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Epidermal growth factor receptor polymorphisms and risk for toxicity in paediatric patients treated with gefitinib

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ABSTRACT

Purpose: To investigate associations between germline genetic variations in the epidermal growth factor receptor (EGFR) and toxicity in paediatric patients treated with gefitinib.

Patients and methods: Gefitinib treatment and toxicity data from five paediatric clinical trials were combined. *EGFR* genotypes evaluated included –191C>A, –216G>T, Arg497Lys and intron 1 CA sequence repeat number. The genetic variations were evaluated for associations with grade one or greater rash or diarrhoea during the first course of treatment.

Results: The analysis included 110 patients, 60 (55%) with grade one or greater rash and 47 (43%) with grade one or greater diarrhoea. Among patients with the –216 GG ($n = 51$), GT ($n = 41$) and TT ($n = 16$) genotypes, grade one or greater rash occurred in 52.9%, 46.3% and 87.5% of patients ($p = 0.003$, recessive model), respectively. Diarrhoea occurred in 27.5%, 58.5% and 43.8% of patients with respective GG, GT and TT genotypes ($p = 0.004$, dominant model). The –191C>A, intron 1 CA repeat number and Arg497Lys genotypes were not significantly associated with either rash or diarrhoea. EGFR –216 and –191 polymorphisms were in linkage disequilibrium ($D' = 0.66$, $p = 0.01$). The haplotype (–191C, –216T) was associated with increased risk for rash ($p = 0.049$), but was not more predictive of rash than the single –216 polymorphism.

Conclusion: These findings indicate that *EGFR* –216G>T genotype is a predictive marker for the development of skin rash and diarrhoea in paediatric patients treated with gefitinib. Continued investigation of relationships between germline EGFR polymorphisms and the efficacy of EGFR inhibitors in paediatric patients is warranted.

1. Introduction

The human epidermal growth factor receptor (EGFR) plays an important and diverse role in cellular signalling with influences on cellular proliferation, apoptosis, angiogenesis and metastasis.[1] In cancer cells, activation of EGFR and subsequent tyrosine kinase phosphorylation of the intracellular domain leads to a series of intracellular signals, resulting in

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increased tumour cell growth, division and resistance to apoptosis.[2] Inhibition of EGFR intracellular signalling with 4-anilinoquinazoline derivatives (e.g. erlotinib and gefitinib), which function as EGFR tyrosine kinase inhibitors (TKIs) has arisen as an effective anticancer treatment strategy.[3]

Despite multiple influences on cancer cell growth and division, inhibition of the EGFR pathway with TKIs only produces clinical responses in subgroups of patients.[4] Factors associated with sensitivity to EGFR TKIs include EGFR amplification and activating mutations influencing the ATP-binding pocket of the tyrosine kinase domain.[5–8] However, neither amplification nor mutational status is sufficient to completely explain clinical responses to EGFR TKIs. The efficacy of anti-EGFR therapy has also been associated with the intensity of skin rash, one of the major side-effects of EGFR-targeted drugs.[9] Germline genetic variation in the expression and function of the *EGFR* gene may, in part, predict the probability of systemic responses such as rash and diarrhoea in patients and may also influence disease response in patients treated with EGFR TKIs.[10–13]

The *EGFR* gene, located at 7p12.3-p.1, contains multiple polymorphic variants.[12] Several of these variants lead to alterations in *EGFR* expression and signalling. The *EGFR* –216G>T polymorphism is located in the promoter region and influences binding of Sp1, a transcription factor essential to *EGFR* expression.[2] The –216T allele increases promoter activity and expression of *EGFR*.[14] Expression is also influenced by the –191C>A polymorphism in the promoter region.[14] Intron 1 contains a dinucleotide CA repeat (CA[*n*]). The number of CA repeats is inversely related to *EGFR* expression, with the most common CA repeat number being 16.[15,16] An additional variation located at codon 497 (Arg497Lys) results in an amino acid change in the extracellular domain of EGFR with considerable effects on ligand binding.[17]

Recent data from adult trials revealed significant associations between germline *EGFR* genetic variations and responses to EGFR TKIs including toxicity and disease response to therapy.[10–13,18] Continuing investigations indicate a growing potential for EGFR TKIs in treating paediatric solid tumours.[19–21] However, the relationship between phenotypic responses to EGFR TKIs and germline genetic variation has not been evaluated in a paediatric population. The aim of this study was to analyse the association between *EGFR* genotypes (*EGFR* –216G>T, *EGFR* –191C>A, intron 1 CA(*n*) and Arg497Lys) and the common toxicities of rash and diarrhoea in paediatric patients treated with the EGFR TKI, gefitinib.

2. Patients and methods

2.1. Study design and treatment

Patients who consented to provide blood samples for pharmacogenetic analysis were included in this study if adequate DNA was available for *EGFR* genotyping, complete toxicity information was available from clinical trial data and at least one full course of gefitinib therapy was completed on one of the five clinical trials evaluating gefitinib in paediatric solid tumours (Table 1). These included three phase one trials in which gefitinib was administered concomitantly with irinotecan. In the other two studies, gefitinib was administered as a single agent. Toxicity and treatment data in this analysis were limited to the first course of treatment, 21 d for studies 1, 2 and 3 and 28 d for studies 3 and 4. St. Jude Children's Research Hospital Institutional Review Board approved the subsequent genotyping and retrospective collation and analysis of clinical trial data. Study personnel who were unaware of the genotyping results performed the chart review for toxicity.

2.2. Drug administration

Gefitinib was administered orally once daily to all patients. In two studies (Nos. 4 and 5) gefitinib was administered as a single agent continuously for 28-day cycles. In three studies (Nos. 1, 2 and 3), gefitinib was administered days 1 to 12 of 21-day cycles, while irinotecan was administered concomi-

Table 1 – Characteristics of clinical trials from which data were derived.

Study number	Disease	Gefitinib dosage	Gefitinib schedule	Irinotecan dosage
1 Phase I (NCT00186979)	Refractory solid tumours	112.5, 150 mg/m^2	Days 1–12	15, 20 mg/m^2[a]
2 Phase I (NCT00132158)	Refractory solid tumours	150 mg/m^2	Days 1–12	10, 15 mg/m^2[b]
3 Phase II (NCT00135135)	Advanced, high-risk neuroblastoma	112.5 mg/m^2	Days 1–12	15 mg/m^2
4 Phase I (NCT00040781)	Refractory solid tumours	150, 300, 400, 500 mg/m^2	Days 1–28	–
5 Phase I/II (NCT00042991)	Newly diagnosed brain stem tumours or incompletely resected supratentorial malignant gliomas	100–375 mg/m^2	Days 1–28	–

[a] Irinotecan was administered intravenously with the exception of one oral dose during the first course.
[b] Irinotecan was administered orally with the exception of one intravenous dose during the first course.