Tumor heterogeneity in the clinic: is it a real problem?

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***Abstract*:** Tumor heterogeneity is one of the major problems limiting the efficacy of targeted therapies and compromising treatment outcomes. A better understanding of tumor biology has advanced our knowledge of the molecular landscape of cancer to an unprecedented level. However, most patients with advanced cancers treated with appropriately selected targeted therapies become resistant to the therapy, ultimately developing disease progression and succumbing to metastatic disease. Multiple factors account for therapeutic failures, which include cancer cells accumulating new molecular aberrations as a consequence of tumor progression and selection pressure of cancer therapies. Therefore, single agent targeted therapies, often administered in advanced stages, are unlikely to have a sufficiently lethal effect in most cancers. Finally, the molecular profile of cancer can change over time, which we are not able to monitor with existing strategies using tumor tissue biopsies as the gold standard for molecular diagnostics. Novel technologies focusing on testing low-risk, easily obtainable material, such as molecular cell-free DNA from plasma, can fill that gap and allow personalized therapy to be delivered in real time.

Keywords: molecular aberrations, targeted therapies, tumor heterogeneity

Introduction

Technological advances in cancer genomics have improved our understanding of the molecular landscape in cancer and introduced a new era of targeted therapies matching appropriately selected molecular targets with novel treatments [Meric-Bernstam and Mills, 2012; Tsimberidou *et al.* 2012]. These discoveries led to major therapeutic breakthroughs in diverse cancers that were historically considered difficult or nearly impossible to treat. Examples, among many, include *BCR-ABL* rearranged chronic myelogenous leukemia (CML), *HER2*-amplified breast cancer, *EGFR*-mutant nonsmall cell lung cancer, *BRAF*-mutant melanoma and others [Druker *et al.* 2001; Slamon *et al.* 2001; Lynch *et al.* 2004; Flaherty *et al.* 2010; Falchook *et al.* 2012a, 2012b].

In addition, understanding the molecular background helped define patient populations for whom a specific targeted therapy would be ineffective or even harmful, such as the use of anti-EGFR monoclonal antibodies in advanced colorectal cancer with *KRAS* mutations or BRAF inhibitors used in patients without *BRAF* mutations [Amado *et al.* 2008; Van Cutsem *et al.* 2009; Hatzivassiliou *et al.* 2010]. Unfortunately, even though therapeutic response, progression-free and overall survival increased, often dramatically, in patients with advanced cancers treated with therapy matching the molecular target, ultimately nearly all patients, with the exception of CML, succumb to their disease despite being treated with appropriately selected targeted therapies. In addition, some studies in advanced colorectal and breast cancer suggested that treatment with drug(s) matching the molecular target might not always lead to improved outcomes [Dienstmann *et al.* 2012; De Mattos-Arruda *et al.* 2013b]. This can be explained by multiple factors including the effect of the tumor microenvironment and tumor heterogeneity. Tumor heterogeneity presents resistant clones that are not responsive to matching targeted therapy. Thus, targeting only one abnormality is not sufficient to be lethal for most, if not all, cancer cells [Engelman *et al.* 2007; Nazarian *et al.* 2010; Janku *et al.* 2011; Holzel *et al.* 2013]. This article delineates the role of

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tumor heterogeneity in advanced cancer and its therapeutic implications.

Tumor heterogeneity

Intratumor genetic heterogeneity has important implications for personalized medicine approaches as it can limit therapeutic efficacy and lead to resistance to therapy. Genomic analysis of tumor relying on archival tumor tissue has been established as the gold standard for molecular profiling [El-Osta *et al.* 2011; Kim *et al.* 2011]. In clinical practice, the source of biological material typically comes from formalin-fixed paraffin-embedded tumor samples obtained during standard of care surgical procedures or biopsies. These samples can be obtained at any point of care, which often is a long time before the indication for targeted therapy becomes relevant. Arguably, the molecular profile of the primary tumor from the initial surgical specimen might significantly differ from the molecular profile in a tumor sample obtained from a biopsy of a metastatic site and might not reflect molecular aberrations accumulated as a consequence of selection pressure caused by applied cancer therapies. In addition, the molecular profile(s) of different metastatic sites might be disparate [Dupont Jensen *et al.* 2011; Gonzalez-Angulo *et al.* 2011; Gerlinger *et al.* 2012].

A study investigating *PIK3CA* mutation status and PTEN expression status in an immunohistochemical analysis of 46 primary breast cancers and 52 breast cancer metastases demonstrated an 18% discordance for *PIK3CA* mutations and 26% for loss of PTEN expression between the primary sample and the metastatic one [Gonzalez-Angulo *et al.* 2011]. In addition, a small study with *PIK3CA* mutation analysis of formalin-fixed paraffin-embedded samples in primary breast cancer revealed three different results for *PIK3CA* mutations status (H1047R, E542K and wildtype *PIK3CA*) depending on the area of the sample that was used as a source of material for DNA isolation [Dupont Jensen *et al.* 2011]. Overall, this study showed concordance in *PIK3CA* mutation status among the primary tumor and corresponding asynchronous metastases in 75% of cases.

More importantly, a seminal paper from the Sanger group from the United Kingdom reported in a systematic way the molecular profile of renal carcinoma in different sites of the primary tumor and samples of metastatic tumor [Gerlinger *et al.* 2012]. In this work, certain molecular aberrations were present in most analyzed sites. However, some aberrations presented only in primary or metastatic sites, which led to the formulation of the tree and branches theory of cancer phylogenesis, which involves mapping the distribution of ubiquitous, shared and private molecular aberrations.

These findings led to skepticism about our ability to address the complexity and heterogeneity of malignant disease, especially in an advanced setting. In addition, experimental models and clinical observations suggested that cytokines and other factors in the extracellular matrix of the tumor microenvironment could modify tumor cell survival and resistance to cancer therapy [Correia and Bissell, 2012]. For instance, in a lymphoma mouse model, several chemotherapeutic agents led to release of the proinflammatory cytokine interleukin-6 from endothelial cells in the thymus owing to genotoxic stress. This process promoted lymphoma cell survival in spatially limited 'chemoresistant niches' [Gilbert and Hemann, 2010].

Conceptually, cancers are deemed to develop according to Darwinian principles when stochastic genetic and epigenetic changes lead to selection of the most viable clones [Turner and Reis-Filho, 2012]. Depending on the level of genetic instability, cancers can be classified as: simple clonal cancers with a low level of intratumoral heterogeneity (e.g. BCR-ABL aberrant CML); complex clonal cancers with a universal driver aberration, but distinct private mutations in diverse subclones; and mosaic cancers, which share some genetic abnormalities. However, variations among subclones are substantial. The latter are the most difficult to treat and most likely to be resistant to targeted and other therapies [Druker *et al.* 2001; Flaherty *et al.* 2010; Turner and Reis-Filho, 2012].

The introduction of high-throughput detection of genomic alterations by massively parallel sequencing, which can be used in formalin-fixed paraffin-embedded archival tumor samples, expanded our capability to detect resistance mutations [Wagle *et al.* 2012]. This approach led to discovery of *MEK1* mutation in a postprogression tumor biopsy from a patient with melanoma with acquired resistance to a BRAF inhibitor [Wagle *et al.* 2011]. Potential strategies that can be used

to overcome the challenges discussed above and improve treatment outcomes are examined below.

Timing of treatment

Introduction of the ABL inhibitor, imatinib, to treatment of *BCR-ABL* rearranged CML led to one of the most striking advances in the targeted therapeutics field. In the pre-imatinib era, CML was usually a fatal disease, with a median survival of 4 years and for which allogeneic stem cell transplant was the only curative approach, and that was feasible only for selected patients [Westin and Kurzrock, 2012]. During the clinical research phase of testing imatinib in CML patients in blast crisis, the results were universally dismal with response rates less than 15% and survival usually not exceeding 1 year [Westin and Kurzrock, 2012]. Although outcomes improved in the accelerated phase of CML, what really made a difference was to move imatinib to the front line of therapy for newly diagnosed CML, whereby expected median survival increased from 4 to 19-25 years [O'Dwyer *et al.* 2002; Westin *et al.* 2012].

If we hypothetically view treating metastatic solid tumors as equivalent to treating a CML blast crisis, it is not surprising that we cannot achieve a cure or long-lasting remissions in these patients, albeit the prevailing perception that CML cannot be compared with other cancers because of its distinct features and biology. However, if we explicate the primary factors leading to success with imatinib in CML they include identification of a validated molecular target, development or identification of a targeted agent capable of inhibiting the target, and moving its use in treatment to early diagnosed disease, which is less likely to accumulate diverse molecular aberrations potentially leading to resistance to therapy [Westin *et al.* 2012].

To test the early treatment concept in solid tumors, clinical trials focusing on appropriately selected targeted therapies with confirmed efficacy in advanced stages of disease need to be carried out in patients with newly diagnosed cancers before metastatic disease develops. As mentioned above, it is plausible that early stage cancers are less likely to suffer from significant heterogeneity and be less prone to therapeutic failure. Even in cases when dramatic or durable effects are not achieved with appropriately selected targeted therapies in newly diagnosed solid tumors, their efficacy can be expected to exceed current findings in the relapsed or refractory setting. Finally, apprehending resistance to targeted therapies is likely to be a far less daunting task in early cancers than in advanced stages that have acquired multiple molecular aberrations.

Targeting critical hubs

It has been accepted that cancer therapy can be successful only if it attacks processes that are absolutely essential for cancer survival but dispensable for normal functionality. Cancer formation is a multistep process, which is associated with the nondeterministic accumulation of genetic abnormalities that drive cancer development [Cho and Vogelstein, 1992]. Plasticity of genetic aberrations, especially in advanced stages, makes the cancer a moving target, which is difficult to eliminate. However, it is plausible that cancer growth can be abrogated if we target pathways critical for cancer cell survival. This 'Achilles heel' theory has been conceptualized in the hypotheses of oncogene addiction and synthetic lethality [Weinstein, 2002; Kaelin, 2005].

One of the proposed approaches for how to overcome tumor heterogeneity is developing combination therapies that can attack multiple targets important for tumorigenesis or resistance to therapy. However, previously summarized data from the Sanger group demonstrating diverse mutations in different areas of tumor challenged the idea of devising effective combinations as it is clearly not feasible to combine drugs to target every single abnormality [Gerlinger *et al.* 2012]. In addition, there is significant interpatient genomic heterogeneity, which makes development of a universally applicable combination therapy impossible [Wood *et al.* 2007].

Cell signaling can be viewed as a network of proteins that mutually influence each other through cross-talk and other mechanisms. It is conceivable, however, that only some proteins serve as critical hubs for cellular function. If this assumption is correct, then targeting these critical hubs could cause a sufficient lethal effect in most cancer cells. To determine which molecular aberrations comprise critical hubs requires a better understanding of system biology, the functional consequences of different molecular aberrations, functional interactions among multiple cancer-related pathways, critical convergence nodes and hubs in cancer circuits. For instance, in

preclinical models of breast cancer cell lines with *PIK3CA* mutations, treatment with the single agent BEZ235, a PI3K/mTOR inhibitor, led to an apoptotic response only in the presence of BIM expression, whereas paclitaxel was similarly effective irrespective of BIM expression [Faber *et al.* 2011]. Also, *PIK3CA* mutations can increase the expression of other factors such as heregulin, which lead to oncogenic pathway activation independent of PI3K [Chakrabarty *et al.* 2010]. Finally, monotherapy with mTOR inhibitors can result in feedback activation of AKT signaling [Carracedo *et al.* 2008; Rodrik-Outmezguine *et al.* 2011]. Similarly, it has been demonstrated in patients with diverse advanced cancers that *PIK3CA* mutations often coexist with simultaneous *KRAS* mutations. Preclinical data and early clinical observations demonstrated that tumors with these combined mutations could respond to combinations of PI3K and MEK inhibitors [Engelman *et al.* 2008]. Finally, BRAF inhibition is effective in advanced melanomas with *BRAF* V600 mutations; however, in advanced colorectal cancer with the same mutation, treatment with the BRAF inhibitor vemurafenib led to dismal outcomes [Flaherty *et al.* 2010; Kopetz *et al.* 2010]. Subsequently, preclinical models in colorectal cancer demonstrated multiple resistance pathways, such as PI3K pathway activation, abnormal methylation, and activation of the EGFR [Prahallad *et al.* 2012; Mao *et al.* 2013].

Monitoring molecular profiles in real time

As previously mentioned, most molecular profiling approaches assess DNA from formalin-fixed paraffin-embedded archival tumor samples obtained during routine therapeutic or diagnostic procedures. A pivotal BATTLE trial in advanced nonsmall cell lung cancer proofed the concept that even lung lesions can be biopsied on a regular basis; however, it did not address the fact that the molecular profile might change over time [Kim *et al.* 2011]. The plasticity of molecular profiles was elegantly demonstrated in a small study in patients with advanced *EGFR*-mutant nonsmall cell lung cancer treated with anti-EGFR tyrosine kinase inhibitors, who underwent sequential biopsies at the time of disease progression [Sequist *et al.* 2008]. Selection pressure from treatment with EGFR inhibitors led to the emergence of secondary aberrations such as an *EGFR* T790M mutation, *MET* amplification or *PIK3CA* mutations. Interestingly, when treatment was changed to standard chemotherapy and the selection pressure toward EGFR was no longer applied, resistance aberrations often disappeared in subsequent biopsies and patients responded again to retreatment with anti-EGFR therapy.

These results underscore the need for molecular profiling in real time, which can be used as a tool for adjusting molecularly targeted therapy regimens to reflect changing cancer genotypes. In addition, the molecular profile of tissue biopsies depends on the site of a biopsy, which can provide inadequate information about underlying aberrations. Collectively, these issues can lead to the mixed response phenomenon when cancer responds to targeted therapy in areas with a targeted molecular aberration present and disease progression in areas where the targeted aberration is absent (Figure 1).

From a practical and safety standpoint, sequential or multiple biopsies can rarely be implemented in routine clinical care because of logistical, financial and ethical barriers. Ideally, tumor biopsies would be needed at the time of restaging scans, possibly even more often. This is not feasible, even in a clinical research setting. Therefore, developing new, noninvasive techniques for garnering the greatest amount of information at multiple time points from the least amount of available biologic material is of paramount importance for furthering personalized therapy [Crowley *et al.* 2013; De Mattos-Arruda *et al.* 2013a]. Furthermore, there is a clear need for a new, inexpensive, and easily obtainable source of material for analysis of tumor molecular aberrations. Cell-free DNA is released to the circulation from cells undergoing apoptosis, necroptosis and active secretion, and has been identified in the plasma of patients with cancer (Figure 2) [Leon *et al.* 1977; Shapiro *et al.* 1983]. Cell-free DNA can also originate from inflammatory cells and other cells in the tumor microenvironment; however, it is assumed that a substantial proportion of cell-free DNA originates from cancer cells [Nawroz *et al.* 1996]. This cell-free DNA can be isolated from plasma or serum samples of patients with advanced cancers.

Because plasma cell-free DNA can originate from multiple tumor sites, arguably its molecular analysis may actually better reflect prevailing molecular aberrations [Forshew *et al.* 2011; Murtaza *et al.* 2013]. In addition, unlike tissue biopsies, obtaining samples of cell-free DNA is a noninvasive approach, with less risk to patients at a lower

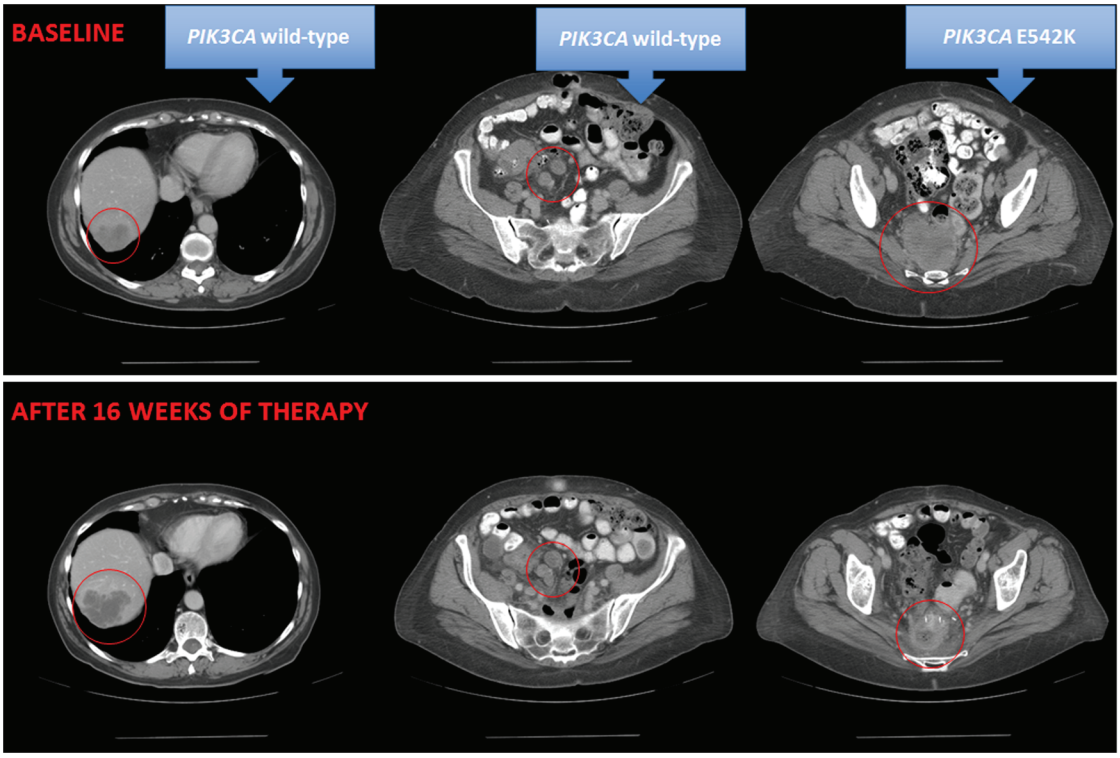

Figure 1. A 59-year-old patient with heavily pretreated advanced ovarian cancer experiencing a mixed response to treatment with an mTORC1 inhibitor reflecting heterogeneous *PIK3CA* mutation status.

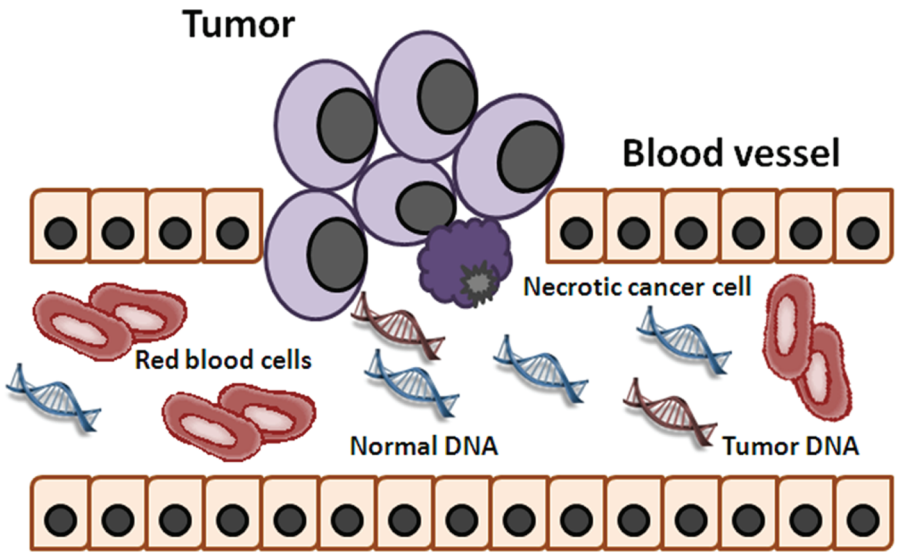

Figure 2. Concept of tumor-derived cell-free DNA released to the circulation.

cost. Furthermore, cell-free DNA can be used to assess biological material at multiple time points and provide valuable information about genetic changes that occur during the disease trajectory, which is not a static process [Sequist *et al.* 2011].

To date, most data on mutation analysis of cell-free DNA has been demonstrated using polymerase chain reaction (PCR) based technologies. A pilot study of 18 patients with metastatic colorectal cancer who were indicated as being candidates

for surgical resection or radiofrequency ablation showed that cell-free DNA from plasma samples can be isolated and oncogenic mutations (*APC*, *KRAS*, *TP53*) can be detected in all tested patients using a PCR-based technology called BEAMing [Diehl *et al.* 2008]. In addition, pharmokinetic analysis of a quantity of mutant copies more accurately predicted disease progression than standard evaluation of serum carcinoembryonic antigen (CEA) levels. Another study tested massive parallel sequencing used to detect rearrangements in circulating tumor DNA from patients with breast and colorectal cancer as markers of residual disease [Leary *et al.* 2010].

Chromosomal instability and rearrangements are universal features of human cancers [Lengauer *et al.* 1998]. Diagnosis of specific chromosomal translocations revolutionized the diagnostics of minimal residual disease in leukemia and lymphoma, while the same concept applied to solid tumors remains controversial [Braun *et al.* 2000; Janku *et al.* 2004, 2008]. Furthermore, two pilot studies in advanced colorectal cancer patients that were wildtype for *KRAS* demonstrated emerging mutant *KRAS* DNA during treatment with anti-EGFR therapy [Diaz *et al.* 2012; Misale *et al.* 2012]. The first study reported that 38% of patients treated with the anti-EGFR monoclonal antibody cetuximab, who were known to have wildtype *KRAS* on the basis of tumor tissue analysis, developed *KRAS* mutations. These mutations were detectable in blood samples, usually between 5 and 6 months following treatment [Diaz *et al.* 2012]. The second study in patients who developed resistance to cetuximab or panitumumab showed the emergence of *KRAS* amplification in one sample and acquisition of secondary *KRAS* mutations in 60% of the cases. *KRAS*-mutant alleles were also detectable in the blood samples of cetuximab-treated patients up to 10 months before disease progression appeared on restaging scans [Misale *et al.* 2012].

Currently, the major barrier to implementing genetic analysis in the clinic is the lack of multiplexing capability with PCR technologies, which are often capable of testing only one mutation at a time. Several attempts were made to use high-throughput, massively parallel next-generation sequencing to detect mutation in plasma cell-free DNA; however, reports uniformly suggested a detection limit of identifying 1 mutant in 100 wildtype alleles at best, which is likely not sufficient for many clinical scenarios [Forshew *et al.* 2012; Dawson *et al.* 2013; Murtaza *et al.* 2013].

Conclusion

Tumor heterogeneity is a significant challenge to the implementation and success of targeting therapies to matched molecular targets into routine clinical care and certainly limits therapeutic outcomes. Various factors and approaches can, if implemented, help overcome this problem. One strategy is moving the use of targeted therapies matching appropriately selected targets to earlier stages of disease, in which fewer molecular aberrations have presumably accumulated and which renders the tumor more sensitive to matched targeted therapy. Second, single agent targeted therapies are unlikely to have a significant impact on most cancers even if an appropriate molecular abnormality is identified. Therefore, identification of critical molecular hubs, which if targeted, can lead to synthetic lethality in most cancer cells. This underscores the necessity of developing rationally designed combinatory therapy strategies. In addition, existing clinical trial models will need to be redefined to reflect individual variations detected through the use of genomic technologies. Third, a molecular profile can change over time as a consequence of the accumulation of molecular aberrations developing during cancer progression and resulting from selection pressure of prior therapies. Therefore, novel technologies are needed to monitor cancer genotypes in real time to allow adjustments in cancer therapy regimens.

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References

Amado, R., Wolf, M., Peeters, M., Van Cutsem, E., Siena, S., Freeman, D. *et al.* (2008) Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 26: 1626–1634.

Braun, S., Pantel, K., Muller, P., Janni, W., Hepp, F., Kentenich, C. *et al.* (2000) Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. *N Engl J Med* 342: 525–533.

Carracedo, A., Ma, L., Teruya-Feldstein, J., Rojo, F., Salmena, L., Alimonti, A. *et al.* (2008) Inhibition of MTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest* 118: 3065–3074.

Chakrabarty, A., Rexer, B., Wang, S., Cook, R., Engelman, J. and Arteaga, C. (2010) H1047r Phosphatidylinositol 3-kinase mutant enhances HER2-mediated transformation by heregulin production and activation of HER3. *Oncogene* 29: 5193–5203.

Cho, K. and Vogelstein, B. (1992) Genetic alterations in the adenoma–carcinoma sequence. *Cancer* 70: 1727–1731.

Correia, A. and Bissell, M. (2012) The tumor microenvironment is a dominant force in multidrug resistance. *Drug Resist Updates* 15: 39–49.

Crowley, E., Di Nicolantonio, F., Loupakis, F. and Bardelli, A. (2013) Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 10: 472–484.

Dawson, S., Tsui, D., Murtaza, M., Biggs, H., Rueda, O., Chin, S. *et al.* (2013) Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N Engl J Med* 368: 1199–1209.

De Mattos-Arruda, L., Cortes, J., Santarpia, L., Vivancos, A., Tabernero, J., Reis-Filho, J. *et al.* (2013a) Circulating tumour cells and cell-free DNA as tools for managing breast cancer. *Nat Rev Clin Oncol* 10: 377–389.

De Mattos-Arruda, L., Oliveira, M., Navarro, A., Vilaro, M., Nuciforo, P. and Vivancos, A. *et al.* (2013b) Molecular profiling of advanced breast cancer patients and benefit obtained from matched targeted therapy in early phase clinical trials. In *Proceedings European Cancer Congress 2013*, Amsterdam.

Diaz, L., Jr., Williams, R., Wu, J., Kinde, I., Hecht, J., Berlin, J. *et al.* (2012) The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 486: 537–540.

Diehl, F., Schmidt, K., Choti, M., Romans, K., Goodman, S., Li, M. *et al.* (2008) Circulating mutant DNA to assess tumor dynamics. *Nat Med* 14: 985–990.

Dienstmann, R., Serpico, D., Rodon, J., Saura, C., Macarulla, T. and Elez, E. *et al.* (2012) Molecular profiling of patients with colorectal cancer and matched targeted therapy in phase 1 clinical trials. *Mol Cancer Ther* 11: 2062.

Druker, B., Talpaz, M., Resta, D., Peng, B., Buchdunger, E., Ford, J. *et al.* (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 344: 1031–1037.

Dupont Jensen, J., Laenkholm, A., Knoop, A., Ewertz, M., Bandaru, R., Liu, W. *et al.* (2011) PIK3CA mutations may be discordant between primary and corresponding metastatic disease in breast cancer. *Clin Cancer Res* 17: 667–677.

El-Osta, H., Hong, D., Wheler, J., Fu, S., Naing, A., Falchook, G. *et al.* (2011) Outcomes of research biopsies in phase I clinical trials: the MD Anderson Cancer Center experience. *Oncologist* 16: 1292–1298.

Engelman, J., Chen, L., Tan, X., Crosby, K., Guimaraes, A., Upadhyay, R. *et al.* (2008) Effective use of PI3K and MEK inhibitors to treat mutant KRAS G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14: 1351–1356.

Engelman, J., Zejnullahu, K., Mitsudomi, T., Song, Y., Hyland, C., Park, J. *et al.* (2007) MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* 316: 1039–1043.

Faber, A., Corcoran, R., Ebi, H., Sequist, L., Waltman, B., Chung, E. *et al.* (2011) BIM expression in treatment-naive cancers predicts responsiveness to kinase inhibitors. *Cancer Discov* 1: 352–365.

Falchook, G., Lewis, K., Infante, J., Gordon, M., Vogelzang, N., Demarini, D. *et al.* (2012) Activity of the oral MEK inhibitor trametinib in patients with advanced melanoma: a phase 1 dose-escalation trial. *The lancet oncology* 13: 782–789.

Falchook, G., Long, G., Kurzrock, R., Kim, K., Arkenau, T., Brown, M. *et al.* (2012) Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. *Lancet* 379: 1893–1901.

Flaherty, K., Puzanov, I., Kim, K., Ribas, A., Mcarthur, G., Sosman, J. *et al.* (2010) Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med* 363: 809–819.

Forshew, T., Murtaza, M., Parkinson, C., Gale, D., Tsui, D., Kaper, F. *et al.* (2012) Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Sci Transl Med* 4: 136ra168.

Gerlinger, M., Rowan, A., Horswell, S., Larkin, J., Endesfelder, D., Gronroos, E. *et al.* (2012) Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med* 366: 883–892.

Gilbert, L. and Hemann, M. (2010) DNA damage-mediated induction of a chemoresistant niche. *Cell* 143: 355–366.

Gonzalez-Angulo, A., Ferrer-Lozano, J., Stemke-Hale, K., Sahin, A., Liu, S., Barrera, J. *et al.* (2011) PI3K pathway mutations and PTEN levels in primary and metastatic breast cancer. *Mol Cancer Ther* 10: 1093–1101.

Hatzivassiliou, G., Song, K., Yen, I., Brandhuber, B., Anderson, D., Alvarado, R. *et al.* (2010) RAF inhibitors prime wild-type RAF to activate the mapk pathway and enhance growth. *Nature* 464: 431–435.

Holzel, M., Bovier, A. and Tuting, T. (2013) Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance? *Nat Rev Cancer* 13: 365–376.

Janku, F., Kleibl, Z., Novotny, J., Tesarova, P., Petruzelka, L. and Matous, B. (2004) Mammaglobin A, a novel marker of minimal residual disease in early stages breast cancer. *Neoplasma* 51: 204–208.

Janku, F., Lee, J., Tsimberidou, A., Hong, D., Naing, A., Falchook, G. *et al.* (2011) PIK3CA Mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers. *PLoS One* 6: e22769.

Janku, F., Srovnal, J., Korinkova, G., Novotny, J., Petruzelka, L., Power, D. *et al.* (2008) Molecular detection of disseminated breast cancer cells in the bone marrow of early breast cancer patients using quantitative RT PCR for CEA. *Neoplasma* 55: 317–322.

Kaelin, W., Jr. (2005) The concept of synthetic lethality in the context of anticancer therapy. *Nat Rev Cancer* 5: 689–698.

Kim, E., Herbst, R., Wistuba, I., Lee, J., Blumenschein, G., Jr., Tsao, A. *et al.* (2011) The battle trial: personalizing therapy for lung cancer. *Cancer Discov* 1: 44–53.

Kopetz, S., Desai, J., Chan, E., Hecht, J., O'dwyer, J., Lee, R. *et al.* (2010) PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors. *J Clin Oncol* 28: abstract 3534.

Leary, R., Kinde, I., Diehl, F., Schmidt, K., Clouser, C., Duncan, C. *et al.* (2010) Development of personalized tumor biomarkers using massively parallel sequencing. *Sci Transl Med* 2: 20ra14.

Lengauer, C., Kinzler, K. and Vogelstein, B. (1998) Genetic instabilities in human cancers. *Nature* 396: 643–649.

Leon, S., Shapiro, B., Sklaroff, D. and Yaros, M. (1977) Free DNA in the serum of cancer patients and the effect of therapy. *Cancer Res* 37: 646–650.

Lynch, T., Bell, D., Sordella, R., Gurubhagavatula, S., Okimoto, R., Brannigan, B. *et al.* (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350: 2129–2139.

Mao, M., Tian, F., Mariadason, J., Tsao, C., Lemos, R., Jr., Dayyani, F. *et al.* (2013) Resistance to BRAF inhibition in BRAF-mutant colon cancer can be overcome with PI3K inhibition or demethylating agents. *Clin Cancer Res* 19: 657–667.

Meric-Bernstam, F. and Mills, G. (2012) Overcoming implementation challenges of personalized cancer therapy. *Nat Rev Clin Oncol* 9: 542–548.

Misale, S., Yaeger, R., Hobor, S., Scala, E., Janakiraman, M., Liska, D. *et al.* (2012) Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 486: 532–536.

Murtaza, M., Dawson, S., Tsui, D., Gale, D., Forshew, T., Piskorz, A. *et al.* (2013) Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. *Nature* 497: 108–112.

Nawroz, H., Koch, W., Anker, P., Stroun, M. and Sidransky, D. (1996) Microsatellite alterations in serum DNA of head and neck cancer patients. *Nat Med* 2: 1035–1037.

Nazarian, R., Shi, H., Wang, Q., Kong, X., Koya, R., Lee, H. *et al.* (2010) Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* 468: 973–977.

O'Dwyer, M., Mauro, M., Kurilik, G., Mori, M., Balleisen, S., Olson, S. *et al.* (2002) The Impact of clonal evolution on response to imatinib mesylate (STI571) in accelerated phase CML. *Blood* 100: 1628–1633.

Prahallad, A., Sun, C., Huang, S., Di Nicolantonio, F., Salazar, R., Zecchin, D. *et al.* (2012) Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 483: 100–103.

Rodrik-Outmezguine, V., Chandarlapaty, S., Pagano, N., Poulikakos, P., Scaltriti, M., Moskatel, E. *et al.* (2011) MTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discov* 1: 248–259.

Sequist, L., Martins, R., Spigel, D., Grunberg, S., Spira, A., Janne, P. *et al.* (2008) First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. *J Clin Oncol* 26: 2442–2449.

Sequist, L., Waltman, B., Dias-Santagata, D., Digumarthy, S., Turke, A., Fidias, P. *et al.* (2011) Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 3: 75ra26.

Shapiro, B., Chakrabarty, M., Cohn, E. and Leon, S. (1983) Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. *Cancer* 51: 2116–2120.

Slamon, D., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A. *et al.* (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344: 783–792.

Tsimberidou, A., Iskander, N., Hong, D., Wheler, J., Falchook, G., Fu, S. *et al.* (2012) Personalized medicine in a phase I clinical trials program: the MD Anderson Cancer Center initiative. *Clin Cancer Res* 18: 6373–6383.

Turner, N. and Reis-Filho, J.. (2012) Genetic heterogeneity and cancer drug resistance. *Lancet Oncol* 13: e178–185.

Van Cutsem, E., Kohne, C., Hitre, E., Zaluski, J., Chang Chien, C., Makhson, A. *et al.* (2009) Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 360: 1408–1417.

Wagle, N., Berger, M., Davis, M., Blumenstiel, B., Defelice, M., Pochanard, P. *et al.* (2012) High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov* 2: 82–93.

Wagle, N., Emery, C., Berger, M., Davis, M., Sawyer, A., Pochanard, P. *et al.* (2011) Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol* 29: 3085–3096.

Weinstein, I. (2002) Cancer. Addiction to oncogenes–the achilles heal of cancer. *Science* 297: 63–64.

Westin, J. and Kurzrock, R. (2012) It's about time: lessons for solid tumors from chronic myelogenous leukemia therapy. *Mol Cancer Ther* 11: 2549–2555.

Wood, L., Parsons, D., Jones, S., Lin, J., Sjoblom, T., Leary, R. *et al.* (2007) The Genomic landscapes of human breast and colorectal cancers. *Science* 318: 1108–1113.