Ipsos Healthcare

Conquering Complexity:
The Coming Revolution in Oncology Biomarker Testing

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I. On the cusp: from evolution to revolution

The clinical management of cancer patients has undergone a remarkable evolution in the past decade, with the concept of personalized medicine now well-entrenched in the treatment paradigm of a significant number of tumor types. Whereas treatment decisions used to rely on a combination of clinical observations, various macro-imaging techniques and general histopathological findings, oncologists now have a range of biomarker tests at their disposal to make a more informed drug choice. Notwithstanding the added complexity such testing brings[1], this approach ultimately benefits physicians and patients alike: treatments which are likely to lead to better response rates and more prolonged responses can be selected based on molecular characteristics exhibited by the patient's tumor. Cancer is a cunning adversary, and fighting back requires an array of approaches that strike at the very heart of this foe: by targeting the mutations that drive and empower it.

However, despite the advent of targeted therapies and the associated rise in the use of companion and complementary diagnostics, we are only just emerging from the initial exploratory stages of oncology biomarker testing. As will be argued in this paper, we are on the cusp of a much more radical revolution, and the molecular diagnostics landscape – and indeed the very way we think about fighting cancer – is set to change dramatically in the coming decades. It is crucial that any company entering this space prepare for this imminent upheaval and plan their launch strategy accordingly.

II. Phase change: from solids to liquids

The central principle at the core of using molecular diagnostics to inform anti-cancer treatment decisions is that the patient's tumor exhibits certain aberrant characteristics that predispose it to interventions at the protein and/or nucleic acid level. These characteristics can be either rare (e.g. ALK rearrangements, seen in 3-7% of NSCLC cancers[2]) or common (e.g. BRAF mutations, seen in ~50% of melanomas[3]), but the key here is that they are, by their very nature, not universal. Generally speaking, these mutations or abnormal expression patterns are present only in the tumor cells: they are somatic mutations, not shared with the rest of the body's cells, and absent from the germline (the body's reproductive cells). There are exceptions, the most notable being BRCA mutations, which are often inherited through the germline and hence present in all of the patient's cells. For the purposes of this discussion, however, we will focus on the more common somatic tumor mutations.

By definition, these mutations are not present in non-malignant cells in the patient's body, and therefore it is no surprise that the traditional approach for tumor biomarker testing for patients with solid cancers relies heavily on obtaining tissue samples from the actual tumor. This, unfortunately, comes with several drawbacks: initial biopsies used in diagnosis don't always contain enough viable tissue for testing (as shown in Figure 1), biopsy samples cannot be stored indefinitely without degrading, there is considerable intra-tumor genetic heterogeneity[4], and re-biopsies mean the patient – often with a poor performance status – needs to undergo another invasive procedure. The latter point is particularly important when it comes to patients who have experienced a disease progression, which may be indicative of the tumor having acquired novel somatic mutations not present at the time the initial biopsy was taken.
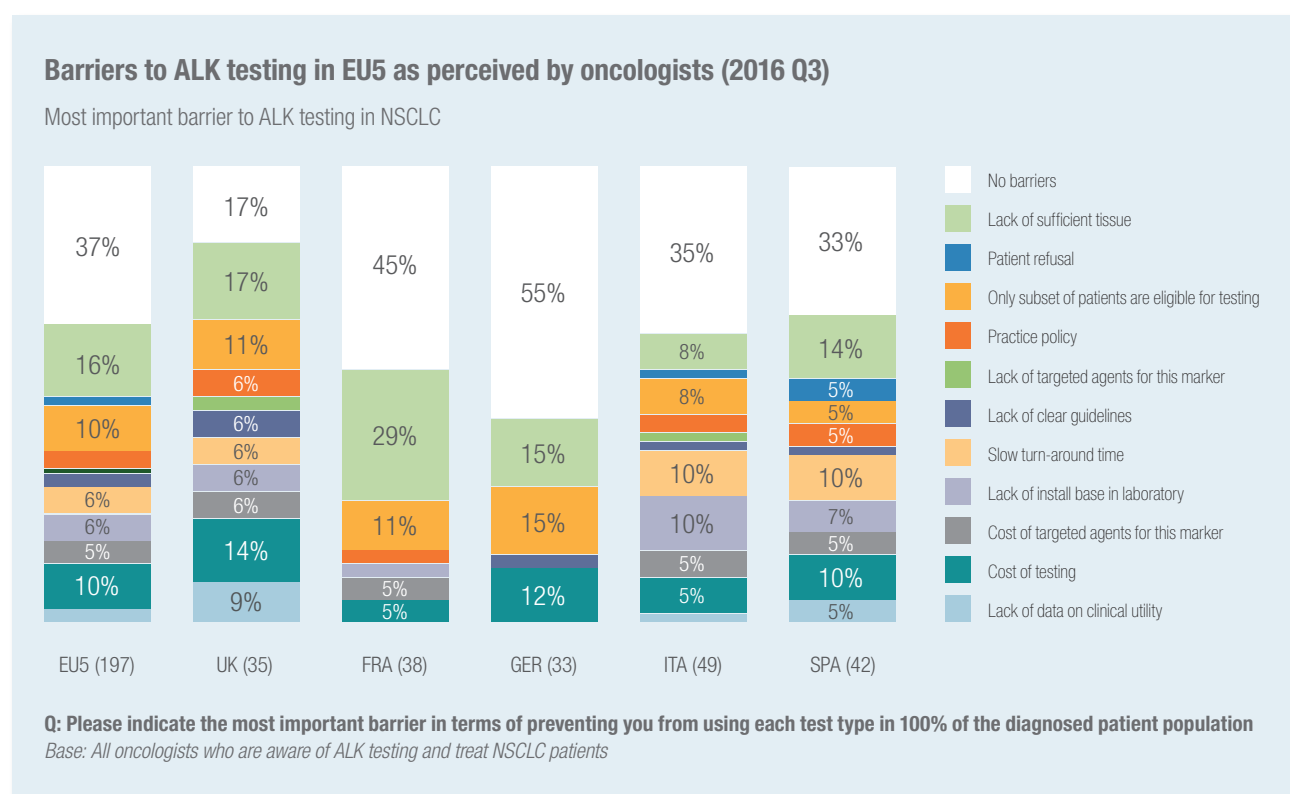
Barriers to ALK testing in EU5 as perceived by oncologists (2016 Q3)

Most important barrier to ALK testing in NSCLC

Legend:
- No barriers
- Lack of sufficient tissue
- Patient refusal
- Only subset of patients are eligible for testing
- Practice policy
- Lack of targeted agents for this marker
- Lack of clear guidelines
- Slow turn-around time
- Lack of install base in laboratory
- Cost of targeted agents for this marker
- Cost of testing
- Lack of data on clinical utility

EU5 (197): 37%, 16%, 10%, 6%, 6%, 5%, 10%
UK (35): 17%, 17%, 11%, 6%, 6%, 6%, 6%, 6%, 14%, 9%
FRA (38): 45%, 29%, 11%, 5%, 5%
GER (33): 55%, 15%, 15%, 12%
ITA (49): 35%, 8%, 8%, 10%, 10%, 5%, 5%
SPA (42): 33%, 14%, 5%, 5%, 5%, 10%, 7%, 5%, 10%, 5%

Q: Please indicate the most important barrier in terms of preventing you from using each test type in 100% of the diagnosed patient population
Base: All oncologists who are aware of ALK testing and treat NSCLC patients

Fig. 1: *Stated main barriers to ALK testing in NSCLC amongst oncologists and other drug-treating specialties in EU5, who were asked to select their single most important barrier when it came to testing for ALK rearrangements in NSCLC. Lack of sufficient tissue was the most frequently stated top barrier for this marker, which tends to come second in the testing sequence. (Source: Ipsos EU Solid Tumors MDx Monitor 2016 Q3; research conducted online August – November 2016)*

For some time, it seemed that this was to be accepted as an inherent limitation of solid tumor biomarker testing, and many tests were approved since the late 90s that relied on this principle of tissue-based testing. Below (Table 1) is a non-exhaustive list of a number of FDA-approved biomarker tests for various solid cancer types, and their respective sample requirements:

Examples of FDA-approved in-vitro diagnostics and their sample requirements

Test name	Manufacturer	FDA approval date*	Cancer type*	Associated drug*	Sample requirements
HercepTest	Dako	May 1998	Breast	Herceptin	Tissues fixed in neutral buffered formalin
cobas EGFR mutation test (v1)	Roche Molecular Systems	May 2013	NSCLC	Tarceva	Formalin-fixed, paraffin-embedded tissue containing at least 10% tumor content by area
Vysis ALK Break Apart FISH probe	Abbott Molecular	August 2011	NSCLC	Xalkori	Formalin-fixed, paraffin-embedded tissue
THxID™ BRAF Kit	bioMérieux	May 2013	Melanoma	Tafinlar/ Mekinist	Formalin-fixed, paraffin-embedded tissue with at least 20mm^2 of tumour area and at least 80% tumor content by area

Table 1: Examples of FDA-approved in-vitro diagnostics and their sample requirements. Source: FDA.gov, manufacturer websites

It turned out, however, that it was possible to recruit one of cancer's main weapons to help fight it. Solid tumors, like breast and lung cancer, begin as small groups of localized malignant cells in the primary organ in which they arose, but ultimately become much more dangerous to the host body by spreading to distant organs. Their relentless drive to replicate means that tumor cells often gain the ability to spread through the human body, in many cases (especially if not treated early enough) resulting in distant metastases. Cancer metastasizes by shedding cells from the primary tumor, which enter the lymphatic system and/or the bloodstream[5] and travel to distant sites where they eventually take hold and replicate. These so-called circulating tumor cells (CTCs) were first observed by Thomas R. Ashworth as early as 1869[6], and it was theorized that it

should one day be possible to detect CTCs and use them for biomarker testing. Furthermore, the – much more recent - discovery of cell-free circulating tumor DNA (ctDNA[7]) and RNA (ctRNA) opened up additional possibilities for detection of mutations in the blood, through so-called "liquid biopsies".

Before that could become a reality, several key challenges needed to be overcome: assays needed to be sensitive and specific enough to detect somatic mutations from very low concentrations of tumor DNA, against a background of non-malignant cells that far outnumber the cancerous cells/nucleic acid[8]. With the improvement in sensitivity of nucleic acid sequencing techniques and an associated drop in cost, this only became a practical possibility in the last few years. Indeed, the first half of this decade saw the beginnings of an increase in clinical trials that investigated testing CTCs and ctDNA for the purpose of selecting the most appropriate cancer treatment, and this culminated in the first FDA approval of a commercial liquid biopsy-based assay in June 2016: the Roche cobas EGFR Mutation Test v2[9]. Prior to this date, the kit was already available and approved for detecting sensitizing EGFR mutations in formalin-fixed paraffin-embedded (FFPE) tissue samples to identify patients eligible for treatment with erlotinib. The label extension allowed the kit to be used for identifying those mutations based on ctDNA. As the FDA approval at the time stated, "this new test may benefit patients who may be too ill or otherwise unable to provide a tumor specimen for EGFR testing."[10]
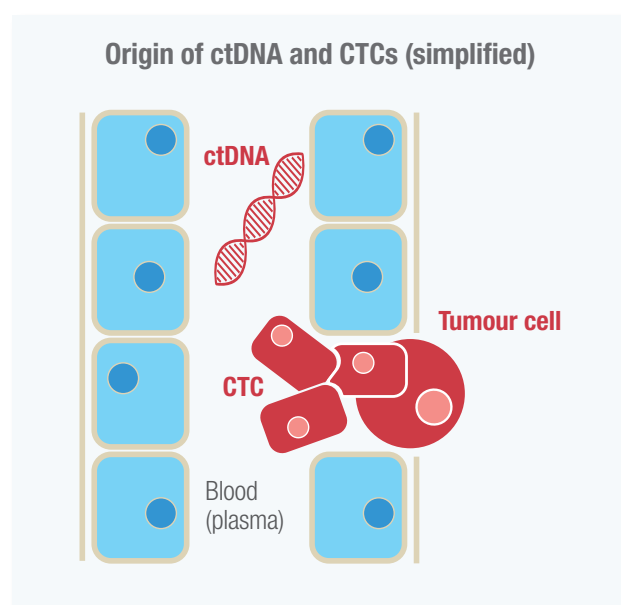

Fig. 2: *Origin of ctDNA and CTCs (simplified). Blue cells represent healthy cells lining the blood vessels, whereas red cells represent malignant material dislodging from the tumor tissue and entering the bloodstream, either as whole cells (CTCs) or as cell-free genetic material (ctDNA)*

An important subsequent development was the further label extension of this test kit as a companion diagnostic for osimertinib three months later, based on the detection of T790M resistance mutations in blood or tissue samples.[11] This is significant, as the clinical and practical benefit of being able to test for (acquired) resistance mutations based on blood samples exceeds that of testing for (initial) sensitizing mutations. As most testing for sensitizing mutations is done at diagnosis, there usually is enough tissue available based on the initial biopsy (or resection) that was performed to confirm the cancer diagnosis and histology, limiting the need for blood-based tests (although there are theoretical advantages in turn-around time over tissue-based testing). On the other hand, testing for resistance mutations should – by definition – be performed after treatment failure, which therefore requires a new sample to be collected in order to detect any newly acquired mutations. Furthermore, there may be a benefit to regularly conducting tests for resistance mutations such as T790M, to track the evolution of the genetic make-up of the tumor during its exposure to targeted therapies. Hence, with liquid biopsies removing many of the barriers relating to re-testing, and with an increasing number of next-generation targeted therapies designed to overcome resistance mutations in development[12], we can expect the use of liquid biopsies for biomarker testing to show steady growth in the coming years from its current low levels (see Fig. 3).
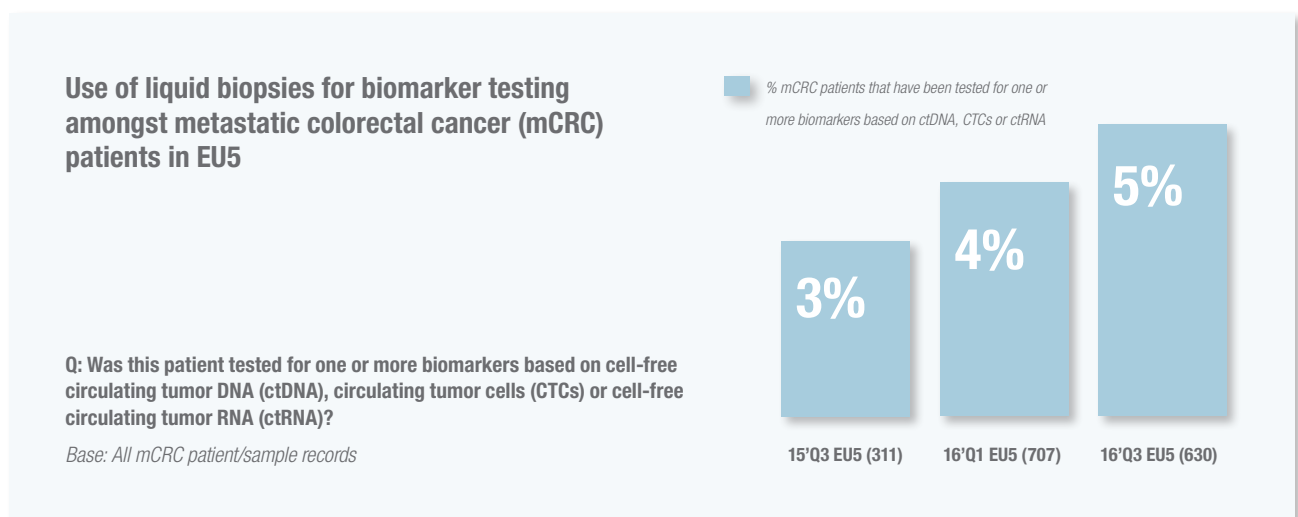
Use of liquid biopsies for biomarker testing amongst metastatic colorectal cancer (mCRC) patients in EU5

% mCRC patients that have been tested for one or more biomarkers based on ctDNA, CTCs or ctRNA

15'Q3 EU5 (311)	16'Q1 EU5 (707)	16'Q3 EU5 (630)
3%	4%	5%

Q: Was this patient tested for one or more biomarkers based on cell-free circulating tumor DNA (ctDNA), circulating tumor cells (CTCs) or cell-free circulating tumor RNA (ctRNA)?

Base: All mCRC patient/sample records

Fig. 3: *Use of liquid biopsies for biomarker testing amongst metastatic colorectal cancer (mCRC) patients in EU5. Data was extracted from real, de-identified patient records, and physicians were asked, for each patient: Was this patient tested for one or more biomarkers based on cell-free circulating tumor DNA (ctDNA), circulating tumor cells (CTCs) or cell-free circulating tumor RNA (ctRNA)? Note that all patient records in this study were tested for at least one biomarker. (Source: Ipsos EU Solid Tumors MDx Monitor 2015 Q3, 2016 Q1, 2016 Q3; research conducted online August – October 2015, March – April 2016, August – November 2016)*

Indeed, since 2015, a range of single-marker tests based on liquid biopsies have been made commercially available (some as non-FDA-approved lab-developed tests), covering a range of different markers and cancer types, as illustrated in Table 2:

Examples of single-marker liquid-biopsy based tests

Test name	Manufacturer	Approval status	Cancer type(s)	Associated drug(s)	Sample requirements
cobas EGFR mutation test v2	Roche Molecular Systems	FDA-approved as CDx in US CE-IVD in EU	NSCLC	Tarceva Tagrisso	2mL plasma (recommended 4-6mL plasma)
Therascreen EGFR Plasma RFQ PCR Kit	Qiagen	Available for CE-IVD use in EU	NSCLC	Iressa	2mL plasma
Target Selector Assays	Biocept	CLIA-certified	NSCLC, Melanoma, Gastric, CRC, Prostate, Breast	Various	4mL plasma
Trovera EGFR / KRAS / BRAF	Trovagene	CLIA-certified	NSCLC, CRC, Pancreatic, Melanoma, Ovarian	Various	Urine or plasma
Genestrat EGFR / KRAS / ALK / BRAF / RET / ROS1	Biodesix	CLIA-certified	NSCLC	Various	Plasma
OncoBEAM RAS	Sysmex Inostics	Available for CE-IVD use in EU	CRC	Erbitux, Vectibix	Plasma
BRACAnalysisCDx	Myriad Genetics	FDA-approved as a CDx in US	Ovarian cancer	Lynparza	Plasma

Table 2: Examples of single-marker liquid-biopsy based tests available in the US and EU, as of April 2017

As innovative and promising as these tests are, despite the radically different sample requirements, they still represent a fundamentally unchanged approach in terms of what markers are being tested: each of the tests listed above looks for mutations/changes in expression levels of single genes or proteins (though some commercial laboratories allow for multiple individual markers to be requested at the same time, provided there is enough liquid in the sample). Cancer, however, is a hugely complex disease (or set of diseases) and involves the interaction between many different such genes and proteins. Single-marker tests, even when used in combination, only scratch the surface of elucidating the molecular mechanics underpinning each patient's tumor.

III. Strength in numbers: from single-marker tests to pan-cancer testing panels

The inherent limitations of single-marker tests are two-fold:

i. **Genes do not act in isolation.** The forces that drive the uncontrolled replication of cancer cells are far more complex than simple on/off switches. While a certain degree of success has been obtained by targeting specific, individual mutations in cancer cells, these approaches fail to address the fact that oncogenesis (the transformation of healthy cells into cancer cells) is a highly complex process that is often the result of a combination of mutations acting together, and that the kinases (a class of enzymes) commonly affected by oncogenetic mutations form parts of intricate signal transduction cascades[13] (signaling pathways within and between cells).

ii. **Not all cancers exhibit mutations commonly found in that cancer type.** Clinical trials have understandably focused on mutations or amplification of genes that are relatively frequently observed in the population of interest. However, given the number of genes upstream and downstream of those common markers, just testing for those particular biomarkers would not identify any of the many potential rare abnormalities. By extension, certain mutations are very common in certain cancer types only (for example, KRAS mutations in colorectal carcinoma), but are rare in other cancers, and are therefore often bypassed in the testing process in favour of more common mutations in those cancers (for example, BRAF mutations in melanoma). The fact that they are less common in those cancers, however, does not mean they are non-existent: KRAS mutations can be found in approximately 0.7% of all melanomas[14].

Until relatively recently, the only feasible way to overcome those limitations was to increase the number of single-marker tests performed on individual cancer tissues: rather than testing melanoma samples for just BRAF mutations, for example, separate BRAF, KRAS, NRAS and PIK3CA mutation tests could be performed to detect less common aberrations and to gain more insight into the specific molecular characteristics of that patient's cancer. However, this approach presents several challenges: it requires more viable tumor tissue, it requires more time and effort, it significantly complicates the testing workflow and reagent/assay requirements, and it multiplies the cost of testing every time an additional marker is added. Because of those limitations, using this approach beyond four or five different genes is usually not feasible in practice.

The rise of next generation sequencing (NGS) — and in particular the continuous drop in price that has occurred in the last decade[15] - has resulted in a way to approach this problem from a different angle: rather than testing each gene with an individual assay, gene panels run on highly advanced equipment aim to sequence a large number of genes simultaneously. The resulting output then provides the physician with a mutational status for each of those many genes in one go, greatly increasing the available information regarding that patient's tumor. While NGS cancer panels have been around for a while, their cost and complexity meant that they were mostly limited to somewhat experimental panels in large academic institutions. More recently however, several companies have launched and marketed commercial testing panels as integrated solutions: doctors are able to send samples for analysis to those companies' dedicated laboratories, and will receive - often very detailed - reports on the genetic characteristics of the tumor, complete with treatment and/or clinical trial recommendations.

What's more, most of those panels are marketed as tumor-agnostic, or pan-cancer, testing panels, meaning that they can be used for any of a wide range of solid (and sometimes also hematological) cancers, since they cover such a large number of genes. The number of commercial pan-cancer testing panels has undergone a veritable explosion in the last few years; Figure 4 shows the market shares of just a selection of those panels currently marketed in the US. Also included is the aggregate total of in-house test panels (panels typically developed by academic institutions for use by their practice only and not available as branded solutions).
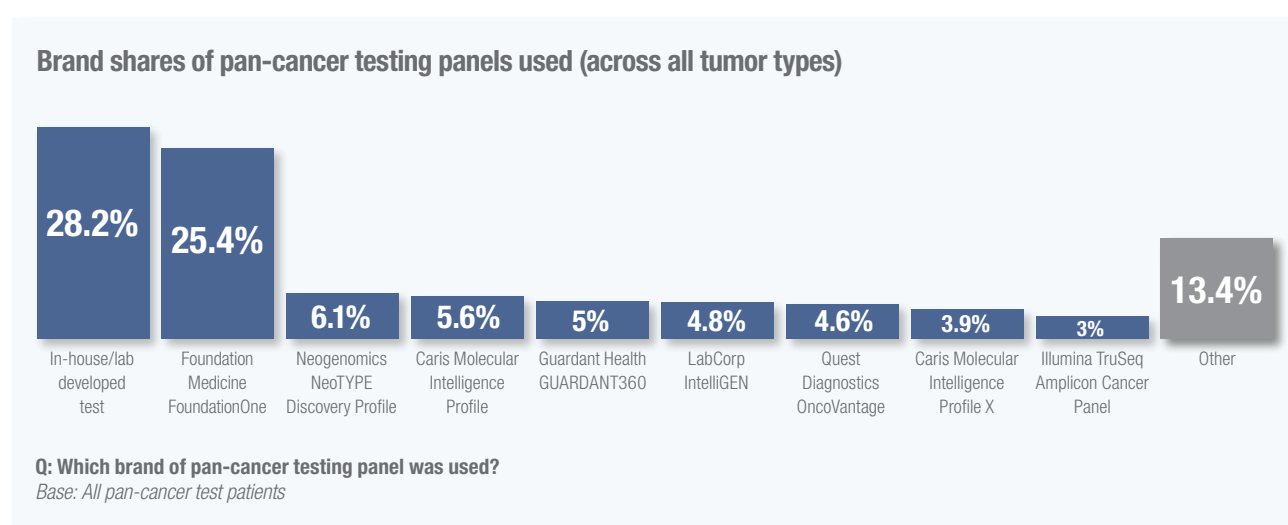
Brand shares of pan-cancer testing panels used (across all tumor types)

28.2%	25.4%	6.1%	5.6%	5%	4.8%	4.6%	3.9%	3%	13.4%
In-house/lab developed test	Foundation Medicine FoundationOne	Neogenomics NeoTYPE Discovery Profile	Caris Molecular Intelligence Profile	Guardant Health GUARDANT360	LabCorp IntelliGEN	Quest Diagnostics OncoVantage	Caris Molecular Intelligence Profile X	Illumina TruSeq Amplicon Cancer Panel	Other

Q: Which brand of pan-cancer testing panel was used?
Base: All pan-cancer test patients

Fig. 4: *Pan-cancer test panel shares for selected commercial panel compared to the total share of in-house test panels. (Source: Ipsos US Pan-Cancer Testing Monitor 2016 Q3; research conducted online September – November 2016)*

IV. The sum of the parts: liquid pan-cancer testing panels

The two major new developments described in the earlier section – clinically useful liquid biopsies and pan-cancer testing panels – arrived on the scene at around the same time. Several diagnostics companies sensed a real opportunity here, and started working on products that sat squarely at the center of where those two new technologies converged: pan-cancer testing panels based on liquid biopsies. The theory behind them is remarkably elegant: if circulating tumor DNA (or ctRNA) can be detected in a cancer patient's blood, and if tumor DNA can be used to conduct pan-cancer testing panels, then a cancer patient's blood should be a viable sample type for conducting such large multi-gene panels.

However, multiple technical hurdles had to be overcome before these liquid biopsy test panels could become a reality. The key challenge to Next Generation Sequencing of ctDNA is that the concentrations of mutated DNA fragments are typically so low (an order of magnitude lower than in cancer tissue samples) that the signal is obscured by the noise inherent in NGS machines. This meant that dramatic improvements in specificity were required to move beyond single marker testing towards sequencing complete exons in multiple gene targets[16].

Multiple diagnostics manufacturers appear to have successfully overcome these challenges, and various such liquid biopsy testing panels are now available at commercial laboratories in the US. Two such examples, both based on ctDNA, are listed below:

• Guardant Health Guardant360[17]

• Foundation Medicine FoundationACT[18]

As of late 2016, these liquid biopsy testing panels made up a small proportion of all pan-cancer testing panels (Fig. 5), which in turn made up a small proportion of all oncology biomarker testing. It is uncertain just how significant their eventual impact will be, but it is certainly an area that will continue to develop further in the coming months and years.
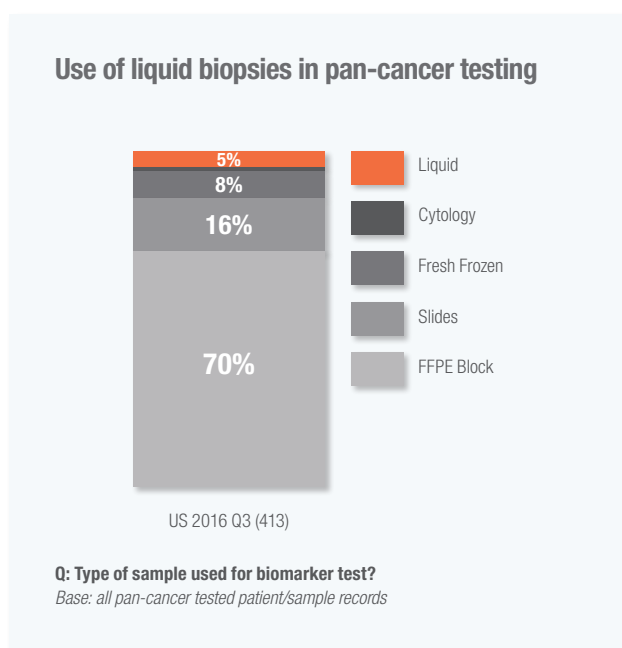
Use of liquid biopsies in pan-cancer testing

5%	Liquid
8%	Cytology
16%	Fresh Frozen
	Slides
70%	FFPE Block

US 2016 Q3 (413)

Q: Type of sample used for biomarker test?
Base: all pan-cancer tested patient/sample records

Fig. 5: *Liquid-biopsy based pan-cancer testing panels vs. tissue-based pan-cancer testing panels (Source: Ipsos US Pan-Cancer Testing Monitor 2016 Q3; research conducted online September – November 2016)*

One of the major perceived drawbacks of pan-cancer testing panels – whether based on solid tumor samples or liquid biopsies – is that they sometimes deliver an unmanageable amount of information, which is not always actionable. Being able to identify rare mutations in cancer types does provide an oncologist with additional insights into the genetic make-up of a cancer patients' malignant cells, but if no drugs are on the market that adequately target those mutations, it potentially leaves both oncologist and patient with more questions than answers. Still, it could facilitate patient selection for and enrolment into novel clinical trials – and indeed, some pan-cancer test panels provide such clinical trial recommendations, sometimes leading to the recommended action (Fig. 6).
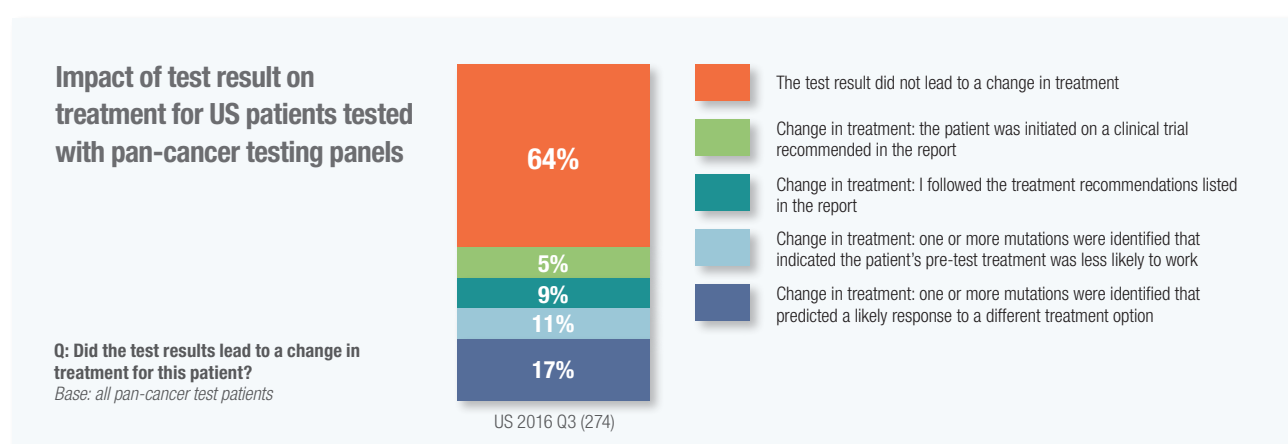
Impact of test result on treatment for US patients tested with pan-cancer testing panels

64%	The test result did not lead to a change in treatment
5%	Change in treatment: the patient was initiated on a clinical trial recommended in the report
9%	Change in treatment: I followed the treatment recommendations listed in the report
11%	Change in treatment: one or more mutations were identified that indicated the patient's pre-test treatment was less likely to work
17%	Change in treatment: one or more mutations were identified that predicted a likely response to a different treatment option

Q: Did the test results lead to a change in treatment for this patient?
Base: all pan-cancer test patients

US 2016 Q3 (274)

Fig. 6: *Impact on treatment for US patients tested with pan-cancer testing panels, based on de-identified patient record data. Over 1 in 3 pan-cancer test panels led to a change in treatment for that patient. (Source: Ipsos US Pan-Cancer Testing Monitor 2016 Q3; research conducted online September – November 2016)*

V. Looking ahead: the coming molecular intelligence revolution

The advances discussed in the previous chapters are, as potentially revolutionary as they may be, still fundamentally variations on the same basic approach: cancer cells or their genetic material (either tissue-bound or suspended in a liquid) are extracted, sent for analysis to a laboratory and analyzed by a pathologist or related specialty, after which a report is generated and sent to the oncologist, who is then required to make a treatment decision. While immensely useful from a clinical perspective, this approach is far from perfect, due to a number of fundamental limitations:

i. **Cancer cells are in a constant state of flux**, by definition undergoing rapid division and often acquiring new mutations in the process[19]. Taking a snapshot at one point in time, or even at various points in the treatment journey, is going to miss a lot of the dynamic detail and identify resistance mutations with a significant amount of delay.

ii. **Genes do not act in isolation.** Even the largest pan-cancer testing panels are limited by the number of genes they cover and by the fact that they usually do not cover epigenetic alterations such as DNA methylation and histone modifications.

iii. **Even the most well-trained pathologists or oncologists are limited by the amount of information they can process**; as mentioned earlier, there is already a risk of information overload with multi-gene pan-cancer test panels, and this issue increases exponentially with the number of genes/codons that are added to a panel: coming up with an appropriate treatment for a cancer based on the mutational and expression status of 1,000 different genes is infinitely more complex than doing so based on a handful of genes.

iv. **Biomarker testing – whether single marker or multi-gene, whether tissue-based or liquid-based – is currently reactive**: apart from hereditary (germline) panels to assess cancer risk, testing is conducted after a cancer diagnosis has been confirmed, and often after it has already metastasized. Detecting acquired tumor mutations in the blood as soon as they arise could theoretically act as an early warning system prior to any symptoms presenting.

Addressing each of the barriers above will require a number of technological advances, but there are clear indications that the whole field of cancer biomarker testing is going to be radically different in the coming decades.

Three major areas of research are likely to make a significant impact:

i. **Labs on a chip, with remarkable sensitivities**, may ultimately be implanted in patients' bodies / bloodstreams, providing a means to continuously monitor biomarker status in real time. Taken one step further, these may be implanted prior to diagnosis, allowing cancer cells to be detected when they first arise[20].

ii. **Continuous improvements in the cost, speed and reliability** of whole genome and whole exome sequencing.

iii. **Perhaps most significantly, the inexorable rise of Artificial Intelligence**. In order to make sense of the enormous amount of data collected by whole genome/exome sequencing, especially when done so on a (semi-)continuous basis, human-based interpretation will necessarily need to be replaced by AI systems. In a recent survey conducted by Healthcare IT News, 35% of healthcare organizations in the US said they plan to leverage AI within two years, with clinical decision support, patient diagnosis and precision medicine among the top areas where respondents perceived AI will have the greatest initial impact[21]. Indeed, AI is already starting to prove itself in helping to recommend successful treatment combinations for specific molecular subtypes, as shown in a recent in vitro study in metastatic BRAF-mutated melanoma[22].

It is therefore far from inconceivable that, at some point in the not-too-distant future, highly sensitive chips in individuals' bodies will detect somatic mutations (or changes in gene expression patterns) as they arise, sending this information in real time to powerful AI software, which will recommend a clinical course of action to be undertaken based on the wealth of molecular diagnostics information continuously transmitted through the chips' sensors. This approach would represent personalized medicine taken to the extreme, and these systems would even continue to improve further through deep learning algorithms.

The next – and perhaps ultimate - step in the more distant future would be pairing this ability with nanobots which would permanently inhabit individuals' bodies, dispensing targeted doses of highly specific drug cocktails, or physically destroying wayward cells, in an extremely localized matter in response to the information passed to them through the chips and AI agents. In this bold - but by no means unrealistic - view of the future, cancer would be destroyed before it has a chance to take hold, meaning it would effectively be cured. While this approach remains a largely theoretical future concept for now, the first small-scale human trials investigating the use of drug-delivering nanobots are already underway[23].

We are on our way to conquering the complexity that makes curing cancer so difficult, but we are not there yet; technology has a lot of catching up to do before such a vision can be realized. However, one thing is for certain: we are only at the very start of an accelerating revolution in molecular diagnostics. To assume that the same logistical touch points, stakeholders, market forces and dynamics that shape the Oncology MDx market now are going to remain relevant in the coming decades would be very risky indeed.

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