A drug response predictor to guide treatment for breast cancer

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Currently, the clinical situation in advanced breast cancer is such that a variety of drugs are available with very little guidance on their selection. Estrogen receptor status, HER2 status and subtypes based on PAM50 can be used to stratify patients to treatment with antiestrogen therapy, anti-HER2 therapy and adjuvant chemotherapy [1]. However, no drug-specific biomarkers are currently available for personalizing treatment to patients.

Personalization – choice of assay & strategy

The original approach of biomarker searches was based on biological knowledge of mechanism of action for each specific drug. In breast cancer, estrogen receptor is used as a simple, single biomarker identified by staining with immunohistochemistry (IHC) and is used to select patients to treatment with selective estrogen-receptor modulator tamoxifen or aromatase inhibitors like letrozole giving an approximately 30% response rate in estrogen receptor positive breast cancer patients [2]. Other examples are topoisomerase 2 to predict efficacy of topoisomerase 2 inhibiting drugs such as epirubicin [3] and *BRCA* mutations being used to guide treatment with PARP inhibitors with response rates varying from 20 to 60% [4]. Lastly, using HER2/ErbB2 levels on IHC can be used to select patients to treatment with trastuzumab, pertuzumab or lapatinib. However, knowing the target is only part of the solution. Trastuzumab given to HER2 positive patients produces a response rate of 25%, not 100% [5].

Many projects are prospectively searching for targetable mutations with next-generation sequencing (NGS) and trying to target these. The dream of finding an extremely efficient drug for a specific mutation has been alive since imatinib became available for chronic myeloid leukemia patients with Philadelphia chromosome mutations [6]. This dream of targeting single mutations is driving projects such as FoundationOne that was launched as an assay searching for targetable mutations [7,8]. Another example is the Copenhagen Prospective cohort with a Phase I study of a variety of mixed tumors selected for targeted anticancer treatments based on mutational status, for example, *BRAF* mutated treated with BRAF inhibitor combination (EGFRi/MEKi) therapy like vemurafenib + panitumumab [9]. The overall response rate from the latter study was only 15% albeit in a heavily pretreated cohort. The NGS gives a thorough deep sequencing of DNA in a very efficient manner. But currently the use of NGS is limited to single-hit mutations or tumor mutational burden and not capturing collective interactions in data.

Whether it is protein with IHC or DNA with NGS, the approach of searching for single biomarkers to select drugs has brought substantial treatment benefits into advanced breast cancer but sadly we have yet to see actual cure. Further, these approaches do not aid the clinician in choosing between drugs when there are several drugs to choose from.

A completely different take on the matter of personalizing treatment in oncology is taken by mimicking a standard strategy from microbiology. Specifically, the idea of growing bacteria in petri dishes and examining specific effect of antibiotics is mimicked. One way is by use of patient-derived xenografts where cells from a patients' cancer are implanted into immunoincompetent mice [10]. Afterward, the mice are treated with different drugs and one can identify drugs effective against that patient's cancer [11]. Patient-derived *in vitro* models either from biopsies

from a patients' cancer making *in vitro* models in 2D [12] or 3D tumor spheroids [13] or as circulating tumor cells explants (CDX) are other similar ideas [14]. These all have the benefit of aiding in the selection between drugs, an important parameter in situations like advanced breast cancer where many drugs are available as standard therapy. Limitations include the rather expensive workflow and dependence of local availability of systems to extract and manage circulating tumor cells and/or keeping tumor cells alive after surgery [10,12].

In the field of selecting between drugs, we have attempted a quite different approach in trying to embrace the complexity of cancer. We apply statistical and computational biology methodologies to huge amounts of data available from standard gene chips. Due to years of standardization efforts, data from gene expression microarrays such as Affymetrix HG-U133 are robust and it is possible to add data from various sources with confidence in the data representing the intratumoral biology. Specifically, we correlate cell growth *in vitro* after treatment with a drug to the baseline gene expression of the cells. The National Cancer Institute 60 cell lines has, for decades, made data on growth inhibition and baseline transcriptome data publicly available [15]. Correlating these data gives a raw outcome of genes upregulated in sensitive cells and genes upregulated in resistant cells for each drug. To assess the biological importance of these genes, we apply a 'clinical relevance' filter of gene expression from 3500 tumors of mixed origin [16]. Only genes presenting as partaking in pathways in the actual tumors will be assessed in the final drug response predictor. The filter reduces irrelevant background gene expression patterns and thus gives a dramatic improvement in the signal-to-noise ratio.

This system primarily focuses on approved drugs without approved biomarkers such as cisplatin, 5-FU and fulvestrant [16,17]. We try to seek out responders for these approved drugs but in theory one can derive a biomarker with this system for any drug with a direct cytotoxic effect on cancer cells. This excludes antibodies and drugs with effects on endothelial cells like VEGFR inhibitor bevacizumab. But with biomarkers specific to a majority of available breast cancer drugs, this system offers the possibility of selecting the most effective drug.

One factor contributing to the efficacy of this system is how robust the transcriptome has turned out to be. We assessed formalin-fixed paraffin-embedded tumor tissue with a median age of 10 years in more than 700 advanced breast cancer patients. This system was in fact able to predict outcome, but only with some drugs. We have shown how treatment with epirubicine was not sensitive to age of the block [18], but also that both assays for fulvestrant and exemestane are sensitive to long-term endocrine treatments [19].

With often more than 200 genes in each drug-specific predictor (drug response predictor) some redundancy is bound to be present. We expect that some genes could be discarded altogether and that possibly only few genes are the culprit genes. Still we believe the gene–gene interactions represented with this system are what ensure interpretation into clinical benefit.

Future perspective

Assays like Affymetrix gene chips appear robust in the aid of prediction in breast cancer. But we also need to consider information on DNA mutations or protein products to get a fuller picture. At the least we need to take advantage of the depth of data and handle the complexity of data and integration of separate data sources. This is complicated in a busy clinical ward. In every hospital, we might need pharmacogenomically trained staff to aid oncologists or even other specialties in regard to choice of and sequence of therapy in the future.

With some variety in efficiency of the markers in prediction of benefit in the clinical real-life situations, more refinement of all methods is called for. With available data from outcome, treatment and assays, the next natural steps are to combine this exploratorily and make hybrid biomarkers, either markers combined with clinical prognosticators (like PAM50 or Endopredict®), or markers refined after clinical assessment. With enough data available, new validations of hybrid markers will then be possible.

Such efforts could be supported by public–private partnerships as public institutions have the data that is needed for the next steps. We propose shared efforts to make a tool to aid in drug selection. Is it possible that the generic compound is more active than the branded new expensive product? Drug developers or owners might find it disruptive and payers will greet such efforts. But we regard it as in everyone's interest to use drugs as specifically as possible.

As many treatment choices are available in metastatic cancer, the urgency to select precisely is obvious. It is a matter of efficient use in regards of effect and toxicity. This could be achieved in many ways but we believe that single mutation searches should be combined with other drug predictors to begin unraveling the eminent complexity of cancer.

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