

Association of creatine kinase and skin toxicity in phase I trials of anticancer agents

V Moreno Garcia^{1,2}, P Thavasu^{1,3}, M Blanco Codesido^{1,2}, LR Molife^{1,2}, J Vitfell Pedersen^{1,2}, M Puglisi^{1,2}, B Basu^{1,2}, K Shah^{1,2}, J Iqbal², JS de Bono^{1,2,3}, SB Kaye^{1,2,3} and U Banerji^{*,1,2,3}

¹Drug Development Unit, Division of Cancer Therapeutics and Division of Clinical Studies, The Institute of Cancer Research, 15 Cotswold Road, Sutton SM2 5NG, UK; ²The Royal Marsden NHS Foundation Trust, Drug Development Unit, Sycamore House, Downs Road, Sutton SM2 5PT, UK; ³Cancer Research UK Cancer Therapeutics Unit, Division of Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton SM2 5NG, UK

BACKGROUND: We investigated the association between skin rash and plasma creatine kinase (CK) levels in oncology phase I trials. **METHODS:** We analysed data from 295 patients treated at our institution within 25 phase I trials which included CK measurements in the protocol. Trials involved drugs targeting EGFR/HER2, m-TOR, VEGFR, SRC/ABL, aurora kinase, BRAF/MEK, PARP, CDK, A5B1 integrin, as well as oncolytic viruses and vascular disrupting agents.

RESULTS: Creatine kinase measurements were available for 278 patients. The highest levels of plasma CK during the trial were seen among patients with Grade (G) 2/3 rash (median 249 U l⁻¹) compared with G1 (median 81 U l⁻¹) and no rash (median 55 U l⁻¹) ($P < 0.001$). There was a significant reduction in CK after the rash resolved (mean 264.2 vs 100.1; $P = 0.012$) in 25 patients, where serial CK values were available. *In vitro* exposure of human keratinocytes to EGFR, MEK and a PI3Kinase/m-TOR inhibitor led to the increased expression of CK-brain and not CK-muscle or mitochondrial-CK.

CONCLUSION: Plasma CK elevation is associated with development of skin rash caused by novel anticancer agents. This should be studied further to characterise different isoforms as this will change the way we report adverse events in oncology phase I clinical trials.

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The patterns of toxicities caused by anticancer agents have changed with the advent of the development of targeted agents, and myelotoxicity is no longer the predominant limiting toxicity. Skin rash is a frequent side-effect of targeted anticancer drugs including drugs inhibiting the EGFR–RAS–RAF–MEK and PI3 kinase–AKT–m-TOR pathways (Figure 1) (Moore *et al*, 2007; O'Donnell *et al*, 2008; Banerji *et al*, 2010; Flaherty *et al*, 2010; Yap *et al*, 2010; Moreno Garcia *et al*, 2011).

Creatine (Cr) and creatine kinase (CK) have an important role in human soft tissue. In the cell, CK catalyses the reversible phosphorylation between ATP and Cr to produce phosphocreatine (PCr) and ADP, acting as a cytosolic energy buffer. Three predominant isoforms of CK exist with different subcellular locations: two cytosolic isoforms (CK-muscle (CK-M) and CK-brain (CK-B)) and one mitochondrial isoform (mitochondrial-CK (mt-CK)) (Wallimann *et al*, 2011). Cytosolic isotypes form homo- or hetero-dimers with tissue specificity: CK-MM (muscle), CK-MB (heart) and CK-BB (brain and skin) (Schlattner *et al*, 2002). During wound healing, keratinocytes overexpress CK-BB to

cope with the increased metabolism caused by nucleotide biosynthesis (Schlattner *et al*, 2002). We hypothesised that skin toxicity caused by novel anticancer agents was associated with keratinocyte stress, thus leading to increased levels of CK in the skin and possibly circulating CK levels in the plasma. We thus aimed to study the association, if any, of plasma CK in patients who developed skin rash in phase I trials.

METHODS

Trial and patient inclusion

This was a retrospective study including 295 consecutive patients treated within phase I clinical trials at the Drug Development Unit, Royal Marsden Hospital, Sutton, UK. We reviewed 104 trial protocols and found 25 that mandated regular plasma CK measurements. All patients consented to individual phase I clinical trials that were approved by the institutional research ethics committee, and this project was reviewed by the Royal Marsden Hospital Audit Committee.

Data collection

Compliance to CK measurement during the trial was assessed. To assess the dynamics of CK, we included several data points:

*Correspondence: Dr U Banerji; E-mail: Udai.Banerji@icr.ac.uk

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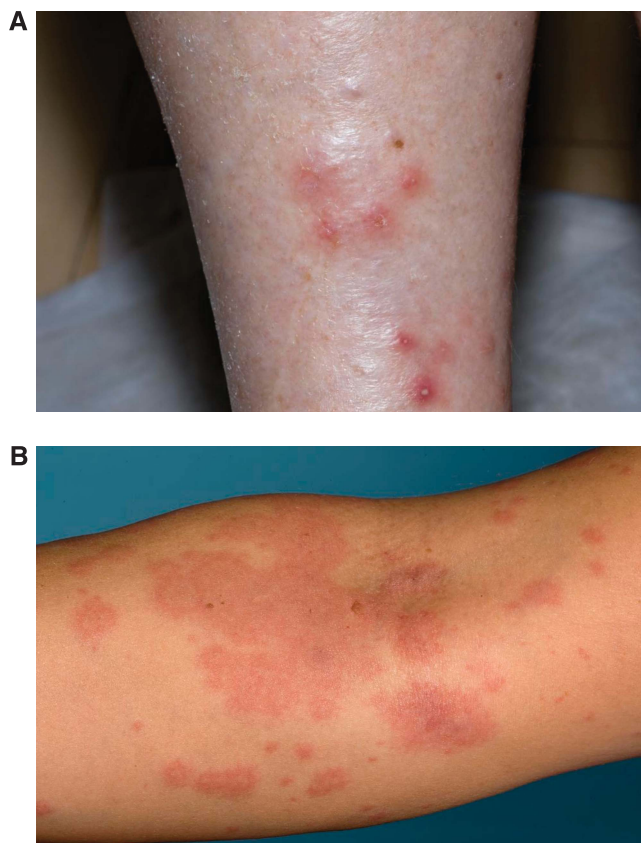


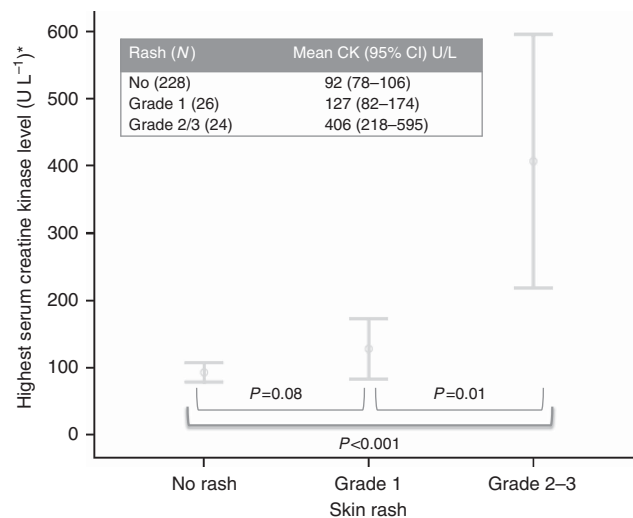
Figure 1 Types of skin rash caused by molecularly targeted agents. Skin rash often causes dose-limiting toxicities in phase I trials of molecularly targeted agents. **(A)** Acneiform skin rash typical of EGFR or MEK inhibitors. **(B)** Maculopapular skin rash typical of PI3K, AKT or m-TOR inhibitors.

baseline CK, first elevation (level and day) and highest level (and day of highest level). A complete list of concomitant medications (such as use of statins) was not available. The baseline plasma CK elevation was defined as levels above 140 U l^{-1} by local laboratory reference ranges. To avoid potential bias due to baseline elevation or significant changes below the threshold of CK elevation, we also calculated the ratio: higher CK-on trial/baseline CK and considered 'increased CK' if the ratio was ≥ 1.5 . The 1.5-fold cutoff was arbitrary. If rash occurred, we attempted to collect CK measurements at time points when the rash resolved, if documented. Representative photographs of skin rash caused by MEK and AKT inhibitors were taken after informed consent from patients and have been anonymised.

Please see Supplementary Data for tissue culture, chemical reagents and western blotting.

Statistical analysis

To compare the difference between changes in plasma CK, and because of the limited number of patients with skin rash, we performed non-parametric tests. Mann-Whitney *U* and Kruskal-Wallis one-way analysis of variance tests were used to compare CK levels with rash when two or three categories were included, respectively. The Wilcoxon test was used to analyse paired CK values at diagnosis and resolution of rash. For categorical variables, χ^2 and Fisher's exact test were used as required. A *P*-value of ≤ 0.05 was considered statistically significant and all tests were two-tailed. SPSS v13.0.1 (IBM, Portsmouth, UK) was used for all the analyses.



*Bars represent 95% confidence interval (CI)

Figure 2 Serum creatine kinase levels and rash grade. Two hundred and seventy-eight patients with available CK measurements were included in the analysis. The highest CK level on study was selected for analysis.

RESULTS

Data from 295 patients were considered in the analysis. CK measurements were available for 278 patients (94%), indicating compliance to protocol. For further analysis of the association of CK and skin rash, only the 278 patients who had CK measurement on file were used. Sixty-four patients (23%) developed a high CK during the study, including 42 (15%) patients who had an $1.5 \times$ elevation compared with baseline during the study. In 25 patients who developed rash, it was possible to find documented paired CK at development and resolution of rash. Fifty patients (18%) developed drug-induced skin rash during the trial (Figure 1).

There was an association between development of rash and CK rise; 17% (39 out of 228) of patients who did not develop skin rash had a CK rise during the trial compared with 35% (9 out of 26) of patients who developed G1 and 67% (16 out of 24) of patients who developed G2/3 rash ($P < 0.001$). The levels of CK during the trial were significantly elevated among patients with G2/3 rash (mean 406 U l^{-1} ; median 249 U l^{-1}) compared with G1 (mean 127 U l^{-1} ; median 81 U l^{-1} ; $P = 0.01$) and no rash (mean 92 U l^{-1} ; median 55; $P < 0.001$; Figure 2). In addition, it was possible to find documented CK when rash developed and when it resolved in 25 cases. There was significant difference in the CK during rash and CK at resolution (mean 264.2 vs 100.1 ; $P = 0.012$).

In vitro analysis

Adult human keratinocytes were exposed to increasing concentrations of an EGFR (gefitinib), PI3K/m-TOR (PI-103) and MEK inhibitor (PD0325901) at increasing doses for 24 h. The expression of CK-B, CK-M and mt-CK was studied in the lysates. There was an increase in the expression of CK-B (Figure 3). CK-M and mt-CK were not detected in the lysates.

DISCUSSION

This is the first time that an association between skin rash and elevated plasma CK in patients treated with different novel anticancer agents has been reported. Further pointers that the association is real are the fact that there was a temporal relationship between the CK levels dropping when the rash

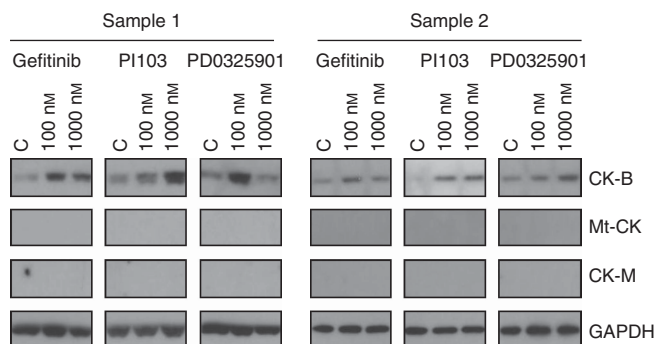


Figure 3 Expression of CK isoforms in human keratinocytes. Expression of the three different isoforms of CK in adult human keratinocytes after exposure to vehicle control and two concentrations (100 nM and 1000 nM) of gefitinib (EGFR inhibitor) and PDO325901 (MEK inhibitor). An increase in CK-B expression was seen after exposure to the three drugs. Mt-CK and CK-M were not detectable in the samples studied. Abbreviation: GAPDH; glyceraldehyde 3-phosphate dehydrogenase.

resolved in a subset of patients where this data was available and the fact that the relationship between skin rash and CK seem to spread across a wide range of tumours and anticancer agents (Supplementary Table 1). However, this study has the limitations of a retrospective analysis and only total CK and not its specific isoforms were measured. CK is known to be elevated in patients treated with a range of drugs such as statins (Stolcpart *et al*, 2010) and anticancer agents, trabectedin (Stoyianni *et al*, 2011) and imatinib (Gordon *et al*, 2010). In most instances these elevations are thought to be due to inflammation in muscles and relate to CK-MM. CK-BB subtype is usually undetectable in serum but can be responsible for 1–18% of the total CK activity (Skogseid *et al*, 1992; Kovesdi *et al*, 2010). It has also been found to be overexpressed in different tumour cells, such as breast (Zarghami *et al*, 1996) and lung (Niklinski *et al*, 1991) cancers. CK elevation has also been reported in patients with toxic epidermal necrosis. However, it was found that CK elevation of CK-BB fraction represented only 3% of the serum total CK whereas 94% was CK-MM (Caux *et al*, 1994). The present analysis includes total plasma CK measurement and does not differentiate between isotypes.

We conducted preclinical experiments to explore this further. We detected a rise in expression of CK-B in keratinocytes after *in vitro* exposure to EGFR, PI3K/m-TOR and MEK inhibitors. This was consistent with previous reports that there was an increased

expression of CK-BB in keratinocytes, when they were under stress (Schlattner *et al*, 2002), or in exudates within blisters (Paavonen *et al*, 1988; Kiistala *et al*, 1989). It is therefore possible that the rise in plasma CK associated with skin toxicity is due to a rise in CK-BB, and this has not been documented before. Future studies should analyse different circulating isoforms of CK to find the source of the rise of plasma CK associated with skin rash.

These findings have several clinical implications. First, at present, a raised CK would be reported as a musculoskeletal adverse or serious adverse event in phase I trials. However, it is possible that these are manifestations of skin toxicity. Second, these findings may give an insight into the treatment of skin rash. It has previously been shown that repair mechanisms in keratinocytes require ATP. The free energy of ATP is stored and transported in the form of PCr, where CK activity can replenish cellular ATP *in situ* and exogenous Cre protected against free radical stress (Lenz *et al*, 2005). On the basis of the observations that skin rash is associated with a raised CK and keratinocytes show increased expression of CK (CK-B) after exposure to different signal transduction agents, it could be hypothesised that topical Cre supplementation (to increase the PCr cytosolic energy buffer in keratinocytes) may be useful to prevent or treat rash caused by novel anticancer agents. Indeed, topical Cre has been investigated for the treatment of skin damage associated with UV light (Lenz *et al*, 2005; Knott *et al*, 2008). In conclusion, this is the first report of an association of skin rash and total circulating CK in the setting of phase I trials of novel anticancer agents. This has a number of clinical implications that include adverse event reporting and the clinical management of rash. These findings merit further prospective evaluation in clinical trials.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary Information accompanies the paper on British Journal of Cancer website (<http://www.nature.com/bjc>)

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