Nominalism in Medicine: the case of personalized medicine or precision medicine

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Introduction

The destiny of medical terminology is sometimes unpredictable. Some terms, originating from a clinical or scientific setting, jealously maintain their proper definition and firmly uphold their technical meaning. Other terms take a different course, because the medical world, as a restricted community, is tempted to present them to the general population as a sign of prestige. So, these terms very quickly risk becoming a symbol of a triumphant Medicine and are exploited as a validation of the so-called medical scientism.

Two terms, currently present in today’s Medicine, need to be reexamined in order to restore them to their correct interpretation. They are personalized medicine and precision medicine. Even if these terms are used today almost as synonyms, there are some subtle but significant differences in their meaning. Personalized medicine is an older term that indicates the tailoring of medical treatment to the individual characteristics of each patient.1 Instead, precision medicine is an emerging field aimed to identify which approach will be effective for which patients based on genetic, environmental, and lifestyle factors. According to the National Research Council, precision medicine2 should be preferred to personalized medicine since the use of word personalized could be misleading and it may imply that treatments and preventions are being developed uniquely for each individual.

While the personalized medicine has been the main goal of clinicians since the beginning of medical sciences, precision medicine rose recently, together with our improved abilities of reading molecular, genetic and epigenetic variables in human beings.

In his 2015 State of Union speech, President Barack Obama invoked the possibility of matching a cancer cure to the genetic code by applying genomics to the entire field of cancer.3 As a result of this presidential proposal, in early 2015, the National Institute of Health (NIH) launched a national precision medicine initiative with the primary goal of rapidly improving prevention, diagnosis and treatment of cancer.4 Its central concept was to integrate individual-level data including genomics, biomarkers, lifestyle and other environmental factors, in order to provide better clinical care for individual patients.5 The precision medicine Initiative was primarily directed to support efforts in cancer genomics through substantial funding to the 2016 NIH and Food and Drug Administration (FDA) budgets. This was done through the creation of a one million-cohort study in the United States.

However, its long-term goal was to advance precision medicine in all areas of health. In other words, Obama had ambitiously hoped to convey through genomics a broader concept of precise tailoring therapies for subcategories of diseases or subpopulations of patients.6

The announcement by President Barack Obama sought also to create a greater and more equitable access to precision medicine advances.

If the NIH-led Precision Initiative is a pioneering participant-centered model prompted to ensure access to leading-edge cancer treatment to all Americans, the current situation in the United States is that less than 5% of cancer adults take part in clinical trials.7 Community oncologists often treat two thirds of their patients8,9 with limited access to new research and related advances. In Europe, where the maximum of scientific knowledge and logistic framework is represented, the
percentage of patients involved in clinical trials is much higher: 37.1% accounts for in Italy.16

To date, the precision medicine Initiative has been a well-defined health policy project, with important political implications. However, in these past two years, the term precision medicine has expanded quickly worldwide and has been used by the medical scientific community in a wider and often inappropriate way. Many studies, in every field of Medicine and in clinical practices such as translational research, often refer to or are preceded by the term precision medicine, in order to add scientific credibility or validity to their publication. In this way, the term precision medicine is misused and overused by the medical community. Thus this term of strong intrinsic value runs the risk of being reduced to a fashionable concept and as a consequence of being kidnapped.16

From this observation, three basic concerns arise on the critical use of these terms: a question of common sense, a subtler theoretical problem and an emphasis on evident ethical issues.

A question of common sense

The core philosophy of precision medicine is not new. It was the major expected clinical derivative of Human Genome Project that President Bill Clinton launched as revolutionary in 2000 and for which Francis Collins called for patience. The concept, as currently promoted, gives the implicit impression that it is arriving just in the nick of time so that doctors at last can start individually treating patients. John Murray, in 2012, had already criticized the concept12 by stating that doctors have been practicing personalized medicine since 400 BC: Hippocrates transferred the treatment of sick people from magical potions and religious incantation to empirical remedies based on careful observation and recording. Until now, intrinsic advocacy of Medicine and its practical and ethical aims have always taken individual variability into account in the prevention and creation of treatment strategies. Physicians always face and still struggle with novel diseases (i.e., pulmonary hantavirus, SARS), and in each instance their professional response has been to refine diagnoses and perfect treatments in order to save individual lives.15 The anti-HIV agents have been rapidly individualized despite the continuous evolution of genetic mutants. The same has occurred for the individuation of Legionella pneumophila as the cause of the Legionnaire’s disease epidemic. In the near future, it is highly probable that we will have a vaccine against the Ebola virus. This is precision medicine.

I firmly believe that doctors have always been practicing personalized medicine and that Medicine has always tried to meet precise and current public health needs. Any temporal boundary to restrict the term to its current involvement in genomics regarding prevention, diagnosis and treatment is misdirecting.

So, even limiting the major breakthrough of implementing genomics to clinical and scientific practices in the field of cancer is deceiving and may conceal the intention to exclude other clinical and research areas from the benefits of the Initiative. In order to avoid an oncological exclusivity in the precision medicine initiative, an inclusion was almost immediately proposed by the cardiovascular community.5

Yang11 raises other important concerns about how precision medicine is presently vigorously promoted. He suspects, on the one hand, that precision medicine could be driven by a selected group of dominant scientific personalities (Yang calls them gurus) who request research funding for genetics and molecular medicine and, on the other hand, by some genomic companies that may exploit this opportunity to earn income for unnecessary gene sequencing. This is not an unrealistic outcome: in several public encounters dealing with health policies, I have heard political representatives of the Italian National Health System (Servizio Sanitario Nazionale, SSN) proposing gene-sequencing screening for the general population!

A theoretical problem

Important theoretical issues argue against the improper meaning of the term precision medicine. There are three major points: i) statistically unproved precision (precision is sacrificed for interpretability); ii) intratumoral genomic heterogeneity; iii) unpredictability of epigenetic role in gene expression.

First point: A National Research Council report explains that in precision medicine the word precision is used in a colloquial sense1 to mean accurate and precise. In a colloquial sense, this also implies a high degree of certainty of a given outcome, as, for example, in a precision-guided missile. Hunter poses the question whether precision medicine will truly usher in an age of diagnostic and prognostic certainty and his answer is that the opposite will probably result.1 New tools for tailoring treatment will demand greater tolerances of uncertainty and a greater facility for calculating and interpreting probabilities that we were used to in the past as physicians and patients.

Hunter bases his comments on an important article of Cardoso et al., on the MINDACT Investigation13 on the use of the genomic test in treatment decisions in patients with early-stage breast cancer.14,15 Patients with early-stage breast cancer are often treated with adjuvant systemic therapy consisting of chemotherapy, endocrine therapy, agents against human epidermal growth factor receptor 2 (HER 2) or combination of these drugs. Treatment decisions are based13 on tumor (hormonal receptor and
HER2 status, tumor grade and size, and lymph-node status) and patient characteristics (age, menopausal status, and performance status). The standard practice has been to prescribe anti-HER2 therapy plus chemotherapy in HER2-positive cancers, chemotherapy alone in triple negative cancers and endocrine therapy, with or without chemotherapy, in hormone-positive HER2-negative cancers.

It is in this hormone-positive HER2-negative subgroup of cancers that the biggest confusion lies. Many patients do well with endocrine therapy alone and thus the adjunction of chemotherapy could cause overtreatment and exposure to the risk of toxic effects from adjuvant therapy without deriving significant benefits. On the other hand, in a subgroup of patients who are at higher risk of relapse, forgoing chemotherapy could mean undertreatment.

Tools that incorporate these features, such as Adjuvant! Online and PREDICT Plus, were created to assist in decision making. However, these algorithms do not take into account the individual biologic characteristics of the tumor. So, in 2007, the use of molecular signatures has been identified and proposed to select patients who could avoid adjuvant chemotherapy. Several genomic tests have been developed to better predict clinical outcome and to determine whether the addiction of adjuvant chemotherapy to endocrine therapy is worthwhile.

The 70-Gene signature test (MammaPrint) is one of the tools (such as Oncotype DX, PAM50) used to examine changes in genetic expression in order to provide prognostic information. The study of Cardoso et al. enrolled a vast cohort of patients - 6693 women - with early-stage breast cancer with the objective of establishing whether by adding the molecular signature (70-Gene Signature test) in defining genomic risk to the classical prognostic clinical criteria (Adjuvant! Online, PREDICT Plus) could lead to adjuvant therapy. The clinical predictors were defined as clinical, C, high (C h) or clinical, C, low (C l). The genomic predictors were defined as genomic, G, high (G h) and genomic, G, low (G l). The information extracted from cancer genomes of the patients included both somatic mutations (cancer genome sequencing) and functional changes that result from these mutations and epigenetic events (gene-expression alterations in tumor).

The patients were allocated, according to their clinical and genomic criteria, into 4 groups. Women at low clinical and genomic risk did not receive chemotherapy, whereas those at high clinical and genomic risk did receive chemotherapy. In patients with discordant risk, either the genomic risk or the clinical risk were used to guide chemotherapy. The primary goal was to assess among patients with a high-risk clinical and a low-risk gene-expression profile (C high, G low) who did not receive chemotherapy, the absence of distant metastases in 5-year follow up. They showed that the 5-year survival rate without distant metastases was 94.7%. Therefore, in this group of patients, the adjuvant chemotherapy could reasonably be avoided being the risk of distant metastases at 5 years 1.5%.

The great value of Cardoso’s study, MINDACT, - apart from being a fantastic collaborative act of researchers from 112 institutions in Europe - is having dared to include in its trial even patients at clinical high risk, as HER2-positive and triple negative, to whom one would usually prescribe a course of chemotherapy and to test whether chemotherapy could be safely omitted for those with low genomic risk.

The conclusion of the study was therefore largely positive, but Hunter, in a more complete examination of the study, points out that to make results interpretable both statistically and clinically, a continuous variable, namely the genomic score derived from 70 separate rate gene-expression analyses, is dichotomized into ‘high’ and ‘low’, in other words precision is sacrificed for interpretability.

The MINDACT study provides an excellent example of complexities surrounding precision medicine and its limitations. More precision brings more uncertainties. With medicine getting more precise, patient care is getting more and more imprecise. When a patient arrives in clinic we may have precise knowledge of the genomic of her cancer but only uncertainties in recommending treatment options.

In the strict scientific sense, precision and uncertainty cannot be compatible, they do not agree with each other. For even more obvious reasons, it is improper to use the term precision medicine in common language and doctors who interpret it correctly find themselves lost. The word precision medicine is currently moving to an interpretation very far from its original sense.

Second point: The tendency to misuse the term precision medicine has been greatly reinforced in oncology by the general introduction of targeted therapy, that is a therapy strictly tailored to the genetic variant found in tumor samples.

Molecular characterization of tumors has permitted targeted treatment, for several types of tumor, to prolong survival rates and improve patient quality of life. As a consequence targeted therapy is now the standard of care and an important component of cancer treatment.

A closer examination of this topic, instead, suggests that there are some problems with the benefits of targeted therapy in patients with cancer. We need a convincing explanation of these problems. In the SHIVA trial - the only randomized controlled trial (RCT) of precision medicine - Le Torneau et al. compared RCTs outcomes of patients treated with tar-
geted drugs selected to match genetic sequences of their tumors with outcomes in patients who received standard therapy (investigator choice) and found no progression-free survival difference between these strategies. In a comment on the results of the SHIVA trial, Tannock and Hickman reached the conclusion that the current clinical evaluation of precision medicine has only limited success.21 The authors attribute this partial failure to two general constitutive aspects: the intratumoral genomic heterogeneity, as a result of a Darwinian evolution of the tumor, and the inherent limitations of molecular target agents.

Let’s examine only the first point, intratumoral genomic heterogeneity.

Biopsy samples from different regions of multiple tumors (or from primary tumor and metastases) have shown substantial heterogeneity. Sequential biopsy samples from tumor sites in the same patient also show considerable genomic heterogeneity: some mutations are present in all sampled cancer cells and are clonal markers of the cancer, while other mutations are unique to subclones that are generated. This observation has led to the hypothesis of a Darwinian model in tumor evolution, which can be represented by a branching tree. As a practical consequence, a molecular analysis of a single biopsy sample from a tumor does not represent other parts of it.22

This heterogeneity is present early in cancer development23 and - this is consequently important - subclones are selected by cancer treatments.24-28

Intratumoral genomic heterogeneity represents a severe limit to the potential targeting of mutated pathways on the basis of molecular analysis of a tumor sample. This represents a critical limit to the central concept of precision medicine and explains the therapeutic failure of very high percentage of anticancer drugs.21

In a more general biologic sense, it underlines that the mutation landscape of the most common cancer is highly complex. The least mutated cancers have on average 0.28 mutations per megabase, the most mutated have 8.15 mutations per megabase.29 In a moderately mutated disease like pancreatic cancer (mean, 2.64 mutations per megabase), many of these mutations are passenger mutations (conferring no survival advantage on the clone), even driver mutation (which propels cancer progression) occurs at low prevalence. A substantial diversity of mechanism involved occurs in pancreatic cancer progression.30

The term precision oncology is therefore overly broad. It describes different strategies in cancer medicine and which activities and actions fall under its domain is unclear. Prasad and Gale19 analyzed the use of this term by searching Google Scholar for precision oncology over 3 intervals and observed that the use of the term has largely changed over time. In the earliest, it described targeted therapies as such as vascular endothelial growth factor inhibitors (bevacizumab) or BCR/ABL 1 inhibitors (imatinib); later it was used to describe the selection of therapies based on data from analysis of biomarkers (for instance, adjuvant chemotherapy guided by the results of genomic testing such as Oncotype DX panel in women with breast cancer in cited MINDACT study). More recently it refers to the use of data from next-generation sequencing to guide therapy. The last definition is directing therapy independently from cancer type and instead by mutations: persons with BRAF V600E mutation treated similarly for acute myeloid leukemia or breast cancer.

The imprecise use of the word precision constitutes almost jargon.19 At least, the definition of precision oncology is an evolving definition.

An attempt to reach the true identity of tumoral cells and monitoring cancer features and behavior through blood examination instead of solid biopsies is currently provided by the development of liquid biopsies.31 Unlike traditional solid-tissue biopsies, liquid biopsies remove the need for invasive procedures, allowing test for signs of cancer from a simple blood-draw. Two kinds of cancer-associated markers can be evaluated by this approach:22,33 circulating tumoral cells (CTC) and circulating tumoral DNA (ctDNA).

CTC are released from the tumor and isolated by peripheral blood but their rarity, short half-life and uncertain specificity in the heterogeneous context of the blood make their isolation and identification challenging, therefore their overall contribution to the diagnosis and to a more appropriate treatment is very small. By contrast, the possibility of detecting tumor specific mutations in the blood circulating tumoral DNA has been proposed as an alternative, minimally invasive diagnostic tool to monitor tumor growth and follow evolution of tumor heterogeneity.

For example liquid biopsy has been employed to determine epidermal growth factor receptor mutation status in European and Japanese patients with advanced non-small cell lung cancer, non-small cell lung cancer.34 Treatments that lead to the death of drug sensitive tumor cells might accelerate the emergence of drug-resistant subpopulations by means of selection of pre-existing tumor subclones or by induction of new genetic mutations.23-25 Liquid biopsy may help foreseeing this phenomenon allowing anticipation of alternative treatment strategies.

Third point: To date, studies on molecular pathology have implied the need for a more comprehensive evaluation involving not only gene sequencing but also gene-expression profiles in order to firmly contribute to the selection of effective drugs to control tumors. In this context the role of epigenetic regulation of gene expression in tumor is very important.35

For a long time, gene expression regulation has
been considered as a mono-dimensional process in which each gene was controlled by the activity of the nearest promoter. Systematic functional analysis of the non-coding genome has revealed that gene expression is far more complicated than expected and requires a widespread regulatory landscape involving specific chromatin architectures and the hierarchical interactions of multiple interspersed regulatory elements (https://www.encodeproject.org). Rapid changes in the epigenetic organization and structure of the non-coding genome allow cancer cells to respond in a very rapid and very efficient way to many intrinsic or external stimuli and to overcome stressful situations.

As well, environmental pesticides and fungicides, stimulant drug exposure and oxidative stresses and increased ROS formation, smoking, diet, physical exercise, and traumatic accidents in pediatric age and adolescence can alter the epigenetic organization of chromatin resulting in dramatic changes in gene expression.

The understanding of the sophisticated mechanisms that cancer cells adopt in achieving changes in gene expression and function, by either mutations or epigenetic events, is continuously improving. In addition to the three most known epigenetic mechanisms of regulation (DNA methylation, histone profile modification such as deacetylation, microRNAs), new researches based on solid principles and experimental validation have discovered other mechanisms, even if not well-defined, such as long non-coding RNAs and circular RNAs which seem to have an equally important role.

Their complex, various and intertwined role on DNA expression cannot be foreseen in a single subject whose biology is greatly conditioned by continuous environmental etiologic inputs. The problem is not a theoretical one. Gene-environment interactions are substantially unpredictable. Even when the maximum number of epigenetic prognostic factors, recognized as influencing, are inserted in a prognostic assessment tool (as in the case of breast cancer), the effect of other possible unknown environmental factors, the mechanism of which genomic expression is still poorly understood, escapes from our evaluation.

In conclusion, the basic conceptual question of whether genotyping accurately predicts or not the individual risk of developing a disease or response to a drug is substantially unsolved. Health is determined both by genetic (internal) and environmental (external) factors. People with the same genetic background in different environments have different phenotypic outcomes. The presence of a particular genetic polymorphism or haploid pattern does not always play a pivotal role in the development of the disease risk or drug response.

Nevertheless, even in this completely theoretical interpretation, precision medicine is imprecise. The role of epigenetics in determining expression of gene, even if fully accepted, is constitutively ill defined and cannot be reduced to a predictable measure. Therefore, is precision medicine impossible?

### Ethical problems

The broad use of these two terms often emphasizes evident ethical issues.

Firstly, the appealing concept of molecular characterization of tumors has been marketed directly to the patient despite a lack of evidence of benefit. As a consequence, as in every medical undertaking, it has generated acute personal expectancies, and not only in patients.

Secondly, the enormous amount of data generated by clinical whole genome or whole exome sequencing and the extent of current uncertainties with respect to data interpretation and disease association is a unique ethical issue. An inherent problem arises in communicating such uncertainties to patients.

If we reflect upon the fact that estimates of disease-risk are based on inherited germline sequencing, we will face an even vaster array of probabilities. Personal required skills in mentally handling this complexity is outright frightening.

Thirdly, ethical issues that will get even worse for clinicians in the future, apart from the labyrinthine informed consent, privacy and unexpected confidential processes, outcomes, such as incidental findings will be particularly worrying.

Obviously to solve these dilemmas, we can rely on previous ethical analyses and recommendations. In any case, the general ethical scenario will not be so easily managed.

### Precision/personalized or individualized?

Let’s consider some lexical objections.

Firstly, during the therapeutic process of so called personalized/precision medicine, drugs act precisely, spatially, physically and chemically on the grossly dominant genetic variant present in a tumor, not on the patient’s organism considered as a whole.

Secondly, adopting a core-word like person when referring to this somatic alteration of the body, such as cancer or other diseases, is open to criticism. In occidental culture, the term person identifies a complex in which physical, biological, psychological and environmental components (in Greek philosophy: bios, psyche, logos, nous) contribute to the realization of a unique entity, which we call personality. In other words, a person is a thinking being who, through will, sentiment and self-consciousness, represents the final stable integrated structure that is man. Differently,
while affecting a body in one of its somatic parts, every disease would be preferably called a disease at/of an individual or of a subject. To be coherent, medicine, which adheres, treats and cures the diseases of the single patient’s body should be named, at the most, individualized medicine instead of personalized.

Conclusive remarks

In summary, a risk of a slippery terminology is very apparent.

In crossing over from a scientific and medical meaning to a political interpretation and back again to a newly altered scientific and medical one, the term precision medicine may migrate from its true meaning. Doctors and scholars must be alert not to fall into the trap of using trendy concepts whose generic appeal may be strong but completely miss the true significance. Our duty as physicians is to convey clarity and truth when dealing with our patients.

References

1. Hunter DJ. Uncertainty in the Era of precision medicine. N Engl J Med 2016;375:711-13.
2. Committee on a framework for developing a new taxonomy of disease. Toward precision medicine: building a knowledge network for biomedical research and a new taxonomy of disease. Washington, DC: National Academy Press; 2011.
3. Cancer Moonshot, Bethesda, MD: National Cancer Institute. Available from: http://www.cancer.gov/research/key-initiatives/moonshot-cancer-initiative
4. Fiore RN, Goodman KW. Precision medicine ethics: selected issues and developments in next-generation sequencing, clinical oncology, and ethics. Curr Opin Oncol 2016;28:83-7.
5. Shah SH, Arnett D, Houser SR, et al. Opportunity for a knowledge network for biomedical research and a new taxonomy of disease. Toward precision medicine: building a knowledge network for biomedical research and a new taxonomy of disease. Washington, DC: National Academy Press; 2011.
6. Ashley EA. The precision medicine initiative: a new national effort. JAMA 2015;313:2119-20.
7. Lowy DR, Collins FS. Changing the trajectory for cancer. N Engl J Med 2016;374:3901-4.
8. Bell JA, Balneaves LG. Cancer patient decision making related to clinical trials participation: an integrative review with implications for patient’s relational autonomy. Support Care Cancer 2015;23:1169.
9. Spandrio JD. Oncology patient-centered medical home. J Oncol Pract 2012;8:475-9s.
10. Osservatorio Nazionale Sperimentazione Clinica (OsSC). 15° Rapporto nazionale 2016. Roma: Agenzia Italiana del Farmaco (AIFA); 2016.
11. Yang Z. Do not let precision medicine be kidnapped. Front Med 2015;9:512-3.
12. Murray J. Personalized medicine: been there, done that, always needs work. Am J Resp Crit Care Med 2015;185:1251-2.
13. Cardoso F, van’t Veer LJ, Bogaerts J, et al. 70-Gene signature as an aid to treatment decisions in early-stage breast cancer. N Engl J Med 2016;375:717-29.
14. Van de Vijver MJ, He YD, van’t Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999-2009.
15. Sorlie T, Perou CM, Tibshirani R, et al. Gene-expression patterns of breast carcinomas distinguish tumor sub-classes with clinical implications. Proc Natl Acad Sci U S A 2001;98:10969-74.
16. Dowsett M, Goldhirsch A, Haynes DF, et al. International Web-based consultation on priorities for translational breast cancer research. Breast Cancer Res 2007;9:R 81.
17. Sotiriou C, Pustzai I. Gene expression signature in breast cancer. N Engl J Med 2009;360:790-800.
18. Gyawali B. MINDACT: mind-blowing uncertainties with precision medicine. eCancerNews; 25 August 2016. Available from: http://ecancer.org/news/10059
19. Prasad V, Gale RP. What precisely is precision medicine - and will it work? TheASCOPost; 25 January 2017. Available from: http://www.ascopost.com/issues/january-25-2017
20. Le Tourneau C, Delord J-P, Goncalves A, et al. Molecularly targeted therapy based on tumor molecular profiling versus conventional: therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 3 trial. Lancet Oncol 2015;16:1324-44.
21. Tannock IF, Hickman JA. Limits to personalized cancer medicine. N Engl J Med 2016;375:1289-94.
22. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumoral heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883-92.
23. Swanton C. Intratumoral heterogeneity evolution through space and time. Cancer Res 2012;72:4875-82.
24. Carreira S, Romanel A, Goodall J, et al. Tumor clonal dynamics in lethal prostate cancer. Sci Transl Med 2014;6:254ra125.
25. Brastianos PK, Carter SL, Santagata S, et al. Genomic characterization of brain metastases reveals branched evolution and potential therapeutic targets. Cancer Discov 2015;5:1164-77.
26. Murugaeus N, Wilson GA, Birkbak NJ, et al. Tracking the genomic evolution of esophageal adenocarcinoma through neo adjuvant chemotherapy. Cancer Discov 2015;5:821-31.
27. Bhang HE, Ruddu DA, Krishnamurty Radhakrishna V, et al. Studying clonal dynamics in response to cancer therapy using high-complexity baarcoding, Nat Med 2015;21:440-8.
28. Yates LR, Gerstung M, Knappskog S, et al. Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med 2015;21:251-9.
29. Kandoth C, Mc Lellan MD, Vandin F, et al. Mutational landscape and significance across 12 major cancer types. Nature 2013;502:333-9.
30. Waddel N, Pajic M, Patch AM, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 2015;518:495-501.
31. Forte VA, Barrak DK, Elhodaky M, et al. The potential for liquid biopsies in the precision medical treatment of breast cancer. Cancer Biol Med 2016;13:19-40.
32. Alix-Panabières C, Pantel K. Clinical applications of cir-
culating tumor cells and circulating tumor DNA as liquid biopsy. Cancer Discov 2016;6:479-91.

33. Normanno N, De Luca A, Gallo M, et al. The prognostic role of circulating tumor cells in lung cancer. Exp Rev Anticancer Ther 2016;16:859-67.

34. Reck M, Hagiwara K, Han B, et al. ctDNA determination of EGFR mutation status in European and Japanese patients with advanced NSCLC: the ASSESS study. J Thor Oncol 2016;11:1682-9.

35. Katz TA, Huang Y, Davidson NE, Jankowitz RC. Epigenetic reprogramming in breast cancer: from new targets to new therapies. Ann Med 2014;46:397-408.

36. Kalda A, Zharkovsky A. Epigenetic mechanism of psychostimulant-induced addiction. Int Rev Neurobiol 2015;120:85-105.

37. Afanas’ev I. Mechanism of superoxide signaling in epigenetic processes: relation to aging and cancer. Aging Dis 2015;3:216-27.

38. Marczylo EL, Amoako AA, Konje JC, et al. Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? Epigenetics 2012;7:432.

39. Haghighi F, Galalfy H, Chen S, et al. DNA methylation perturbations in genes involved in polyunsaturated fatty acids biosynthesis associated with depression and suicide risk. Front Neurol 2015;6:92.

40. Recchioni R, Marcheselli F, Antonicelli R, et al. Physical activity and progenitor cell-mediated endothelial repair in chronic heart failure: is there a role of epigenetics? Mech Ageing Dev 2016;159:71-80.

41. Brown WM. Exercise-associated DNA methylation changes in skeletal muscle and the importance of imprinted genes: a bioinformatic meta-analysis. Br J Sports Med 2015;49:1567-78.

42. Marques Rocha JL, Milagro FI, Mansego ML, et al. LINE-1 methylation is positively associated with healthier lifestyle but inversely related to body fat mass in healthy young individuals. Epigenetics 2016;11:49-60.

43. Mendell JT. Targeting a Long Noncoding RNA in breast cancer. N Engl J Med 2016;374:2287-9.

44. Coote JH, Joyner M. Is precision medicine the route to a healthy world? Lancet 2015;385:1617.

45. Joyner MJ, Paneth N. Seven questions for personalized medicine. JAMA 2015;314:999-1000.

46. Gray SW, Cronin A, Bair E, et al. Marketing of personalized cancer care on the Web: an analysis of Internet websites. J Natl Cancer Inst 2015;107:5.