Understanding Precision Medicine in Oncology

Since scientists first sequenced the human genome nearly 20 years ago, we have all been increasingly enthusiastic about the potential for precision medicine to better diagnose and treat diseases. In oncology, targeted therapies against specific mutations have already demonstrated this promise, though only in the relatively limited settings where these therapies exist and testing is done as part of the standard of care (like HER2 in breast or EGFR in lung).

Most cancer patients do not have the opportunity to get tested or to receive appropriate targeted therapies. So, accelerating pharmaceutical and diagnostic development has enormous potential to improve outcomes and save lives.

In this paper, we further explore the challenges and potential of evolving and integrating precision medicine into oncology. This paper consists of three parts:

• Part I: The Science Behind Molecular Testing
• Part II: Current Clinical Utility and Commercial Challenges
• Part III: Future Co-Evolution of Diagnostics and Pharma

Part I – The Science Behind Molecular Testing

Overview of Molecular Alterations and Testing Technology

Cancer occurs when healthy cells experience a cascade of genetic errors (mutations) that shift normal biology in specific ways that drive malignancy. Examples include uncontrolled growth or the inability to repair DNA damage resulting in additional mutations. Disrupting these malignancy pathways without irrevocably damaging healthy tissues is the overarching therapeutic goal for all cancers. At its core, precision medicine oncology involves identifying and targeting cancer-specific molecular alterations.

Cancer is a particularly difficult disease to treat because each cancer arises in a unique fashion due to random mutations over time, and then tumor cells evolve to become resistant to whatever treatment is attempted. A treatment that is targeted to only part of a tumor, or to an older version of that tumor, will inevitably fail. This evolution leaves a “trail” of molecular alterations that tell the story of how the tumor formed, grew, and spread, and the core challenge of precision medicine is to efficiently and accurately measure these alterations in order to direct a more personalized treatment strategy.

To accurately detect molecular alterations, three types of molecules must be considered: DNA, mRNA, and protein.

Key Terms:

Antibody: a type of protein which binds very selectively to another specific protein (used to detect mutated proteins and measure protein expression levels)

DNA: linear strand of base pairs; the sequence of which encodes genetic information used to build proteins

Diagnostic: a test for molecular alterations that indicates or confirms a disease or specific molecular alteration

Gene: a stretch of DNA that encodes a protein

Gene / Genomic (or DNA) Sequencing: process of reading and reporting out the DNA or mRNA sequence (to check for mutations)

Molecular Alteration: any change in a protein or its precursors that impedes its normal biological function. This includes both mutations that change the function of a protein, as well as significant increases or decreases in the amount of the protein.

Molecular Test: a test that examines DNA, mRNA, or protein to detect molecular alterations

mRNA: a mobile copy of a gene that is transported throughout the cell to make proteins; the amounts of mRNA loosely correspond to protein amounts

Protein: a chain of amino acids (built based on a DNA/mRNA sequence) folded into a 3-dimensional molecule that works as a cellular machine to carry out biological processes

Precision medicine: collecting information about molecular alterations and incorporating it into treatment decision-making

Predictive vs Prognostic test: Predictive tests indicate whether a particular treatment is more or less likely to work for the tumor, whereas prognostic tests provide information about the likely course of disease (treatment agnostic).
proteins. This requirement is rooted in the fundamentals of biology – the mis-regulations that drive cancer can be reflected at any one or more points in the following stepwise process of translating DNA sequence into mRNA into protein, so alterations in any or all three can contribute to a given tumor’s mis-regulation(s):

While we frequently refer to DNA or genes when discussing mutations, it is changes in protein function or dramatic differences in the amount of a non-mutated protein (either loss of expression, or over-expression) that are the molecular alterations ultimately responsible for cancer biology. We can therefore classify precision medicine tests based on which type of protein mis-regulation they can detect: A change in protein function, or a change in protein amount. Each of these can be detected at various levels (DNA, mRNA, and / or protein). To detect a previously unknown molecular alteration, multiple types of tests may be necessary.
Precision medicine test design therefore involves both a choice of molecule type (DNA, mRNA, protein) as well as what is being measured – an alteration in sequence (impacting protein function) or a change in expression levels (protein amount). The choice of molecule has implications on the capabilities of the testing technology.

### DNA Tests (Sequencing / Methylation / FISH)

These technologies detect and report out the presence or amount of a specific DNA sequence to identify any alterations within that gene. This includes changes to the sequence within a gene (mutations), as well as deletions, duplications, or fusions of portions of the gene. A DNA mutational test can be designed to test for one specific mutation that is common or suspected (like BRAF V600E testing in melanoma), or hundreds done in parallel to look more broadly for multiple mutations (like the pan-tumor Foundation One panel).

There is also a specific type of sequencing that can be done to detect DNA methylation, which is a chemical tag on the DNA itself near the gene that means the associated gene is “silenced” (won’t be expressed). This provides indirect evidence of changes in protein amount.

A Fluorescence In Situ Hybridization (FISH) test is when a fluorescent tag is used to mark specific sequences of DNA. It can detect deletions or amplifications, both of which predict changes in protein amounts.

Broad panels that search for numerous alterations can report out a list of all the mutations identified, as well as an overall count of how many alterations are seen in the entire genome. This is commonly referred to as genomic stability. Tumors that have numerous mutations are said to have a high tumor mutational burden (TMB), or to be microsatellite instable (MSI-hi). This measure predicts prognosis and also response to checkpoint inhibitor immunotherapies.

As compared to the other types of molecular tests, DNA tests require very little sample to test. They’re also fast and easy to interpret. If a test comes back positive for a known mutation, it is also likely to be clinically significant because an alteration within a gene will predictably impact the associated protein. However, a negative result does not mean that the associated protein is not involved in tumor formation or growth, because many times a mis-regulated protein does not have a mutation in its gene but is rather being impacted by another molecular alteration that is not necessarily detected by a given test.

DNA tests have been quite well-integrated into clinical practice in a few limited settings where certain mutations are common and where targeted drugs are available, for example non-small cell lung cancer and melanoma, where specific mutations (EGFR and ALK for lung; BRAF for melanoma) are near-universally tested for. DNA methylation tests are also used to indicate prognosis as well as sensitivity to certain treatments (MGMT methylation in brain tumors). The broader panels are also used in rare tumors or in other settings where the clinician or patient desires a more comprehensive search for mutations.

<table>
<thead>
<tr>
<th>Test Types*</th>
<th>Clinical Examples</th>
<th>Mis-Regulation Detected</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutations / Fusions</td>
<td>BRAF V600E (melanoma); BRC-ABL (leukemias)</td>
<td>Detects changes in <strong>Protein Function</strong> (for gene tested)</td>
<td>Fast, easy, and (can be) inexpensive</td>
<td>Highly predictive DNA alterations are rare</td>
</tr>
<tr>
<td>Deletions / Amplifications / FISH</td>
<td>1p 19q codeletion (gliomas); ALK amplification (lung)</td>
<td>Detects changes in <strong>Protein Amount</strong> (for gene tested)</td>
<td>Detects highly predictive alterations</td>
<td>Can miss important protein mis-regulations not present within the gene</td>
</tr>
<tr>
<td>Methylation</td>
<td>MGMT methylation (gliomas)</td>
<td>Estimates changes in <strong>Protein Function</strong> and <strong>Amount</strong> (whole tumor)</td>
<td>Outputs are not subjective (y/n, quantitative)</td>
<td></td>
</tr>
<tr>
<td>Genomic Stability</td>
<td>Tumor Mutation Burden (TMB); Microsatellite Instability (MSI)</td>
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*Panel tests (like Foundation One, Guardant 360, Caris) measure one or more these for numerous genes in parallel.
Gene Expression (mRNA) Testing

This technology measures the amount of mRNA of a gene. Like DNA sequencing, it is also relatively fast and inexpensive, although slightly more technically challenging than DNA sequencing. The interpretation is significantly more challenging because it is a relative and only semi-quantitative measure: It requires choosing a reference set of genes that is believed to be relatively stable to determine if the mRNA of interest is lower or higher than “normal”. Most gene expression tests therefore use a panel approach that tests dozens or hundreds of genes at once, so that any abnormal expression will be more obvious.

In terms of clinical utility, gene expression testing is typically bypassed in favor of DNA testing (which is easier) and protein testing (which more definitively reports out protein amounts). One notable exception is panel tests that help estimate the risk of recurrence for early stage breast and prostate tumors.

Gene expression tests do have enormous utility in research and drug development that may someday translate into broader clinical use, as they offer much more comprehensive snapshots of biology than either DNA or protein tests. In these settings gene expression tests are frequently used to better understand subtypes of tumors and their underlying biology. This can lead to new targets for pharmaceutical development, as well as suggesting avenues for more personalized treatments.

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</tr>
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<tbody>
<tr>
<td>Gene (mRNA) Expression</td>
<td>Multi-gene Panel</td>
<td>Indicates changes in Protein Amount (for genes tested)</td>
<td>Relatively fast; More comprehensive snapshot of tumor biology</td>
<td>Complexity of test interpretation; Challenges in proving clinical utility</td>
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<tr>
<td>(“microarray”)</td>
<td>(“microarray”)</td>
<td>Informs broadly on Tumor Biology</td>
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<tr>
<td>Oncotype Dx in breast and prostate; Mammogram in breast</td>
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Protein Assays (IHC)

This technology, called immunohistochemistry or IHC, uses antibodies that bind to the protein of interest and can then be visualized or otherwise measured to provide a semi-quantitative measurement of target protein quantity. Antibodies can also detect specific protein mutations, such as an antibody to a mutated protein called IDH1 R132H is used in gliomas as a diagnostic and prognostic marker.

Protein assays are more technically challenging and expensive than either DNA or mRNA tests. This is because they are dependent on antibodies, which must be generated in cell culture or in live animals (and are harder to standardize and more likely to suffer from manufacturing issues). They also require significantly more sample to test. While DNA and mRNA can be extracted from minute, even single cell amounts, protein tests require much more tissue that must also be processed in a time-consuming way. Another challenge in protein assays is that because the antibodies are typically attached to fluorescent markers, the visible spectrum limits how many can be tested at once (most microscopes can only scan for a few distinct colors at once). Therefore, protein assays typically focus on just one or a few targets per test.

That said, because proteins are the effectors of biology, a direct measure of protein mis-regulation is very clinically relevant. Similar to DNA testing, there are several specific clinical settings where protein tests are already quite well integrated into clinical practice, most notably in breast cancer where ER, PR, and HER2 antibody testing is used to diagnose subtypes and direct targeted therapy treatment.
Molecular Alteration Tests Side-by-Side

Each type of testing technology measures different types of molecular alterations and provides its own snapshot of the underlying biology, and each has its pros and cons from both a scientific and a practical perspective. Roughly speaking, protein tests are the most biologically informative but also the most technically challenging and expensive. Single gene DNA-based tests cast a rather narrow net, as the known actionable mutations are few and far between. But, they’re much cheaper and provide a result faster. Typically, clinicians will start with a limited set of tests that are specific to the known biology of that tumor type. Then if they don’t detect anything, they may progress to broader panels in search of something rarer but still actionable.

Overview of Sample Sources and Implications

So, one key question is which type of molecular alteration to test for (DNA, mRNA, or protein). However, there is also the question of where and how to obtain the sample material to use for testing.

Sample source matters because
- Tumors can contain different cell populations in different spots
- The overall set of mutations and alterations within the tumor changes in response to treatment and over time during tumor growth

So, both the location of the sample as well as how recently it was obtained are critical considerations when interpreting the data.
In the liquid cancers (leukemias and lymphomas), testing is relatively straightforward as the tumor cells are easily collected from the blood and can be tested frequently to track response and progression. Conversely, solid tumor testing has inherent challenges due to its invasive nature, as well as the typically small amounts of tissue recovered, some of which must be reserved for pathology testing.

There is also a temporal issue: if the treatment works initially but the tumor ultimately becomes resistant and begins growing again or metastasizes, another biopsy is not always done, meaning there is no up-to-date sample for testing. Even if a biopsy is done, it is not always retested for molecular alterations. This gap in testing risks missing key information about resistance mechanisms that could be addressed with targeted approaches.

The emerging technology of testing Circulating Tumor Cells (CTCs), which are shed from solid tumors and end up circulating in the bloodstream, is an intriguing workaround to the issues surrounding solid tumor testing. This technology enables identifying and purifying CTCs from a typical blood draw (based on molecular tags seen on cancer cells but not healthy cells). Once purified, the CTCs are used for DNA-based tests. The Guardant 360 test which measures a broad panel of mutations, as well as more targeted tests like the Cobas T790M test, are already in clinical use for this purpose. However, not all tumors shed identifiable CTCs at the same rate, so a negative result in a CTC-based test requires a follow-up test to confirm using a standard biopsy.

Because molecular alterations arise in various and sometimes unpredictable ways during the course of tumor formation and growth, each of the various testing technologies has its own unique value in the clinical setting. In a world with limited resources (tissue for testing, as well as financial cost of the tests), the clinical challenge is deciding which test or which sequence of tests to pursue. In Part II, we delve into the inter-related challenges of how to design these tests to maximize clinical utility, and how clinicians currently navigate this complex landscape.

Part II – Current Clinical Utility and Commercial Challenges

Over the past few decades in oncology, targeted therapies—and the tests that identify which tumors will be sensitive to them—have significantly advanced the standard of care in several key settings. However, precision medicine will not achieve its full potential in oncology without broader success in pharmaceutical and diagnostic development, and better integration of both into clinical practice.
Current Application of Precision Medicine in Oncology

Currently, precision medicine is used in oncology in three distinct manners: prognostic, predictive (direct), and predictive (indirect). While all three types of test focus on classifying the underlying biology of the tumor, the clinical utility of each is somewhat distinct.

Simply put, prognostic tests provide additional information about disease characteristics beyond what pathology tests can show, whereas predictive tests indicate sensitivity to a particular intervention - whether it will have a higher (or lower) likelihood of effectiveness for this particular tumor.

Prognostic tests provide information about the likely course of disease under the standard treatment paradigm. For example, the Oncotype Dx test uses a panel of 21 mRNA expression tests to categorize early stage breast tumors into high, intermediate, or low risk of recurrence after standard treatment. In later-stage breast cancers, the absence of both HER2 and ER/PR protein overexpression is used to categorize tumors as “Triple Negative”, with a worse prognosis.

Prognostic tests do not suggest a specific treatment but can help guide overall treatment strategy and set expectations for the patient and loved ones. Ideally, prognostic tests will evolve over time to become predictive as new treatments are developed.

**Prognostic**

Categorizes tumors into groups with known correlations to particular outcomes (likelihood of recurrence, survival time, etc)

**Considerations:**
- Typically is not binary (yes / no) but rather involves a cut-off threshold for gene or protein expression, which means interpretation can be complex
- Datasets are constructed from samples that may not be very large or representative, so individual variation can complicate interpretation (ex: a prognostic test built of samples from Northern European men may not be as relevant for Asian women)
- Does not indicate any particular treatment or deviation from standard of care

**Examples:**
- Oncotype Dx classifies risk of recurrence for early stage breast cancer based on a 21 gene expression test
- HER2 and ER/PR negative breast cancers are categorized as Triple Negative Breast Cancer, which are more aggressive and difficult to treat with worse overall outcome

**Opportunity:** Convert test to Predictive by developing targeted drugs or tailored interventions that work better in particular groups
Predictive tests also categorize tumors based on patterns of molecular alterations, but unlike prognostic tests they suggest specific treatment approaches to address the underlying biology. Predictive tests indicate treatments that can be classified as direct or indirect.

Direct targeted drugs are those that directly interact with and negate the harm of mutated or mis-regulated proteins. For example, the drug Tagrisso binds specifically to the T790M mutation on the protein EGFR, which arises as a mechanism of resistance to first-line EGFR inhibitors in non-small cell lung cancer. Other examples include the BRAF inhibitor drugs, Zelboraf or Tafinlar, used in melanoma to specifically target the BRAF V600E mutation. Another common example is the HER2 targeted drugs (like Herceptin) which are used to block HER2 overexpression if it is identified at the gene or protein level in breast cancer. Typically, direct targeted drug efficacy is very well-correlated with the predictive marker.

### Predictive (Direct)

Identifies tumors with specific molecular alterations that can be directly targeted therapeutically

**Considerations:**
- Can either be definitive (mutation is present or absent) or more semi-quantitative (expression is over threshold x in y% of tumor cells); for the latter the determination of the thresholds can impact predictiveness
- Approved directly targeted therapies are relatively rare, although the mutations to be targeted are sometimes quite common in specific settings

**Examples:**
- EGFR T790M mutation in non-small cell lung cancer can be blocked by Tagrisso
- BRAF V600E mutated protein in melanoma can be blocked by Zelboraf or Tafinlar
- Overexpressed HER2 protein in breast can be blocked by Herceptin

**Opportunity:** Increase testing rates (to identify all people who would benefit)

An indirect predictive test still indicates whether a particular therapy is more (or less) likely to work, but it does not measure the target directly. Instead, it surveys a separate but strongly-correlated molecular alteration that is an indirect predictor of response. This can be another protein that operates nearby in the same oncogenic pathway. For example, the MEK inhibitor drugs Cotellic or MeKininist are often used in BRAF V600E positive melanoma. They work indirectly by suppressing the overactivity of MEK, which occurs when BRAF has the V600E mutation.

Indirectly predictive tests can also illuminate the underlying biology rather than a single protein misregulation. For example, tumors that have a BRCA mutation are unable to repair certain types of DNA damage. In this setting, drugs called PARP inhibitors can be used to block their back-up repair mechanisms. This can dramatically improve the efficacy of DNA-damaging systemic treatments like radiation or chemotherapy.

The reason to differentiate direct vs indirect in predictive tests is that a direct test is typically easier to interpret and has a higher correlation with efficacy. When either designing or choosing between tests, a direct predictive marker is preferable, if feasible.
Checkpoint immunotherapies are an interesting case of a therapy that can be indicated by either a direct or indirect predictive test, depending on the molecular alteration identified. If a tumor has PDL1 overexpression, then the PD1/PDL1-targeted checkpoint inhibitors (ex: Keytruda, Opdivo, Tecentriq) can directly suppress the inappropriate activity of the PDL1 checkpoint.

If a tumor has high tumor mutation burden (TMB), this indirect marker indicates that there are numerous mutations throughout the genome that make it more likely for the immune system to recognize and attack the cancer. In such a case, checkpoint inhibitors are more likely to kickstart the process of immune activation. PDL1 expression and TMB therefore illuminate different underlying biologies and are independent predictors of response to checkpoint immunotherapies.

In general, directly predictive tests and their associated targeted therapies are the gold standard (since the drugs act directly on the alteration measured by the test). Indirect predictive tests can also be quite useful, but only if there is a high enough correlation between test result and drug efficacy. Prognostic tests have relatively limited utility in clinical decision-making. However, they can be quite helpful to patients trying to appropriately manage their expectations for the course of their disease.

Integration of Precision Medicine into Clinical Oncology Practice

Currently, precision medicine is fully integrated into the standard of care for cancer in only a few settings, those in which:

- Particular molecular alterations are relatively common, and
- Predictive test:drug pairings can successfully target that alteration
There are also two recent examples of drugs that have achieved pan-tumor indications based purely on predictive testing:

- **VITRAKVI** for any solid tumor with NTRK gene fusion
- **Keytruda** for any solid tumor with high TMB / genomic instability (Microsatellite Instability High or MMR deficient).

A wide breadth of tumor types have at least some element of precision medicine integrated into the standard of care. Within each tumor type, however, there is high variability in how many patients receive testing. This can be driven by:

- Narrow indications (for example testing is only done in certain lines of therapy or for patients who have already received other specific treatments)
- Gaps in physician awareness
- Limited patient access to tests even within approved indications

In addition to the very targeted tests described above, broader panel tests are emerging that typically include numerous genetic alterations (DNA testing). Some can also include a few common protein expression alterations assessed by IHC. These tests identify molecular alterations that can indicate approved, off-label, or experimental (clinical trial) treatments.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Standard of Care Predictive Test / Drug Pairings (from NCCN Guidelines)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td><strong>PDL1</strong> expression: use PD1 checkpoint inhibitors like Keytruda, Tecentriq if high</td>
</tr>
<tr>
<td><strong>Breast</strong></td>
<td><strong>HER2</strong> expression: use HER2 Inhibitors like Herceptin if high&lt;br&gt;<strong>ER/PR</strong> expression: use Estrogen Inhibitors, Aromatase Inhibitors, CDK4/6 Inhibitors if high&lt;br&gt;<strong>BRCA</strong> mutation: use PARP Inhibitors like Lynparza if present&lt;br&gt;<strong>Multigene expression</strong> (Oncotype test) predicts resistance to chemo</td>
</tr>
<tr>
<td><strong>Colorectal</strong></td>
<td><strong>KRAS/NRAS</strong> mutation: avoid EGFR inhibitors like Vectibix or Erbitux if present&lt;br&gt;<strong>Microsatellite Instability</strong>: use PD1 checkpoint inhibitors like Keytruda or Opdivo if high</td>
</tr>
<tr>
<td><strong>Gastric</strong></td>
<td><strong>PDL1</strong> expression: use PD1 checkpoint inhibitors like Keytruda, Tecentriq if high&lt;br&gt;<strong>Microsatellite Instability</strong>: use PD1 checkpoint inhibitors like Keytruda, Tecentriq if high</td>
</tr>
<tr>
<td><strong>Leukemias</strong></td>
<td><strong>Risk classification</strong> (based on molecular alterations) is used to select chemo regimen&lt;br&gt;<strong>FLT3</strong> mutation: use Rydabt if present&lt;br&gt;<strong>IDH</strong> mutation: use IDH inhibitors like Tesetax if present</td>
</tr>
<tr>
<td><strong>Lung (non-small cell)</strong></td>
<td><strong>ALK</strong> gene fusion: use ALK inhibitors like Xalkori if present&lt;br&gt;<strong>EGFR</strong> mutation: use EGFR inhibitors like Tarceva if present&lt;br&gt;<strong>PDL1</strong> expression: use PD1 checkpoint inhibitors like Keytruda, Opdivo, Tecentriq if high</td>
</tr>
<tr>
<td><strong>Melanoma</strong></td>
<td><strong>BRAF</strong> mutation: use BRAF/MEK inhibitor combo like Zelboraf+Cotellic if present&lt;br&gt;<strong>PDL1</strong> expression: use PD1 checkpoint inhibitors like Keytruda, Opdivo if high</td>
</tr>
<tr>
<td><strong>Ovarian</strong></td>
<td><strong>BRCA</strong> mutation: use PARP inhibitors like Lynparza if present</td>
</tr>
<tr>
<td><strong>ANY (pan-tumor approval)</strong></td>
<td><strong>NTRK</strong> fusion: use TRK Inhibitor Vitraki&lt;br&gt;<strong>Microsatellite Instability/TMB</strong>: use PD1 inhibitor Keytruda if high</td>
</tr>
</tbody>
</table>
These broader panel tests are not yet officially part of the standard of care. However, academic centers use them rather often, and some have their own in-house versions (ex: UCSF500 gene panel). They’re also sometimes used in rare cancers or later lines of therapy where there is the hope of identifying a rare but actionable alteration. Finally, these tests are sometimes requested by patients interested in proactively seeking treatment options beyond the standard of care.

For these panel tests, the interpretation is almost always simple 1:1 mapping of regimens to single alterations (use – or don’t use - Drug X because of Mutation Y). In the cases where multiple molecular alterations are discovered, the clinician receives a list of alterations and drugs or clinical trials to consider. The clinician is then responsible for interpreting which results are most meaningful and prioritizing treatment options accordingly. Needless to say, this can be quite challenging.

The broader panel tests also include many alterations that are not fully predictive. They may provide prognostic information, hint at underlying biology that could be addressed therapeutically, or have targeted therapies in clinical trials.

**Key Challenges in Advancing Precision Medicine**

To advance precision medicine, we must accelerate pharmaceutical development, diagnostic development, and integration into clinical practice. Simply put, we need more and better targeted drugs, more predictive tests to guide their usage, and better uptake of both the tests and drugs in clinical practice.

The underlying barriers to achieving all the goals above relate to scientific and technical issues, clinical development challenges, and commercial issues. Some may be solvable given enough resources and time; others (like developing a targeted drug for a particular alteration) may ultimately prove intractable.

One critical unmet need is better pharmaceutical approaches to target known drivers of malignancy. For example, overactivity of the protein MET, driven by gene amplifications and/or protein overexpression, is a common molecular alteration in many tumors, particularly lung, colorectal, and liver. None of the numerous attempts to develop MET inhibitors in clinic have yet succeeded. This has not been a failure of drug design per se (the drugs do inhibit MET protein as intended). Rather, it indicates some gap in scientific understanding – either MET protein overactivity is not truly a primary driver of malignancy, or perhaps clinical efficacy will require more complex approaches. These could include combinatorial or sequential treatments to address other parts of the MET pathway, a better predictive test to identify likely responders, etc.

To achieve clinical efficacy with regards to this pathway—and others where drug development has so far underperformed—we need a deeper understanding of tumor biology that will clarify a better approach. Perhaps that would be a different type of inhibitor, a combination treatment strategy, or a pattern of background genetics that creates susceptibility, etc.
In parallel, diagnostic development has struggled to support some drugs and classes of drugs where we either have only indirect predictive tests, or none. The immunotherapies are a notable example. Both the checkpoint inhibitors as well as the promising but less developed vaccine, virus, and cell-based therapy approaches show a distinctive “long tail” response curve where a small percentage of recipients have an exceptionally durable (even curative) response to treatments. However, the majority suffer the side effects of these therapies but receive no real benefit.

While tumor mutation burden and PDL1 expression are considered predictive for response to checkpoint inhibitors, they are not well-correlated enough for many clinicians to risk undertreating. Instead, they treat everyone, knowing that there can still be some responders in the “low” test result category.

There are also some therapies which are targeted in the sense that they directly block a protein, but alterations of that protein do not appear to be predictive. Avastin and the other VEGF inhibitors are a notable example where diagnostic tests have never been successfully developed. Better predictive tests would result in a more favorable benefit/risk ratio for these drugs, improving outcomes for more patients as well as cost savings where expensive drugs can be safely avoided.

The underlying barriers to diagnostic development are typically associated with test design choices. If a test is intended to complement an existing drug, then the molecular alteration is likely already known. However, diagnostic test designers may still need to choose which type of assay (DNA, mRNA, protein) will provide the best balance between accuracy and feasibility. For example, protein tests inherently require more sample and more qualitative interpretation, so DNA-based tests may be preferred when possible.

For the broader, panel-based DNA tests that are not aligned to a particular drug, there is the question of which genetic alterations to include. There are ~20,000 genes in the human genome, each of which can exist in numerous mutated or otherwise forms. Panel tests typically restrict themselves to a list of a few hundred or dozens of genetic alterations that are either prognostic or predictive / targetable.

The challenge is that new information is constantly coming out, and updates to tests—and their interpretation—must be carefully considered. Updating too frequently can confuse consumers. Updating too infrequently can let the tests get outdated.

The other somewhat open question is how slightly modifying existing tests will impact their FDA approval and reimbursement status. Will they need to re-demonstrate clinical utility with each new version?
Understanding Precision Medicine in Oncology

As clinicians attempt to integrate drugs and tests into their practice, they face barriers of awareness, access, and interpretation. Even in cases where testing is part of the standard of care and has been for some time (for example EGFR testing in lung), the testing rate is still not 100%. Ongoing consumer education of both physicians and patients will continue to be required to maximize testing rates.

Given the complexity of precision medicine testing technology, clinicians are challenged to decide whether to test, and which test to use. There are some clear-cut contexts where a companion diagnostic is labeled for use to select a specific drug. Examples include the Cobas blood-based DNA sequencing test that detects the EGFR T790M mutation (which the drug Tagrisso can target) or the various PDL1 tests marketed for use with checkpoint inhibitor immunotherapies. However, in the majority of treatment settings, the standard of care guidelines do not recommend specific precision medicine tests, so it’s at the discretion of the clinician.

Even if the clinician and/or the patient is aware of and wants to test, there may be access barriers associated with feasibility of obtaining an appropriate sample. For example, a progression may be identified on an MRI but a biopsy is not done because the pathology is already inferred.

The more common access barrier is reimbursement and cost, particularly for those tests that have not provided clinical utility nor obtained FDA approval. Many testing companies provide financial support to patients as needed, but this is not sustainable for growth. A related barrier can arise when a test identifies an actionable alteration, but the drug is not approved in that indication. This can create both logistical and financial hurdles to accessing the drug off-label.

Interpretation is a very challenging barrier in certain settings. Specifically, it’s challenging in settings where testing is done but the results are either too vague (nothing much actionable) or too complicated (multiple actionable targets identified).

In the case of the former, a clinician can always revert to the standard of care, but there may be a missed opportunity of an actionable alteration wasn’t tested for or that wasn’t recognized as potentially actionable in the read-out. For example, a set of several alterations within the same pathway might indicate that the pathway should be targeted, but a clinician would have to make that inference on their own because test reports typically only provide 1:1 alteration:drug information.

In the latter case, if more than one obviously actionable alterations are identified, the test reports still only provide the 1:1 information, i.e., a list of each alteration identified along with appropriate drugs or clinical trials for each. The reports do not, however, provide any relative prioritization that would support a clinician’s decision between two or more options.
Maximizing the potential of precision medicine will require co-evolution of diagnostic and pharma to boost the utility of both, as well as a higher order understanding of how to integrate test results into clinical practice. In our next installment, we will explore how diagnostic and pharma companies can lead the advancement of precision medicine.

Part III – Future Co-Evolution of Diagnostics and Pharma

Better integration of personalized cancer treatments into clinical practice will immensely improve outcomes for patients. But, to achieve the full potential of precision medicine, both pharma and diagnostics must accelerate and broaden their scope of precision medicine beyond their currently limited settings and ensure successful integration into clinical practice. Below, we focus on the future landscape and the requirements for both diagnostics and pharma companies to achieve broader success.

Evolution of Scientific Understanding and Clinical Application

To transform cancer from a progressively fatal disease to one that is curable (or at least manageable), we need better:

• Drugs / regimens
• Predictive tests to guide treatment strategy
• Integration into clinical practice to bring these pharmaceutical and diagnostic tools together
A deeper and more comprehensive scientific understanding of tumor biology and drug mechanisms of action is a foundational requirement for all three of these pillars.

At the general level, we know that a consistent set of drivers cause tumors to form and spread. Each arises as the result of one or more genetic mutations or other molecular alterations. This framework, known as the “Hallmarks of Cancer”, identifies key drivers of malignancy in solid tumors such as:

- Unrestrainable growth
- Angiogenesis (the ability to grow new blood vessels to feed a growing tumor)
- Invasion of other tissues
- Blocking of cell death signals
- Immune system avoidance

While every tumor must have each of these hallmarks (otherwise it would not be cancerous), each tumor develops them via a unique signature of molecular alterations which can also change over time in response to treatment and other environmental pressures.

Some of these molecular alterations are well understood, relatively common in certain settings, and have targeted therapies available to address them. For example, unrestrained growth from HER2 overexpression in breast cancer can be blocked by Herceptin. However, for most tumors, we either cannot definitively identify the molecular alterations or do not have appropriate drugs to treat them.

To translate this general understanding to the personalized level, we need better detection of which molecular alterations are responsible for driving malignancy in each individual tumor. To do that, we need a more comprehensive “dictionary” of all the molecular alterations (and patterns of alterations) that drive malignancy. Importantly, this cannot just be a simple list of all alterations present in a tumor, because many of them are so-called “secondary” or “passenger” mutations which are incidental and not responsible for malignancy (targeting them will not help kill the cancer).

Broader development and adoption of liquid biopsy-based tests will help appropriately refine our dictionary of driver alterations, because the ease of liquid biopsy will enable frequent testing. This information about kinetics can be mapped to treatment and disease response to show which alterations are leading indicators of disease progression. For example, if alteration A appears right before a tumor stops responding to treatment and begins growing, it could be a driver of resistance to treatment.

This general and comprehensive understanding of malignancy drivers would then inform molecular test design such that each individual tumor can be exhaustively mapped for the specific molecular alterations that drive its malignancy. In turn, this detailed understanding of a given tumor will enable a more personalized treatment strategy.

1. Identify comprehensive set of molecular alterations for each key driver of malignancy
2. For each patient, identify which molecular alterations are present
3. Design personalized treatment strategy

In addition to a more comprehensive understanding of which molecular alterations drive malignancy so that we can detect them via diagnostic tests, we must also evolve regimens to ensure we can effectively target these alterations. A deeper understanding of mechanisms of action—which proteins and pathways a drug impacts—will help researchers and clinicians explore beyond the labeled indications to identify more applications for the drugs we already have. This will also likely involve a shift from simple 1:1 drug to alteration mapping to more complex combinatorial regimens that simultaneously address multiple drivers of malignancy to more effectively suppress the tumor. Complex regimens require more than just developing more and better targeted drugs. They also require appropriately adjusting regimens with existing drugs to take advantage of our deeper knowledge of personalized tumor biology. Current targeted therapy regimens are aimed at one alteration or pathway at a time. This is typically one drug, or sometimes two where they target two points in the same pathways. An example includes checkpoint inhibitor combos that target both PD1/PDL1 and CTLA (Opdivo and Yervoy), or BRAK/MEK combos that block that pathway in BRAF V600E melanomas. These combos can be very effective when the pathway they target is the primary driver of malignancy. However, this 1:1 mapping of simple regimens to single alterations is not always effective, likely because other alterations are driving malignancy in those resistant tumors.

One challenge of clinical application will be detecting and measuring all the important drivers of malignancy. However, another key challenge will be designing more complex and adaptive regimens to effectively kill evolving tumors without overwhelming side effects or toxicity from the drugs themselves. This may involve:

- Adjusting regimens before tumor resistance fully takes hold (using information gathered from liquid biopsies or other blood-based markers)
- More personalized therapies such as cancer vaccines or repurposed drugs or combos identified using in vitro screens of the patient’s own tumor cells

Both pharma and diagnostic companies will play key roles in successful evolution to this new precision medicine treatment paradigm.

Key Requirements for Success: Pharma

Both pharmaceutical and diagnostic companies must develop their products in a complementary fashion, as well as collaborate to drive clinical integration of both tests and drugs / regimens. Pharmaceutical companies will continue to allocate their efforts across three types of development:

- Maximize launched drugs (broaden indications)
- Evolve existing targeted therapies (next gen drugs)
- Introduce novel targeted therapies
Pharmaceutical company research and development teams can leverage deeper scientific knowledge of tumor biology and drugs, as well as emerging clinical data to explore:

- New indications or patient subsets for their existing drugs
- How to integrate existing drugs into new and effective regimens

For already approved drugs, one major challenge is that after the patent exclusion expires, there is typically generic competition that results in a lowered price (and profitability) for a drug. So, pharma companies must timebound their investments in label expansions accordingly.

That said, it may be that future regulatory landscapes will better incentivize new labels or regimens even past the original expiration date. Or, generics manufacturers might expand their efforts into clinical development to maximize their own profits. Clinicians and other researchers may also step in to further drive expanded development of generics into other “off-label” indications.

In addition to existing drugs, pharmaceutical companies will need to develop new drugs - balancing their investment across improved “next generation” drugs for already drugged targets, as well as developing effective drugs for novel targets. The latter is higher risk, higher reward because previous efforts to drug these targets have not worked. However, if an effective drug can be found, it could have blockbuster potential.

To successfully develop, launch, and market existing drugs in new indications or regimens as well as novel drugs, pharmaceutical companies must continue to demonstrate clinical efficacy. Development teams must successfully select the “right” patient population where the drug / regimen will prove better than the standard of care, and this decision-making will be improved by learnings from precision medicine. Pharmaceutical companies can work towards success in both directions – either starting with an intractable disease type and interrogating the molecular alterations to design a drug / regimen or taking a drug / regimen and scanning across tumor types and subsets for the most likely “sensitive” group.

### Key Requirements for Success: Diagnostics

Diagnostic companies face similar decisions around maximizing the utility of the platforms and tests they already have vs attempting to shift towards something more novel. In general, there is a movement away from companion diagnostics (single tests paired with single drugs) towards more universal platform-based testing, which will prove beneficial for whichever companies develop the “winning” options. It may be that the market remains somewhat fragmented for the long-term, particularly if no one develops a successful cross-technology platform or service, i.e., one that incorporates not just broad genomic testing, but mRNA and protein expression testing. At the moment, Caris Life Sciences is the only major player that offers all three types of testing – not on the same technical platform but as part of one service.

Another impactful trend is towards liquid biopsy for solid tumors; if Guardant, Foundation, and others are able to successfully develop and expand technologies across tumor types, it will open up enormous opportunities, not just for the diagnostic companies but for advancing pharmaceutical development and clinical practice based on learnings from these tests. In parallel, it will also be helpful to obtain frequency of alterations (what percentage of tumor cells contain patterns of alterations), vs just the presence or absence of alterations. As these tests are done over time, this tumor kinetics information will be hugely helpful in predicting response or progression and adapting the treatment accordingly.
As diagnostic companies shift towards a broader, more universal platform type model for their tests, they will have to work with regulatory agencies to forge a new path for coverage and reimbursement. In addition to the classic requirements of demonstrating clinical utility and value, diagnostic companies will also have to negotiate how to handle updates of both the technology and the test interpretations – what is the right frequency and is there a threshold of change that would trigger a new review by regulatory agencies? If diagnostic tests can include not just predictors of efficacy, but predictors of toxicity, this will likely help with reimbursement as well.

In parallel, diagnostic companies can continue to explore other business models. Such models might focus on leveraging the data they obtain regarding novel predictive molecular alterations to partner with pharma companies and other researchers.

Key Requirements for Success: Clinical Integration

Finally, both pharmaceutical and diagnostic companies must ensure their drugs and tests are appropriately and successfully integrated into clinical practice. This requires not just awareness of the various products but also better access and interpretation.

Pharmaceutical and diagnostic companies will continue to need to drive awareness of their products. But, particularly in a fragmented marketplace, clinicians will increasingly look to more “impartial” parties, such as guidelines bodies or broad, impartial collaborations.

One recent example is the Blueprint PD-L1 IHC Assay Comparison project. There has been market confusion with the numerous PD-1/PD-L1 targeted drugs have reached the markets with various paired diagnostic PD-L1 tests, and the Blueprint project is providing a direct comparison between the diagnostics (by testing each one on the same samples to see which ones provide similar or different results). All the major diagnostic and pharma manufacturers are participating and the ultimate goal of the program is to provide not just interchangeability information, but head-to-head comparison of clinical outcomes as well.

In terms of access, it will obviously be easier if pharmaceutical and diagnostic companies can obtain traditional coverage for their products. But in situations where clinicians or patients must pursue reimbursement on an individual basis, manufacturers can provide appropriate guidance and logistical support for off-label reimbursement requests, which can be quite logistically burdensome for individual practitioners.

In addition to clear guidelines for use from manufacturers and guideline bodies such as the NCCN, tumor molecular boards may emerge as a key resource for interpretation. This model is not focused on tumor types as are traditional tumor boards, but rather includes various specialists who may be disease-focused (e.g., a lung oncologist) or pan-tumor specialists (e.g., a molecular biologist or radiologist). Ideally, these tumor boards would be available broadly (not just in major institutions) and the learnings would be captured and made available, potentially even as part of automatically-generated diagnostic test reports.
Snapshot of the Future

We are already seeing successful integration of precision medicine in clinical oncology in several settings. One notable example is Genomic Health’s Oncotype Dx tests. These use a set of mRNA expression tests to refine the likelihood of recurrence for breast and prostate cancer beyond what can be determined by histological analysis. The breast test can also be used to determine which tumors are likely chemo-resistant (and chemo can therefore be skipped as it’s unlikely to provide any benefit).

Certain experimental immunotherapies are also precision-medicine based, for example personalized cancer vaccines. These are not vaccines in the classical disease prevention sense, but rather involve determining which alterations in an individual’s tumor are most likely to trigger immune response, and the creation of a unique “vaccine” that exposes the immune system to those alterations in hopes of triggering an immune response to eradicate the tumor.

These vaccines are under clinical investigation and are also in relatively common use in for-profit clinics in Europe, where they are combined with other immunotherapy treatments like checkpoint inhibitors in hopes of boosting response. While these clinics have not traditionally done a great job of publishing their outcomes data, if these approaches demonstrate consistent efficacy, they should begin to publicize it more accordingly.

Finally, there is also the in vitro screening of compound libraries against tumor cell samples. This treatment is offered via clinical trials (ex: https://clinicaltrials.gov/ct2/show/NCT02654964) and also for-profit clinics. It is a complementary approach to genomic screening that bypasses the hunt for molecular alterations in favor of directly testing cultured tumor cells for sensitivity to a large library of approved drugs. When these results are combined with knowledge of molecular alterations in the same tumor cells, we might identify new predictive markers of response.

Furthermore, we expect precision medicine to expand not just in oncology but across medicine more broadly. Learning from oncology will inform best practices in how to apply precision medicine for other aspects of health and wellness, for example:

• Predicting response, resistance, and toxicity for all drugs (not just in oncology)
• Screening and monitoring for various conditions via liquid biopsy
• Refining knowledge about disease susceptibility based on germline variants, enabling preventative treatments

We have already made major advancements in leveraging knowledge about the human genome and its connection to health and disease. With successful expansion of precision medicine oncology through both pharmaceutical and diagnostic company advancements, we anticipate many more.
New Ideas. Better Results.

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