ABRF 2011

Technologies to Enable Personalized Medicine

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The theme of this year’s ABRF meeting in San Antonio is ‘Technologies to Enable Personalized Medicine’ and the role that ABRF core laboratories may play in the discovery of these key enablers. The reason we have chosen this theme becomes clear when we look at the safety and efficacy of medicines currently being prescribed. In 2008, 294 B$ was spent on new medicines, however, in only about 50% of the cases (averaged over all therapeutic areas, Figure 1) did these medicines have the appropriate therapeutic effect. In addition to wasting nearly ~150 B$, there were two million adverse events reported to the FDA (not to mention the ones not reported) and over 100,000 deaths. Aside from the concomitant pain and suffering, it is estimated that these events cost the nation an additional 45 - 135 B$ in increased medical costs, in addition to the effect of loss of worker productivity on the economy, as a whole.

Differential safety and efficacy

These are big numbers and demand our attention. This differential response to medicines has its origins in the fact that we are genetically different, we have made different life style choices and we have had different environmental experiences. The current hypothesis is that if we understood, or could in some way measure, what makes us physiologically different, we could segregate or stratify patients into responders or non-responders for a particular therapy (Figure 2). We would then prescribe particular medicines only to the responder group. This is the origin of the phrase, personalized medicine (3).

Correspondingly, if such differentiators are developed, they can be used to select patients for clinical trials, making the development of new medicines more cost efficient.
Gate Keepers

The gate keepers to personalized medicine will be the tools which allow us to measure those physiological (genotypic and phenotypic) differences, i.e., diagnostics and biomarkers. As a result, there has been a large effort to develop these tools using any approach that works. These include, gene mutations, gene expression patterns, proteins, proteomic patterns, metabolomics, histology, imaging, physician’s clinical observations, self-reported patient surveys (4). Indeed, personalized medicine is now on the national agenda of both the NIH and the FDA (3).

As an example, Figure 3 show data from a PCR assay that detect mutations in exons 18-21 of the EGF receptor. Mutations in the EGFR have been associated with a differential response to the anticancer agent, Gefitinib (5-7). This diagnostic assay is now commercially available from Genzyme or DxS (8).

Other companion diagnostics for cancer therapeutics are shown in Figure 4.

![Figure 3. Genzyme provides a PCR diagnostic that identifies mutations in the EGFR of NSCLC tumors. This assay allowed the patients in to be segregated on this basis. Patients with mutations in either exons 18-21 responded better to Gefitinib (8).](image)

<table>
<thead>
<tr>
<th>Approved Drug</th>
<th>Mechanism</th>
<th>Approved Companion Diagnostic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herceptin (trastuzumab)</td>
<td>Targets HER2 to treat metastatic breast cancer</td>
<td>HER2 immunohistochemistry tests, HER2 gene amplification tests</td>
</tr>
<tr>
<td>Erbitux (cetuximab)</td>
<td>Targets EGFR to treat metastatic colorectal cancer</td>
<td>EGFR immunohistochemistry test</td>
</tr>
<tr>
<td>Gleevec (imatinib)</td>
<td>Targets the cell-surface tyrosine kinase receptor c-kit in gastrointestinal stromal tumors</td>
<td>c-kit immunohistochemistry test</td>
</tr>
</tbody>
</table>

* EGFR denotes epidermal growth factor receptor, and HER2 human epidermal growth factor receptor type 2.

![Figure 4. Examples of FDA-Approved Drugs and Companion Diagnostics in Clinical Practice (3).](image)
As mentioned, such an EGFR status assay would be powerful in detecting the 10% of NSCLC patients that harbor these specific mutations and might therefore respond to Gefitinib. In a pivotal Phase 3 clinical trial, it was shown, using the diagnostic as a stratification tool, that patients with EGFR mutations had 24.9% progression free survival with Gefitinib, compared to progression free survival of 6.7% for patients who were EGFR mutation free. This result helped Gefitinib win approval for mutant EGFR NSCLC patients.

Diagnostics can take many forms

Biomarkers and diagnostics can be gene mutations, gene expression patterns, proteins, proteomic patterns, metabolomics, histology, imaging, physician’s clinical observations, self-reported patient surveys. A useful example where many of these tools are brought into play is in breast cancer.

As shown in Figure 5, patient stratification occurs in a number of such steps: mammogram or self exam, biopsy or tissue imaging, definitive diagnosis (tumor size, nodal status, ER, PR and HER2 status, histology), and surgery. With this data in hand, a 21 gene expression signature can be obtained from a tumor sample and the likelihood of tumor recurrence can be determined. Depending on the answer here, the choice of adjuvant therapy can be made.
Figure 5. A series of stratification steps in the diagnosis and treatment of breast cancer. From www.oncotypedx.com

<table>
<thead>
<tr>
<th>Variable</th>
<th>MammaPrint</th>
<th>Oncotype DX</th>
<th>Theros</th>
<th>MapQuant Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provider</td>
<td>Agenda</td>
<td>Genomic Health</td>
<td>Biotheranostics</td>
<td>Ipsogen</td>
</tr>
<tr>
<td>Type of assay</td>
<td>70-Gene assay</td>
<td>21-Gene recurrence score</td>
<td>2-Gene ratio of HOPX13 to IL17R (H/I) and molecular grade index</td>
<td>Genomic grade</td>
</tr>
<tr>
<td>Type of tissue sample</td>
<td>Fresh or frozen</td>
<td>Formalin-fixed, paraffin-embedded</td>
<td>Formalin-fixed, paraffin-embedded</td>
<td>Fresh or frozen</td>
</tr>
<tr>
<td>Technique</td>
<td>DNA microarrays</td>
<td>Q-RT-PCR</td>
<td>Q-RT-PCR</td>
<td>DNA microarrays</td>
</tr>
<tr>
<td>Centralized laboratory?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Indication</td>
<td>To aid in prognostic prediction in patients ≥61 yr of age with stage I or II, node-negative disease with a tumor size of ≤3 cm</td>
<td>To predict the risk of recurrence in patients with ER-positive, node-negative disease treated with tamoxifen; to identify patients with a low risk of recurrence who may not need adjuvant chemotherapy</td>
<td>To stratify ER-positive patients into groups with a predicted low risk or high risk of recurrence and a predicted good or poor response to endocrine therapy</td>
<td>To re-stratify grade 2 tumors into low-risk grade 1 or high-risk grade 3 tumors, specifically for invasive, primary, ER-positive grade 2 tumors</td>
</tr>
<tr>
<td>Level of evidence (1-IV)</td>
<td>III</td>
<td>II</td>
<td>III</td>
<td>III</td>
</tr>
<tr>
<td>FDA clearance</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Availability</td>
<td>Europe and United States</td>
<td>Europe and United States</td>
<td>United States</td>
<td>Europe</td>
</tr>
</tbody>
</table>

Figure 6. Commercially available genomic assays for the prediction of clinical outcome in patients with breast cancer (10)

Extend this approach to more complex signatures

The above examples demonstrate that in some cases the knowledge of a single nucleotide change, or the use of several fixed genetic markers, will allow the clinically useful stratification of patient populations. However, a wider and more flexible analysis of gene expression ought to produce a more robust and more broadly applicable methodology. One such approach is outlined below (Figure 7). The advantage of such an approach is that hundreds of signals can be used to construct an expression based classifier, giving it predictive power, while minimizing the effects of errors in individual signals. Selventa then extends this approach to model the underlying molecular mechanisms.
These tools give researchers potential mechanistic insight into why non-responders do not respond. This might be useful for designing therapeutic approaches for this outlier group. Approaches like this could be extended to include other omics data.

**More complex than it appears**

From these examples that we see that genomic assays can be used to stratify populations in a clinical trial, to demonstrate efficacy in a subpopulation and subsequently to identify patients who will receive the medicine as therapy. But, will such genomic assays work across the spectrum of diseases and therapeutic areas?

A result that bears on this comes from the work of Tim Spector at the Department of Twins Research and Genetic Epidemiology at Kings College in London. He has observed that for any analyte that can that be quantitated in the blood of identical twins (humans that are genetically identical), the heritability of blood levels between twin A and twin B is only 50-60%. For grosser aspects of phenotype – freckles, blood pressure, asthma, obesity and IQ, the heritability ranges from 90 to 40%, again averaging at about 60% across traits (11).

This observation demonstrates that there can be a non-obvious connection between genotype and phenotype, with some important exceptions, as mentioned above.
Assay phenotype directly

So, if genotype may not always be a straightforward guide to phenotype, why not assay phenotype directly? It has long been felt that nearly every protein in the body appears in the serum at some concentration, and that this repository would be a valuable source of biomarkers and eventually diagnostics, if only it could be mined efficiently and effectively. But, as Leigh Anderson pointed out some time ago, the useful protein concentration range in serum is at least 12 orders of magnitude (12, 13) and despite great

![Figure 8. SOMAmers are aptamers designed to bind blood analytes. The affinity range bridges that of mass spectrometry and ELISAs. 850 analytes can be quantitated from 14 uL of serum in a highly multiplexed manner. Figure courtesy SomaLogic, modified from (12).](image-url)

excitement early on, due to detection limits, MS will probably not be the tool of choice for this task (14). In the meantime, SomaLogic has developed an aptamer-based technology that will quantitate 850 blood analytes in a highly multiplexed manner from 14 uL of serum. This technology is infinitely scalable (10,000 analytes could be measured – the rate limiting step is proteins to raise the aptamers against), bridges the concentration ranges of both MS and ELISA and has a lower limit of detection of $10^{-14}$ M (Figure 8)(15, 16). So, this approach has the potential to deeply mine serum for biomarkers of disease, disease progression, and the safety and efficacy of new medicines, and so could be tool to stratify patient populations, based on phenotype. Time will tell. In Figure 9, below, we can see the sort of data that is starting to appear.
A broader role

In the gefitinib example discussed above, the value of molecular diagnostics as a tool for patient stratification in clinical trials was clear. However, the application of molecular probes is much wider, and their application to multiple steps in drug development will have broad impact relieving the high attrition in the process (see Figure 10, below).

Currently, the failure rate in drug development overall is about 99% (from idea to drug to market). The failure rate from first in man (Phase 1) to registration is about 89%, though this varies with therapeutic area, see Figure 11 (17).
Figure 11. (a) Success rates from first in man to registration. The overall success rate is 11%. (b) Reasons for attrition in 1991 and 2000. CNS, central nervous system; PK, pharmacokinetics (17).

This high failure rate is the reason that the current amortized cost of putting a new drug on the market is estimated to be $3.9 B (18). Any effort or technology that reduces this cost will have huge impact and will be financially rewarded at a significant level.

**Strategic and economic considerations**

After having made the case that personalized or stratified medicine is a desired end, we then have to ask the question, are all drug markets stratifiable? This issue has been dealt with nicely be Mark Trusheim and colleagues (19). For markets to stratify, one or several of the follow key factors must maintain. First there must be variability in the underlying pathobiology, i.e., diabetes probably has many origins. Second, and related to the above, there must be multiple relevant drug targets. Third, there must be differential ADME (absorption, distribution, metabolism and excretion) pathways. Fourth, there must be differential mechanisms leading to resistance. Finally, this must all result in multiple treatment options with differential disease outcomes and, importantly and relevant to our discussion, there must be a logistically and medically acceptable clinical biomarker. It is clear that not all disease areas will meet these criteria, at least at our current state of knowledge.

But let’s assume that enough of these conditions hold to allow stratification (questionable for some pathologies), there are still several important issues (19).

1. **Attractive economics?** The decrease in the patient population needs to be matched by an increase in cost for the therapy. Is the impact on the pathology enough to justify higher cost?
2. **Can stratified medicines maintain industry economics?**
Attractive Economics

The financial concerns in going from an empirical to a stratified medicine are outlined in Figure 13 (19). Here we see that if initial decreased market share is compensated for by the medicine’s becoming the preferred therapy for an underserved patient population and by increased patient compliance in this group, a stratified therapy may make economic sense. This is by no means clear for all indications and the issue will have to be decided on a case by case basis. What’s interesting in the American healthcare system, is that it will be payers, health insurance companies and pharmaceutical benefit managers, as well as clinicians and regulators, who will be making these judgments.

In Table 1, Trusheim and colleagues do a first order economic analysis (19). With reasonable assumptions about market trends, they show that stratified medicines may yield an increased payback ratio (gross margin divided by development costs) of 4.3, compared to the trending value of 3.0. With more optimistic assumptions, this value may increase to 9.6. So, in principle, market stratification does make economic sense. Key to these projections is a robustly stratifiable market, as discussed above. Time will tell how well these projections hold up.

![Figure 13. Potential effects of stratified medicine on the economic value of therapies.](image)

Use of a diagnostic reduces the size of the treated patient population and thereby the potential market, but enhanced efficacy/safety increases market share as the therapy becomes the preferred treatment. Further market expansion occurs as underserved patients enter the market encouraged by the greater certainty of outcome should they qualify. Last, improved long term compliance through diagnostic feedback and improved tolerability can further increase market size. From (19).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Potential drug development futures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Today</td>
</tr>
<tr>
<td>Patent life*</td>
<td>20</td>
</tr>
<tr>
<td>Development time*</td>
<td>10</td>
</tr>
<tr>
<td>Development cost</td>
<td>$1</td>
</tr>
<tr>
<td>Sales life*</td>
<td>10</td>
</tr>
<tr>
<td>Average yearly sales</td>
<td>$0.5</td>
</tr>
<tr>
<td>Gross margin</td>
<td>80%</td>
</tr>
<tr>
<td>Lifetime gross profit</td>
<td>$4</td>
</tr>
<tr>
<td>Payback ratio</td>
<td>4</td>
</tr>
</tbody>
</table>

*In years. *Gross margin divided by development costs.

From (19)
Technologies to enable personalized medicine – ABRF 2011

In the above discussion, we have seen examples of biomarker discovery approaches based on genotype and phenotype. The reason that we have chosen this as a theme for ABRF2011, is that society is currently paying a high price, both in terms of pain and suffering and national treasure, for the lack of these stratification tools. Moreover, the omic technologies underlying these discovery approaches are the stock and trade of ABRF members and member labs. With this enormous skill base at its disposal, ABRF members are well positioned to make significant contributions to this important national issue. The fine structure analysis in Figure 10 points to many potential entry points for improvement of this process.

The opening plenary lecture by Raju Kucherlapati will put these global issues into historic and current perspective in a talk entitled ‘Personalized Medicine - Opportunities and Challenges’. In the first of the daily plenaries, Larry Gold will then talk about ‘Unlocking Biomarker Discovery: Unbiased Human Proteomics at High Scale, Sensitivity, and Accuracy’. In the second daily plenary, Eric Green will talk about genomic tools in talk entitled, ‘En Route to the Era of Genomic Medicine’. Finally, David de Graaf will talk about combining these tools in a clinical environment in a talk entitled, ‘Computational Systems Biology Approaches to Identify Mechanistic Response Biomarkers in a Clinical Population’.

These talks and focus, in addition to the usual ABRF fare of practical presentations on methods and issues, will provide and exciting three days of thought provoking discussion both in the lecture rooms and in the hallways. Come, enjoy, talk, network and then go home and pursue your work with new vision and focus.

References:


