Pathol. Oncol. Res. (2016) 22:35–40
DOI 10.1007/s12253-015-9970-3

ORIGINAL ARTICLE

Feasibility of Targeting *PIK3CA* Mutations in Head and Neck Squamous Cell Carcinoma

Julie A. Theurer[1,2,3,7] · **William Stecho**[4] · **John Yoo**[1,5,6] · **Keith Kwan**[4,6] · **Bret Wehrli**[4,6] · **Vimla Harry**[4] · **Morgan Black**[1] · **Nicole Pinto**[1] · **Eric Winquist**[5,6] · **David Palma**[3,5,6] · **Suzanne Richter**[5,6] · **John W. Barrett**[1,3,5,6] · **S. Danielle MacNeil**[1,5,6] · **Kevin Fung**[1,5,6] · **Christopher J. Howlett**[4] · **Anthony C. Nichols**[1,3,5,6]

Received: 3 March 2015 / Accepted: 4 August 2015 / Published online: 14 August 2015

Abstract *PIK3CA* is the only frequently-mutated, directly druggable oncogene in head and neck squamous cell carcinoma (HNSCC). However, it is unclear if a molecularly-driven intervention trial can be launched successfully, particularly within a single-institution setting secondary to the infrastructure necessary for mutation detection, mutation prevalence, and patient willingness to participate. This study aimed to evaluate 1) local frequency of *PIK3CA* activating mutations in HNSCC, 2) timeliness of our mutation-profiling clinical pathway, and 3) patients' willingness to enroll in a novel neoadjuvant drug trial. Tissue biopsies of 25 consecutive cases of HNSCC were tested for activating *PIK3CA* mutations at three mutational hotspots by real-time polymerase chain reaction. Mutations prevalence and number of working days accrued in determining *PIK3CA* mutational status were calculated. In addition, 30 HNSCC patients were surveyed prospectively regarding their willingness to participate in a hypothetical drug trial. Survey data were summarized descriptively. 4 of 25 (16 %) tumors harbored a *PIK3CA* activating mutation, including one at codon E542K, two at codon E545K/D, and one at codon H1047R. On average, this result was obtained in approximately 15 working days (range, 9–24 working days). The majority of patients surveyed (70 %) indicated their willingness to participate in a targeted *PIK3CA* trial. This study provides evidence that within a single institution, *PIK3CA* activating mutations can be detected with expected frequency, with sufficient timeliness and sufficient patient interest to mount a targeted intervention trial that may lead to improved tumor response in selected HNSCC patients.

Keywords *PIK3CA* · Targeted therapeutics · Biomarkers · Head and neck squamous cell carcinoma · Feasibility study

Drs. Howlett and Nichols contributed equally to this manuscript.

✉ Julie A. Theurer
jtheurer@uwo.ca

[1] Department of Otolaryngology – Head and Neck Surgery, Schulich School of Medicine & Dentistry, Western University, 800 Commissioners Rd. E., London, ON N6A 5W9, Canada

[2] School of Communication Sciences and Disorders, Faculty of Health Sciences, Western University, Elborn College, London, ON N6G 1H1, Canada

[3] Lawson Health Research Institute, 750 Base Line Rd., London, ON N6C 2R5, Canada

[4] Department of Pathology and Laboratory Medicine, Schulich School of Medicine & Dentistry, Western University, Dental Sciences Building, London, ON N6A 5C1, Canada

[5] Department of Oncology, Western University, 790 Commissioners Rd. E., London, ON N6A 5W9, Canada

[6] London Health Sciences Centre, 800 Commissioners Rd. E., London, ON N6A 5W9, Canada

[7] Department of Otolaryngology – Head and Neck Surgery, London Health Sciences Centre – Victoria Hospital, Rm B3-451, 800 Commissioners Rd E., London, ON N6A 5W9, Canada

Introduction

Head and neck squamous cell carcinomas (HNSCCs) represent the 6th most common cancer worldwide, with approximately 600,000 new cases per year [1]. Despite advancements in treatment technologies over the last 3 decades, HNSCC survival rates have not significantly improved, with 5-year survival of approximately 50 % [2]. New strategies are needed to improve outcomes. Targeted therapy directed against activating mutations and fusions in oncogenes has shown tremendous potential to achieve these improved outcomes in other cancers such as lung cancer and melanoma [3]. Similar

strategies can be applied to HNSCC for patients with tumors harboring targetable mutations.

Early whole exome sequencing studies of HNSCC identified *PIK3CA* as the only targetable frequently mutated oncogene in HNSCC [4, 5]. We have previously demonstrated that these alterations are significantly more common in human papilloma virus (HPV)-positive versus negative disease [6]; *PIK3CA* is often the only mutated oncogene in HPV-positive tumors [7]. Recently, The Cancer Genome Atlas (TCGA) HNSCC study revealed very frequent genomic point *PIK3CA* mutations in 21 % of cases with higher frequency noted in HPV-positive samples (37 % versus 17 %) [8]. Advanced stage HNSCCs harbor a myriad of PI3K pathway mutations, including *PIK3CA*, implicating this pathway in both tumor development and disease progression [7]. Preclinical studies have indicated that cell lines and xenografts with *PIK3CA* mutations respond preferentially to *PIK3CA* inhibitors, particularly BYL719, a highly α specific *PIK3CA* inhibitor [7, 9, 10]. Based on these findings, a preoperative window of opportunity phase II trial of a selective *PIK3CA* inhibitor for patients with tumors containing *PIK3CA* mutations may represent the next logical step to test the activity of *PIK3CA* inhibitors with direct pathological assessment of tumor response.

The purpose of this study was to determine the feasibility of conducting a future single-institution window of opportunity trial of targeted therapy for *PIK3CA* mutations in head and neck cancer patients undergoing primary surgical resection. We explored feasibility through prospective examination of: 1) the frequency of *PIK3CA* mutations in our local HNSCC patient population, 2) the timeliness of real-time *PIK3CA* mutation testing in a clinical context, and 3) patients' hypothetical willingness to receive a targeted therapeutic in a neoadjuvant setting.

Methods

Real-Time *PIK3CA* Mutational Testing

Study approval was obtained from Western University's Research Ethics Board. All referrals to our regional cancer center's Head and Neck Multidisciplinary Team between December 2013 and March 2014 were screened to identify cases of biopsy-proven mucosal squamous cell carcinomas. Thirty-three specimens meeting our requirements were requisitioned for molecular analysis using clinically-validated real-time polymerase chain reaction (PCR) testing.

Tissue curls (2 × 10 μm thick) sectioned from each tumor block were deparaffinized in 1 ml xylene, and subsequently hydrated with decreasing concentrations of ethanol. Genomic DNA was extracted from the deparaffinized tissue using the QIAmp DNA FFPE kit (QIAGEN, Manchester, UK). The *PIK3CA* mutational hotspot (H1047R, E542K, E545K/D) profile was determined using real-time PCR employing the PI3K Mutation Test Kit (QIAGEN, Manchester, UK). Genomic DNA from HNSCC cell lines (Detroit562 (H1047R), PCI6A (E545K), and a validated patient sample (E542K)) served as positive controls for each mutation type. *PIK3CA* DNA from exon 15 served as the internal control for assessing DNA quality and for normalization purposes. Real-time PCR and data analyses were completed using ABI 7500 Fast Real-Time PCR system (Applied Biosystems®, Foster City, CA, USA). Summary statistics were generated to report the frequency of *PIK3CA* mutations, and relationships between mutation status and clinico-demographic variables were analyzed by two-sided Fisher's exact tests. Clinico-demographic data was collected relative to patients' age, gender, disease site, tumor and nodal staging, and p16 status. P16 staining, which served as a surrogate marker for HPV status, was available for 11 of the 25 specimens as it was only completed routinely for oropharyngeal cancer specimens. Differences were considered statistically significant if $p < 0.05$. Statistical analyses were performed using SPSS statistics package (v22, SPSS, Inc., Chicago, IL, 2013).

Timeline of Real-Time *PIK3CA* Testing

The calendar dates corresponding to the following timeline milestones were recorded for all 25 specimens that underwent *PIK3CA* mutational testing: requisition of the specimen, receipt of the specimen in anatomical pathology, receipt of tissue curls in molecular pathology, and availability of *PIK3CA* mutational testing result. Using this information, the number of working days (WD) associated with specimen acquisition, anatomical pathology review (including cutting of tissue curls), and molecular analysis (deparaffinization, DNA extraction, detection of *PIK3CA* hot spot mutations) were calculated using Microsoft Excel.

Patient Willingness

Patients presenting to the London Regional Cancer Program's Head and Neck Multidisciplinary Team for HNSCC assessment and treatment planning between August 2013 and October 2013 were screened for participation in a prospective survey study. Patients were eligible to participate based on the following inclusion criteria: histologic diagnosis of upper aerodigestive tract squamous cell carcinoma, first-ever cancer diagnosis, and treatment-naïve. Patients provided written informed consent prior to participation in this study, which was approved by Western University's Review Board for Health Science research involving human subjects.

Following their multidisciplinary team assessment, participants were provided with brief verbal and written information regarding the role of genetic mutations in carcinogenesis, the

potential to detect individual tumor-specific *PIK3CA* mutations, and the side-effect profile of an un-named Health Canada-approved PI3K inhibitor. Patients subsequently answered the following hypothetical question: "If your tumor was tested today and had a *PIK3CA* mutation, would you be willing to take a chemotherapy drug while you are waiting for your surgery or radiation therapy?" Categorical ('yes', 'no') answers were recorded and analyzed descriptively. Participants were prompted to provide subjective reflections related to their categorical answer, and these responses were recorded qualitatively.

Results

PIK3CA Mutations are Present in Consecutive Cases of HNSCC

Of the 33 consecutively acquired archival specimens, 76 % (25/33) contained sufficient material for analysis and were confirmed to be invasive squamous cell carcinoma (versus carcinoma in situ or high-grade dysplasia). All specimens were screened by two pathologists. Real-time PCR testing revealed four *PIK3CA* mutations in the 25 specimens, for a frequency of 16 %. These mutations occurred at codons E542K (n = 1), E545K/D (n = 2), and H1047R (n = 1). Patient demographics and *PIK3CA* mutation status associated with the archival tumor specimens are presented in Table 1. Mutation status was not associated with age, gender, tumor site, tumor or nodal stage, or HPV status. Fifty percent of the *PIK3CA* mutations occurred in two of six p16 positive tumors (33 %).

Timeline of Real-Time *PIK3CA* Mutational Testing

Overall, mean number of WD from specimen requisition to *PIK3CA* result for the 25 specimens was 19.1 (SD = 7.1 WD; range, 9–38 WD). Ten tumors were regarded as 'pilot' specimens to initiate the clinical molecular pathway. Mean number of WD to achieve *PIK3CA* status for this early cohort was 24.8 (SD = 6.0 WD; range, 19–38 WD). For the remaining 15 specimens, mean number of WD from requisition to *PIK3CA* result was 15.3 (SD = 5.0 WD; range, 9–24 WD; Fig. 1) - a statistically significant decrease in pathway timeliness from the 10 pilot specimens ($p < 0.001$, Student's t test). The average number of WD for specimen acquisition, pathology review, and molecular analysis for the latter 15 specimens was 1.4 (SD = 1.3 WD; range, 0–4 WD), 6.9 (SD = 5.3 WD; range, 0–19 WD), and 6.9 (SD = 2.7 WD; range, 4–10 WD), respectively.

Table 1 Patient demographics and *PIK3CA* mutation status

Patient characteristic	*PIK3CA* Mutation Status		*P* Value[a]
	Negative (n = 21)	Positive (n = 4)	
Age, y			
< 60	5	2	0.55
≥ 60	16	2	
Sex			
Male	17	2	0.23
Female	4	2	
Site			
Oral cavity	9	2	
Oropharynx	6	2	
Nasopharynx	2	0	0.80
Larynx	2	0	
Hypopharynx	2	0	
T classification			
1	6	1	
2	6	2	0.61
3	2	1	
4	7	0	
N classification			
0	9	1	
1	0	1	
2	10	0	0.48
3	1	0	
X	1	0	
Overall tumor stage			
I	3	0	
II	1	1	0.47
III	1	1	
IV	16	2	
HPV (p16)[b]			
Negative	2	1	
Positive	6	2	0.39
Unknown	13	1	

Abbreviations: y, years; HPV, human papillomavirus

[a] Fisher's exact test (two-tailed)

[b] p16 status was available for n = 11 specimens

Patients Were Willing to Accept a Novel Treatment in Addition to Standard of Care

Of 61 patients screened between August 2013 and October 2013, 30 (49 %) met the inclusion criteria, and all 30 (100 %) were willing to participate. In response to the hypothetical question posed to elicit patients' willingness to receive an experimental targeted drug therapy, 21 of 30 (70 %) participants indicated they would be willing to take a novel chemotherapy drug while awaiting definitive treatment. Seven participants

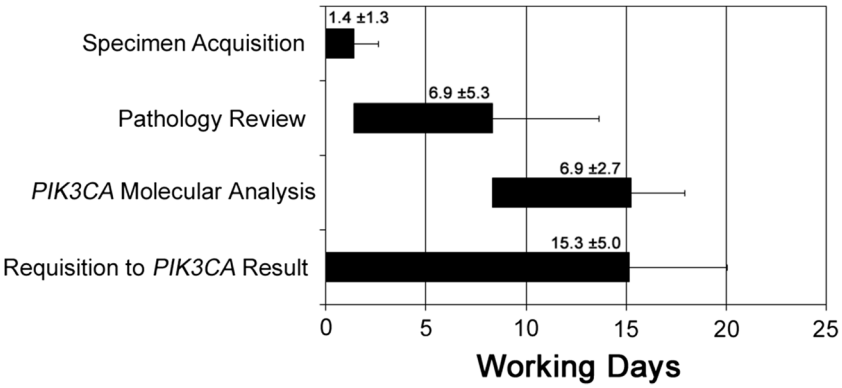

Fig. 1 Timeline of clinical *PIK3CA* mutational analysis pathway. Mean number of working days (± SD) associated with specimen acquisition, pathology review, *PIK3CA* molecular analysis, and the complete mutational analysis pathway (specimen requisition to *PIK3CA* result) for the final 15 specimens. Error bars denote one standard deviation

(23.3 %) responded that they would not be willing to take a targeted drug, while two participants (6.7 %) were unsure how to respond to this hypothetical question. When prompted to qualify these categorical responses, participants who responded 'yes' to the hypothetical question indicated that they "wished to do everything possible to fight the cancer" and they wanted "to help others facing this cancer in the future". For those who indicated they would be unwilling to take a targeted drug, reasons such as "I do not want to be a guinea pig", "I just want the standard treatment", and "I cannot afford to lose any more weight [as a side-effect of the drug]" were provided. Participants who were unsure how to respond to the hypothetical question wanted "[the] doctor to decide [their] treatment" or described being "too overwhelmed with the diagnosis to make a decision".

Discussion

Low cure rates and high treatment-related toxicities continue to drive the search for improved HNSCC treatment strategies. Genomically-directed therapy is one of the most promising anticancer strategies, and is already the standard of care for certain cancers including BRAF-mutant melanoma and EGFR-mutant lung cancer. Presently, the *PIK3CA* oncogene appears to be the most promising, potentially actionable mutation in HNSCC. The current feasibility study was motivated primarily by the goal of carrying out a phase II trial in patients with HNSCC harboring activating *PIK3CA* mutations.

Previous molecularly-driven therapeutic trials posit that targeted agents hold the most therapeutic benefit for patient populations selected on the presence of specific genetic mutations [3, 11–13]. To this end, we sought to determine the potential for identifying *PIK3CA* mutations in our local HNSCC population. The frequency of *PIK3CA* hotspot mutations in our consecutive tumor specimen series (16 %) fell within the range of rates reported previously in the literature. Studies have documented mutations in 6 %-34 % of primary HNSCC tumors [4–8, 14, 15], with higher prevalence (28 %-56 %) reported in HPV-positive tumors [6–8]. Consistent with these findings, half of our mutations were noted in two of the six p16-positive oropharyngeal tumors (33 %).

A second major goal of this study was to quantify the turnaround time necessary to obtain results of the *PIK3CA* mutation analysis. The ability to identify actionable mutations efficiently so that targeted therapy can be initiated and sustained to provide clinically sufficient neoadjuvant exposure to the drug may be challenging and self-limiting. However, even short courses of pre-operative treatment (e.g., ≤2 weeks) with targeted agents have demonstrated measurable response [16, 17]. Given a median time from initial consultation to surgery at our institution of 3.2 ± 2 weeks (Nichols et al., personal communication), timeliness of access to mutational analysis results is of paramount importance in order to allow our desired 14 days of treatment preoperatively without delaying surgery. Tran et al. [18] reported obtaining genomic profiling results with a median time of 21 calendar days, with range of 7–63 days between patient consent and final molecular profiling report. Our data revealed an achievable average timeframe of 15 WD to obtain *PIK3CA* mutational status, with a range of 9–24 WD. Unlike Tran's study, our pathway incorporated time associated with the acquisition of archived specimens housed either at external community care centres, or at geographically separate campuses of our tertiary care hospital. Examination of pathway timeliness across the relatively short duration of this study demonstrated that early optimization efforts resulted in significantly shorter turnaround times, and with integration of the *PIK3CA* assay into routine clinical use, we believe the turnaround time will continue to drop dramatically. This illustrates the utility of a feasibility project to facilitate critical examination of potential clinical system constraints that could be of consequence in clinical research endeavors.

Identification of potentially actionable mutations sets the stage for examining therapeutic benefit of targeted drugs, but presupposes that patients will be willing to be enrolled in preclinical drug trials. To date, few patients with identified potentially actionable mutations receive targeted therapies [11], perhaps in part due to lack of access to clinical trials [19]. However, when trials are available and accessible,

patient factors may influence recruitment [20, 21]. As part of planning for a pre-clinical drug trial targeting *PIK3CA* mutations, we felt it was prudent to examine willingness of treatment-naïve patients to accept a novel neoadjuvant therapy in relation to a recent cancer diagnosis. In the present study, an overwhelming majority of patients indicated that they would join a drug trial prior to initiation of definitive treatment. These results mirror previously reported clinical cancer trial participation data in which approximately 80 % of cancer patients accept randomization into clinical trials [20]. When probed further, our patient population indicated they would accept trial enrollment in order to "do everything possible to fight the cancer" and "to help others facing this cancer in the future". These reflections corroborate findings by others, who describe the gain of personal benefit and altruism as significant motivators for study participation [20, 22]. Patients who decline trials are reportedly more likely to prefer that the doctor choose the treatment approach. Similar sentiments were described by patients in the current study.

Conclusions

The unacceptable low survival rates of HNSCC, and the potential treatment-related toxicities and functional consequences experienced by HNSCC survivors, drives the search for novel therapeutic approaches. Investigation of efficacious therapeutic approaches for specific prevalent genetic mutations is best undertaken in a setting in which mutations are identifiable, patients can be accrued to trial, and intervention can be administered in a manner that allows for measurement of possible therapeutic benefit. We have confirmed that: 1) *PIK3CA* mutations are present in our local HNSCC patient pool, 2) mutation profiling can be incorporated into a clinical care pathway, and 3) patients are willing to be enrolled in investigations of novel agents for both personal and altruistic gain.

Acknowledgments

Author contributions: Drs. Theurer and Nichols had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Compliance with Ethical Standards

Conflict of Interest Dr. Nichols holds an investigator initiated grant from Novartis to study a PI3K inhibitor.

Funding London Regional Cancer Program Catalyst Grant (ACN).

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127(12):2893–2917
2. Loyo M, Li RJ, Bettegowda C, Pickering CR, Frederick MJ, Myers JN, Agrawal N (2013) Lessons learned from next-generation sequencing in head and neck cancer. Head Neck 35(3):454–463
3. Jameson JL, Longo DL. Precision medicine – personalized, problematic, and promising. N Engl J Med. 2015; epub ahead of print,
4. Agrawal N, Frederick MJ, Pickering CR, Bettegowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang J, Wang J, Zhang N, El-Naggar AK, Jasser SA, Weinstein JN, Treviño L, Drummond JA, Muzny DM, Wu Y, Wood LD, Hruban RH, Westra WH, Koch WM, Califano JA, Gibbs RA, Sidransky D, Vogelstein B, Velculescu VE, Papadopoulos N, Wheeler DA, Kinzler KW, Myers JN (2011) Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. Science 333(6046):1154–1157
5. Stransky N, Egloff AM, Tward AD, Kostic AD, Cibulskis K, Sivachenko A, Kryukov GV, Lawrences MS, Sougnex C, McKenna A, Shefler E, Ramos AH, Stojanov P, Carter SL, Voet D, Cortés ML, Auclair D, Berger MF, Saksena G, Guiducci C, Onofrio RC, Parkin M, Romkes M, Weissfeld JL, Seethala RR, Wang L, Rangel-Escareño C, Fernandez-Lopez JC, Hidalgo-Miranda A, Melendez-Zajgla J, Winckler W, Ardlie K, Gabriel SB, Meyerson M, Langer ES, Getz G, Golub TR, Garraway LA, Grandis JR (2011) The mutational landscape of head and neck squamous cell carcinoma. Science 333(6046):1157–1160
6. Nichols AC, Palma DA, Chow W, Tan S, Rajakumar C, Rizzo G, Fung K, Kwan K, Wehrli B, Winquist E, Koropatnick J, Mymryk JS, Yoo J, Barrett JW (2013) High frequency of activating PIK3CA mutations in human papillomavirus-positive oropharyngeal cancer. JAMA Otolaryngol Head Neck Surg 139(6):617–622
7. Lui VW, Hedberg ML, Li H, Vangara BS, Pendleton K, Zeng Y, Lu Y, Zhang Q, Du Y, Gilbert BR, Freilino M, Sauerwein S, Peyser ND, Xiao D, Diergaarde B, Wang L, Chiosea S, Seethala R, Johnson JT, Kim S, Duvvuri U, Ferris RL, ROmkes M, Nukui T, Kwok-Shing Ng P, Garraway LA, Hammerman PS, Mills GB, Grandis JR (2013) Frequent mutation of the PI3K pathway in head and neck cancer defines predictive biomarkers. Cancer Discov 3(7): 761–769
8. The Cancer Genome Atlas Network (2015) Comprehensive genomic characterization of head and neck squamous cell carcinomas. Nature 517:576–582
9. Nichols AC, Black M, Yoo J, Pinto N, Fernandes A, Haibe-Kains B, Boutros PC, Barrett JW (2014) Exploiting high-throughput cell line drug screening studies to identify candidate therapeutic agents in head and neck cancer. BMC Pharmacol Toxicol 15:66
10. Elkabets M, Pazarentzos E, Juric D, Sheng Q, Pelossof RA, Brook S, Benzaken AO, Rodon J, Morse N, Yan JJ, Liu M, Das R, Chen Y, Tam A, Wang H, Liang J, Gurski JM, Kerr DA, Rosell R, Teixidó C, Huang A, Ghossein RA, Rosen N, Bivona TG, Scaltriti M, Baselga J (2015) AXL mediates resistance to PI3K inhibition by activating the EGFR/PKC/mTOR axis in head and neck and esophageal squamous cell carcinomas. Cancer Cell 27(4):533–546

11. Mendelsohn J, Tursz T, Schilsky RL, Lazar V (2011) WIN consortium–challenges and advances. Nat Rev Clin Oncol 8(3):133–134
12. Arteaga CL, Baselga J (2012) Impact of genomics on personalized cancer medicine. Clin Cancer Res 18(3):612–618
13. Engelman JA (2009) Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 9(8):550–562
14. Murugan AK, Hong NT, Fukui Y, Munirajan AK, Tsuchida N (2008) Oncogenic mutations of the PIK3CA gene in head and neck squamous cell carcinomas. Int J Oncol 32(1):101–111
15. Qiu W, Schonleben F, Li X, Ho DJ, Close LG, Manolidis S, Bennett BP, Su GH (2006) PIK3CA mutations in head and neck squamous cell carcinoma. Clin Cancer Res 12(5):1441–1446
16. Guix M, Granja Nde M, Meszoely I, Adkins TB, Wieman BM, Frierson KE, Sanchez V, Sanders ME, Grau AM, Mayer IA, Pestano G, Shyr Y, Muthuswamy S, Calvo B, Krontiras H, Krop IE, Kelley MC, Arteaga CL (2008) Short preoperative treatment with erlotinib inhibits tumor cell proliferation in hormone receptor-positive breast cancers. J Clin Oncol 26(6):897–906
17. Harper-Wynne CL, Sacks NP, Shenton K, MacNeill FA, Sauven P, Laidlaw IJ, Rayter Z, Miall S, Howes A, Salter J, Hills MJ, Lowe FM, A'Hern R, Nasiri N, Doody D, Iqbal J, Dowsett M (2002) Comparison of the systemic and intratumoral effects of tamoxifen and the aromatase inhibitor vorozole in postmenopausal patients with primary breast cancer. J Clin Oncol 20(4):1026–1035
18. Tran B, Brown AM, Bedard PL, Winquist E, Goss GD, Hotte SJ, Welch SA, Hirte HW, Zhang T, Stein LD, Ferretti V, Watt S, Jiao W, Ng K, Ghai S, Shaw P, Petrocelli T, Hudson TJ, Neel BG, Onetto N, Siu LL, McPherson JD, Kamel-Reid S, Dancey JE (2013) Feasibility of real time next generation sequencing of cancer genes linked to drug response: results from a clinical trial. Int J Cancer 132(7):1547–1555
19. Johnson DB, Dahlman KH, Knol J, Gilbert J, Puzanov I, Means-Powell J, Balko JM, Lovly CM, Murphy BA, Goff LW, Abramson VG, Crispens MA, Mayer IA, Berlin JD, Horn L, Keedy VL, Reddy NM, Arteaga CL, Sosman JA, Pao W (2014) Enabling a genetically informed approach to cancer medicine: a retrospective evaluation of the impact of comprehensive tumor profiling using a targeted next-generation sequencing panel. Oncologist 19(6):616–622
20. Jenkins V, Farewell V, Farewell D, Darmanin J, Wagstaff J, Langridge C, Fallowfield L (2013) TTT steering committee. Drivers and barriers to patient participation in RCTs. Br J Cancer 108(7):1402–1407
21. Fouad MN, Lee JY, Catalano PJ, Vogt TM, Zafar SY, West DW, Simon C, Klabunde CN, Kahn KL, Weeks JC, Kiefe CI (2013) Enrollment of patients with lung and colorectal cancers onto clinical trials. J Oncol Pract 9(2):e40–e47
22. Catt S, Langridge C, Fallowfield L, Talbot DC, Jenkins V (2011) Reasons given by patients for participating, or not, in phase 1 cancer trials. Eur J Cancer 47(10):1490–1497