Chapter 2
Overview of Oncology Biomarkers

Mitsukuni Suenaga, Heinz-Josef Lenz, and Stefan J. Scherer

Abstract Biomarkers, whether predictive or prognostic of disease, are an essential element of every modern targeted oncology drug development program. Because they can provide information about the mechanism of drug action, carcinogenesis, and patient characteristics specific to both disease and treatment, they offer the opportunity to individualize therapies and to realize potential of personalized medicine. This chapter provides an introduction to biomarkers, their definition and collection, with emphasis on the utility in colon, breast and lung cancers.

Keywords Biomarker • Predictive marker • Prognostic marker • Pharmacogenomics • Patient stratification • Patient selection • Precision medicine

1 Overview

In oncology, reliable biomarkers are crucial to realize individualized treatment for cancer patients. Biomarkers represent biological characteristics of patients or tumors in various cancer types that identify carcinogenesis mechanisms, individual genetic variations, or pharmacogenomics such as pharmacokinetics and pharmacodynamics. Finally, detected molecular biology-based biomarkers can serve as specified markers for tailor-made treatment especially with molecular-targeting agents.

M. Suenaga
Department of Gastroenterological Chemotherapy, Cancer Institute Hospital of Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo, 135-8550, Japan
e-mail: m.suenaga@jfcr.or.jp

H.-J. Lenz
USC Norris Cancer Center, 1441 Eastlake Ave Suite 3456, Los Angeles, CA, 90089, USA
e-mail: LENZ@med.usc.edu

S.J. Scherer (✉)
VP Global Head Correlative Science, Novartis Pharmaceuticals Corporation, One Health Plaza, East Hanover, NJ, 07936-1080, USA
e-mail: Stefan.Scherer@novartis.com

Biomarkers are generally divided into "predictive" and "prognostic" factors (Nalejska et al. 2014). Predictive markers provide optimal treatment indication with the likelihood of response to an applied chemotherapeutic therapy as well as of treatment-related side effects. By contrast, prognostic markers confer identification of patients with different clinical outcomes derived from somatic mutation, germline polymorphisms, change in DNA methylation, serum cytokine levels, expression of micro-RNA (miRNA) as well as circulating tumor cells (CTCs) (Mehta et al. 2010).

Thus, identification of biomarkers that highly correspond to clinical outcomes or antitumor effect of chemotherapy is a crucial concern in clinical practice when treating cancer patients.

2 Prognostic Marker

Prognostic biomarkers are objectively measurable and act as an intrinsic manner in both patients and tumors and also independent of treatment that provide useful information to the physicians about the likely clinical outcome. In advanced or metastatic cancers, overall survival is the most common prognostic marker (Nalejska et al. 2014). Furthermore, prognostic factors are attributed to assess the tumor staging such as likelihood of the lymph node or distant metastasis at the point of diagnosis of cancer, preoperative screening process, and decision of application of adjuvant chemotherapy to patients who underwent curative tumor resection with respect to risk of cancer relapse (James et al. 2007; Cohen et al. 2009; Coate et al. 2009). Thus, prognostic markers can be used for patient selection who receive benefit from cancer treatment in any tumor stages, but should not be employed to predict treatment efficacy.

Prognostic biomarkers in specific tumor type are identified by molecular analysis for gene expression, gene polymorphism, mutation, DNA methylation variation, CTC, or miRNA in the peripheral blood. Serum or plasma cytokine levels derived from the host or tumor can also become prognostic factors (Hegde et al. 2013).

3 Predictive Marker

Predictive markers are characterized as more practical during cancer treatment that provides information on the likelihood of benefit achieving objective response to treatment. Thereby, in general, predictive markers are used for identification of specific patient groups who are most likely to benefit from treatment, as well as therapeutic decisions. Somatic mutations are the most common predictive markers as shown in epidermal growth factor receptor (EGFR) signaling-related genes such as *KRAS*, *BRAF*, or *EGFR1* (Amado et al. 2008; Van Cutsem et al. 2011). Analysis of the expression of RNA and miRNA or determination of methylation status is recently more focused on detecting good responders to treatment (Ouchi et al. 2015; Perez-Carbonell et al. 2015).

4 Biomarkers in Various Cancers

In several common cancer types, predictive and prognostic markers have been successfully used to predict a response to treatment given to patients by genetic analysis mentioned above. Some examples in major solid tumors are shown below.

4.1 Colon Cancer

4.1.1 Predictive Marker

Epidermal growth factor receptor (EGFR) is a target of anti-EGFR monoclonal antibodies (cetuximab and panitumumab) in the treatment with metastatic colorectal cancer (mCRC). Although mechanism of the drugs is inhibiting downstream EGFR signaling and approximately 70 % of EGFR expression in CRC reported, EGFR expression has not been shown to correlate with efficacy of the anti-EGFR monoclonal antibodies (Cunningham et al. 2004).

Further analyses on genes in the EGFR signaling pathway demonstrated that such anti-EGFR antibodies could be effective only in mCRC harboring *KRAS* and *NRAS* [exon 2 (codons 12 and 13), exon 3 (codons 59, 61)], *BRAF*(V600E), and *PIK3CA* (exon 20) as wild type (De Roock et al. 2010). In addition, *PTEN* is known as a tumor suppressor gene inhibiting PI3K-Akt signaling that indirectly diminishes response to anti-EGFR antibodies with its mutation (Perrone et al. 2009; Sartore-Bianchi et al. 2009; De Roock et al. 2011). The latest guidelines indicate that clinical use of the anti-EGFR antibodies should be considered only in extended RAS (*KRAS* and *NRAS*) wild-type mCRC patients (Allegra et al. 2016; Sorich et al. 2015).

4.1.2 Prognostic Marker

Familial adenomatous polyposis (FAP) is a familial syndrome, in which mutation of *APC* tumor suppressor gene predisposes the patients to adenoma or adenocarcinoma from the normal epithelium in the gastrointestinal tract. Annual screening or investigation of the family history is strongly recommended in patients with *APC* gene mutation or family history of FAP (Plawski and Slomski 2008).

Mismatch repair deficiency (dMMR) has been shown to involve many somatic mutations acting in a prognostic manner and also as predictive showing less response to 5-fluorouracil in adjuvant therapy (Sargent et al. 2010). Recently, a study demonstrated that dMMR was dramatically associated with enhanced response to programmed death 1 (PD-1) immune checkpoint inhibitor. In this mean, dMMR acts as a predictive marker and will be surely focused on its correlation with the immune microenvironment widely in many types of cancer (Le et al. 2015).

4.2 Breast Cancer

4.2.1 Predictive Marker

Breast cancer is the one that has been most investigated for biomarkers because of its characterization showing precise response to both biologic agents and hormone therapy. Hormone receptors such as estrogen receptor (ER) and progesterone receptor (PR) are targets of hormone therapy and expression of these genes serve as predictive markers in breast cancer (Chung and Christianson 2014; Dowsett et al. 2006), and current guidelines indicate clinical use of the hormone therapy as both adjuvant and in metastatic setting in specific patients with hormone receptor positive tumor (https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf). On the other hand, HER2 is the target of HER2 inhibitor including RTKs (trastuzumab, lapatinib, pertuzumab, and T-DM1). As a predictive factor, HER2-negative tumor does not respond to trastuzumab, as observed in different types of cancers with metastatic breast cancer and advanced gastric or gastroesophageal junction cancer (Bang et al. 2010; Blackwell 2010).

4.2.2 Prognostic Marker

The hormone receptors and HER2 status also serve as prognostic markers in breast cancer. HER2-positive tumors are significantly associated with poor survival compared to those without HER2 overexpression in breast cancer and possibly in gastric cancer (Rüschoff et al. 2010; Hofmann et al. 2008). Although the frequency of HER2 expression is around 20 % in both cancer types, HER2 testing is routinely underwent to provide benefit and to avoid unnecessary harmfulness to patients under the current clinical guideline (https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf).

4.3 Lung Cancer

4.3.1 Predictive Marker

In patients with non-small-cell lung carcinoma (NSCLC), *EGFR* kinase mutations in exons 19 or 21 are routinely tested to decide the indication of tyrosine kinase inhibitors (gefitinib and erlotinib) because of high sensitivity to these agents compared with normal gene status (Heuckmann et al. 2012). Although the frequency of mutation is small (around 5 %) in NSCLC, anaplastic lymphoma kinase (*ALK*) gene rearrangement leading to the constitutive expression and activation of ALK fusion protein has become a promising target of ALK inhibitor (crizotinib) (Camidge et al. 2012). However, recent studies demonstrated secondary ALK kinase mutations in relation to drug resistance by ALK fusion gene amplification, EGFR, or KIT

activation (Gainor et al. 2013). Ceritinib is approved as next-generation ALK inhibitor in patients confirmed with crizotinib-resistant tumor. Recently, p-glycoprotein overexpression was revealed as crizotinib resistance mechanism in ALK-rearranged NSCLC patients (Katayama 2015). Thus, testing for EGFR mutations and ALK gene rearrangement is standardized in the treatment decision for NSCLC. However, further drug-resistant tumors will still remain as an unavoidable issue along with novel drug development.

4.3.2 Prognostic Marker

Excision repair cross-complementation group (ERCC1) protein was reported as a predictive and prognostic factor that participates in the DNA repair in the nucleotide excision repair pathway caused by cisplatin. Highly expressed ERCC1 tumor was revealed to provide longer survival in patients who did not receive adjuvant therapy; by contrast, only low-expressed ERCC1 tumor was associated with good outcome in patients receiving adjuvant chemotherapy (Huang et al. 2016). As focused on CRC, *KRAS* mutation has been investigated in NSCLC as relevant biomarker to date and found to be poor prognostic marker of NSCLC (Zhu et al. 2008). Although abnormalities of these genes are conceivable to be prognostic markers with respect to their critical role in each signal transduction pathway, most of the results have not been validated for their true clinical values.

5 Timing of Biomarker Measurement

Although the impact of biomarkers is marvelous, testing them with inappropriate timing may provide a risk of false-positive or false-negative results that misleads the physician to incorrect choice of patients or treatment. We also recognize that biomarker characterization including pharmacodynamics of agents or gene status can be changeable by previous treatment or other extrinsic stimulation. In that regards, preoperative study is considered as one of the most reasonable tools to evaluate the true functions of biomarkers (Marous et al. 2015).

Candidate biomarkers discovered in small population study such as phase II trials are finally verified in randomized clinical trials that are stratified by the biomarkers. There are two types of biomarker study in clinical trial. An integral biomarker directly reflects its impact on clinical endpoints because treatment arms are stratified by the biomarker with enrolling patients randomly to each arm. By contrast, integrated biomarkers are obtained after the prospective randomized trial met the primary endpoint, at least meaning that biomarkers are not crucial factor directing treatment (Mankoff et al. 2014). However, most common biomarker approved for use in clinical practice is an integrated biomarker derived from additional research of clinical trials, because the large amount of time and cost will be carried on researchers.

Therefore annual review of the availability of biomarkers and amendment of guideline are necessary to avoid unfavorable outcome in patients who undergo biomarker-dependent treatment.

6 Future Perspective

Biomarker research has remarkably progressed in oncology and accelerates novel drug development. Analysis of DNA methylation and miRNA are recent topics in several types of cancer (Nalejska et al. 2014). On the other hand, technology of DNA and RNA sequencing, quantification of RNA, and SNP genotyping have been developed and provide us the opportunity to analyze numerous number of genes at one testing in short period such as genome-wide association study (GWAS) (Mehta et al. 2010; Easton et al. 2007). However, GWAS covers only common SNPs revealed as predictive or prognostic factors in treatment efficacy and carcinogenesis even though it examines more than 500,000 SNPs at once, and we thereby should recognize the disadvantage of GWAS as reward for amount of examination cost. Therefore, we should think more deeply about the candidate gene-related pathway before executing whole genome sequencing. If a hypothesis is well considered and biologically reasonable, conventional SNP analysis may be enough and defeat whole genome sequencing in terms of likelihood to find out specific biomarker as well as cost benefit.

In conclusion, recent biomarker research has been remarkably progressed and assisted early drug development especially molecular-targeting agents in oncology. To evaluate the true value of candidate or approved biomarker, the timing of testing and change of characterization by the environment such as previous treatment should be always considered when choosing patients and deciding treatment strategy.

References

Allegra CJ, Rumble RB, Hamilton SR, Mangu PB, Roach N, Hantel A, Schilsky RL (2016) Extended RAS gene mutation testing in metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. J Clin Oncol 34:179–185

Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, Chang DD (2008) J Clin Oncol 26:1626–1634

Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK, ToGA Trial Investigators (2010) Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 376:687–697

Blackwell KL, Burstein HJ, Storniolo AM, Rugo H, Sledge G, Koehler M, Ellis C, Casey M, Vukelja S, Bischoff J, Baselga J, O'Shaughnessy J (2010) Randomized study of Lapatinib alone or in combination with trastuzumab in women with ErbB2-positive, trastuzumab-refractory metastatic breast cancer. J Clin Oncol 28:1124–1130

Camidge DR, Bang YJ, Kwak EL, Iafrate AJ, Varella-Garcia M, Fox SB, Riely GJ, Solomon B, Ou SH, Kim DW, Salgia R, Fidias P, Engelman JA, Gandhi L, Jänne PA, Costa DB, Shapiro GI, Lorusso P, Ruffner K, Stephenson P, Tang Y, Wilner K, Clark JW, Shaw AT (2012) Activity and safety of crizotinib in patients with ALK-positive non-small-cell lung cancer: updated results from a phase 1 study. Lancet Oncol 13:1011–1019

Chung C, Christianson M (2014) Predictive and prognostic biomarkers with therapeutic targets in breast, colorectal, and non-small cell lung cancers: a systemic review of current development, evidence, and recommendation. J Oncol Pharm Pract 20:11–28

Coate LE, John T, Tsao MS, Shepherd FA (2009) Molecular predictive and prognostic markers in non-small-cell lung cancer. Lancet Oncol 10:1001–1010

Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse MA, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW, Meropol NJ (2009) Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. Ann Oncol 20:1223–1229

Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351:337–345

De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S (2010) Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 11:753–762

De Roock W, De Vriendt V, Normanno N, Ciardiello F, Tejpar S (2011) KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol 12:594–603

Dowsett M, Houghton J, Iden C, Salter J, Farndon J, A'Hern R, Sainsbury R, Baum M (2006) Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status. Ann Oncol 17:818–826

Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R; SEARCH collaborators, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schürmann P, Dörk T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X; kConFab; AOCS Management Group, Mannermaa A, Kosma VM, Kataja V, Hartikainen J, Day NE, Cox DR, Ponder BA (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447(7148):1087–1093.

Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, Katayama R, Pawlak A, Mino-Kenudson M, Yeap BY, Riely GJ, Iafrate AJ, Arcila ME, Ladanyi M, Engelman JA, Dias-Santagata D, Shaw AT (2013) ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. Clin Cancer Res 19:4273–4281

Hegde PS, Jubb AM, Chen D, Li NF, Meng YG, Bernaards C, Elliott R, Scherer SJ, Chen DS (2013) Predictive impact of circulating vascular endothelial growth factor in four phase III trials evaluating bevacizumab. Clin Cancer Res 19:929–937

Heuckmann JM, Rauh D, Thomas RK (2012) Epidermal growth factor receptor (EGFR) signaling and covalent EGFR inhibition in lung cancer. J Clin Oncol 30:3417–3420

Hofmann M, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, Ochiai A, Rüschoff J, Henkel T (2008) Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology 52:797–805

https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

Huang ZL, Cao X, Luo RZ, Chen YF, Zhu LC, Wen Z (2016) Analysis of ERCC1, BRCA1, RRM1 and TUBB3 as predictors of prognosis in patients with non-small cell lung cancer who received cisplatin-based adjuvant chemotherapy: a prospective study. Oncol Lett 11:299–305

James CR, Quinn JE, Mullan PB, Johnston PG, Harkin DP (2007) BRCA1, a potential predictive biomarker in the treatment of breast cancer. Oncologist 12:142–150

Katayama R, Sakashita T, Yanagitani N, Ninomiya H, Horiike A, Friboulet L, Gainor JF, Motoi N, Dobashi A, Sakata S, Tambo Y, Kitazono S, Sato S, Koike S, John Iafrate A, Mino-Kenudson M, Ishikawa Y, Shaw AT, Engelman JA, Takeuchi K, Nishio M, Fujita N (2015) P-glycoprotein mediates Ceritinib resistance in anaplastic lymphoma kinase-rearranged non-small cell lung cancer. EBioMedicine 3:54–66

Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA Jr (2015) PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 372:2509–2520

Mankoff DA, Pryma DA, Clark AS (2014) Molecular imaging biomarkers for oncology clinical trials. J Nucl Med 55:525–528

Marous M, Bièche I, Paoletti X, Alt M, Razak AR, Stathis A, Kamal M, Le Tourneau C (2015) Ann Oncol 26:2419–2428

Mehta S, Shelling A, Muthukaruppan A, Lasham A, Blenkiron C, Laking G, Print C (2010) Predictive and prognostic molecular markers for cancer medicine. Ther Adv Med Oncol 2:125–148

Nalejska E, Mączyńska E, Lewandowska MA (2014) Prognostic and predictive biomarkers: tools in personalized oncology. Mol Diagn Ther 18:273–284

Ouchi K, Takahashi S, Yamada Y, Tsuji S, Tatsuno K, Takahashi H, Takahashi N, Takahashi M, Shimodaira H, Aburatani H, Ishioka C (2015) DNA methylation status as a biomarker of anti-epidermal growth factor receptor treatment for metastatic colorectal cancer. Cancer Sci 106:1722–1729

Perez-Carbonell L, Sinicrope FA, Alberts SR, Oberg AL, Balaguer F, Castells A, Boland CR, Goel A (2015) MiR-320e is a novel prognostic biomarker in colorectal cancer. Br J Cancer 113:83–90

Perrone F, Lampis A, Orsenigo M, Di Bartolomeo M, Gevorgyan A, Losa M, Frattini M, Riva C, Andreola S, Bajetta E, Bertario L, Leo E, Pierotti MA, Pilotti S (2009) PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. Ann Oncol 20:84–90

Plawski A, Slomski R (2008) APC gene mutations causing familial adenomatous polyposis in Polish patients. J Appl Genet 49:407–414

Rüschoff J, Dietel M, Baretton G, Arbogast S, Walch A, Monges G, Chenard MP, Penault-Llorca F, Nagelmeier I, Schlake W, Höfler H, Kreipe HH (2010) HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. Virchows Arch 457:299–307

Sargent DJ, Marsoni S, Monges G, Thibodeau SN, Labianca R, Hamilton SR, French AJ, Kabat B, Foster NR, Torri V, Ribic C, Grothey A, Moore M, Zaniboni A, Seitz JF, Sinicrope F, Gallinger S (2010) Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. J Clin Oncol 28:3219–3226

Sartore-Bianchi A, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S, Di Nicolantonio F, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A (2009) PIK3CA

mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted mono-clonal antibodies. Cancer Res 69:1851–1857

Sorich MJ, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS (2015) Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. Ann Oncol 26:13–21

Van Cutsem E, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P, Ciardiello F (2011) Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol 29:2011–2019

Zhu CQ, da Cunha SG, Ding K, Sakurada A, Cutz JC, Liu N, Zhang T, Marrano P, Whitehead M, Squire JA, Kamel-Reid S, Seymour L, Shepherd FA, Tsao MS, National Cancer Institute of Canada Clinical Trials Group Study BR.21 (2008) Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 26:4268–4275