Support Care Cancer (2017) 25:2179–2185
DOI 10.1007/s00520-017-3623-x

ORIGINAL ARTICLE

Placebo-controlled phase II study of vitamin K3 cream for the treatment of cetuximab-induced rash

Jesper Grau Eriksen[1] · **Inger Kaalund**[1] · **Ole Clemmensen**[2] · **Jens Overgaard**[3] · **Per Pfeiffer**[1]

Received: 28 July 2016 / Accepted: 6 February 2017 / Published online: 15 February 2017

Abstract
Purpose Cetuximab inhibits the epidermal growth factor receptor (EGFR), and papulopustular eruptions is a frequent side effect. Vitamin K3 (menadione) has preclinically shown to be a potential activator of the EGFR by phosphorylating the receptor (pEGFR). The present randomised study investigated the effect of a vitamin K3 cream on cetuximab-induced rash.
Materials and methods Thirty patients were included in this double-blinded placebo-controlled trial. Patients receiving cetuximab 500 mg/m^2 every second week plus chemotherapy for metastatic cancer were included. In each patient, vitamin K3 cream and placebo were applied twice daily on two separate areas of the skin of minimum 10 × 10 cm for up to 2 months. Papulopustular eruptions were evaluated clinically and monitored by clinical photos. Skin biopsies, from ten patients taken before and after 1 month of treatment from each treatment area, were stained for EGFR and pEGFR.
Results Application of vitamin K3 cream twice daily during treatment with cetuximab did not reduce the number of papulopustular eruptions, and this was independent of the use of systemic tetracycline. No significant changes in the staining of EGFR or pEGFR were observed in the skin of the vitamin K3-treated area compared to the placebo area.
Conclusion The present data do not support any clinical or immunohistochemical benefit of using vitamin K3 cream for cetuximab-induced rash.

Keywords Skin toxicity · Rash · EGFR-inhibitor · Cetuximab · Phase II

Introduction

Epidermal growth factor receptor (EGFR) inhibitors are commonly used in combination with chemotherapy [1] or radiotherapy [2]. One of the more frequently used inhibitors is the monoclonal antibody cetuximab, approved by the European Medicines Agency (EMA) in 2004 for the treatment of irinotecan-resistant metastatic colorectal cancer and in 2006, in combination with radiotherapy, for the treatment of locally advanced pharyngeal or laryngeal head and neck cancer [3].

Skin toxicity with papulopustular eruptions is one of the most common side effects of EGFR-inhibitor treatment and is seen in 80–90% of the patients. In approximately 10% of the cases, a severe grade 3–4 skin reaction is registered [4]. The rash is frequently described as acneiform eruptions although it has nothing to do with acne vulgaris. Skin biopsies have shown that the papulopustules represent inflamed hair follicles, and as such a folliculitis, even though sterile [5]. It is often located to the areas with oily skin like the nose, cheeks, chin, chest and back but can also be seen as a more widespread rash [6]. It usually appears within the first week of EGFR-inhibitor treatment and peaks after 2 to 3 weeks. The rash causes significant impact on quality of life in one-third of the patients, and it is reported to cause discontinuation of treatment in up to 17% of the patients [7–9].

Electronic supplementary material The online version of this article (doi:10.1007/s00520-017-3623-x) contains supplementary material, which is available to authorized users.

✉ Jesper Grau Eriksen
jesper@oncology.dk

[1] Department of Oncology, Odense University Hospital, 5000 Odense C, Denmark

[2] Department of Clinical Pathology, Odense University Hospital, Odense, Denmark

[3] Department Of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark

The pathogenesis has yet to be clarified but involves inhibition of EGFR-driven growth of keratinocytes, cell migration and cytokine release [9]. Although several symptom-relieving treatments have been tested, no treatment specifically directed against the EGFR inactivation has yet been found [10]. Vitamin K3 (menadione), a synthetic pro-drug of vitamin K, has been suggested to be able to re-phosphorylate EGFR—even during treatment with EGFR-inhibitors [11], and encouraging data on reduced papulopustular eruptions during EGFR-inhibitor therapy have been reported with vitamin K1 cream [10, 12–14].

The aim of the present study was to test the possible benefit of a topical vitamin K3 cream on cetuximab-induced rash in a double-blinded placebo-controlled phase II design.

Materials and methods

Patients

A total of 30 patients were included. Patients were eligible for the study when treated for metastatic cancer with cetuximab 500 mg/m^2 every second week plus chemotherapy, at least 18 years old; no other diseases that could affect evaluation of the study drug (including chronic skin disease), no concomitant treatment with vitamin K or vitamin K antagonists and no known hypersensitivity to menadione. No concomitant anti-inflammatory topical or systemic therapy was allowed except for systemic tetracycline. Tetracycline was allowed as it was considered unethical not to have any possibility to relieve the skin symptoms during the study.

Study design

Patients entered consecutively after signed consent. For every patient, two separate equally sized areas were chosen on either the chest or on the back. Each of them at least 10 × 10 cm was selected (Fig. 1). According to the randomisation, one area was treated with placebo cream, the other with vitamin K3 cream twice daily; thus, the patient served as his/her own control in a randomised double-blinded way.

Topical treatment was initiated either as prophylactic treatment concomitantly with the start of cetuximab or initiated when treatment-induced papulopustular eruptions were observed. The patients received two identical tubes marked ‘Right side’ or ‘Left side’. The content of the tubes was blinded for the patient and the medical staff and randomly contained either vitamin K3 or placebo. The key to this randomisation was performed by the pharmacy and kept sealed until the clinical database was completed and locked for further changes. Treatment lasted for a maximum of 2 months, and a follow-up was performed 14 days after the end of treatment. Photographs were taken of the test sites at baseline, after 1 week, after 2 weeks and then every second week throughout the study, and the number of inflamed follicles were counted and registered. At every visit, patients were asked to bring the cream tubes which were weighed (without patient’s knowledge) to monitor if the patient used approximately the same amount of cream from each tube (SUPPLEMENTARY 1).

As an optional part of the clinical study, patients were asked permission that one skin punch biopsy was taken from each of the treatment areas at baseline and after 1 month of treatment. Four biopsies were taken altogether from each patient.

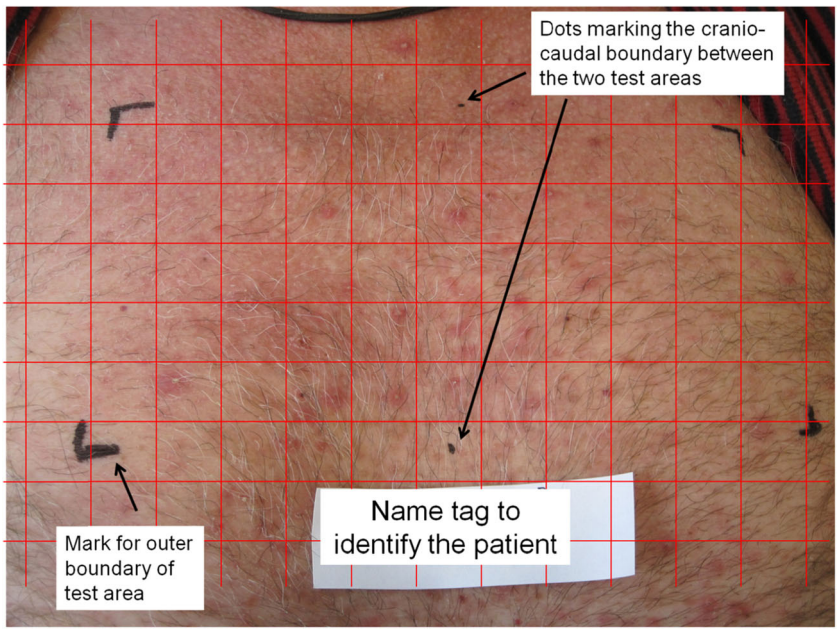

Fig. 1 An example of a patient with two equally sized test areas for placebo treatment and vitamin K3 treatment

Vitamin K3 and placebo cream

No commercial product with an exact amount of vitamin K3 was available on the marked at the time of initiation of the study. Therefore, the study cream was manufactured at Glostrup Pharmacy, Copenhagen, Denmark, which is certified by the Ministry of Health, to produce extemporaneous products. The placebo cream consisted mainly of purified water (78.5%), sorbitol (7%), cetylanum (5%), paraffin liquid (5%) and glycerol 85% (4%). The vitamin K3 cream consisted of placebo cream with 56.5 mg purified vitamin K3 (ROVIMIX®, DSM Nutritional Products Ltd., Basel) per 100 mL placebo cream, corresponding to 1.5 mM/L. This corresponded to 1/10 of the dose used in animal experiments [11]. The cream was tested on nude mice by a certified laboratory to secure that it did not induce tumour growth or skin reactions. For a detailed description, see SUPPLEMENTARY 2.

Study endpoints

The primary endpoint was a possible decrease in the number of inflamed follicles at the vitamin K3 side compared to the placebo side after 4 weeks of treatment. The secondary endpoint was to investigate any possible side effects of vitamin K3 cream.

Assessment of papulopustular eruptions

Photos taken under standardised conditions were evaluated by placing the photo in a counting grid, and the number of papulopustular eruptions was counted on each side. Ten out-of-trial photos were used as training set for I.K. counting follicles twice with 1-week interval. Inter-observer variation was estimated by comparing counts with J.G.E. Bland-Altman plots for intra- and inter-observer variation showed an acceptable agreement but with increasing uncertainty, with increasing number of follicles counted (SUPPLEMENTARY 3). Study photos were evaluated without knowledge of the clinical data.

Analysis of safety data

At every visit during the study, patients were evaluated for possible side effects using CTCAE 4.0, and regular blood tests were done including vitamin K-dependent coagulation factors and haptoglobin as haemolysis has been described in infants given vitamin K3.

Immunohistochemistry

Biopsies were formalin-fixed and paraffin-embedded. Immunohistochemistry was performed for EGFR (EGFR mouse antibody, clone 3C6, Ventana Medical Systems) and phospho-EGF-receptor (pEGFR, rabbit antibody, clone 53A5, Cell Signalling Technology) using the hospital standard protocols for antigen retrieval and visualisation. Scoring of expression was performed using a semi-quantitative scale [15]. Examples of stainings are shown in SUPPLEMENTARY 4.

Statistical analysis

Intra- and inter-observer variation was evaluated with Bland-Altman plots and linear regression. Patient and tumour characteristics were evaluated using descriptive statistics. Changes in the number of follicular eruptions comparing placebo side with vitamin K3 side after 4 weeks were evaluated using paired sample *t* test after testing for normal distribution using QQ-plots.

Ethical considerations

Approval for manufacturing vitamin K3 cream was obtained from the Ministry of Health, and the clinical study was approved by the Regional Scientific Ethical Committees for Southern Denmark, the Danish Health and Medicines Authority and the Danish Data Protection Agency. Patients were enrolled after oral and written informed consent, and the study was conducted according to the Helsinki Declaration. The study was registered at with EudraCT 2009-016591-68 and ClinicalTrials.gov Identifier: NCT01094444. Animal experiments complied with the ARRIVE guidelines and were carried out in accordance with EU Directive 2010/63/EU for animal experiments.

Results

Patient population

Thirty patients were enrolled in the study. Eighteen patients had photos of sufficient quality for evaluation of the papulopustular eruptions. The drop-out of patients is shown in the CONSORT diagram (Fig. 2). No significant differences were found, regarding patient and treatment characteristics for the whole cohort of 30 patients enrolled and the 18 patients that were fully evaluable (Table 1).

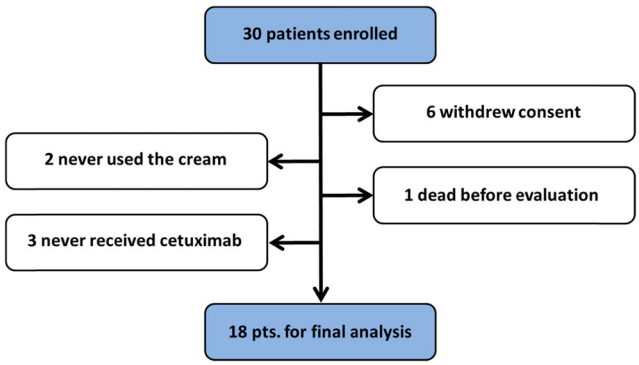

Fig. 2 CONSORT diagram of patients in the study

Table 1 Selected patient and treatment characteristics

	Entire cohort $n = 30$	Evaluable cohort $n = 18$
-Male	23 (77%)	15 (83%)
-Female	7 (23%)	3 (17%)
Median age in years	63.5 (range 30–78)	64.0 (range 30–74)
Performance status		
-PS 0–1	28 (94%)	18 (100%)
-PS 2	1 (3%)	0
-Unknown	1 (3%)	0
Disease		
-Rectum	12 (40%)	8 (44%)
-Colon	10 (33%)	5 (28%)
-Pancreas	3 (10%)	3 (17%)
-Oesophagus	1 (3%)	1 (6%)
-Head and neck	3 (10%)	0
-Unknown primary	1 (3%)	1 (6%)
Biopsy	10 (33%)	8 (44%)
Start K3 upfront	17 (57%)	12 (67%)
Start K3 when rash	11 (37%)	6 (33%)
Never started	2 (6%)	NR
Median cycles cetuximab	6 (range 1–6)	6 (range 3–6)
Tetracycline		
-Yes	21 (70%)	14 (78%)
-No	9 (30%)	4 (22%)

Treatment delivery

Seventeen out of the 18 patients received at least four courses of cetuximab corresponding to full treatment during the 2 months of active use of placebo/vitamin K3 cream. The median number of doses of cetuximab was six (Table 1). The 18 patients used placebo/vitamin K3 cream throughout the study period (SUPPLEMENTARY 1). The mean total amount of cream used at each test area was 44.9 g (±4.4 g) versus 46.3 g (±4.4 g), respectively. Thus, there is no significant difference ($p = 0.9$). All, except two patients, returned the surplus cream at the end of the study.

Efficacy

Figure 3a shows the mean number of papulopustular eruptions counted in each test area (vitamin K3 versus placebo) for every visit during the 2-month treatment and at the 14-day follow-up (week 10). The mean number of elements was 4.9 (placebo) versus 5.1 (vitamin K3) at baseline ($p = 0.9$), increasing to 11.1 (placebo) versus 14.1 (vitamin K3) at 2 weeks ($p = 0.5$), declining to 8.9 (placebo) versus 7.3 (vitamin K3) at 6 weeks ($p = 0.7$). At week 4 which was the primary endpoint, no difference at all was found: 6.1 (placebo) versus 6.3 (vitamin K3). In fact, at any time point during the study, there was no significant difference between placebo and vitamin K3 areas among 18 evaluable patients. Stratification of the patients into a group treated with the cream from the start of treatment with cetuximab (Fig. 3b) and a group that started treatment when papulopustular eruptions became apparent (Fig. 3c) rendered similar results.

All but four patients received tetracycline for inhibiting secondary infection with *Staphylococcus aureus*. These four patients had significantly less papulopustular eruptions in the fourth and sixth week of the study ($p < 0.05$). However, no significant difference in the number of eruptions at the placebo side versus the vitamin K3 side was noticed, week by week, among the 14 patients receiving tetracycline or the four patients not receiving systemic antibiotics.

Side effects

No vitamin K3-related serious adverse events were reported during the study. However, when evaluating the patients after termination of the study, it seemed (judged from photos—SUPPLEMENTARY 5) that one patient might have experienced a rash only located to the vitamin K3 treatment side (after 8 weeks of treatment). It was not possible to confirm this, but vitamin K3 has been known to cause dermatitis after long-term usage. The patient died of disease progression and judged by the records; the rash was not permanent and did not bother the patient. No significant or clinically relevant

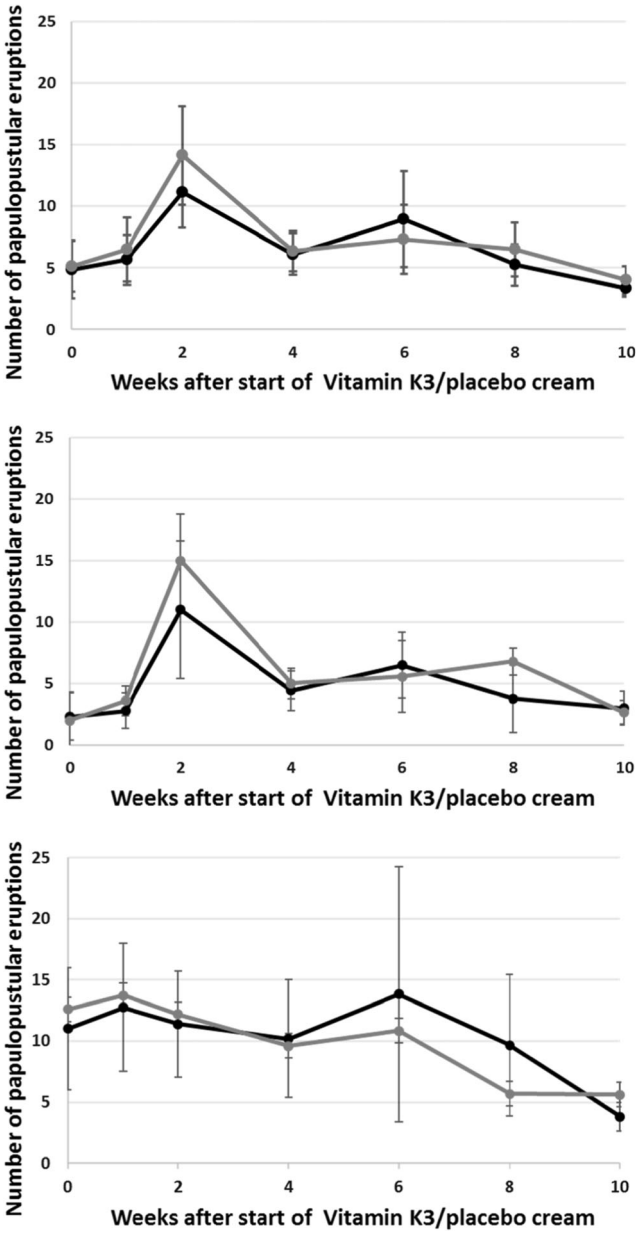

Fig. 3 **a** The number of counted papulopustular eruptions in the placebo (*black line*) and vitamin K3 (*grey line*) area for every visit during the study. **b** The same only for the group of patients starting the cream at the same time as cetuximab was initiated whereas **c** shows the same as **a** and **b** but only for the group of patients starting placebo and vitamin K3 cream when cetuximab-induced rash was apparent

changes in vitamin K3-dependent coagulation factors or in haptoglobin levels were recorded for any of the patients throughout the study period (data not shown).

Immunohistochemistry

Ten patients accepted to have skin punch biopsies taken from both test areas at baseline and after 4 weeks of treatment. A trend towards less expression of EGFR was seen at the placebo side compared to the K3 side. However, if vitamin K3 was able to rephosphorylate EGFR, then the same pattern should be found for pEGFR, which was not the case. For none of the markers EGFR, nuclear and cytoplasmic pEGFR, any significant change in expression was found (SUPPLEMENTARY 6).

Discussion

Papulopustular eruptions are a frequent side effect to treatment with EGFR-inhibitors [4], and several approaches to relive symptoms or to decrease the duration and severity of the cetuximab-induced rash exist [16]. Promising results on activation of EGFR by re-phosphorylation of the receptor [17, 18], even under the influence of EGFR-inhibitors [11], have been published. However, only few clinical studies have yet been published. They all use vitamin K1 [12–14] in a non-blinded setting, thus making the interpretation of a possible benefit difficult. In spite of this, the use of vitamin K1 cream for skin care during treatment with EGFR-inhibitors has become common in some institutions. Therefore, we decided to explore the possible clinical effect in a phase II double-blind controlled study using the patient as his/her own control. The present study showed that application of a vitamin K3 cream twice daily during treatment with cetuximab did not reduce the number of papulopustular eruptions and that this result was independent of the use of systemic tetracycline. These clinical results were supported by the fact that no change in the pEFGR staining of the skin biopsies from the treated areas was observed. Finally, the patients themselves reported no clear preference for placebo or vitamin K3 cream (data not shown).

The randomised double-blinded approach is one of the strengths of this study, but weakened by only two-thirds of the patients being evaluable.

Vitamin K3 was preferred in this study to other vitamin K compounds, as menadione has been reported to be more potent in terms of re-phosphorylation of the EGFR compared to vitamin K1 or K2 [11]. Furthermore, it is stable, lipophilic and has a small molecular size favouring permeation of the skin. However, in general, permeation might be difficult with only one-third of vitamin K reaching the dermal layer [19]. Only one-tenth of the concentration of vitamin K3 used in animal studies [11] was used in the present study. The reason for this was to avoid toxic skin reactions [20], and the dose chosen was still three times the concentration reported to yield full re-phosphorylation of the EGFR in experimental systems [11]. Some of the patients in the study started the cream prophylactically, whereas others waited for the papulopustular eruptions to appear. Apparently, it had no influence on the results of the study which is in line with other reports on prophylactic versus active treatment of EGFR-inhibitor-induced skin toxicity [21].

The of today published clinical reports with encouraging results are all on vitamin K1, although vitamin K3 is reported to be more potent for introducing re-phosphorylation [11]. A study comparable with the present using vitamin K3 (ClinicalTrials.gov identifier: NCT01393821) and randomising patients to either placebo or 0.1% menadione lotion to the facial skin has been completed but is not yet published. The vitamin K3 cream in the present study corresponds to 0.05% menadione, which have to be kept in mind when results are available for comparison. Recently, the authors to one of the preclinical papers of EGFR re-phosphorylation with vitamin K3 have questioned their own data and retracted their paper [22], which might give further support for our results.

In the present study, we used biweekly cetuximab [23] instead of the standard weekly regime. Although it seems to result in less severe rash, there is no reason to believe that a possible effect of vitamin K3 cream would have been different with a weekly schedule of cetuximab.

In conclusion, the present data do not support any clinical benefit of using vitamin K3 cream, 0.05% for cetuximab-induced rash, nor do they reveal any immunohistochemical evidence of increased re-phosphorylation of EGFR in the skin. We suggest that the routine use of topical vitamin K be it either vitamin K1 or K3, as part of skin care during EGFR-inhibitor treatment, must await the results of further clinical trials.

Acknowledgements We acknowledge the skilled work of Mrs. Inger Marie Horsman, Inger Marie Thuesen and Dorthe Grand, conducting the animal experiments at Dept. of Experimental Clinical Oncology, Aarhus, Denmark.

The study was supported by a grant from "A.P. Møller og Hustru Chastine Mc-Kinney Møllers Fond til almene Formaal; Fondet til Lægevidenskabens Fremme" and Merck KGaA, Darmstadt, Germany. The study was designed, conducted and the data analysed independently of the company. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors.

Compliance with ethical standards

Conflicts of interest The study was supported by a grant from a public foundation "A.P. Møller og Hustru Chastine Mc-Kinney Møllers Fond til almene Formaal; Fondet til Lægevidenskabens Fremme" and the commercial company Merck KGaA, Darmstadt, Germany. The study was designed, conducted and the data analysed independently of the company. The authors are fully responsible for the content of this manuscript, and the views and opinions described in the publication reflect solely those of the authors. None of the authors have any conflicts of interest to report related to the present study. The authors allow the journal to review the data if requested.

References

1. Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A et al (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351(4):337–345
2. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB et al (2006) Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 354(6):567–578
3. Pfeiffer P, Qvortrup C, Eriksen JG (2007) Current role of antibody therapy in patients with metastatic colorectal cancer. Oncogene 26(25):3661–3678
4. Su X, Lacouture ME, Jia Y, Wu S (2009) Risk of high-grade skin rash in cancer patients treated with cetuximab–an antibody against epidermal growth factor receptor: systemic review and meta-analysis. Oncology 77(2):124–133
5. Robert C, Soria JC, Spatz A, Le CA, Malka D, Pautier P et al (2005) Cutaneous side-effects of kinase inhibitors and blocking antibodies. Lancet Oncol 6(7):491–500
6. Agero AL, Dusza SW, Benvenuto-Andrade C, Busam KJ, Myskowski P, Halpern AC (2006) Dermatologic side effects associated with the epidermal growth factor receptor inhibitors. J Am Acad Dermatol 55(4):657–670
7. Li T, Perez-Soler R (2009) Skin toxicities associated with epidermal growth factor receptor inhibitors. Target Oncol 4(2):107–119
8. Hu JC, Sadeghi P, Pinter-Brown LC, Yashar S, Chiu MW (2007) Cutaneous side effects of epidermal growth factor receptor inhibitors: clinical presentation, pathogenesis, and management. J Am Acad Dermatol 56(2):317–326
9. Lacouture ME (2006) Mechanisms of cutaneous toxicities to EGFR inhibitors. Nat Rev Cancer 6(10):803–812
10. Ocvirk J, Heeger S, McCloud P, Hofheinz RD (2013) A review of the treatment options for skin rash induced by EGFR-targeted therapies: evidence from randomized clinical trials and a meta-analysis. Radiol Oncol 47(2):166–175
11. Perez-Soler R, Zou Y, Li T, Ling YH (2011) The phosphatase inhibitor menadione (vitamin K3) protects cells from EGFR inhibition by erlotinib and cetuximab. Clin Cancer Res 17(21):6766–6777
12. Ocvirk J (2010) Management of cetuximab-induced skin toxicity with the prophylactic use of topical vitamin K1 cream. Radiol Oncol 44(4):265–266
13. Pinta F, Ponzetti A, Spadi R, Fanchini L, Zanini M, Mecca C et al (2014) Pilot clinical trial on the efficacy of prophylactic use of vitamin K1-based cream (Vigorskin) to prevent cetuximab-induced skin rash in patients with metastatic colorectal cancer. Clin Colorectal Cancer 13(1):62–67
14. Tomkova H, Pospiskova M, Zabojnikova M, Kohoutek M, Serclova M, Gharibyar M et al (2013) Phytomenadione pretreatment in EGFR inhibitor-induced folliculitis. J Eur Acad Dermatol Venereol 27:514–519
15. Eriksen JG, Steiniche T, Alsner J, Askaa J, Overgaard J (2004) The prognostic value of epidermal growth factor receptor is related to tumor differentiation and the overall treatment time of radiotherapy in squamous cell carcinomas of the head and neck. Int J Radiat Oncol Biol Phys 58:561–566
16. Lacouture ME, Anadkat MJ, Bensadoun RJ, Bryce J, Chan A, Epstein JB et al (2011) Clinical practice guidelines for the prevention and treatment of EGFR inhibitor-associated dermatologic toxicities. Support Care Cancer 19(8):1079–1095
17. Abdelmohsen K, Patak P, Von MC, Melchheier I, Sies H, Klotz LO (2004) Signaling effects of menadione: from tyrosine phosphatase inactivation to connexin phosphorylation. Methods Enzymol 378:258–272
18. Beier JI, Von MC, Sies H, Klotz LO (2006) Activation of ErbB2 by 2-methyl-1,4-naphthoquinone (menadione) in human keratinocytes: role of EGFR and protein tyrosine phosphatases. FEBS Lett 580(7):1859–1864
19. Lopes LB, Speretta FF, Bentley MV (2007) Enhancement of skin penetration of vitamin K using monoolein-based liquid crystalline systems. Eur J Pharm Sci 32(3):209–215

20. Camarasa JG, Barnadas M (1982) Occupational dermatosis by vitamin K3 sodium bisulphite. Contact Dermatitis 8(4):268
21. Wehler TC, Graf C, Mohler M, Herzog J, Berger MR, Gockel I et al (2013) Cetuximab-induced skin exanthema: prophylactic and reactive skin therapy are equally effective. J Cancer Res Clin Oncol 139(10): 1667–1672
22. Perez-Soler R, Zou Y, Li T, Ling YH (2013) Retraction: the phosphatase inhibitor menadione (vitamin K3) protects cells from EGFR inhibition by erlotinib and cetuximab. Clin Cancer Res 19:4901–4902
23. Pfeiffer P, Nielsen D, Bjerregaard J, Qvortrup C, Yilmaz M, Jensen B (2008) Biweekly cetuximab and irinotecan as third-line therapy in patients with advanced colorectal cancer after failure to irinotecan, oxaliplatin and 5-fluorouracil. Ann Oncol 19(6):1141–1145