deletions and a corresponding lack of protein expression and if tumors with these alterations could be candidates for therapeutic strategies based on the inhibition of the de novo AMP synthesis pathway.

Materials and Methods

Patients. Frozen tumor samples from 64 MCL patients diagnosed between 1989 and 2002 in the Department of Pathology from Hospital Clinic, Barcelona and the Institute of Pathology in Wurzburg, Germany were studied. The samples corresponded to 48 typical MCL tumors and 16 blastic variant samples. Fresh tissue was obtained at the moment of biopsy, embedded in OCT Tissue Tek (Sakura, Finetek Europe, Zoeterwoude, NL), frozen at −40°C in 2-methyl-butanol, and stored at −80°C. An informed consent was obtained from each patient of the both institutions according to their Ethical Committees.

Cell lines. Cell lines carrying the t(11;14)(q13;q32) translocation were studied: Granta 519, REC-1, NCEB-1, JeKo-1, and JVM-2. The genetic and molecular characteristics of these cell lines have previously been described (20–22).

JVM-2, REC-1, and NCEB-1 cell lines (0.5 × 10⁶ cells/mL) were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mmol/L glutamine, 50 μg/mL penicillin/streptomycin (Life Technologies, Inc., Paisley, United Kingdom), and 100 μg/mL normocin (Anaxa Biosystems, Inc., Köln, Germany) at 37°C in a humidified atmosphere containing 5% carbon dioxide. JeKo-1 cell line was incubated at the same conditions but supplemented with 20% FCS and Granta 519 was cultured at 0.5 × 10⁶ cells/mL in DMEM culture medium. Cell cultures were periodically tested for mycoplasma and all experiments were conducted in mycoplasma-free cells. Cells were incubated in the absence (CT) or presence of l-alanosine (20-100 μmol/L) and the 5′-methylthioadenosine analogue 9-β-D-erythrofurano-syladenine (EFA; 100 μmol/L) and Salmedix, Inc., San Diego, CA).

p16 and MTAP deletion analysis. Genomic DNA was obtained using proteinase K/RNase treatment and phenol/chloroform extraction. To study deletions of the p16 and MTAP locus, real-time quantitative PCR was done under universal real-time standard conditions with the ABI Prism 7900 (Applied Biosystems, Foster City, CA) in a total reaction volume of 25 μL using 25 ng of genomic DNA. Primers and probes used for MTAP and p16 analysis have previously been published (23, 24). At least three replicates were done for each sample. Albumin and β-actin were used as control genes. The calculated copy numbers of MTAP and p16 were normalized by the copy number of each control gene, and the relative values were obtained using the ΔΔCT method (User Bulletin #2, Applied Biosystems). Three control DNA samples obtained from peripheral blood lymphocytes of healthy donors were used to establish the cutoff ratio for p16 and MTAP deletions. The normalized ratio of MTAP and p16 genes to control gene is expected to be close to 1 if no deletions were present and close to 0 for homozygous deletions. Considering the potential for contamination of MCL tumor samples with normal cells, values <0.4 were judged to be deleted in MTAP or p16 genes.

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissue was available in 52 of the MCL cases. In the rest of our cases, other fixation methods were used (8-5, Bouin) that were not suitable for a proper immunostaining. MTAP immunostaining was done using a mouse monoclonal antibody (clone 6.9.5) and a staining technique developed by Salmedix (United Kingdom). Three control DNA sections were deparaffinized by routine techniques. Antigen retrieval was done with heat-induced epitope retrieval procedure, incubating the tissue sections in BORG buffer (pressure cooker) at 120°C for 3 minutes, followed by trypsin incubation for 1 minute at room temperature. Slides were washed thrice in PBS in PBS and incubated with Labeled Polymer from the EnVision Plus detection Kit (DAKO) for 30 minutes at room temperature. The slides were then incubated with 20 μg/mL of the primary antibody or the appropriate negative reagent control for 30 minutes at room temperature. The slides were washed thrice in PBS and incubated with the Cell Titer-Glo Luminescent Cell Viability Assay (Promega Corporation, Madison, WI), which indicates the presence of metabolizable ATP. Detection was done with the EnVision Plus detection Kit (DAKO) using diaminobenzidine tetrahydrochloride solution (Dako) for 5 minutes. Tissue sections were thoroughly washed with tap water and counterstained with Harris hematoxylin solution.

The proliferative activity of tumors was determined in 58 MCL cases by the immunohistochemical detection of Ki67 using the MIB monoclonal antibody (Immunotech, Marseille, France) at 1:400 dilution. Antigen retrieval was done with 10% EDTA solution (pH 9) in a pressure cooker. Detection was done with the EnVision Plus detection Kit (DAKO) using diaminobenzidine tetrahydrochloride as chromogen (25).

Analysis of MTAP promoter-associated CpG island methylation status. We established the MTAP gene CpG island methylation status by PCR analysis of bisulfite-modified genomic DNA using two procedures. First, methylation status was analyzed by bisulfite genomic sequencing of multiple clones as previously described (26). The second analysis used methylation-specific PCR (27) using primers specific for either the methylated or modified unmethylated DNA. Primer sequences of MTAP for the unmethylated reaction were 5′-GAAGGA-TAATTTTGGTTTTGTTGTT-3′ (sense) and 5′-AACATCTCAAAATTCTCACAA-3′ (antisense), and for the methylated reaction, 5′-ATATAATTCCGTTTTGCTCCG-3′ (sense) and 5′-GACACCTCCCC-AATTCTCCGCG-3′ (antisense). The annealing temperature for both unmethylated and methylated reactions was 58°C. DNA from normal lymphocytes was used as a positive control for unmethylated alleles and DNA from normal lymphocytes treated in vitro with 5-aza 2′-deoxycytidine as a positive control for methylated alleles. PCR products were loaded onto nondenaturing 2% agarose gels, stained with ethidium bromide, and visualized under UV light.

Protein extraction and Western blot analysis. Total protein extracts of the five MCL cell lines were obtained as previously described (28). One hundred micrograms of protein were separated on SDS-polyacrylamide gels and transferred to Immobilon membranes (Millipore, Bedford, MA). Western blot was done using the monoclonal MTAP antibody (clone 6.9.5) at 1:1,000 dilution. Antibody binding was detected using a chemiluminescence detection system (Amersham, Buckinghamshire, United Kingdom) and the Image Gauge Reader Software (Las3000, Fujifilm, Tokyo, Japan). Equal amounts of protein were confirmed using α-tubulin as a control protein.

Quantitation of intracellular ATP levels. ATP levels were measured with the Cell Titer-Glo Luminescence Cell Viability Assay (Promega Corporation, Madison, WI), which indicates the presence of metabolically active cells. Cells were incubated in a final volume of 100 μL of culture medium. After 24 hours, the same volume of Cell Titer-Glo Reagent was added to each test well. The mixture was incubated for 10 minutes and the luminescence was analyzed using a luminometer.
Detection of apoptotic cells. Membrane translocation of phosphatidylserine residues was quantified by surface Annexin V binding as previously described (28). Cytotoxicity was measured as the percentage of Annexin V− and propidium iodide−positive cells. Changes in mitochondrial transmembrane potential (ΔΨm) were evaluated by staining with 1 nmol/L 3,3′, dihexyloxocarbocyanine iodide [DiOC6(3); Molecular Probes, Eugene, OR] and reactive oxygen species production was determined by staining with 2 μmol/L dihydroethidine (Molecular Probes) as previously described (28). Briefly, cells were incubated with dyes for 30 minutes at 37°C, washed, resuspended in PBS, and analyzed by flow cytometry. A total of 10,000 cells per sample were acquired in a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Experiments were done in triplicate.

For the detection of intracellular proteins by flow cytometry, cells were fixed in 80% ethanol for 5 minutes at 4°C, centrifuged, and washed twice in PBS. Cells were incubated for 20 minutes at room temperature, followed by goat anti-rabbit-FITC (SuperTechs, Bethesda, MD) or goat anti-mouse-FITC (DAKO, Glostrup, Denmark), and analyzed in a FACScan. Western blot analysis for poly(ADP-ribose) polymerase (Roche Diagnostics, Mannheim, Germany), caspase-3 (BD PharMingen) was done as previously described (21).

Cell cycle analysis. Cells were fixed in 80% ethanol for 5 minutes at 4°C, centrifuged, and washed twice in PBS. Cells were incubated for 15 minutes at room temperature in a citrate-phosphate buffer (1:24), centrifuged, resuspended in 0.25 mL propidium iodide (5 mol/L; Sigma Chemicals), and RNase A (100 μg/mL; Sigma Chemicals), and incubated for 10 minutes in the dark. The percentage of cells in G0-G1, S, and G2-M and the presence of a sub-G0 peak were evaluated with ModFit LT software (Verity Software House, Inc., Topsham, MA) as previously described (21).

Results

MTAP and p16 gene deletions. Exon 8 of the MTAP gene and exon 2 of the p16 gene were amplified by quantitative PCR to detect gene deletions using genomic DNA from five cell lines carrying the translocation t(11;14)(q13;q32). Homozygous deletion of the MTAP gene was detected only in Granta 519 cells. In the other MCL cell lines, no changes were seen compared with normal lymphocytes. Granta 519 and REC-1 cell lines showed a homozygous p16 deletion and JeKo-1 displayed a heterozygous p16 deletion (Table 1).

To determine whether MTAP was also deleted in primary MCL, we analyzed by quantitative PCR the DNA from 64 tumors. MTAP homozygous deletions were detected in 9 (14%) tumors, 5 (11%) of them typical and 4 (25%) of blastoid variant MCL. p16 homozygous deletions were observed in 8 (13%) of these cases, 5 of them typical and 3 blastoid variants. These results are summarized in Table 2.

MTAP protein expression. MCL cell lines were next screened for MTAP protein expression by Western blot. Concordant with results from the genetic study, a band of ~30 kDa was detected in denaturing conditions, corresponding to a subunit of the trimeric 90-kDa MTAP holoenzyme in REC-1, JVM-2, NCEB-1, and JeKo-1 cell lines, whereas no MTAP expression was observed in Granta 519 cell line (Fig. 1A). These results were confirmed by immunohistochemistry. Cytoplasmic staining was detected in wild-type MTAP MCL cell lines whereas lack of MTAP labeling was observed in Granta 519 cells (Table 1). Figure 1B showed a representative immunostaining pattern in wild-type MCL cell lines (JVM-2) and MTAP-deleted cells (Granta 519).

MTAP protein expression was examined immunohistochemically in 52 primary MCL tumors. Biopsies were considered MTAP positive when a cytoplasmic staining was observed in tumor cells and MTAP negative when tumor cells showed no immunoreactivity whereas normal cells (histiocytes and endothelial cells) showed immunostaining, hence serving as an internal positive control. Nuclear positivity of tumor cells was detected in some of the cases (Fig. 1C). Cytoplasmic MTAP

<table>
<thead>
<tr>
<th>MCL variant</th>
<th>MTAP protein expression (n = 52)</th>
<th>MTAP gene (n = 64)</th>
<th>p16 gene (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>Wt</td>
<td>Del</td>
</tr>
<tr>
<td>Typical</td>
<td>34 of 38 (89%)</td>
<td>43 of 48 (89%)</td>
<td>43 of 48 (89%)</td>
</tr>
<tr>
<td>Blastoild</td>
<td>10 of 14 (71%)</td>
<td>12 of 16 (75%)</td>
<td>13 of 16 (81%)</td>
</tr>
<tr>
<td>Total</td>
<td>44 of 52</td>
<td>55 of 64</td>
<td>56 of 64</td>
</tr>
</tbody>
</table>

Abbreviations: wt, wild-type; del, deleted; IHC, immunohistochemistry.
staining was detected in 44 (85%) cases, 34 (89%) being typical and 10 (71%) blastoid variant MCL. Complete loss of MTAP immunolabeling was seen in 8 (15%) tumors, 4 (11%) of them typical, and 4 (29%) blastoid variants (Table 2). Six of these eight tumors lacking MTAP protein expression had a codeletion of the MTAP and p16 genes, one case showed a deletion of MTAP but not p16, and one tumor had no deletions of either genes (Table 2). We confirmed that the case with nondeleted p16 showed two p16 signals by fluorescence in situ hybridization. Furthermore, p16 mRNA expression levels analyzed by quantitative PCR were similar to other cases with wild-type p16 and MTAP genes (data not shown). The methylation analysis of MTAP promoter of the case with no expression of the protein and apparent nondeleted genes revealed a wild-type configuration with no evidence of hypermethylation (data not shown).

Correlation between MTAP gene alterations, proliferation, and survival. Survival information was available in 41 cases (32 typical and 9 blastoid variant MCL). The median survival...
in this series was 41 months (range, 1-136 months), being 20 months in blastoid and 61 months in typical MCL variants. MTAP gene deletions were detected in five of these patients (two blastoid and three typical variants). Patients with MTAP gene deletion had a significant shorter overall survival (mean, 16.1 months) than patients with wild-type MTAP tumors (mean, 63.6 months; Fig. 2A). Similarly, lack of MTAP protein expression had a significant predictive value for shorter survival, with a mean of 20 months for patients with loss of MTAP expression and 63.2 months for cases with normal MTAP expression (Fig. 2B).

Because proliferation index is the main prognostic factor in MCL, we compared the proliferation index of these tumors with MTAP gene deletion and protein expression. The proliferative index that best predicts survival of the patients in this series was a Ki67 >35%. We found a statistically significant correlation between the proliferation index and MTAP gene deletions. Eight of nine (89%) cases with MTAP gene deletion were included in the group of high proliferation index (P < 0.005). Similarly, all cases with lack of MTAP protein expression by immunohistochemistry were included in the high Ki67 proliferation index group (P < 0.005). Four of the five cases with codeletion of MTAP and p16 genes and typical histology were included in the high proliferation index group, with overall survival ranging from 7.7 to 28 months and a mean of 18.4 months (P < 0.005). In a multivariate analysis, including the proliferative index and MTAP or p16 deletions, only proliferative index kept independent significative value as survival predictor.

1-Alanosine induced cytotoxicity in MCL cells. As MTAP is necessary for AMP synthesis, we analyzed whether cells from MCL with lack of MTAP expression may be sensitive to the inhibition of de novo pathway. For this purpose, we incubated MTAP wild-type (REC-1 and JVM-2) and MTAP-deleted (Granta 519) MCL cell lines with t-alanosine, a selective inhibitor of this pathway.

After incubation with several doses of t-alanosine (20-100 μmol/L), a decrease in intracellular ATP levels was detected. This effect was dose and time dependent. Figure 3A and B showed the decrease of ATP levels after t-alanosine incubation for 24 hours in two representative MCL cell lines, one with a MTAP deletion (Granta 519) and one with a wild-type MTAP (JVM-2). To verify the specificity of the observed intracellular ATP depletion, we used a “rescued strategy” by preincubating the selected cell lines with EFA, a MTAP substrate, before the exposure to t-alanosine. It has recently been described that EFA at the same concentrations as t-alanosine rescued MTAP-deleted Granta 519 (MTAP-deleted) than in JVM-2 (MTAP-expressing) cells with wild-type MTAP (JVM-2) were preincubated with EFA 100 μmol/L. In contrast, EFA did not rescue MTAP-deleted cells (Granta 519) from t-alanosine-induced cell cycle arrest (Fig. 3C).

1-Alanosine induces activation of the mitochondrial apoptotic pathway. After incubation with 100 μmol/L t-alanosine for 48 hours, the decrease of the ATP levels was associated with membrane translocation of phosphatidylserine residues and activation of mitochondrial apoptotic pathway, characterized by a loss of mitochondrial transmembrane potential (∆Ψm), reactive oxygen species production, conformational changes of Bax and Bak, and activation of caspase-3 (Fig. 4A). Furthermore, a decrease of procaspases 9, 8, and 3 and proteolysis of poly(ADP-ribose) polymerase were also observed by Western blot (Fig. 4B). These typical characteristics of activation of the mitochondrial apoptotic pathway were more pronounced in Granta 519 (MTAP-deleted) than in JVM-2 (MTAP-expressing) cell lines after 48 hours of treatment with 100 μmol/L t-alanosine.
cells. All these changes were reversed by preincubation of MTAP-positive cell lines with the 5'-methylthioadenosine analogue EFA 100 μmol/L, but not in the case of Granta 519 cell line (Fig. 4A and B).

Discussion

MCL is an aggressive B-cell neoplasm with a very poor prognosis that frequently develops resistance to current chemotherapy regimens (3, 4). For this reason, novel therapeutic strategies taking advantage of the biological alterations of the tumor might be useful to improve the outcome of the patients. In this sense, the recent introduction of proteasome inhibitors as antineoplastic agents has provided evidence of their potent in vitro effect against MCL, offering a promising approach in the treatment of patients with refractory MCL (22, 30, 31). Rapid disease progression and poor response to therapy particularly occur in patients with blastoid variants of MCL, high number of chromosomal alterations, or high proliferative index (2). One of the most common genetic alterations in this subset of aggressive tumors is the deletion of 9p21 targeting the INK4a-ARF locus encoding for the cyclin-dependent kinase-4 inhibitor p16INK4a and the murine double minute-2 regulator p14ARF (10). Interestingly, 100 kb telomeric to these genes is the MTAP locus encoding for the salvage of adenine and methionine synthesis. Cells lacking MTAP rely exclusively on the de novo pathway for the purine synthesis of these elements and might therefore be candidates to treatments based on the inhibition of the purine salvage pathway (36, 37).

In this study, we have shown that MTAP gene deletion is a relatively frequent phenomenon in MCL occurring in 14% of the cases, in 11% of cases with typical morphology, and in 25% of the blastoid variants. Our study also shows a high correlation between deletion of p16 and MTAP genes, with just one case harboring a deletion of MTAP and wild-type p16. This case showed a lack of MTAP protein expression by immunohistochemistry confirming the deletion of the MTAP gene. Although most deletions in this chromosomal region target the INK4a-ARF locus (38–40), these results indicate that 9p21 deletions may also involve other genes in this region (41).

The immunohistochemical analysis of MTAP protein expression showed a good concordance with the status of the gene. Only one case had a discordant negative expression for the protein whereas the genomic analysis showed no deletion of the gene. This suggests that other mechanisms may be involved in silencing MTAP gene expression. In this regard, although hypermethylation of the MTAP promoter region has recently been described (39, 42, 43), we could not detect MTAP hypermethylation in our MCL cases. The close correlation between MTAP gene deletion and lack of protein expression indicates that this is the main mechanism for inactivation of MTAP in these lymphomas. This observation is concordant

Fig. 3. Cytotoxicity of l-alanosine in MCL. Intracellular ATP levels were determined in Granta 519 (A) and JVM-2 (B) after 24 hours of incubation without (CT) or with different doses of l-alanosine (l-Ala; 20-100 μmol/L). Preincubation of these cells with EFA, at the same doses as l-alanosine, preserved ATP levels in the MTAP-positive cell line (JVM-2). C, analysis of DNA content. Cells from Granta 519 and JVM-2 were incubated for 48 hours with medium alone (CT), l-alanosine (100 μmol/L), or l-alanosine + EFA (100 μmol/L). DNA content was quantified as described in Materials and Methods.
with previous findings indicating that the INK4a-ARF locus is commonly inactivated by homozygous deletions in MCL whereas hypermethylation, although present in other lymphomas, is uncommon in MCL (44, 45).

**MTAP deletions and loss of protein expression in this series of MCL were significantly associated with higher proliferation indices for these tumors and shorter survival of the patients. This phenomenon is most probably due to the close correlation between the deletion of MTAP gene and INK4a-ARF locus in these tumors, and suggests that the immunohistochemical detection of MTAP may be a good surrogate marker of the inactivation of the whole locus. Interestingly, some recent studies have indicated that MTAP by itself may also act as a tumor suppressor gene and its inactivation may contribute to the progression of the tumors. Thus, reintroduction of MTAP in a breast cancer cell line in which the gene was deleted abolished the anchorage-independent cell growth and inhibited the tumorigenesis of the cell lines (46). Similarly, a forced expression of MTAP induced a strong reduction in the invasive potential in melanoma cell lines (32). In addition, inactivation of MTAP has also been involved in an indirect inhibition of the STAT1 pathway (47).

The inactivation of MTAP gene in MCL cells with high proliferative index and clinical aggressive behavior provides a tumor-specific biochemical feature that could be targeted using inhibitors of the de novo AMP synthesis pathway, such as 1-alanosine.

Several clinical trials have been conducted in the past with 1-alanosine, but in tumors where the deletion of MTAP was not documented (48, 49). There is evidence that MTAP-deficient tumors, unable to salvage adenine from 5’-methylthioadenosine, are more dependent on de novo AMP synthesis.

In this study, we reported that in MCL cell lines, 1-alanosine is cytotoxic against MTAP-negative and MTAP-positive MCL cell lines, as it has been described in other models (18, 50). We also showed that 1-alanosine induces the typical features of activation of the mitochondrial apoptotic pathway. Furthermore, we described that EFA, a new MTAP substrate analogue, rescued wild-type MTAP cells from 1-alanosine toxicity. EFA has been described as a salvage agent for MTAP-positive cells to enhance the therapeutic effect of 1-alanosine because the MTAP substrate provides a source of adenine for normal cells (29).

In summary, MCL cases displaying MTAP gene deletions and lack of protein expression are associated with poor prognosis. Moreover, MTAP analysis may help to identify patients who might benefit from therapeutic inhibition of de novo AMP synthesis pathway. Our results give background to the use of a combination of 1-alanosine and EFA as treatment of MTAP-deficient MCL cells.

**References**

Lack of Methylthioadenosine Phosphorylase Expression in Mantle Cell Lymphoma Is Associated with Shorter Survival: Implications for a Potential Targeted Therapy

Silvia Marcé, Olga Balagué, Luis Colomo, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/12/12/3754

Cited articles
This article cites 49 articles, 26 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/12/12/3754.full.html#ref-list-1

Citing articles
This article has been cited by 7 HighWire-hosted articles. Access the articles at:
/content/12/12/3754.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.