Improved \textit{EGFR} mutation detection using combined exosomal RNA and circulating tumor DNA in NSCLC patient plasma

A. K. Krug\textsuperscript{1†}, D. Enderle\textsuperscript{1†}, C. Karlovich\textsuperscript{2}, T. Priewasser\textsuperscript{1}, S. Bentink\textsuperscript{1}, A. Spiel\textsuperscript{1}, K. Brinkmann\textsuperscript{1}, J. Emenegger\textsuperscript{1}, D. G. Grimm\textsuperscript{1}, E. Castellanos-Rizaldos\textsuperscript{3}, J. W. Goldman\textsuperscript{4}, L. V. Sequist\textsuperscript{5}, J.-C. Soria\textsuperscript{6,7}, D. R. Camidge\textsuperscript{8}, S. M. Gadgeel\textsuperscript{9,10}, H. A. Wakelee\textsuperscript{11}, M. Raponi\textsuperscript{2}, M. Noerholm\textsuperscript{1} & J. Skog\textsuperscript{3*}

\textsuperscript{1}Exosome Diagnostics GmbH, Martinsried, Germany; \textsuperscript{2}Clovis Oncology Inc., San Francisco; \textsuperscript{3}Exosome Diagnostics Inc, Waltham; \textsuperscript{4}University of California Los Angeles, Los Angeles; \textsuperscript{5}Massachusetts General Hospital, Boston, USA; \textsuperscript{6}Institut Gustave Roussy, Villejuif; \textsuperscript{7}University Paris-Sud, Paris, France; \textsuperscript{8}University of Colorado, Denver; \textsuperscript{9}Karmanos Cancer Institute, Detroit; \textsuperscript{10}Wayne State University, Detroit; \textsuperscript{11}Stanford University, Stanford, USA

\textsuperscript{*}Correspondence to: Dr Johan Skog, Exosome Diagnostics, Inc, 266 Second Ave, Suite 200, Waltham, MA 02451, USA. Tel: +1-617-588-0500; Fax +1-617-588-0580; E-mail: johan@exosomedx.com

\textsuperscript{†}Both authors contributed equally as first authors.

\textbf{Background:} A major limitation of circulating tumor DNA (ctDNA) for somatic mutation detection has been the low level of ctDNA found in a subset of cancer patients. We investigated whether using a combined isolation of exosomal RNA (exoRNA) and cell-free DNA (cfDNA) could improve blood-based liquid biopsy for \textit{EGFR} mutation detection in non-small-cell lung cancer (NSCLC) patients.

\textbf{Patients and methods:} Matched pretreatment tumor and plasma were collected from 84 patients enrolled in TIGER-X (NCT01526928), a phase 1/2 study of rociletinib in mutant \textit{EGFR} NSCLC patients. The combined isolated exoRNA and cfDNA (exoNA) was analyzed blinded for mutations using a targeted next-generation sequencing panel (EXO1000) and compared with existing data from the same samples using analysis of ctDNA by BEAMing.

\textbf{Results:} For exoNA, the sensitivity was 98\% for detection of activating \textit{EGFR} mutations and 90\% for \textit{EGFR} T790M. The corresponding sensitivities for ctDNA by BEAMing were 82\% for activating mutations and 84\% for T790M. In a subgroup of patients with intrathoracic metastatic disease (M0/M1a; \(n = 21\)), the sensitivity increased from 26\% to 74\% for activating mutations (\(P = 0.003\)) and from 19\% to 31\% for T790M (\(P = 0.5\)) when using exoNA for detection.

\textbf{Conclusions:} Combining exoRNA and ctDNA increased the sensitivity for \textit{EGFR} mutation detection in plasma, with the largest improvement seen in the subgroup of M0/M1a disease patients known to have low levels of ctDNA and poses challenges for mutation detection on ctDNA alone.

\textbf{Clinical Trials:} NCT01526928

\textbf{Key words:} liquid biopsy, exosomes, ctDNA, exoRNA, NSCLC, \textit{EGFR}

\textbf{Introduction}

A growing understanding of the molecular complexity of cancer and the role of oncogenic drivers has ushered in the current era of targeted therapies [1]. The molecular analysis required to inform appropriate choice of targeted therapy is typically carried out on tumor tissue acquired from biopsy at diagnosis. However, tumor biopsy has several limitations, including the invasiveness of the procedure and the risk of false-negative results due to tumor heterogeneity or low tumor cellularity. In addition, as many as 49\% of advanced or metastatic non-small-cell lung cancers (NSCLC) do not have accessible tumor tissue [2, 3]. The noninvasive identification of tumor-associated mutations through body fluids such as blood or urine thus represents an attractive alternative to tissue-based methods [4, 5].
Circulating tumor DNA (ctDNA), the component of circulating cell-free DNA (cfDNA) released from tumor sites into the blood of cancer patients, has shown great promise as an alternative to tumor tissue in NSCLC and other cancer indications [6]. This has led to the recent regulatory approval in the United States and Europe of blood-based EGFR companion diagnostic tests [7, 8]. Moreover, highly sensitive and selective technologies have been developed to overcome the inherent challenge posed by the very low fraction of tumor-derived DNA relative to wild-type that is often found in the blood of cancer patients [9]. In this regard, BEAMing (Beads, Emulsion, Amplification, and Magnetics), a technology based on digital PCR, can identify mutations at a mutant allele fraction (MAF) of down to 0.02% and has previously been demonstrated to be among the most sensitive for mutation detection from cfDNA [10], and next-generation sequencing (NGS) technologies are now reaching similar levels (0.05% MAF) [11]. One remaining hurdle is the identification of somatic alterations in patients whose tumor biology does not result in sufficient levels of ctDNA to allow detection. The proportion of patients with detectable ctDNA varies by indication [12], stage of disease [12, 13], tumor burden and other clinical characteristics [14]. In NSCLC, patients with activating EGFR mutations, including deletions in exon 19 and the L858R point mutation in exon 21, are eligible for treatment with various EGFR inhibitors and the first FDA-approved liquid biopsy is available [15]. In patients with relapsed NSCLC, where third-generation EGFR tyrosine kinase inhibitors (EGFR-TKI) are in development or approved for the EGFR T790M resistance mutation, the false-negative rate for T790M is often >30% due to limiting levels of ctDNA in the blood of this patient population [16, 17]. Accordingly, the first FDA-approved Roche cobas® EGFR Mutation Test v2 for T790M achieves a sensitivity of only 58.4% and a specificity of 80.4% [7].

One means to overcome the challenge of mutation detection in patients with limiting ctDNA could be to include the tumor-associated RNA derived from exosomes. Exosomes are small vesicles that are actively released by living cells, including tumor cells [4, 18] and which carry RNA, DNA and proteins [19–22]. The use of exosomal RNA (exoRNA) to identify somatic mutations of tumor origin has previously been demonstrated [19], but the extraction of both exoRNA and ctDNA combined (exoDNA) has not been explored yet and may offer advantages over ctDNA alone. An important feature of exosomes is that nucleic acids in the lumen of these vesicles are protected from nucleases present in plasma and other biofluids, which allows for isolation of intact, good quality RNA [23]. Additionally, exosomes are actively released from living cells as opposed to ctDNA, which is released from dying cells through apoptosis or necrosis [24]. We speculate that the additional mutations present on the exoRNA as well as their origin from actively growing tumor cells could help to improve a plasma-based test for EGFR mutations.

In this study, we analyzed 84 plasma samples from stage IIIB and IV NSCLC patients enrolled in TIGER-X (NCT01526928), a phase 1/2 trial of the third-generation EGFR inhibitor rociletinib. We co-isolated exosomes and ctDNA and extracted the nucleic acids and used a targeted NGS assay (EXO1000) to screen for EGFR mutations. The primary objective was to investigate if the co-isolation of exoRNA and ctDNA would increase copy number and sensitivity of EGFR mutation detection, especially in those patients known to be EGFR mutation positive by tumor biopsy but not identified as such by ctDNA analysis alone. The data were compared with matching tissue data and with matching ctDNA results obtained previously with BEAMing. We also investigated whether changes in EGFR mutation levels during treatment was associated with treatment outcome, by analyzing plasma collected at baseline and after 15 days of therapy.

Materials and methods

Patient selection and collection of plasma samples

All patients (n = 84) were enrolled in TIGER-X (NCT01526928), a phase 1/2 trial of rociletinib in advanced NSCLC patients, and had documented evidence of an activating mutation in the EGFR gene [25] from central laboratory tissue testing (Supplementary material S1, available at Annals of Oncology online). Matched baseline plasma and tissue biopsy samples were collected within 28 days before initiating therapy. A subgroup of patients (subgroup A, n = 56) was chosen from the entire TIGER-X patient population (N = 548) using a randomizer (https://www.random.org). Additional analytically challenging patients (n = 28) were included without randomization that had previously been determined to have low amounts of EGFR T790M ctDNA by BEAMing (< 10 copies/mL). All patients signed an Ethics Committee/Institutional Review Board (EC/ IRB)–approved consent form before enrolment. Further details of the TIGER-X study design have been published previously [25, 26].

Blood samples were collected in K2 EDTA tubes (up to 4 × 6 mL VACuette), processed into plasma within 30 min (1800 g for 10 min at 18–23 °C), and stored at −70 °C or below until further processing.

Plasma nucleic acid extraction and analysis

Extraction and analysis of the plasma samples was carried out in one central laboratory by Exosome Diagnostics GmbH. Plasma samples were prefiltered using a 0.8 μm filter to remove cellular material, platelets and other large debris. Plasma exoNA (exosomal DNA and RNA, along with present ctDNA) was co-isolated from the samples using ExoLution™ Plus extraction technology (Exosome Diagnostics, Inc.) on a median plasma input of 3 mL per patient. ExoLution™ Plus uses spin-columns capturing both cell-free DNA and extracellular vesicles smaller than 0.8 μm in diameter, followed by nucleic acid purification. The captured exosomes are roughly 50–200 nm in size and enriched in known exosomal protein markers [27]. The exoNA was reverse transcribed using VILO™ cDNA Synthesis Kit (Invitrogen) and the cDNA/cDNA mixture was analyzed using EXO1000, a custom, targeted NGS assay (Supplementary material S2, available at Annals of Oncology online). During this process, molecular barcodes, attached to the exon targeting assays, were used for individual sample identification. The libraries were sequenced using 150 bp paired-end reads on a MiSeq System (Illumina CA, USA). Samples were called positive for a mutation if the allelic frequency and copy number passed the thresholds of a predefined assay-specific background model.

For ctDNA analysis by BEAMing (Sysmex Inostics GmbH, Germany) and the cobas® EGFR mutation test v2 (Roche Molecular Systems, Inc.), the ctDNA extraction and subsequent analysis was carried out in separate laboratories as described previously [10, 28]. All laboratories were blinded to the EGFR status and patient characteristics of the samples at the time of analysis.

Results

Patient characteristics

The subjects in this study (n = 84) fell into three partially overlapping subgroups as illustrated in Figure 1. Patients in subgroup A (n = 56) were chosen to be representative of the TIGER-X study population, and an additional n = 28 analytically challenging
Patients were selected based on prior BEAMing data to make up subgroup B ($n=50$) consisting of cases with low-copy T790M (<10 copies/mL). Patients in subgroup C ($n=21$) all have intrathoracic metastatic disease (M0/M1a), a clinical feature which has been shown previously to be especially challenging for EGFR mutation detection using ctDNA [13, 17]. Patient demographics of the three subgroups were similar to each other and to the patient demographics of the entire clinical study population (Table 1).

**Concordance of tumor and plasma mutations**

All 84 plasma samples were tested with EXO1000, using exoNA, and compared with existing data by BEAMing, using ctDNA only. In 54 samples with valid tumor tissue, from subgroup A, the sensitivity of using exoNA (98%, 53/54) was significantly higher ($P=0.004$, Supplementary material S3, available at Annals of Oncology online) than ctDNA (82%, 44/54) for activating EGFR mutations. Similarly, the sensitivity for the T790M mutation was higher for exoNA (90%, 44/49) than for ctDNA (84%, 41/49) (Table 2). This difference in sensitivity between the two methods increased in subgroup B ($n=50$), with sensitivity 81% (39/48) for exoNA and 58% (28/48) for ctDNA for activating EGFR mutations ($P=0.003$), and 61% (23/38) over 53% (20/38) for T790M ($P=0.5$, Supplementary material S4, available at Annals of Oncology online). The difference between the two approaches was greatest in subgroup C ($n=21$) of patients with intrathoracic (M0/M1a) disease, where the sensitivity for activating mutations was 74% (14/19) for exoNA and only 26% (5/19) for ctDNA ($P=0.003$), and 31% (5/16) and 19% (3/16), respectively, for T790M, although not significant ($P=0.5$, Table 2). We also

**Figure 1.** NSCLC patient subgroups. Overview of the $n=84$ patients in this study, in three partially overlapping subgroups: the TIGER-X representative subgroup A, the low copy subgroup B and the M0/M1a subgroup C.
compared a subset of 22 cases to data previously generated using the cobas® EGFR Mutation Test v2 on ctDNA and, consistent with the results from BEAMing, found the sensitivity to be lower than when using exoNA (Supplementary material S5, available at Annals of Oncology online).

The increase in sensitivity when using exoNA was not accompanied by a significant difference in specificity (Supplementary material S6, available at Annals of Oncology online). Notably, among three patients with L858R-negative/del19-positive tumor results and for which exoNA detected L858R, one was also L858R-positive by ctDNA, suggesting that this case was indeed L858R-positive in plasma and might rather have been wrongly assessed by tissue analysis (Supplementary material S7, available at Annals of Oncology online).

Despite the observed differences in sensitivity between the two methods, both had a good overall agreement and especially in patients with a mutation negative or invalid result from tumor, both plasma tests agreed in the majority of cases (Supplementary materials S8 and S9, available at Annals of Oncology online).

### Table 1 Patient characteristics

| Groups analyzed with exoNA | TIGER-X Representative Subgroup A | Low Copy Subgroup B | M0/M1a Subgroup C |
|----------------------------|-----------------------------------|---------------------|------------------|
| N = 548*                  | n = 56                            | n = 50              | n = 21           |
| Median age                | 63 years                          | 63 years            | 60 years         |
| Female                    | 68%                               | 79%                 | 82%              |
| Asian ethnicity           | 21%                               | 16%                 | 14%              |
| ECOG PS grade 0           | 31%                               | 23%                 | 33%              |
| M0 or M1a                 | 27%³                             | 18%                 | 42%              | 100%            |

*The total number of patients treated at therapeutic doses.
³n = 461 patients were assessable for M stage analysis.

ECOG PS, Eastern Cooperative Oncology Group Performance Status.

### Table 2 Concordance between tumor and plasma EGFR status

| Tissue Biopsy Result | Activating⁴ | T790M | Activating | T790M |
|----------------------|-------------|-------|------------|-------|
| TIGER-X Representative Subgroup A (n = 56 total, 54 with valid tumor status) | | | | |
| exoNA (EXO1000)⁵     | +           | 53    | 44         | Sensitivity (exoNA) | 98% | 90% |
| ctDNA (BEAMing)⁵     | +           | 1     | 5          | Sensitivity (ctDNA) | 82% | 84% |
|                     |             |       |            |                   |
| M0/M1a Subgroup C (n = 21 total, 19 with valid tumor status) | | | | |
| exoNA (EXO1000)⁵     | +           | 14    | 5          | Sensitivity (exoNA) | 74% | 31% |
| ctDNA (BEAMing)⁵     | +           | 5     | 11         | Sensitivity (ctDNA) | 26% | 19% |

⁴All activating mutations were EGFR L858R or del19.
⁵P-value (exoNA versus ctDNA) is 0.004 for activating mutations and 0.25 for T790M.

Quantitative comparison of exoNA (EXO1000) versus ctDNA-only (BEAMing)

We next explored the extent to which the addition of exoRNA impacted the overall quantity of EGFR mutations in plasma. Overall, there was a good correlation between plasma EGFR mutant copy numbers in exoNA compared with ctDNA (Figure 2A). However, there were more false negatives in the BEAMing analysis, concordant with some patients having no or very few detectable mutations on ctDNA alone (Figure 2B). There was a significantly higher number of activating EGFR mutation copies in exoNA (234 copies/mL plasma) than in ctDNA alone (24 copies/mL plasma) (Figure 2C). Also, T790M mutation copies were significantly more abundant in exoNA (12 copies/mL plasma) than in ctDNA (6 copies/mL plasma). For both T790M and activating EGFR mutations, the MAF were higher in exoNA than in ctDNA only (Supplementary material S10, available at Annals of Oncology online). Overall, this is in agreement with other studies where the gene copy number as well as the mutation...
Detection is higher on exoNA than on ctDNA, even when analyzed on the same EXO1000 platform (Supplementary material S11, available at Annals of Oncology online).

**Prediction of treatment response using mutation levels in exoNA**

In addition to mutation detection for stratification, liquid biopsies also have the potential to monitor patients’ response to treatment (4) or predicting treatment outcome early, potentially allowing for a change in treatment regimen. To demonstrate the feasibility of such a diagnostic test in our dataset, we examined changes in plasma mutation levels within the first 2 weeks of treatment. Using such a diagnostic test in our dataset, we examined changes in the patients that continue on treatment (100% Negative Predictive Value (NPV), 45% specificity; see Supplementary material S12, available at Annals of Oncology online).

**Discussion**

Tissue biopsy remains the primary method for molecular genotyping in NSCLC but liquid biopsies are becoming an important complement. The limitations of tissue biopsies are well known and include the risk of bleeding, infection and other complications for the patient [29]. The average cost of a lung biopsy has been reported at $14,587 (19% of patients with biopsies had some adverse events) [30]. Recent studies have highlighted the feasibility of liquid biopsies [4, 31], and the FDA recently approved the first ctDNA-based plasma test for EGFR mutations [7]. ctDNA, circulating tumor cells (CTCs) or exosomes derived from tumor cells have all been explored as a means to identify somatic alterations of tumor origin from body fluids [21, 32, 33]. In this study, we have investigated the benefit for liquid biopsies of co-isolating the nucleic acids carried in exosomes, originating from living cells, and ctDNA released by dying cells [18, 24]—in contrast to current liquid biopsy applications that only use ctDNA. To our knowledge, this is the first report that investigates the added value of using a combined exoRNA and ctDNA (exoNA) extraction.

A plasma test that seeks to detect low, emerging fractions of the tumor, monitor recurrence or diagnose early stages of disease is likely to benefit from an isolation method that increases the available molecules by co-extracting exoRNA and ctDNA. One of the subgroups in this study was enriched for low-copy plasma ctDNA samples. In this challenging subgroup of low-copy samples, the sensitivity was much higher using exoNA (81% compared with 58% for ctDNA), and this difference was even more pronounced in subgroup C of patients with intrathoracic disease (M0/M1a cases), showing 74% sensitivity on exoNA for activating mutations compared with 58% on ctDNA.

The low sensitivity of plasma ctDNA-based methods for mutation detection in intrathoracic disease patients (M0/M1a) has been reported previously using a number of different techniques ranging from BEAMing [17], PNA-ZNA-PCR [13], NGS [34], droplet digital PCR (ddPCR) [35], to qPCR [35, 36]. Intrathoracic disease constitutes approximately one-third of all advanced NSCLC cases [17, 37], so this improved performance with exoNA is critical to maximize the clinical sensitivity of the liquid biopsy assay. However, the generally good concordance of both platforms in this study highlights the feasibility of liquid biopsies as an alternative to tissue testing.

A consistently lower sensitivity was observed for the T790M mutation than for activating mutations by both plasma tests in
this study, in line with previous reports [17, 35]. This points to a generally lower allelic load and lower copy number of the T790M resistance mutation compared with the corresponding EGFR driver mutation in plasma, reflecting intra- and intertumoral heterogeneity [38].

Another distinctive feature of the T790M mutation is the apparent lower specificity when compared with tissue. Out of 11 cases that were reported as T790M negative in tumor by central laboratory testing, only 4 were negative both by analysis of exoNA and cDNA alone. For the remaining seven cases, one or both plasma tests detected T790M, as expected for patients progressing after first-line treatment with EGFR-TKI. The occurrence of a relatively high rate of T790M tumor negatives that are consistently plasma positive has been observed before [35, 39] and suggests that tumor heterogeneity or divergent metastatic lesions [40, 41] lead to false-negative results in tissue. While this may well be specific to the emergence of T790M resistance, localized tumor sampling is likely to limit the use of tissue biopsies also in other cases. Ultimately, interventional studies comparing tissue biopsy and liquid biopsy will be needed to establish the clinical utility of both approaches.

The exoNA-based liquid biopsy platform described in this study can be used and will add significant value for any cancer patient where a liquid biopsy is appropriate. It could be especially beneficial in cases with low levels of nucleic acids in circulation, such as patients with low tumor burden, intrathoracic disease or for early detection of cancer.

Acknowledgements

We would like to thank Peter Morello and Seth Yu for review of the manuscript.

Funding

None declared.

Disclosure

AKK, DE, TP, KB, DGG, ECR, JS and MN are employees and shareholders of Exosome Diagnostics, Inc. JCS and JWG received advisory fees from Clovis Oncology. All remaining authors have declared no conflict of interest.

References

1. Gadgeel SM. Personalized therapy of non-small cell lung cancer (NSCLC). Adv Exp Med Biol 2016; 890: 203–222.
2. Kim ES, Hirsh V, Mok T et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet 2008; 372(9652): 1809–1818.
3. Thompson JC, Yee SS, Troxel AB et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next generation sequencing of cell-free circulating tumor DNA. Clin Cancer Res 2016; 22(23): 5772–5782.
4. Brock G, Castellanos-Rizaldos E, Hu L et al. Liquid biopsy for cancer screening, patient stratification and monitoring. Transl Cancer Res 2015; 5: 200–209.
5. Reckamp KL, Melnikova VO, Karlovich C et al. A highly sensitive and quantitative test platform for detection of NSCLC EGFR mutations in urine and plasma. J Thorac Oncol 2016; 11(10): 1690–1700.
6. Douillard JY, Ostoros G, Cobo M et al. Gefitinib treatment in EGFR mutated Caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. J Thorac Oncol 2014; 9(9): 1345–1353.
7. Cobas EGFR Mutation Test v2; FDA Summary of Safety and Effectiveness Data; 2016; https://www.accessdata.fda.gov/cedh_docs/pdf15/P150044B.pdf (2 January 2018, date last accessed).
8. CE-IVD marking for therascreen EGFR RQG Plasma PCR kit; 2015 [press release]; https://corporate.qiagen.com/newsroom/press-releases/2017/20150112_therascreen_Bb.
9. Underhill HR, Kitzman JO, Hellwig S et al. Fragment length of circulating tumor DNA. PLoS Genet. 2016; 12(7): e1006162.
10. Diehl F, Li M, Dressman D et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci U S A 2005; 102(45): 16368–16373.
11. Newman AM, Britman SV, To J et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med 2014; 20(5): 548–554.
12. Bettegowda C, Sausen M, Leary RJ et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med 2014; 6(224): 224ra24.
13. Tseng JS, Yang TY, Tsai CR et al. Dynamic plasma EGFR mutation status as a predictor of EGFR-TKI efficacy in patients with EGFR-mutant lung adenocarcinoma. J Thorac Oncol 2015; 10(4): 603–610.
14. Tie J, Kinde I, Wang Y et al. Circulating tumor DNA as an early marker of therapeutically responsive patients with metastatic colorectal cancer. Ann Oncol 2015; 26(8): 1715–1722.
15. Cobas EGFR Mutation Test v2; FDA Summary of Safety and Effectiveness Data; https://www.accessdata.fda.gov/cedh_docs/pdf13/P130047B.pdf (2 January 2018, date last accessed).
16. Oznard GR, Thress KS, Alden RS et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. J Clin Oncol 2016; 34(28): 3375–3382.
17. Karlovich C, Goldman JW, Sun JM et al. Assessment of EGFR mutation status in matched plasma and tumor tissue of NSCLC patients from a phase I study of rociletinib (CO-1686). Clin Cancer Res 2016; 22(10): 2386–2395.
18. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013; 200(4): 373–383.
19. Skog J, Wurdinger T, van Rijn S et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008; 10(12): 1470–1476.
20. Thakur BK, Zhang H, Becker A et al. Double-stranded DNA in exosomes: a novel biomarker in cancer detection. Cell Res 2014; 24(6): 766–769.
21. McKiernan J, Donovan MJ, O’Neill V et al. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. JAMA Oncol 2016; 2(7): 882–889.
22. Demory Beckler M, Higginbotham JM, Franklin JL et al. Proteomic analysis of exosomes from mutant KRAS colon cancer cells identifies intercellular transfer of mutant KRAS. Mol Cell Proteomics 2013; 12(2): 343–355.
23. Enderle D, Spiel A, Coticchia CM et al. Characterization of RNA from exosomes and other extracellular vesicles isolated by a novel spin column-based method. PLoS One 2015; 10(8): e0136133.
24. Jahr S, Hentze H, England C et al. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 2001; 61(4): 1659–1665.
25. Sequist LV, Rolle L, Allen AR. Rociletinib in EGFR-mutated non-small-cell lung cancer. N Engl J Med 2015; 373(6): 578–579.
26. Sequist LV, Soria JC, Camidge DR. Update to rociletinib data with the RECIST confirmed response rate. N Engl J Med 2016; 374(23): 2296–2297.
27. Mohrmann L, Huang H, Hong D et al. Liquid biopsies using plasma exosomal nucleic acids and plasma cell-free DNA and clinical outcomes of
patients with advanced cancers. Clin Cancer Res 2018; doi: 10.1158/1078-0432.CCR-17-2007.
28. Weber B, Meldgaard P, Hager H et al. Detection of EGFR mutations in plasma and biopsies from non-small cell lung cancer patients by allele-specific PCR assays. BMC Cancer 2014; 14: 294.
29. Wiener RS, Schwartz LM, Woloshin S, Welch HG. Population-based risk for complications after transthoracic needle lung biopsy of a pulmonary nodule: an analysis of discharge records. Ann Intern Med 2011; 155(3): 137–144.
30. Lokhandwala T, Bittoni MA, Dann RA et al. Costs of diagnostic assessment for lung cancer: a medicare claims analysis. Clin Lung Cancer 2017; 18(1): e27–e34.
31. Alix-Panabières C, Pantel K. Clinical applications of circulating tumor cells and circulating tumor DNA as liquid biopsy. Cancer Discov 2016; 6(5): 479–491.
32. Schwaederle M, Husain H, Fanta PT et al. Use of liquid biopsies in clinical oncology: pilot experience in 168 patients. Clin Cancer Res 2016; 22(22): 5497–5505.
33. Danila DC, Heller G, Gignac GA et al. Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res 2007; 13(23): 7053–7058.
34. Uchida J, Kato K, Kukita Y et al. Diagnostic accuracy of noninvasive genotyping of EGFR in lung cancer patients by deep sequencing of plasma cell-free DNA. Clin Chem 2015; 61(9): 1191–1196.