Exploratory Study Report Synopsis

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EGFR Mutation Blood Test Evaluation and Assessment of Changes in Sensitivity Resulting from Initiation of First Line Treatment of Advanced Adenocarcinoma of the Lung Diagnostic (EMERALD) Study

Study dates: First subject enrolled: 28 June 2013
Last subject last visit: 17 July 2014

Phase of development: Therapeutic exploratory (II)

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This study was performed in compliance with Good Clinical Practice, including the archiving of essential documents.

Study centre(s)

This study was conducted at 4 sites in Australia and the primary investigators were:

- Dr Nick Pavlakis, Royal North Shore Hospital, Reserve Road, St Leonards, NSW, 2065.
- Prof. Michaeal Boyer, Royal Prince Alfred Hospital, Missenden Road, Camperdown, NSW, 2050.
- Dr Fiona Abell, Calvary Mater Newcastle, Cnr Edith and Platt Streets, Waratah, NSW, 2298.
- Dr Janine Lombard, Newcastle Private Hospital, 14 Lookout Road, New Lambton Heights, NSW, 2305.

Publications

There are none at the time of writing this report.

Objectives and criteria for evaluation

The primary objective of this study was to determine the concordance between EGFR mutation status reports (i.e. positive or negative) obtained for the same patients via tissue and blood-based testing.

The secondary objectives of this study were:

- To determine the proportion of patients reporting EGFR mutation positive on tissue report and negative on plasma in the pre-treatment setting.
- To determine the proportion of patients reporting EGFR mutation positive on plasma report and negative on tissue in the pre-treatment setting.
- To determine the prevalence of EGFR mutations determined by tissue for all patients enrolled and amongst those with evaluable tissue.

The exploratory objectives of this study were:

- To investigate the proportion of tissue EGFR positive and plasma EGFR negative patients (pre-treatment) who test plasma EGFR positive after commencement of treatment.
- To determine the proportion of patients reporting EGFR mutation positive and negative for blood plasma was the same in the pre-treatment and post-treatment setting.

Study design

This study evaluates the level of concordance that exists between EGFR mutation status (positive or negative) via tissue and blood-based testing.

Patients provided two blood samples during the course of the study for validation of a blood-based test for EGFR mutation. Blood samples were collected before drug therapy and another taken between 14 to 21 days after drug therapy commenced. Blood samples were sent to a

central laboratory where DNA was extracted and analysed for epidermal growth factor receptor (EGFR) gene mutations.

Target subject population and sample size

The EMERALD study enrolled patients with Stage IIIB or IV adenocarcinoma of the lung. Data from publications of previous blood based testing suggests that the sensitivity of this platform likely to be between 50 and 75% and the tissue based testing was estimated at 80-

95%. A sample size of 185 was adequate to provide a minimum of 80% power at the expected testing platform sensitivity levels quoted.

The study design included an initial feasibility phase (n=40) to determine if (1) recruitment to the full sample size of 185 patients was possible and (2) concordance of EGFR gene mutation positive blood results vs. EGFR gene mutation positive results was above 30%. Despite a decision to extend the timeframe of this initial phase, recruitment of patients was below the threshold to continue the study. At study discontinuation 12 months after initiation of the study, only 30 patients had been enrolled.

Statistical methods

Statistical analyses were performed using SAS® software (Version 9.4). All statistical tests were performed at the 5% significance level unless otherwise stated.

The primary endpoint evaluated the level of concordance between EGFR mutation status reports (i.e. positive or negative) obtained for the same patients via tissue and blood-based testing using the Kappa statistic. The diagnostic accuracy of the blood based EGFR mutation status was measured by comparing the results from the blood-based tests and tissue results using a number of diagnostic measures: sensitivity, specificity, positive predictive value (PPV) and negative predictive value. The 95% confidence intervals for the sensitivity, specificity and predictive values were calculated using the Clopper-Pearson method.

Subject population

All 30 patients enrolled into the study had a tissue biopsy confirming their EGFR status and their pre-treatment EGFR mutation status confirmed with blood plasma. Of these patients, 28 had their post-treatment EGFR mutation status confirmed with blood plasma. These patients were recruited by 4 centres and 2 patients withdrew, one patient withdrew due to progression of their disease and the other patient died.

There was an equal distribution of of males and females and the majority of patients were of white race (86.7%). The median age was 65.5 years (range: 42 years to 84 years) and only 3 (10%) patients never smoked. The mean BMI was 25.3kg/m^2 (SD: 5.05 kg/m^2) and there were 7 (23.3%) patients with a ECOG PS of 0, 22 (73.3%) patients with a ECOG PS of 1, and 1 (3.3%) patient with a ECOG PS of 2. The majority of physical examination systems were reported as normal for each patient at baseline.

Adenocarcinoma (NOS) was the histological cancer type for 29 (96.7%) patients and adenocarcinoma: brochoalveolar was the histological cancer type for 1 (3.3%) patient. Malignant pleural infusion was absent for the majority of patients (80%). There were twenty-five (83.3%) patients with stage IV classification and five (16.7%) with stage IIIb classification.

Summary of efficacy results

Primary Outcome: Concordance between EGFR biopsy and blood plasma

Table S1 evaluates the level of concordance between EGFR mutation status reports obtained for the same patients via tissue and blood-based testing pre and post treatment using this minimal dataset.

Table S1: Contingency Table of Plasma EGFR Mutation Reports Pre and Post Treatment and Tissue Biopsy

Protocol: EMERALD
Population: Intent-to-Treat

	Tissue Biopsy		
	Negative	Positive	Total
Blood Plasma (Pre Treatment)			
Negative	24 (80.00%)	1 (3.33%)	25 (83.33%)
Positive	0 (0.00%)	5 (16.67%)	5 (16.67%)
Total	24 (80.00%)	6 (20.00%)	30 (100.00%)
Blood Plasma (Post Treatment)			
Negative	22 (78.57%)	3 (10.71%)	25 (89.29%)
Positive	0 (0.00%)	3 (10.71%)	3 (10.71%)
Total	22 (78.57%)	6 (21.43%)	28 (100.00%)

Of the 30 patients reporting pre-treatment EGFR biopsy and blood plasma results, there were 24 (80.0%) patients who were EGFR negative. There were 5 patients (16.7%) who had EGFR positive results for both the biopsy and blood plasma. However, there was one (3.3%) patient with EGFR positive via tissue biopsy testing and EGFR negative for blood plasma.

Of the 28 patients reporting pre-treatment EGFR biopsy and post-treatment blood plasma results, there were 22 (78.6%) patients who were EGFR negative. There were 3 patients (10.7%) who had EGFR positive results for both the biopsy and blood plasma. There were 3 (10.7%) patients with EGFR positive via tissue biopsy testing and EGFR negative via blood plasma testing. Zero patients had a negative tissue biopsy and a positive blood plasma (pre and post treatment) result.

The Kappa statistic was used to test concordance between EGFR mutation status reports using the pre-treatment tissue biopsy and blood based testing. The kappa coefficient was 0.8889 (95% CI: 0.6761 – 1.000) which is highly significant (P-value: <0.001). This implies there

was substantial agreement in the EFGR status between the two testing methods for pre-treatment tissue biopsy and pre-treatment blood based testing.

The kappa coefficient was 0.6111 (95% CI: 0.2280 – 0.9942) which is significant (P-value: 0.0004) for determining EGFR status using pre-treatment tissue biopsy and post-treatment blood testing. This also implies there was moderate agreement between the two testing methods for pre-treatment tissue biopsy and post-treatment blood based testing.

The diagnostic accuracy of the blood based EGFR mutation status was measured by comparing the results from the blood-based tests and tissue results using a number of diagnostic measures: sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

- Sensitivity refers to the test's ability to correctly detect patients who are identified as EGFR mutation positive. The proportion of patients with a positive tissue biopsy who were correctly identified as EGFR mutation positive based on the pre-treatment blood plasma result was 83.3% (95% CI: 35.88% to 99.58%).
- Specificity refers to the test's ability to correctly detect patients who were identified as EGFR mutation negative. The proportion of patients with a negative tissue biopsy who are correctly identified as EGFR mutation negative based on pre-treatment blood plasma result was 100% (95% CI: 85.75% to 100.00%).
- The positive predictive value is the proportion of patients with a positive pre-treatment blood plasma result who were correctly predicted as EGFR mutation positive based on the tissue biopsy. The estimate was 100% and 95% CI: 47.82% – 100.00%.
- The negative predictive value is the proportion of patients with a negative pre-treatment blood plasma result who were correctly predicted as EGFR mutation negative based on the tissue biopsy. The estimate was 96% and 95% CI: 79.65% – 99.90%.

Secondary Efficacy Variables

Table S1 summarises the results for the secondary endpoints.

Table S2: Summary of Secondary Endpoints

Protocol: EMERALD
Population: Intent-to-Treat

Endpoint	Estimate
EGFR Mutation (+) on the tissue and (-) on blood plasma	16.7 (%)
EGFR Mutation (+) on blood plasma and (-) on tissue	0.0 (%)
Prevalence of EGFR mutations on tissue	20.0 (%)

- The proportion of patients reporting EGFR mutation positive on the tissue report and negative on plasma in the pre-treatment setting was 16.7%.
- The proportion of patients in the pre-treatment setting reporting EGFR mutation positive via plasma and negative via tissue was 0.0%.
- The prevalence of EGFR mutations determined by tissue for all patients enrolled and amongst those with evaluable tissue was 20% in the pre-treatment setting.

Exploratory Variable

For blood plasma, the proportion of patients reporting EGFR mutation positive and negative was summarised for the same patient in the pre-treatment and post-treatment setting (Table S3).

Table S3: Contingency Table of Blood Plasma Pre and Post Treatment

Protocol: EMERALD
Population: Intent-to-Treat

	Blood Plasma (Post Treatment)		
Blood Plasma (Pre Treatment)	Negative	Positive	Total
Negative	23 (82.14%)	0 (0.00%)	23 (82.14%)
Positive	2 (7.14%)	3 (10.71%)	5 (17.86%)
Total	25 (89.29%)	3 (10.71%)	28 (100.00%)

Subjects E0302007 and E0303016 did not have a post treatment blood sample measurement

Of the 28 patients reporting pre and post blood plasma results, there were 23 (82.14%) patients who were EGFR negative in both. There were 3 patients (10.71%) who had EGFR positive results for both pre and post blood plasma. However, there were 2 (7.14%) patients with EGFR positive pre-treatment and EGFR negative post-treatment.

The kappa coefficient was 0.7113 (95% CI: 0.3422 – 1.000) and the p-value for the McNemars test was P= 0.1573. This implies there is insufficient evidence to conclude agreement in the EFGR status between the pre and post-treatment blood based testing.

Summary of safety results

There were no serious adverse events reported during the study. There was one patient death reported during the study due to disease progression and was not related to the study procedures.

As a result of slow recruitment in the initial feasibility phase, the decision was made not to continue with the study.