67. Greenhalgh J, Bagust A, Boland A et al. Erlotinib and gefitinib for treating non-small cell lung cancer that has progressed following prior chemotherapy (review of NICE technology appraisals 162 and 175): a systematic review and economic evaluation. Health Technol Assess 2015; 19: 1–134.

68. de Lima Lopes G, Jr, de Souza JA, Barrios C. Access to cancer medications in low- and middle-income countries. Nat Rev Clin Oncol 2013; 10: 314–322.

69. Glaxo Smith Kline. Health for All. 2014. http://no.gsk.com/media/274688/health-for-all-factsheet.pdf (June 2016, date last accessed).

70. Ward A. Cancer drugs cut as UK budget clampdown bites. Financial Times 2015. http://www.ft.com/intl/cms/s/0/01ac9236-5327-9211e9235-b9029-b9239d9250a9274fd9214.html#axzz9234AJ9298R9234rW (June 2016, date last accessed).

71. Ottersen OP, Dasgupta J, Blouin C et al. The political origins of health inequity: prospects for change. Lancet 2014; 383: 630–667.

72. Boseley S. Pig Pharma's Worst Nightmare. The Guardian 2016; Jan 26 2016. http://www.theguardian.com/society/2016/jan/2026/big-pharmas-worst-nightmare (June 2016, date last accessed).

73. Murphy E. Panel: Compulsory Licensing Could Address High-Priced Medicines in Europe. Intellectual Property Watch 2015. http://www.ip-watch.org/2015/2005/2028/speakers-compulsory-licensing-could-address-high-priced-medicines-in-europe/ (June 2016, date last accessed).

74. El Said M, Kapczynski A. Access to medicines: the role of intellectual property law and policy. Global Commission on HIV and the Law Working Paper, 2011; 19. http://hivlawcommission.org/index.php/report-working-papers?task=document.viewdoc&id=86 (June 2016, date last accessed).

75. World Trade Organization. Compulsory Licensing of Pharmaceuticals and TRIPS. https://www.wto.org/english/tratop_e/trips_e/public_health_faq_e.htm (June 2016, date last accessed).

76. Herget G. WTO approves TRIPS amendment on importing under compulsory licensing. HIV AIDS Policy Law Rev 2006; 11: 23–24.

77. Kapp C. World Trade Organisation reaches agreement on generic medicines. New deal will make it easier for poorer countries to import cut-price generic drugs made under compulsory licensing. Lancet 2003; 362: 807.

78. Beall R, Kuhn R. Trends in compulsory licensing of pharmaceuticals since the Doha Declaration: a database analysis. PLoS Med 2012; 9: e1001154.

79. Wise J. Patent wars: affordable medicines versus intellectual property rights. BMJ 2014; 348: g1533.

80. Chaudhuri S. The larger implications of the Novartis-Glivec judgment. Econ Polit Wkly 2013; 48: 10–12.

Consensus on precision medicine for metastatic cancers: a report from the MAP conference

C. Swanton[1,2], J.-C. Soria[3,4], A. Bardelli[5,6], A. Biankin[7,8,9], C. Caldas[10,11,12], S. Chandarlapaty[13], L. de Koning[14], C. Dive[15], J. Feunteun[16], S.-Y. Leung[17], R. Marais[18], E. R. Mardis[19], N. McGranahan[1], G. Middleton[20,21], S. A. Quezada[22], J. Rodón[23], N. Rosenfeld[10], C. Sotiriou[24] & F. André[4]*

[1]Translational Cancer Therapeutics Laboratory, The Francis Crick Institute, London; [2]UCL Hospitals and Cancer Institute, London, UK; [3]Drug Development Unit, Gustave Roussy, Villejuif; [4]Department of Medical Oncology, INSERM Unit U981, Faculté de medicine Paris-Sud XI, Kremlin-Bicêtre, Villejuif, France; [5]Department of Oncology, University of Torino, Candiolo, Torino; [6]Candiolo Cancer Institute—FPO, IRCCS, Candiolo, Torino, Italy; [7]Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Bearsden, Glasgow, UK; [8]South Western Sydney Clinical School, Faculty of Medicine, University of New South Wales, Liverpool, New South Wales, Australia; [9]West of Scotland Pancreatic Unit, Glasgow Royal Infirmary, Glasgow; [10]Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Cambridge; [11]Department of Oncology, University of Cambridge, Addenbrooke's Hospital, Cambridge; [12]Cambridge Experimental Cancer Medicine Centre and NIHR Cambridge Biomedical Research Centre, Cambridge, UK; [13]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, USA; [14]Department of Translational Research, Institut Curie, PSL Research University, Paris, France; [15]Clinical and Experimental Pharmacology, Cancer Research UK Manchester Institute, The University of Manchester, Manchester, UK; [16]Stabilité Génétique et Oncogenèse, Université Paris-Sud, Gustave-Roussy, Villejuif, France; [17]Department of Pathology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong; [18]Molecular Oncology Group, Cancer Research UK Manchester Institute, The University of Manchester, Manchester, UK; [19]McDonnell Genome Institute, Washington University School of Medicine, St Louis, USA; [20]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham; [21]Department of Oncology, University Hospitals Birmingham NHS Foundation Trust, Birmingham; [22]Cancer Immunology Unit, University College London Cancer Institute, University College London, London, UK; [23]Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; [24]Breast Cancer Translational Research Laboratory—BCTL (ULB 290), Institut Jules Bordet, Université Libre de Bruxelles (ULB), Brussels, Belgium

Recent advances in biotechnologies have led to the development of multiplex genomic and proteomic analyses for clinical use. Nevertheless, guidelines are currently lacking to determine which molecular assays should be implemented in metastatic cancers. The first MAP conference was dedicated to exploring the use of genomics to better select therapies in the treatment of metastatic cancers. Sixteen consensus items were covered. There was a consensus that new technologies

Correspondence to: Prof. Fabrice Andre, Institut Gustave Roussy Villejuif—Medicine, INSERM U981, France. Tel: +33-142114371; E-mail: fandre@igr.fr, fabrice.andre@igr.fr

like next-generation sequencing of tumors and ddPCR on circulating free DNA have convincing analytical validity. Further work needs to be undertaken to establish the clinical utility of liquid biopsies and the added clinical value of expanding from individual gene tests into large gene panels. Experts agreed that standardized bioinformatics methods for biological interpretation of genomic data are needed and that precision medicine trials should be stratified based on the level of evidence available for the genomic alterations identified.

Key words: precision medicine, consensus, biomarkers

introduction

Recent advances in biotechnologies have led to the development of multiplex genomic and proteomic analyses for clinical use. Although this jump in the capacity to dissect biology in individuals looks appealing, its adoption, implementation and dissemination require careful examination of levels of evidence by experts. Molecular tests are being used in two different ways in patients with metastatic cancer. First, they can identify patients who are eligible for biomarker-driven therapeutic trials, such as phase I/II trials testing targeted therapies. This first application will hereafter be referred to as 'molecular screening'. Secondly, they can identify patients who are eligible for a specific therapy in the context of daily practice.

The MAP (Molecular Analyses for Personalized medicine) conference brought together worldwide experts with the aim to reach a consensus on recent advances in the field of Personalized Medicine for cancer therapy. The first edition was held in Paris, during 23–24 October 2015, under the sponsorship of ESMO, CRUK and UNICANCER. The conference was attended by more than 400 participants from 38 countries and focused on 'tailoring therapy for metastatic cancers'. The conference consensus was developed by a panel consisting of 20 experts in the field. The list of questions was sent to the conference speakers; their answers were integrated in a first version of the consensus and discussed during the conference. This first version of the consensus was sent to speakers and the consensus altered according to their suggestions. The consensus covered five topics: methods for driver identification, validated drivers in frequent diseases, multigene assays to improve outcome, circulating DNA and other applications of genomics (resistance, heterogeneity, DNA repair defects). The overall aim of the consensus (summarized in Table 1) is to report the state of the art, together with levels of evidence, in the field of cancer precision medicine. The goal is not to provide clinical guidelines for the oncologist, an exercise that requires a different method.

The level of evidence was defined according to the Evaluation of Genomic Applications in Practice and Prevention [3]. Analytical validity refers to the capacity to make accurate and reliable measurements of the biomarker. Clinical validity refers to the ability of the test to accurately and reliably identify or predict a relevant end point. Clinical utility evaluates whether a treatment decision based on a genomic test results in an improved clinical outcome. The quality of evidence is ranked from inadequate to adequate to convincing.

driver identification

The identification of drivers of cancer progression in patients has clearly and dramatically improved the outcomes of many cancer patients. There is a general consensus that identifying and, when therapeutics exists, targeting the drivers of cancer progression improves outcome.

Whenever possible, it is better to assess the molecular portrait at the time of treatment, and to avoid archival samples. This is particularly relevant for genomic alterations involved in resistance to a previous therapy (EGFRT790M, ESR1 mutations …).

does next-generation sequencing have convincing analytical validity?

Several studies have evaluated the analytical validity of next-generation sequencing (NGS) to accurately detect mutations and copy number alterations (CNAs). As illustration, Frampton et al. [4] reported the analytical validity of a panel of 287 cancer-related genes. A total of 118 samples were tested for mutations and 185 for CNAs. High concordance was observed between the genomic profiling using NGS and standard assays, both for mutation detection and identifying CNAs. The panel agreed that NGS has convincing analytical validity to detect variants and copy number changes. This consensus item only covers the technology of NGS, not the bioinformatics aspects.

in the context of molecular screening programs, is it worth testing genes outside the catalog of known cancer-related genes?

Several efforts have been made to define a list of cancer-related genes. Using the most recent definition, it is considered that around 300 genes could be involved in cancer development and progression in a recurring manner [5]. While there is still some room to discover new cancer-related genes [5], the panel did not recommend placing a patient onto a clinical trial based on a genomic alteration located outside a known cancer-related gene. Indeed, molecular screening programs should not aim to discover new cancer-related genes, but rather to offer first validation steps about their clinical relevance. Nevertheless, the molecular data generated in the context of these screening programs are valuable for ancillary translational research.

Cancer 'drivers' include oncogenes and tumor suppressor genes (TSGs). While biological interpretation of hotspot mutations in oncogenes is relatively straightforward, the biological interpretation of CNAs and alterations of TSGs continues to be controversial. In particular, gene amplification does not always lead to protein overexpression whereby an over-abundance of the protein causes disruption in control of normal cellular growth and division. Similarly, most tumor suppressors are thought to act by virtue of deletion, gene silencing by mutation or other alterations in their expression, but in some cases, tumor suppressors can also carry activating mutations such as in TP53.

Table 1. Summary of consensus items

	Consensus
Driver identification	
Does NGS have convincing analytical validity?	NGS has convincing analytical validity to detect variants and copy number changes
In the context of molecular screening programs, is it worth testing genes outside the catalog of known cancer-related genes?	It is not recommended to place patients onto a clinical trial based on a genomic alteration located outside known cancer-related genes
In a molecular screening program, which criteria suggest that a gene amplification is involved in cancer progression?	The best criteria to define 'driver gene amplification' are those defined by Santarius et al. [1]
Is the loss of function of the second allele required to consider a tumor suppressor gene as a driver?	For most of the genes, it is currently not possible to conclude whether the loss of the second allele of a tumor suppressor gene is a mandatory event to drive oncogenesis. Current data suggest that it could be gene and tissue site-specific. More data, especially in patients treated with matched therapy, are needed
Validated drivers in frequent diseases	
Breast cancer	- ER, PR, Her2, BRCA1/2 are required in daily practice
	- The optimal gene panel for breast cancer clinical trials should detect AKT1, PIK3CA, PTEN, ESR1 mutations and FGFR1 amplification, in addition to the five previously mentioned markers
Lung cancer	- EGFR mutations, ALK and ROS1 rearrangements should be tested in daily practice
	- At least 20 genes should be tested in molecular screening programs to drive patients onto therapeutic trials: mutations in EGFR, BRAF, HER2, KRAS, PI3KCA, NTKR, ALK, MET (ex 14), AKT1, BRCA1/BRCA2, HRAS, NRAS; rearrangement status of ALK, ROS1, NTRK; amplification of RET, MET and EGFR; aberrations (mutations or amplifications) in FGFR1/2/3, NOTCH1/NOTCH2
Gastric cancer	- Her2 should be tested in daily practice
	- At least 11 genomic alterations should be included in molecular screening programs (ERBB2, FGFR2, MET, KRAS, CDK4, CDK6, CDKN2A, EGFR, PIK3CA, PTEN, RNF43)
	- PDL1 expression and MSI should also be tested as selection criteria to enter clinical trials
Multigene assays to improve outcome	There is no evidence that the use of large panels of genes improves outcome in patients with metastatic cancer, when compared with standard panels
	To facilitate interpretation and comparison of clinical trial results, each trial should at least report the level of evidence for the selected genomic alterations being tested
	Academic molecular screening programs using multigene panels increase the likelihood of access to a therapy matched to genomic alteration, if carried out in the context of a large phase I program
Circulating tumor DNA (ctDNA)	
Can ctDNA substitute biopsies to select patients with a genomic alteration?	ctDNA has convincing analytical validity to detect hotspot mutations using digital PCR. Further work is needed to validate the detection of mutations in TSGs or copy number alterations using NGS
Can ctDNA identify patients who present a high risk of relapse?	More data are needed before this approach can be recommended
Beyond driver identification: other applications of genomics	
Detection of subclonal alterations involved in resistance	Currently, there is no evidence based on clinical trials that treating a patient based on the detection of subclonal alteration improves outcome
Detection of clonally dominant alterations involved in resistance	Detecting and targeting a molecular mechanism of resistance could lead to an objective response, as has been seen for the EGFR T790M mutation [2]
Methods to assess intratumor heterogeneity (ITH)	Whole-exome sequencing or high coverage multigene panels carried out on multiple biopsy sites is currently the standard procedure to assess ITH. ctDNA is a promising tool to assess ITH
The development of protein-based multiplex assays in the field of personalized medicine	There is a need to develop efforts to integrate phospho-protein assays in precision medicine programs in order to complement genomics and to identify predictors for drugs targeting pathways

in a molecular screening program, which criteria suggest that a gene amplification is involved in cancer progression?

Gene amplification can lead to overexpression of oncogenes that drive cancer progression. For example, ERBB2 amplifications have been shown to be drivers of cancer progression in around 15% of breast cancer cases [6]. One of the current controversies in the field of personalized medicine is how to identify a gene amplification in individual patients that leads to cancer progression. The panel agreed that the best definition of 'driver gene

amplification' is the one defined by Santarius et al. [1]. Using this definition, a gene amplification could be a driver if it leads to gene overexpression, if the amplicon includes a low number of genes, if inherited mutation of that gene predisposes to the same cancer type, if gene overexpression/down-regulation causes biological effects and if the gene has been confirmed as an oncogene in animal models. The best evidence to show that a gene amplification is involved in cancer progression is if a therapy that targets the protein product leads to an objective response (OR) in a clinical trial in patients where the amplification is present, but not in patients without it.

is the loss of function of the second allele required to consider a TSG as a driver?

The loss of a TSG has been shown to mediate malignant transformation [7]. In the initial model proposed by Knudson [7], the two alleles of TSGs have to be lost (through mutations, deletion or loss of function) to generate inactivation of the protein and promote oncogenesis. While TSGs are not directly actionable, their loss generates pathway activation that can subsequently be targeted by therapies. For example, TSC1/2 loss activates mTOR and has been associated with subependymal giant-cell astrocytomas in tuberous sclerosis. Targeting mTOR in this disease led to OR in 75% of patients included in a phase II trial [8]. Synthetic lethality is another strategy to treat patients who present with loss of a TSG, as shown by the OR observed in patients with BRCA-deficient cancers treated with PARP inhibitors [9].

With the exception of TSC1/2 loss, there are not many examples of targeted therapies given according to the loss of a TSG. There are several reasons for this. First, targeting a pathway could be less effective than targeting an oncogene. Secondly, it has been difficult to define loss of TSGs from a companion diagnostic perspective. One open question is whether the loss of function of the second allele of a TSG is a mandatory event to drive oncogenesis. Indeed, in some models, like PTEN and breast cancer, the loss of a single allele is sufficient to generate cancer [10]. Optimal detection of somatic alterations on TSG requires profiling of normal DNA.

Overall, the panel agreed that, for most of the genes, it is currently not possible to conclude whether the loss of the second allele of a TSG is a mandatory event to drive oncogenesis. Current data suggest that it could be gene and tissue site-specific. More data, especially in patients treated with matched therapy, are needed.

validated drivers in frequent diseases

Three frequent diseases (breast, lung and gastric cancers) were discussed during the conference. The clinical questions considered were: which genes should be routinely tested in daily practice? How many genes should be tested in the context of molecular screening programs?

As mentioned in several guidelines, five markers are currently tested to indicate breast cancer therapy. These include expression of the estrogen receptor (ER), progesterone receptor (PgR) and Her2 proteins, together with BRCA1 and BRCA2 mutations [11]. The optimal panel for breast cancer clinical trials should detect AKT1, PIK3CA, PTEN, ESR1, ERBB2 mutations and FGFR1 amplification, in addition to the five previously mentioned markers.

In lung cancer, tests for EGFR mutations and ALK and ROS1 rearrangement status should be carried out in daily practice. The panel proposed testing at least 20 genes in molecular screening programs that aim to drive patients onto therapeutic trials [mutations in EGFR, BRAF, HER2, KRAS, PI3KCA, NTKR, ALK, MET (ex 14), AKT1, BRCA1/BRCA2, HRAS, NRAS; rearrangement status of ALK, ROS1, NTRK; amplification of RET, MET and EGFR; aberrations (mutations or amplifications) in FGFR1/2/3, NOTCH1/NOTCH2].

Finally, in gastric adenocarcinoma, Her2 is the only target that is currently evaluated in the context of daily practice. The panel agreed that >10 genomic alterations should be included in molecular screening programs dedicated to gastric cancers (ERBB2, FGFR2, MET, KRAS, CDK4, CDK6, CDKN2A, EGFR, PIK3CA, PTEN, RNF43). In addition, PDL1 expression and MSI should be tested as selection criteria to enter clinical trials.

multigene assays to improve outcome

The panel agreed that there is no evidence so far that the use of large panels of genes improves outcome in patients with metastatic cancer, when compared with smaller and validated panels.

In individuals, the level of evidence that a genomic alteration is involved in cancer progression can vary from 'biological interpretation without supporting data' (level IV) to 'evidence from clinical trials' (level I) [12]. The inclusion of patients presenting genomic alterations with very different levels of evidence is one of the major limitations of trials testing the clinical utility of multigene panels. Indeed, it is expected that a clinical trial that includes only validated genomic alterations (level I) would easily show an improvement in patient outcome, while those including a majority of 'putative' alterations (level IV, including new variants on oncogenes) will struggle to report any improvements in outcomes. To facilitate interpretation and comparison of clinical trial results, the panel agreed that each trial should at least report the level of evidence for the selected genomic alterations being tested. Several levels of evidence scales are available [12–14]. These level of evidence scales are consistent and there is no reason to favor one over the others.

Finally, the panel agreed that academic molecular screening programs using multigene panels increase the likelihood of access to a therapy matched to genomic alteration, if carried out in the context of a large phase I program. Changes in the design of clinical trials for targeted therapies are certainly increasing the chances for therapeutic access.

circulating tumor DNA

can circulating tumor DNA substitute biopsies to select patients with a genomic alteration?

It has been shown that circulating tumor DNA (ctDNA) can be detected in the blood of most metastatic cancer patients [15–17]. The panel agreed that ctDNA has convincing analytical validity to detect hotspot mutations using digital PCR. This is based on several studies that compared the detection of mutations in biopsies versus in plasma. For example, Thierry et al.

[18, 19] reported high accuracy when using ctDNA to detect mutations in KRAS and BRAF in colorectal tumors. Similar observations have been seen with AKT1 and ESR1 mutations. The threshold that defines a clonally dominant alteration is not yet consensual. More efforts are needed to implement NGS for ctDNA-based assays [20–22]. While progress is rapid, suitable methods need to be applied, and more evidence is required to support the validity of liquid biopsies for detection of clinically relevant mutations in TSGs or CNAs.

can ctDNA identify patients who present a high risk of relapse?

Studies have suggested that detection of residual ctDNA post-therapy is associated with worse outcome, for example, in early breast cancer patients [23]. It has also been shown that KRAS mutations emerge in the blood of patients who receive anti-EGFR antibodies months before radiographic relapse [24, 25]. While these results are extremely promising, more data are needed before this approach can be recommended.

other applications of genomics: resistance and intratumor heterogeneity

While the identification of drivers is the most frequent application of precision medicine in 2015, there are several other applications with important perspectives.

Detecting subclonal alterations involved in resistance could theoretically allow an early introduction of new therapy. Several studies have shown that ctDNA can detect minor subclonal alterations before they drive resistance (EGFR, T790M, ESR1, K-Ras). Nevertheless, there is currently no evidence based on clinical trials that treating a patient based on the detection of subclonal alterations improves outcome. It must be pointed out that purity of the samples and percentage of cancer cells must be taken into account when analyzing subclonal alterations.

Detecting a clonally dominant genomic alteration involved in resistance could allow patients to be treated with an individualized approach to overcome resistance. Several phase I/II trials [2] have shown that detecting and targeting a molecular mechanism of resistance could lead to an OR. For example, in the AURA trials [2], AZD9291 was associated with 59% OR in patients presenting with the EGFR T790M mutation, a genomic alteration involved in resistance to gefitinib and erlotinib [2]. In contrast, the response rates fell to 23% in patients without the T790M mutation [2]. These results led to the approval of AZD9291 by the FDA for patients presenting with the T790M mutation diagnosed using an FDA-approved test (cobas® EGFR Mutation Test v2, PCR-based assay, Roche Molecular Systems, Inc.).

Genomic studies have shown that intratumor heterogeneity (ITH) is present in many cancers [26]. Some studies have suggested that heterogeneity, measured by the existence of multiple subclonal alterations, could be associated with poor outcome [27]. Nevertheless, the lack of standard methods to assess ITH is currently limiting the capacity to explore its clinical implications. The panel agreed that whole-exome sequencing or high coverage multigene panels carried out on multiple biopsy sites is currently the standard procedure to assess ITH. Defining an

optimal method for ITH scoring still requires some research. New methods to assess ITH should be compared with this gold standard approach. The panel agreed that ctDNA is a promising tool to assess ITH [28].

Currently, genomics cannot explain cancer progression in all patients. For example, expression of ER and AR are validated targets in breast and prostate cancers, but there is no detectable alteration at the DNA level in most patients. One important perspective in the field of personalized medicine is the development of protein-based multiplex assays, like reverse-phase protein array, to quantify protein expression and activation. There is a body of evidence suggesting that a mutation in a driver kinase/TSG does not always lead to activation of the corresponding pathway. For example, mutations in PIK3CA do not necessarily correlate with an active pathway at the time of diagnosis [29]. Selecting drugs based on mutation status only is therefore incomplete. One major limitation of protein-based assays is the lack of analytical validity and lack of guidelines for sample processing. Another is the relative lack of sensitivity compared with genomic assays. The panel agreed that there is a need to develop efforts to integrate phospho-protein assays in precision medicine programs in order to complement genomics and to identify predictors for drugs targeting pathways (mTOR, CDK4 inhibitors).

conclusion

The conclusion of this first consensus conference was that new technologies have convincing analytical validity and the use of small panels of biomarkers is required for optimal cancer care. Nevertheless, there is not yet sufficient evidence that using large gene panels improves patient outcome. Randomized trials are currently addressing this question. The next MAP consensus conference will be held in London, during 23–24 September 2016. Among many items, this new consensus will discuss which genomic alterations should be screened in patients with colon, prostate cancer and sarcoma, together with precision medicine for immunotherapeutics and models of implementation. Regarding this latter point, speakers will debate about whether one universal panel of genes should be implemented across diseases, or whether each disease should have its own panel.

funding

disclosure

references

1. Santarius T, Shipley J, Brewer D, Stratton MR, Cooper CS. A census of amplified and overexpressed human cancer genes. Nat Rev Cancer. 2010; 10(1): 59–64.

2. Remon J, Planchard D. AZD9291 in EGFR-mutant advanced non-small-cell lung cancer patients. Future Oncol 2015; 11(22): 3069–3081.

3. Teutsch SM, Bradley LA, Palomaki GE et al. The evaluation of genomic applications in practice and prevention (EGAPP) initiative: methods of the EGAPP Working Group. Genet Med 2009; 11(1): 3–14.

4. Frampton GM, Fichtenholtz A, Otto GA et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013; 31(11): 1023–1031.

5. Lawrence MS, Stojanov P, Polak P et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature 2013; 499(7457): 214–218.

6. Lavaud P, Andre F. Strategies to overcome trastuzumab resistance in HER2-overexpressing breast cancers: focus on new data from clinical trials. BMC Med 2014; 12: 132.

7. Godbout R, Dryja TP, Squire J, Gallie BL, Phillips RA. Somatic inactivation of genes on chromosome 13 is a common event in retinoblastoma. Nature 1983; 304 (5925): 451–453.

8. Krueger DA, Care MM, Holland K et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. N Engl J Med 2010; 363: 1801–1811.

9. Tutt A, Robson M, Garber JE et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 2010; 376(9737): 235–244.

10. Berger AH, Knudson AG, Pandolfi PP. A continuum model for tumour suppression. Nature 2011; 476(7359): 163–169.

11. Cardoso F, Costa A, Norton L et al. ESO-ESMO 2nd international consensus guidelines for advanced breast cancer (ABC2). Ann Oncol 2014; 25(10): 1871–1888.

12. Andre F, Mardis E, Salm M et al. Prioritizing targets for precision cancer medicine. Ann Oncol 2014; 25(12): 2295–2303.

13. Van Allen EM, Wagle N, Stojanov P et al. Whole-exome sequencing and clinical interpretation of formalin-fixed, paraffin-embedded tumor samples to guide precision cancer medicine. Nat Med 2014; 20(6): 682–688.

14. Dienstmann R, Jang IS, Bot B, Friend S, Guinney J. Database of genomic biomarkers for cancer drugs and clinical targetability in solid tumors. Cancer Discov 2015; 5(2): 118–123.

15. Dawson SJ, Tsui DW, Murtaza M et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013; 368(13): 1199–1209.

16. Bettegowda C et al. Detection of circulating tumour DNA in early- and late- stage human malignancies. Sci Transl Med 2014; 6: 224ra224.

17. Diehl F, Schmidt K, Choti MA et al. Circulating mutant DNA to assess tumor dynamics. Nat Med 2008; 14(9): 985–990.

18. Thierry AR, Mouliere F, El Messaoudi S et al. Clinical validation of the detection of KRAS and BRAF mutations from circulating tumor DNA. Nat Med 2014; 20(4): 430–435.

19. Siravegna G, Mussolin B, Buscarino M et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med 2015; 21(7): 795–801.

20. Rothé F, Laes JF, Lambrechts D et al. Plasma circulating tumor DNA as an alternative to metastatic biopsies for mutational analysis in breast cancer. Ann Oncol 2014; 25(10): 1959–1965.

21. Couraud S, Vaca-Paniagua F, Villar S et al. Noninvasive diagnosis of actionable mutations by deep sequencing of circulating free DNA in lung cancer from never-smokers: a proof-of-concept study from BioCAST/IFCT-1002. Clin Cancer Res 2014; 20(17): 4613–4624.

22. Frenel JS, Carreira S, Goodall J et al. Serial next-generation sequencing of circulating cell-free DNA evaluating tumor clone response to molecularly targeted drug administration. Clin Cancer Res 2015; 21(20): 4586–4596.

23. Garcia-Murillas I, Schiavon G, Weigelt B et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. Sci Transl Med 2015; 7(302): 302ra133.

24. Misale S, Yaeger R, Hobor S et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 2012; 486(7404): 532–536.

25. Diaz LA, Williams RT, Wu J et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012; 486(7404): 537–540.

26. Gerlinger M, Rowan AJ, Horswell S et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012; 366(10): 883–892. Erratum in: N Engl J Med 2012; 367(10): 976.

27. Landau DA, Carter SL, Stojanov P et al. Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. Cell 2013; 152(4): 714–726.

28. De Mattos-Arruda L, Weigelt B, Cortes J et al. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: a proof-of-principle. Ann Oncol 2014; 25(9): 1729–1735.

29. Michaut M, Chin SF, Majewski I et al. Integration of genomic, transcriptomic and proteomic data identifies two biologically distinct subtypes of invasive lobular breast cancer. Sci Rep 2016; 6: 18517.