STUDY SYNOPSIS

Name of Sponsor/Company

AstraZeneca

Study Number

BCIRG 103 (1839 IL/0219)

Title

A presurgical study to evaluate molecular alterations that occur in human breast cancer tissue and normal skin after short term exposure to ZD1839 (Iressa™) and to correlate these alterations with pharmacokinetic parameters

Study period

Patient registration: 15 Jul 2003 to 22 Dec 2004

Patient treatment: 17 Jul 2003 to 10 Feb 2005

Phase of the Study II

Objectives

The primary objective was to identify the molecular alterations which occur in human breast cancer tissue after short term exposure to ZD1839 (IRESSA™).

The secondary objectives were:
- To evaluate the molecular effects of short term ZD1839 exposure in normal skin tissue and on EGFR signaling pathways in non-tumor cells (skin tissue).
- To correlate the pharmacokinetic parameters with molecular alterations detected in breast cancer tissue and normal skin following study treatment.
- To evaluate the tolerability and safety of short term exposure to a daily oral single dose of 250 mg ZD1839 administration.

For patients having consented to the optional sub-study, another secondary objective was to determine the intra-tumoral concentrations of ZD1839 and to correlate these with pharmacokinetic parameters and molecular alterations detected in breast cancer tissue.

Study design

This was a prospective, open-label, non-comparative study.

Number of subjects planned/enrolled

The study was designed to analyze 40 fully evaluable patients (including clinical evaluability, pharmacokinetic, tumor and skin samples evaluability).

The non-evaluability rate was initially estimated to be 20%. A maximum of 50 patients were to be recruited in order to obtain 40 fully evaluable patients.

In June 2004, on the basis of the actual non-evaluability rate (41%), the maximum number of recruited patients was increased to 65 in order to obtain 40 fully evaluable patients.

The sponsor decided to stop the patient recruitment on 30 December 2004. A total of 59 patients were actually enrolled in the study.

Study population

Women, with histologically-proven, operable and untreated adenocarcinoma of the breast.

Treatments

ZD1839 250 mg was administered orally once daily for between 14 and 45 days.

Analyses variables

Primary evaluation parameter

The primary evaluation parameter was the measure of molecular alterations in human breast cancer tissue. This was performed by comparing the tissue samples obtained prior to the start of study treatment and at the time of definitive surgery.

Microarray technology was used to identify the abundance of a wide variety of genes at mRNA level. Evaluation of ER mRNA, PR mRNA, HER2 mRNA from pre-treatment samples enabled categorisation of patients into their molecular subtypes. Expression of genes that altered following ZD1839 treatment were inferred from comparison of the post-ZD1839 samples to the pre-ZD1839 samples. Three molecular response groups were identified based on changes in Ki67 and related cell cycle gene sets as follows: (1) molecular proliferation, (2) molecular inhibition, and (3) no change.

Secondary evaluation parameter

Secondary evaluation parameters included :

- Measure of molecular alterations in normal skin tissues. This was performed by comparing the tissue samples obtained prior to the start of study treatment and at the time of definitive surgery.
- The steady state trough plasma concentration ($C_{ss\ min}$). It was calculated from plasma samples obtained just before the last dose of ZD1839 and 24 hours later.

The optional sub-study parameter was the measure of ZD1839 concentration in human breast cancer tissues collected at the time of definitive surgery.

In addition when sufficient tissue remained, skin samples were assayed to determine ZD1839 concentration at the time of definitive surgery.

Safety Parameters

Adverse events were reported according to the National Cancer Institute Common Toxicity Criteria, version 2.0 (NCI-CTC v 2.0) and its corresponding grading system. All terms were coded using the MedDRA terminology (version 6.1) and their severity was reported as 1: mild, 2: moderate, 3: severe, and 4: life-threatening.

Hematology and blood chemistry toxicity corresponding to abnormal blood counts, renal or hepatic functions during treatment were analyzed according to the NCI-CTC v 2.0.

Statistical methods

The molecular alterations in tumor and skin were determined by comparisons between pre-treatment and post treatment samples. The other variables included steady state trough plasma concentration (Css min) as well as concentration of ZD1839 in tumor and skin. Relations between the parameters were explored graphically in the first instance. Further analyses by regression techniques were conducted if the data suggested a correlation.

Results – Study Subjects

The fifty nine patients registered in the trial all met the eligibility criteria. Seven patients (12%) did not receive study treatment as per protocol: 1 patient withdrew consent and did not receive study treatment, 3 patients received less than 14 days of treatment, 1 patient did not receive 10 consecutive days of study treatment before surgery and 2 patients had definitive surgery more than one day after completing study treatment. Analyses were to be conducted on patients who received study treatment as per protocol and for whom adequate samples were available:

- 39 patients (66%) are evaluable for molecular alterations in tumor samples
- 52 patients (88%) are evaluable for molecular alterations in skin samples
- 43 patients (73%) are evaluable for pharmacokinetic parameters

IHC for the majority of parameters could not be performed with confidence due to the inability to validate the assays due to the lack of suitable reagents and protocols.

Results – Biomarkers

Microarray technology identified a large number of genes at the mRNA level, which changed following short-term exposure to ZD1839. Comparison of the gene expression changes (including Ki67 at the mRNA level) with those observed in a previously collected panel of breast cancer cell lines enabled molecular growth response to ZD1839 exposure to be categorized as molecular proliferation, no change, or molecular inhibition (Table S1):

- Amongst patients with ER mRNA+ disease, molecular inhibition was primarily seen in patients having weak/low PR mRNA levels, with very little inhibition in the ER mRNA+/PR mRNA strong subgroup. Molecular inhibition was also seen in some HER2 mRNA+ patients
- Molecular proliferation was observed in ER mRNA+ tumours with strong PR mRNA. No proliferation was seen in the HER2 mRNA+ subgroups.
- The categorisation of patients in terms of ER and PgR status as originally defined by mRNA level was subsequently confirmed at the protein level by IHC.
- These findings support the hypothesis emerging in the literature, that lower PR levels in ER+ breast cancer may be a marker for a tumour that is dependent on peptide growth factor signaling and thus is likely to be sensitive to EGFR TKIs

Table S1: Molecular response by ER/PR/HER2 status

Tumour classification	Inferred molecular growth response to gefitinib based on mRNA Ki67 and cell cycle gene sets			
	inhibition	No change or unclassified	proliferation	total
ER+, PR strong/moderate (by mRNA)	2 (14%)	6 (43%)	6 (43%)	14
ER+, PR weak/negative (by mRNA)	5 (31%)	11 (69%)	0	16
ER-,PR-	2 (29%)	1 (14%)	4 (57%)	7
HER2+	2 (33%)	4 (67%)	0	6
Total	11 (26%)	22 (51%)	10 (23%)	43

Evaluation of molecular effects of ZD1839 on the EGFR signaling pathways in both tumour and normal skin at the protein level was not possible due to the inability to validate IHC assays for the phosphorylated proteins.

No correlation was observed between Ki67 by immunohistochemistry and ZD1839 concentrations, although limitations of the assay may have compromised this analysis. However, no correlation was seen between Ki67 by mRNA change and ZD1839 concentration either.

From the optional sub-study, concentrations in both tumour and skin were higher than in plasma, with no relationship observed between skin and tumour concentrations. The mean ratio of concentrations, skin:plasma and tumour:plasma were 10.7 and 38.3 respectively.

Results – Safety

Fifty eight patients (98.3%) received study treatment and were therefore evaluable for safety analyses. At least one adverse event was reported for forty five (77.6%) patients. Thirty five patients (60.3%) experienced at least one adverse event related to study treatment.

The most common adverse events related to study treatment (regardless of severity grade), in the order of decreasing frequency, were: rash (31.0%), diarrhoea (19.0%), pruritus (12.1%), nausea (6.9%) and fatigue (5.2%). Other adverse events related to study treatment were infrequent (< 5%).

Adverse events related to study treatment resolved upon treatment completion except for three patients (mild fatigue and skin toxicity).

The majority of drug related AE were mild (CTC grade 1). The only serious adverse events related to study treatment were reported for patient 062001 (grade 3 diarrhoea and grade 2 rash/ desquamation). These also represent the only case of treatment discontinuation due to adverse event. Moreover, treatment interruption because of AE was reported for 2 patients (skin toxicities).

Hematological and biochemical parameters were tested as per protocol for fifty six patients (96.5%). Anemia was reported in six patients, one of whom developed grade 3 anaemia between baseline and pre-surgical samples and persisted in a further follow up sample. No explanation was found for this. Alkaline phosphatase, creatinine and total bilirubin levels were reported as elevated (grade 1) in four patients. ALAT and ASAT activities were elevated in six and four patients respectively. One patient developed grade 3 increases in ASAT and ALAT activities but the date of sampling suggested that these investigations had been performed after her operation. Tissue damage during surgery was therefore the likely explanation.

There were no reports of deaths or second cancers during the course of this study. Overall, the safety profile was consistent with those reported from previous studies of ZD1839 at the dose of 250mg/day.