Early Magnesium Reduction in Advanced Colorectal Cancer Patients Treated with Cetuximab Plus Irinotecan as Predictive Factor of Efficacy and Outcome

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Abstract

Introduction: Magnesium plays a role in a large number of cellular metabolic reactions. Cetuximab is able to induce hypomagnesemia by interfering with magnesium (Mg^{2+}) transport in the kidney. We designed this trial to investigate if Mg^{2+} serum level modifications may be related with clinical response and outcome in advanced colorectal cancer patients during treatment with cetuximab plus irinotecan.

Experimental Design: Sixty-eight heavily pretreated metastatic colorectal cancer patients were evaluated for Mg^{2+} serum levels at the following time points: before; 6 hours; and 1, 7, 14, 21, 50, and 92 days after the start of treatment.

Results: Basal Mg^{2+} median levels were significantly decreased just 7 days after the first anticancer infusion and progressively decreased from the 7th day onward, reaching the highest significance at the last time point ($P < 0.0001$). Twenty-five patients showed a reduction in median Mg^{2+} circulating levels of at least 20% within the 3rd week after the first infusion. Patients with this reduction showed a response rate of 64.0% versus 25.6% in the nonreduced Mg^{2+} group. The median time to progression was 6.0 versus 3.6 months in the reduced Mg^{2+} group and in that without reduction, respectively ($P < 0.0001$). Overall survival was longer in patients with Mg^{2+} reduction than in those without (10.7 versus 8.9 months).

Conclusions: Our results confirm that cetuximab treatment may induce a reduction of Mg^{2+} circulating levels and offer the first evidence that Mg^{2+} reduction may represent a new predictive factor of efficacy in advanced colorectal cancer patients treated with cetuximab plus irinotecan.

Magnesium (with Mg referring to the total elemental content and Mg^{2+} to its ionized form) is a critical cofactor in many enzymatic reactions important for physiologic functions, such as nucleic acid metabolism, protein synthesis, and energy production (1, 2) Moreover, it seems to play a role in tumor biology such as in the regulation of oxidative stress (3), carcinogenesis (4), tumor progression (5), and angiogenesis (6).

With regard to the regulation of cell proliferation, there is convincing evidence that Mg deficiency induces growth arrest by affecting the expression levels of cell cycle regulatory proteins, including p27, p21, cyclins, and CDKs. At the same time, Mg can regulate other steps of cell proliferation such as protein synthesis, DNA duplication, and mitosis (4). Interestingly, mitochondria have all the characteristics to be considered as Mg stores, as they possess specific channels to take up Mg from the cytosol. In this regard, there is a challenging possibility that Mg participates not only in ATP synthesis but also in the apoptotic pathway (2).

Plasma and cellular Mg^{2+} concentrations are both tightly controlled, although regulation of its balance is poorly understood at the cellular and molecular levels.

Mg^{2+} homeostasis is determined by intestinal absorption and renal excretion; thus, the kidney plays a key role in Mg^{2+} handling.

Because epidermal growth factor receptor (EGFR; also known as c-erb1 or HER1) is strongly expressed in the kidney, particularly in the ascending limb of the loop of Henle where 70% of filtered magnesium is reabsorbed, EGFR blockade may interfere with magnesium transport (7).

Very recently, Groenestege et al. defined EGF as an autocrine/paracrine magnesiotropic hormone that regulates renal Mg^{2+} reabsorption by regulating the activity of the Mg^{2+}-permeable channel TRPM6 (transient receptor potential cation channel, subfamily M, member 6). The authors identified a point mutation in pro-EGF that disrupts sorting of the protein to the

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basolateral membrane of distal convoluted tubule cells in kidney nephrons (8). As a consequence, inhibition of the EGFR by anti-EGFR antibodies led to suppressed activity of TRPM6 and renal Mg^{2+} wasting.

Inhibition of the EGFR has become a key part in the treatment of various cancer types, either as monotherapy or in combination with chemotherapy (9, 10).

Cetuximab is a chimeric IgG1 monoclonal antibody that binds to EGFR with high specificity and with a higher affinity than epidermal growth factors, blocking ligand-induced phosphorylation of EGFR.

Clinical trials have shown that cetuximab is synergistic with chemotherapy for patients with metastatic colorectal cancer. As a result, cetuximab is now regarded as part of standard treatment practice in this disease (9, 11).

A previous study reported that patients with colorectal cancer treated with cetuximab may develop a Mg^{2+} wasting syndrome with hypomagnesemia and inappropriate urinary excretion. Because EGFR is strongly expressed in the kidney, particularly in the ascending limb of the Henle's loop where 70% of filtered Mg^{2+} is readsorbed, it was suggested that cetuximab blockade of EGFR could interfere with Mg^{2+} transport, leading to hypomagnesemia in most patients (12).

We aimed to assess the occurrence of magnesium serum levels modifications in advanced colorectal cancer patients treated with a weekly combination of cetuximab plus irinotecan and to evaluate the existence of a correlation with clinical response and outcome during treatment.

Materials and Methods

Study design and patients eligibility criteria. Patients were considered eligible for the study if they had a histologically confirmed colorectal adenocarcinoma (resected or not) associated with distant metastases (with or without local relapse). Magnesium circulating levels were analyzed in a population of colorectal cancer patients receiving cetuximab + irinotecan as third-line anticancer treatment and resistant to oxaliplatin- and irinotecan-based chemotherapy.

We included in the study patients older than 18 years, affected by stage IV and histologically confirmed colorectal adenocarcinoma. Immunohistochemical evidence of EGFR expression measured semiquantitatively (>0 on a scale of 0, 1+, 2+, or 3+) in a single reference laboratory (University Campus Bio-Medico, Rome, Italy) was required. These measurements were performed and graded using a commercially available kit (EGFRpharmDx; Dako Corporation) according to the manufacturer's instructions. Standard criteria for anticancer treatment suitability for all patients were used. In particular, renal function was evaluated and only patients within the reference range (serum creatinine 0.8-1.44 mg/dL) were included.

Patients were considered ineligible for accrual when they had reported fever (body temperature >38.0°C) during the last week before study entry or had received any radiotherapy, chemotherapy, immunotherapy, or growth factors during the last 4 wk before study accrual. Moreover, if patients received radiotherapy or growth factors during our study, they were excluded from the final evaluation as well. Patients recently (<1 wk) or simultaneously treated with chronic steroid–based therapy and with acute or chronic infections or inflammatory diseases were considered ineligible for the study. Malabsorption syndromes, uncontrolled diabetes, genetic magnesium wasting syndromes (such as, Gitelman and Bartter syndrome), drugs, and toxins intake (such as, ethanol, loop diuretics, thiazide, cisplatin, cyclosporine) were also established criteria for ineligibility.

Before being considered in this study, all patients had a documented disease progression after two standard anticancer regimens: one oxaliplatin-based chemotherapy regimen (capecitabine + oxaliplatin or FOLFOX IV regimen as first line) and one irinotecan-based chemotherapy (FOLFIRI regimen as second line) for at least 2 mo. Informed consent was obtained from all the subjects enrolled in the study.

Treatment plan. Cetuximab was given at a loading dose of 400 mg/m^2, followed by weekly infusions of 250 mg/m^2. Irinotecan was administered weekly at the dose of 90 mg/m^2. A histamine receptor antagonist and atropine (0.25 mg) were given as premedication before every infusion. No corticosteroids were routinely administered, and those patients treated with steroids for any reason after the accrual were excluded. A standard antiemetic drug was always given in the premedication and in the following days according to the physician's judgment. All the patients were treated until disease progression or until unacceptable toxic effects occurred.

Modifications to cetuximab dose were made only in cases of toxic effects to the skin and to irinotecan dose in cases of hematologic or nonhematologic toxic effects. Tumor response was evaluated every 8 wk by the use of consistent imaging techniques (computed tomography or magnetic resonance imaging). Assessment was done by the investigators according to the Response Evaluation Criteria in Solid Tumors. Adverse events were recorded according to the National Cancer Institute Common Toxicity Criteria version 2.0.

Electrolyte evaluation. Venous blood was drawn just before the beginning of the 1st day and 1, 21, 50, and 92 d after cetuximab + irinotecan infusion. Samples were rapidly centrifuged for 10 min at 3,500 rpm and serum stored at -80°C until tested. Moreover, standard hematologic variables, including renal function, were tested before every single course.

Serum magnesium and calcium concentrations were measured at baseline and at any time point referred by use of a colorimetric assay (xylidyl blue and arsenazo III method, respectively) in the Konelab 30i automatic analyzer (Dasit s.p.a.) in a single reference laboratory (University Campus Bio-Medico, Rome, Italy). The reference values and standard laboratory deviations for magnesium and calcium were as follows: Mg^{2+} 1.9 to 2.5 mg/dL, SD 0.15; Ca^{2+} 8.1 to 10.4 mg/dL, SD 0.575. Serum calcium concentrations were corrected for hypoalbuminemia. All patients have at the moment of the analysis all serum magnesium measurements; therefore, none was excluded from the final analysis.

Statistical analysis. Basal magnesium levels were compared with the values observed at 1, 21, 50, and 92 d after the start of cetuximab plus irinotecan treatment using Wilcoxon's test for nonparametric-dependent continuous variables. A linear regression model was used to perform the correlation between magnesium and calcium levels and at each time points.

The time to progression (TTP) analysis was calculated as the period from the date of starting the treatment to the first observation of disease progression or to death from any cause within 60 days after the start of treatment or the most recent tumor assessment. The overall survival (OS) time was calculated as the period from the date of starting treatment until death from any cause or until the date of the last follow-up, at which point data were censored. TTP and OS were both determined by Kaplan-Meier product-limit method (13).

Stratified permutation tests were carried out to explore the association between tumor response and acne-like rash and between tumor response and magnesium modifications. Moreover, the differences in terms of TTP and OS according to the presence and severity of acne-like rash and the presence of magnesium reduction were evaluated by the log-rank test (14). The Cox proportional hazards model was applied to the multivariate survival analysis (15).

Patients were stratified according to magnesium reduction into two groups to perform the survival analysis and the correlation with clinical response. In particular, we decided to stratify patients into those who developed a reduction ≥20% or <20%. The cutoff point was decided

after having calculated the median value of percentage magnesium reduction. Moreover, to evaluate the correlation between acne skin rash and survival and clinical response, we compared patients who developed a grade 0-1 rash versus patients with a grade 2-3 rash. We have made this decision after having calculated the median value of rash grade in all the cohort, to separate the patients' population into two equivalent groups.

The cutoff point for survival data was July 2007; for safety data, the cutoff point was March 2007. SPSS software (version 13.05, SPSS, Inc.) was used for statistical analysis. A P value of <0.05 was considered to indicate statistical significance.

Results

Sixty-eight consecutive patients (35 males, 33 females), ages 27 to 82 years (median age, 65 years), with advanced colorectal cancer were included in the study. All patients matched all the inclusion criteria. Patient characteristics are shown in Table 1.

Magnesium analysis. The median magnesium basal value showed a statistically significant decrease just 7 days after the start of cetuximab plus irinotecan anticancer treatment. This effect enhanced during the following analyzed time points as reported in Table 2. The lowest levels of magnesium were achieved at the last time point (92 days after the first cetuximab infusion), with a median value of 1.75 mmol/L versus 2.12 mmol/L when compared with the basal time ($P < 0.0001$).

Table 1. Baseline characteristics of the patients

Patient characteristics	No. patients (%)
Total number	68 (100%)
Male/female	35/33 (51.4%/48.5%)
Age (y)	
Median	65
Range	27-82
Performance status	
Median	1
Range	1-2
Primary tumor site	
Colon	42
Rectum	26
No. metastatic sites	
1	21 (30.8%)
2	25 (36.7%)
3+	22 (32.3%)
Sites of metastases	
Liver	31 (45.5%)
Lung	20 (29.4%)
Nodes	16 (23.5%)
Local	12 (17.6%)
Other	19 (27.9%)
Prior adjuvant therapy	
None	23 (33.8%)
FU/LV	45 (66.1%)
First-line regimen	
XELOX	41 (60.3%)
FOLFOX	27 (39.7%)
Second line regimen	
FOLFIRI	68 (100%)
EGFR expression	
Score 1	21 (30.8%)
Score 2	29 (42.6%)
Score 3	18 (26.4%)

Abbreviations: FU/LV 5-FluoroUracil and folinic acid; XELOX, oxaliplatin and capecitubine; FOLFOX, oxaliplatin and 5-Fluororacil (bolus and continuous infusion) plus folinic acid; FOLFIRI, irinotecan and 5-Fluouracil (bolus and continuous infusion) plus folinic acid.

Table 2. Magnesium modification at different time points

Time points	Median (95% CI)	*P*
Basal levels	2.12 (2.03-2.22)	—
6 h	2.10 (2.0226-2.21)	0.666
1 d	2.15 (2.06-2.24)	0.956
7 d	2.05 (1.93-2.74)	0.020
14 d	2.00 (1.91-2.12)	0.005
21 d	1.96 (1.84-2.03)	0.003
50 d	1.91 (1.73-2.04)	0.001
92 d	1.75 (1.57-1.87)	<0.0001

Abbreviation: 95% CI, 95% confidence interval.

At any of the programmed time points, 65 of 68 patients showed a reduction of magnesium serum levels; 25 of them showed at least 20% reduction with respect to the basal level. Only 3 patients during any of the programmed time points developed a hypomagnesemia G1 according to the Common Toxicity Criteria (all of them at the last time point). No cases of hypomagnesemia higher than G1 were recorded.

Significant correlation in a linear regression model was noted, as expected, between basal calcium serum levels and basal magnesium serum levels (β regression coefficient = 4.230; $P = 0.009$). Calcium levels progressively decreased during cetuximab-based therapy, maintaining the correlation at any time points (data not shown). However, the calcium reduction was not clinically significant and only one patient developed a grade 1 hypocalcemia during anticancer treatment.

Correlation of response and survival with magnesium reduction. An early magnesium reduction of at least 20% respect to the basal value (between the 1st and the 3rd week after the first infusion) represents a significant predictive factor for response. Those patients who developed a decrease of magnesium serum levels within the 1st and 3rd weeks after the start of treatment showed a higher response rate compared with patients who did not show an equal reduction (64.0% versus 25.6% $P = 0.004$). Moreover, TTP was higher in the patient group with reduced magnesium than in the other group (6 months versus 3.9 months; $P < 0.0001$). Moreover, stratifying patient population according to the rank of early magnesium reduction (≤10%, 10-20%, and ≥20%), we identified that the rank of magnesium reduction may also represent a predictive factor for TTP, as described in Table 3.

Hence, the early reduction of magnesium serum levels represents a significant prognostic factor of OS. In fact, those patients with a magnesium reduction ≥20% had a longer OS than those without (10.7 versus 8.9 months; $P = 0.021$). These results are shown Table 4 and in Figs. 1 and 2

Correlation of response and survival with rash. A correlation between the presence and severity of the acne-like rash and tumor response was shown. In particular, patients with a grade 3 rash showed a higher response rate (57.7%) versus those patients with a grade 0, 1, and 2 (28.6%), with a statistically significant difference ($P = 0.033$). Moreover, comparing patients who developed a grade 3 acne-like rash with the others, a statistically significant correlation was recorded

Table 3. TTP according to the entity of magnesium reduction

Magnesium reduction (%)	No. patients	Median TTP (95% CI)
≤10	21	3.8 (3.1-4.4)
10-20	22	5.1 (4.7-5.7)
≥20	25	6.0 (4.3-9.9)

between the acne-like rash and TTP: 6.00 (5.53-6.44) versus 4.01 (3.75-4.45), $P = 0.002$. No statistically significant difference in terms of overall OS between the two groups was identified. These data are summarized in Table 4 and are shown in Figs. 1 and 2.

Multivariate analysis of survival. Performing a multivariate analysis of TTP, the early magnesium reduction preserved the statistical significance whereas the acne-like skin rash lost its significance, even if the *P* value was borderline. In detail, the calculated relative risk of progression in the group of patients with a >20% early magnesium modification is 0.187 (95% confidence interval, 0.103-0.339) with a *P* value of 0.001. The relative risk of progression for the group of patients with G3 skin rash was 0.501 (95% confidence interval, 0.311-1.088) compared with patients without this reduction ($P = 0.090$).

Discussion

We evaluated serum magnesium concentrations in patient affected by metastatic colon cancer during EGFR-targeting antibody therapy; as a second step, we investigated the existence of a correlation with clinical response and outcome during treatment. Magnesium circulating levels were assessed at the basal level and at different time points until 92 days after the start of treatment. It is noteworthy that magnesium plays a key role in many physiologic functions, such as nucleic acid metabolism, protein synthesis, and energy production (1, 2); interestingly, it also seems to play a role in tumor biology (16, 17).

Because EGFR is strongly expressed in the ascending limb of the loop of Henle, EGFR blockade may interfere with magnesium transport (7).

Recently, Groenestege and colleagues identified a point mutation in pro-EGF that disrupts sorting of the protein to the basolateral membrane of distal convoluted tubule cells in kidney nephrons (8). As a consequence, inhibition of the EGFR by anti-EGFR antibodies as cetuximab might lead to renal Mg^{2+} wasting.

A previous study reported that patients with colorectal cancers treated with cetuximab may develop a Mg wasting syndrome with hypomagnesemia and inappropriate urinary excretion (12).

Tejpar et al. confirmed these findings, reporting that EGFR-targeting antibodies (cetuximab and panitumumab) may lead to magnesium wasting in patients affected by colon cancer (18). Another study reported a retrospective analysis of magnesium decreasing during cetuximab treatment and, in particular, a severe hypomagnesemia was associated with treatment duration (19).

Our results confirm the previous findings by showing a progressive decrease in serum magnesium concentrations in our patients during EGFR-targeting treatment. We monitored magnesium serum concentrations during a period of 92 days in a homogeneous cohort of patients affected by metastatic colon rectal cancer (all patients were treated with the same anticancer regimen).

In the present study, hypomagnesemia G1 occurred only in 3 patients and in all of them at the last time point (92 days). Our data show that the incidence of significant hypomagnesemia is less frequent than that observed in previous study by Tejpar and colleagues, even if our observation time for hypomagnesemia is shorter than that studied by Tejpar. Interestingly, although the two studied populations are different, most importantly, our population is more homogeneous and treated only with one anticancer regimen; the

Table 4. Influence of magnesium reduction and skin toxicity on tumor response, TTP, and OS

		P
Magnesium reduction		
Tumor response, no. patients (%)		
≤20% reduction of magnesium levels	11/43 (25.6%)	0.004
>20% reduction of magnesium levels	16/25 (64.0%)	
TTP (mo), median (95% CI)		
≤20% reduction of magnesium levels	3.90 (3.15-4.04)	<0.0001
>20% reduction of magnesium levels	6.00 (5.65-6.57)	
OS (mo), median (95% CI)		
≤20% reduction of magnesium levels	8.90 (7.697-10.103)	0.021
>20% reduction of magnesium levels	10.7 (10.045-11.355)	
Skin toxicity		
Tumor response, no. patients (%)		
Grade 0-1-2	12/42 (28.6%)	0.033
Grade 3	15/26 (57.7%)	
TTP (mo), median (95% CI)		
Grade 0-1-2	4.01 (3.75-4.45)	0.002
Grade 3	6.00 (5.53-6.44)	
OS (mo), median (95% CI)		
Grade 0-1-2	9.70 (8.488-10.912)	0.229
Grade 3	9.89 (0.808-10.183)	

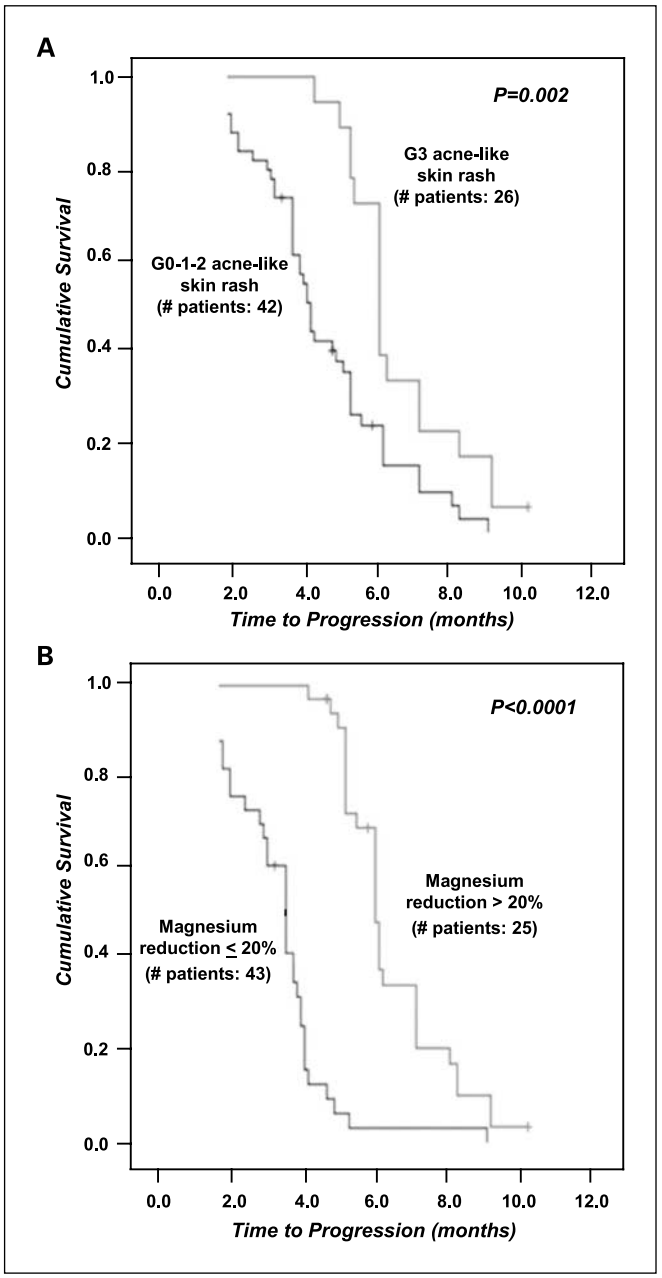

Fig. 1. Kaplan-Meier survival plots for TTP in advanced colorectal cancer treated with cetuximab plus irinotecan according to the presence of severe acne-like rash (*A*) and magnesium modifications (*B*).

median time of onset of hypomagnesemia in the report by Tejpar is very close to our last time point. Dissimilar eating habits might be responsible of this discrepancy.

Finally, for the first time in the literature, we evaluated the existence of a correlation between the occurrence of magnesium serum level reduction and clinical response/outcome (in terms of TTP and OS) during cetuximab treatment.

Clinical studies of cetuximab in metastatic colorectal cancer failed to reveal an association between clinical outcome and EGFR protein expression as measured by immunohistochemistry (20, 21). Furthermore, clinical responses have been shown in patients with undetectable EGFR protein expression (22) and somatic mutations in the EGFR tyrosine kinase domain are associated with sensitivity to the tyrosine kinase inhibitors but not to cetuximab (23, 24).

On the contrary, *KRAS* (a downstream gene of EGFR) mutation is associated with resistance to cetuximab and a shorter survival in EGFR-positive metastatic colorectal cancer patients treated with this therapy. Thus, KRAS mutation status might allow the identification of patients who are likely to benefit from cetuximab and avoid a costly and potentially toxic administration of this treatment in nonresponder patients (25, 26).

Moreover, some other EGFR downstream genes seem to be related to sensitivity to anti-EGFR–based anticancer therapy. The overexpression of the EGFR ligands epiregulin and amphiregulin seems to predict antitumor activity resulting

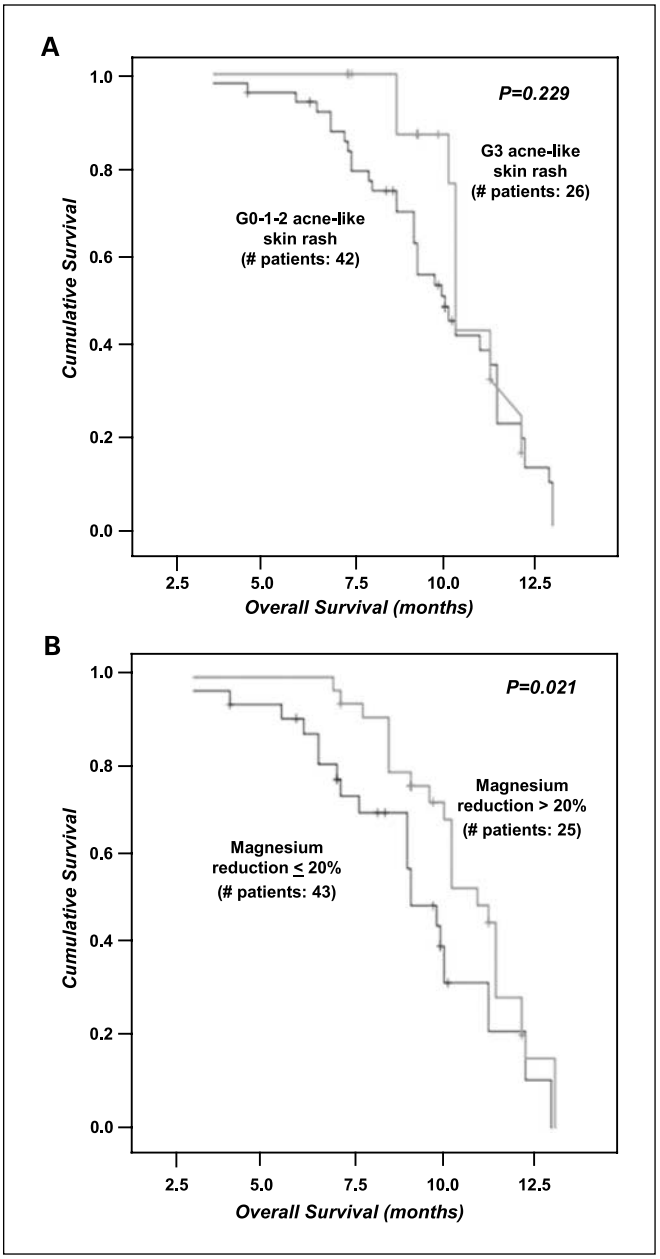

Fig. 2. Kaplan-Meier survival plots for OS in advanced colorectal cancer treated with cetuximab plus irinotecan according to the presence of severe acne-like rash (*A*) and magnesium modifications (*B*).

from cetuximab therapy (27). Moreover, Scartozzi and colleagues showed that tumors that constitutively and aberrantly express nuclear factor-κB are more likely to be refractory to cetuximab and irinotecan than those that do not show nuclear expression of this transcriptional factor (28). Finally, the loss of PTEN protein expression is also associated with nonresponsiveness to cetuximab (29).

The effects of Mg^{2+} on microvascular endothelial cell functions that could contribute to the regulation/deregulation of angiogenesis are rather intricate. Therefore, the reduction of magnesium circulating levels could justify the previous reported reduction of vascular endothelial growth factor serum levels in colorectal cancer patients (30, 31). Consequently, magnesium reduction could be involved in the complex cross-talk between EGFR pathway and angiogenesis.

In conclusion, our results confirm that cetuximab treatment may induce a reduction of magnesium serum levels and offer the first evidence in considering magnesium decrease as a new predictor factor of efficacy and outcome in colorectal cancer patients treated with cetuximab + irinotecan.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Dai LJ, Ritchie G, Kerstan D, Kang HS, Cole DE, Quamme GA. Magnesium transport in the renal distal convoluted tubule. Physiol Rev 2001;81:51–84.
2. Romani AM, Scarpa A. Regulation of cellular magnesium. Front Biosci 2000;5:720–34.
3. Kumar BP, Shivakumar K, Kartha CC. Magnesium deficiency-related changes in lipid peroxidation and collagen metabolism *in vivo* in rat heart. Int J Biochem Cell Biol 1997;29:129–34.
4. Anastassopoulou J, Theophanides T. Magnesium-DNA interactions and the possible relation of magnesium to carcinogenesis. Irradiation and free radicals. Crit Rev Oncol Hematol 2002;42:79–91.
5. Rubin H. Central roles of Mg2+ and MgATP2- in the regulation of protein synthesis and cell proliferation: significance for neoplastic transformation. Adv Cancer Res 2005;93:1–58.
6. Lapidos KA, Woodhouse EC, Kohn EC, Masiero L. Mg(++)-induced endothelial cell migration: substratum selectivity and receptor-involvement. Angiogenesis 2001;4:21–8.
7. Whang R, Hampton EM, Whang DD. Magnesium homeostasis and clinical disorders of magnesium deficiency. Ann Pharmacother 1994;28:220–6.
8. Groenestege WM, Thebault S, van der Wijst J, et al. Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. J Clin Invest 2007;117:2260–7.
9. Cunningham D, Humblet Y, Siena S, et al: Cetuximab monotherapy and cetuximab plus irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004; 351:337–45.
10. Vincenzi B, Santini D, Rabitti C, et al. Cetuximab and irinotecan as third-line therapy in advanced colorectal cancer patients: a single centre phase II trial. Br J Cancer 2006;94:792–7.
11. Saltz LB, Meropol NJ, Loehrer PJ, Sr., Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 2004; 22:1201–8.
12. Schrag D, Chung KY, Flombaum C, Saltz L. Cetuximab therapy and symptomatic hypomagnesemia. J Natl Cancer Inst 2005;97:1221–4.
13. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53: 457–81.
14. Peto R, Pike MC, Armitage P, et al. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. Br J Cancer 1977;35:1–39.
15. Cox DR. Regression models and life tables. J R Stat Soc 1972;34:187–220.
16. Maier JA, Malpuech-Brugere C, Zimowska W, Rayssiguier Y, Mazur A. Low magnesium promotes endothelial cell dysfunction: implications for atherosclerosis, inflammation and thrombosis. Biochim Biophys Acta 2004;1689:13–21.
17. Wolf FI, Maier JA, Nasulewicz A, et al. Magnesium and neoplasia: from carcinogenesis to tumor growth and progression or treatment. Arch Biochem Biophys 2007;458:24–32.
18. Tejpar S, Piessevaux H, Claes K, et al. Magnesium wasting associated with epidermal-growth-factor receptor-targeting antibodies in colorectal cancer: a prospective study. Lancet Oncol 2007;8:387–94.
19. Fakih MG, Wilding G, Lombardo J. Cetuximab-induced hypomagnesemia in patients with colorectal cancer. Clin Colorectal Cancer 2006;6:152–6.
20. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337–45.
21. Saltz LB, Meropol NJ, Loehrer PJ, Sr., et al. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 2004;22:1201–8.
22. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005;23:1803–10.
23. Janne PA, Engelman JA, Johnson BE, et al. Epidermal growth factor receptor mutations in non-small-cell lung cancer: Implications for treatment and tumor biology. J Clin Oncol 2005;23:3227–34.
24. Tsuchihashi Z, Khambata-Ford S, Hanna N, et al. Responsiveness to cetuximab without mutations in EGFR. N Engl J Med 2005;353:208–9.
25. Lièvre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 2006;66:3992–5.
26. Lièvre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 2008;26:374–9.
27. Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 2007;25:3230–7.
28. Scartozzi M, Bearzi I, Pierantoni C, et al. Nuclear factor-kB tumor expression predicts response and survival in irinotecan-refractory metastatic colorectal cancer treated with cetuximab-irinotecan therapy. J Clin Oncol 2007;25:3930–5.
29. Frattini M, Saletti P, Romagnani E, et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. Br J Cancer 2007; 97:1139–45.
30. Vincenzi B, Santini D, Russo A, et al. Circulating VEGF reduction, response and outcome in advanced colorectal cancer patients treated with cetuximab plus irinotecan. Pharmacogenomics 2007;4:319–27.
31. Vincenzi B, Santini D, Russo A, et al. Angiogenesis modifications related with cetuximab plus irinotecan as anticancer treatment in advanced colorectal cancer patients. Ann Oncol 2006;17:835–41.