Changes in plasma EGFR mutation levels in response to therapy

For 51 patients in the full study cohort, a plasma sample collected 15 days after treatment initiation was available to assess early changes in EGFR mutant plasma levels in response to treatment with rociletinib. Sufficient plasma was available to conduct an analysis with the EXO1000 platform only. All reported responses were investigator-assessed and confirmed responses per RECIST1.1 guidelines.

Among the 9 patients with progressive disease (PD) as best confirmed RECIST1.1 response, 7 showed modest to no decrease in the level of circulating activating EGFR mutation by EXO1000 after 15 days of treatment (Supplement Fig. S12.1). Of note, for 2 cases with an unexpectedly robust decrease in plasma mutation levels, also the radiographic scans showed a maximal 50% decrease in target lesions. However, both patients were classified as having PD as best response because of the development of new lesions in the CNS. Patients with PD as best response showed a small, non-significant decrease from baseline (median of 670 copies/mL) to Day15 (median of 145 copies/mL) for activating mutations (Wilcoxon matched-pairs signed rank test: p = 0.37). In contrast, patients with partial response (PR) as best confirmed RECIST1.1 response (n = 20) showed a stronger, significant decrease (p <0.001) in the level of activating mutations from baseline (median = 685 copies/mL) to Day15 (median = 10 copies/mL). The data for the T790M resistance mutation follows the same trend as for activating mutations (Supplementary Fig. S2B), with the most significant decrease in detected mutations observed for patients with PR as best response (p <0.001).
Supplementary Figure S12.1. Association between EGFR mutations and response to treatment.

Plasma mutation level measured in exoNA (EXO1000) of patients with active disease after first line treatment (baseline) and after 15 days of 500 mg / 625 mg / 750 mg BID HBr rociletinib. All reported responses were investigator-assessed and confirmed per RECIST 1.1 guidelines.

(A) Changes in activating EGFR mutation levels in plasma for each RECIST category. Dashed lines = patients that developed brain lesions on treatment and are classified with PD, although their target lesions shrank by more than 30%. (B) Changes in EGFR T790M levels in plasma for each RECIST category. Dashed lines = patients that developed brain lesions on treatment and are classified with PD, although their target lesions shrank by more than 30%. p-values were derived from a Wilcoxon signed-rank test.

Abbreviations: PD = progressive disease; SD = stable disease, PR = partial response, RECIST = Response Evaluation Criteria In Solid Tumors; ND = not detected; ns = not significant
Statistical Methods for the diagnostic test

We based the following analysis on activating EGFR mutations only, because T790M wildtype clones can emerge as the dominant source of tumor regrowth upon progression, thus confounding the original signal of response to the treatment. Receiver operating characteristics (ROC) curve analysis was used to assess the clinical performance of the EXO1000 liquid biopsy test platform to predict response to therapy. A confirmed tumor shrinkage in target lesions of greater than or equal to 30%, which is identical to the threshold criteria required for “Response” in target lesions by Response Evaluation Criteria in Solid Tumors (RECIST v1.1), was defined as binary cut-point for the purposes of this exploratory analysis. The relative change of activating EGFR mutant copy numbers pre- and post-Day15 rociletinib treatment was evaluated for its ability to predict significant tumor shrinkage by ROC analysis (Supplementary Figure 12.2). The binary cut-point was chosen as the percentage of activating EGFR mutation change that gave the highest specificity for identifying non-responders, while maintaining a negative predictive value (NPV) of 100%.
Supplementary Figure S12.2. Receiver operating characteristic (ROC) curve analysis

Evaluation of using exoNA (EXO1000) to predict treatment outcome using two consecutive plasma measurements. (A) A ROC curve analysis was performed, based on the decrease in copies of activating EGFR mutations in plasma on Day15 (in percent of baseline) to predict the objective response to rociletinib treatment. In this analysis, response was defined as shrinkage in the sum of the longest diameters (SLD) of target lesions of ≥ 30%, and the resulting ROC curve has an area under the curve (AUC) of 0.85. To define a cut-point for the reduction of circulating mutations to predict patients with a 30% tumor shrinkage the highest specificity for identifying non-responders with a NPV of 100% should have been maintained. The cut-point identified with the ROC curve was at 28% mutant copy decrease from baseline to Day15. (B) Performance of exoNA (EXO1000) in predicting tumor shrinkage in SLD of target lesions in 51 patients. A cut-point of 28% mutant copy decrease was chosen to predict significant tumor shrinkage (≥ 30%). This cut-point would enable us to identify 45% of patients that would have no benefit of the treatment (9/20) with a NPV of 100% (9/9), while not removing any patient who responded to rociletinib (0/31).
Figure S12.3. Response prediction by exoNA-based liquid biopsy (EXO1000). The waterfall plot displays % shrinkage in tumor target lesions. Bars are colored for predicted responders (grey bars: high reduction in mutant copies; pink bars: low or no reduction in mutant copies). Response prediction was performed by EXO1000 at Day15. All reported responses were investigator-assessed and confirmed per RECIST1.1 guidelines.