

## **Targeted Diagnostics: From Four Humors to Four Nucleotides**

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In medicine it has long been the rule that the cure should fit the ailment. The diagnosis of disease has come a long way from the first systematic attempts by Hippocrates and others to determine the correct treatment based upon observing the disturbance of the balance among four humors (yellow bile, black bile, phlegm, and blood). From these first steps to codify and attribute a specific cause to a disease, a variety of diagnostic tools have been developed and employed, including standard clinical findings (body temperature, blood pressure, pulse, etc.), diagnostic imaging (X-ray, CAT scan, MRI) and laboratory tests (blood glucose, liver enzymes, blood gases). The increasing sophistication and power of these diagnostic tools has been directed toward establishing the manifestations of a disease in a patient with a view toward determining the correct means of treating that individual. William Osler, the 19<sup>th</sup> century Canadian physician who is often described as the Father of Modern Medicine, said, "The good physician treats the disease; the great physician treats the patient who has the disease". The notion that care of the individual should be directed toward the person, not the disease, has reached the point that we have moved from diagnosis based upon the four humors to molecular medicine, which seeks to select the proper treatment based upon differences in the sequence and expression of particular genes, comprised of permutations of four nucleotides.

The development of molecular medicine has brought us to a different perspective on diagnosis and treatment of diseases that integrates knowledge of the patient with the development and utilization of new means of treatment. This view has been considered variously as personalized medicine, targeted therapeutics, theranostics, companion diagnostics, as well as targeted diagnostics. This integrated approach to diagnosis and treatment has profound consequences with regard to basic research, laboratory medicine, drug development, and routine clinical practice where there is a growing body of treatments and tools available in an atmosphere where demands for efficiency and economy are becoming ever more pressing.

It is worth considering the underlying assumption that treatment of disease is dictated by diagnosis. It has long been appreciated that a set of symptoms as experienced by the patient and observed by the physician may have more than one cause. For example, an infectious disease such as pneumonia can be caused by a large number of agents. One treatment may not be effective for all patients if there are a variety of causes for the same constellation of symptoms, clinical findings and lab results. In many such cases diagnostic techniques have often been developed to determine the appropriate treatment for a particular infectious agent. A more telling problem is assumption that all individuals will respond identically to the same treatments, or manifest the same symptoms as part of the same disease process. It is often the case that even when the etiology of a disease is the same, *e.g.* HIV infection or even the current version of influenza, the course of the disease will differ among members of the same

population. As our understanding of human genetics, and more specifically the particular subtle differences among us, has grown these variations have become easier to observe and in some, cases, rationalize in a way that can inform effective treatment and public health measures.

In a sense, there has been a continuous process of refining diagnosis and treatment since the recognition of the existence of specific microbes which cause infectious diseases, or different genetic changes which underlie various cancers. As it has been recognized that particular microbes respond to different antibiotics, or different tumors respond to different chemotherapeutic regimens, the standard of care has generally improved. Most recently, largely as a result of advances in molecular diagnostic testing and knowledge, there has been a dramatic shift in our knowledge of the subtle differences in etiological agents, disease processes, and the differences among individuals which affect treatment response. This knowledge of the molecular basis of disease has had profound consequences upon the interface between these diagnostic assays and the development of new drugs.

One area where the consequences of molecular testing have had the greatest impact is in the treatment of various cancers. A brief consideration of breast cancer serves to illustrate how there has been a paradigm shift in how diagnosis and treatment are approached. In the simplest sense treatment of cancer involves the elimination of the tumor cells with some combination of surgery and chemotherapy. At the level of pharmaceutical interventions, historically the drugs used generally worked by targeting a particular

characteristic of cancer cells. Most often, the ability of cancer cells to grow more rapidly than most normal tissues led to the use of cytotoxic treatments which preferentially kill tumor cells, while leaving most normal cells relatively undisturbed. In essence, the purpose of diagnosis was to identify the disease, while the mechanism of treatment was to target a process characteristic of the disease state. This approach did not involve any knowledge of the particular biology of the tumor, let alone the individual patient. The first instance where this situation began to shift was with breast cancer. Knowledge of human physiology suggested that breast tumors were likely to be dependent upon the same hormonal signals that influence the normal proliferation of breast tissue. For this reason, it was not uncommon for women diagnosed with breast cancer to have their ovaries surgically removed in order to slow the growth of tumor cells that might be dependent on estrogen, the hormone that drives proliferation of mammary glands. Having had the tumor removed, along with normal tissue that could potentially promote the growth of residual cancer cells, the patient was left to be treated with drugs to kill any growing cells that might serve as a seed for recurrence of the cancer.

The development of drugs, notably tamoxifen, which acted as anti-estrogens, chemically preventing the action of estrogen, made it possible to treat patients, without removal of normal tissue. The standards of care became long-term use of anti-estrogen therapy to prevent recurrence of the tumor. At the same time, techniques were introduced which allowed a pathologist examining breast tissue samples to determine whether or not a specific patient's tumor was

actively expressing estrogen receptors, the molecules on the surface of breast cells that allow them to respond to estrogen. In this way the analysis of the tumor was able to go beyond the question of whether or not it was a particular type of breast cancer, to address the critical issue of whether or not a given tumor expressed a potentially “druggable” target. This was the first instance of targeted diagnostics, an approach based upon diagnosis and treatment being coordinated processes. The concept that effective treatment requires knowledge of the presence and nature of a drug target in an individual patient or their abnormal tissue has become an essential part of the drug development process and medical care in recent years.

The general category of targeted treatment includes personalized medicine which recognizes that individuals may differ in specific ways that influence the metabolism of various compounds. This view has fueled the development of personalized genetics where specific and characteristic changes in the genome are cataloged and related to response to specific drugs, or a predisposition to the development of particular diseases. This area will not be discussed in any detail here, as the focus of this paper is on how understanding of biological molecules and metabolic pathways characteristic of disease processes influences the drug development process, from target selection to the regulatory approval process, and ultimately patient management. Human cancers result from a series of progressive changes in the cellular genetic material and its expression. The alterations that are involved in a particular type of cancer often differ among patients with microscopically (anatomically)

indistinguishable tumors, and provide a powerful example of how molecular biology, diagnostics, and pharmaceutical research can work together to provide optimal patient care.

The recognition that there are specific molecules whose expression is altered in some subset of patients with a given disease, *e.g.* estrogen receptor in the majority of breast cancers, suggested that these might serve as suitable targets for drugs which specifically treat those patients. It seemed reasonable to expect that drugs that have a target that is essential to the biology of a particular tumor would be less likely to affect “normal” cells and tissues, and thus be better tolerated by the patient. This is similar to the way that antibiotics, such as penicillin, whose target molecules such as cell wall structures are absent in human cells, have little in the way of side-effects in most people.

The stratification of patients on the basis of the presence or absence of a particular target molecule can also serve as a way to avoid unnecessary treatment of patients who are unlikely to be responsive. The consequences of this for the development and utilization of drugs are rather profound. Individuals who are determined to likely be non-responders, should benefit by avoiding unnecessary delays and side-effects which could delay or compromise effective treatment. From the standpoint of a pharmaceutical company, selection of sub-populations of patients that are likely to respond to treatment increases the likelihood that a drug will be approved. That is, rather than a drug which is equivalent to the current standard of care used for all patients, a smaller ready market and approval can be found for a drug that is more efficacious in one

group of patients, however small. Ascertaining the size of this population, *i.e.*, the frequency that the drug target is present and distinctly expressed in a general population of patients, early in the process of identifying druggable targets can provide essential information as to which candidate drugs to move along the drug pipeline.

Herceptin, a drug used in the treatment of breast cancer, is a good example of how such a selection process might be designed and how it can serve to identify potential markets, in the form of drug-responsive patients. It was recognized that the amount of a cell surface protein, termed HER2/neu, is increased in a subgroup of breast cancer patients with a poor prognosis. That is, HER2 was recognized as a prognostic marker, one that could be used in predicting the course of disease. Genentech recognized this possibility, and developed monoclonal antibodies that targeted the HER2 protein. One of these came to be designated Herceptin. The initial clinical trials of Herceptin failed to show any specific effect when used on women with metastatic breast cancer, though as expected the side effects were minimal compared to the traditional anti-cancer drugs which caused nonspecific damage to growing cells and tissues. Efficacy was only demonstrated, and FDA approval only came, when patients were stratified based upon whether or not they expressed increased amounts of the HER2 protein. The approval for the use of Herceptin required a companion diagnostic to establish that it would be used only in those patients who might show some benefit.

The concept that drugs are designed for a specific target is by no means a new one. The conjunction of molecular biology and drug development that brought Herceptin and its companion diagnostic is just the beginning of a new chapter in this story. Not surprisingly, given the complexity of the regulatory circuits that are active in normal and cancer cells, it has become necessary to consider not just the target molecule itself, but also its biological activity. Many pharmaceutical companies have embarked on major programs to develop drugs that interact with a particular molecule that might be essential to growth of specific tumors. It has become clear that the success of these drug development programs is likely to require “intelligent targeted diagnostics” that recognize that presence of the target is not sufficient; it must be active as well. A major focus for drug development over the last ten years has been drugs that inhibit the activity of epidermal growth factor receptor (EGFR), a protein related to HER2, which is over-expressed in many solid tumors. Several drug companies including Amgen and ImClone have developed, been granted FDA approval, and begun to distribute anti-EGFR drugs for treatment of a variety of tumors, including colon, breast, and lung cancer. The activity of EGFR in regulating cellular growth involves complex regulatory circuits where EGFR on the surface of a cell activates downstream effectors which influence proliferation of cells. One such effector molecule is termed K-ras, and it has recently been recognized that patients whose tumors do not express active K-ras do not respond to anti-EGFR drugs. Most recently the FDA has begun to consider requiring that data on the “genomic profile” of the tumors of patients be included in clinical trials. It may

become policy that such information provided as part of the development and approval processes. It would appear that the FDA is leaning toward requiring the co-development of drugs with any companion diagnostic or genomic profiles to be used to identify the presence of the biologically active target of the drug.

It is certainly the case that the emergence of molecular approaches to the identification of drug targets may serve to support the identification of the target populations for specific new drugs. While this may accrue to the advantage of drug makers seeking new markets, it is worth noting that there are barriers to the acceptance of this approach. A major consideration is the cost in time and patient accrual expenses for subjects that because of their biological profiles are not included in phase 1 (toxicity), phase 2 (response), and phase 3 (efficacy) data sets as part of FDA applications. In the case of drugs where the target is relatively rare, this can devolve into a great deal of expense to gain approval for a drug which will find a very small market. This argues that appraisals of target molecule distribution be part of the early evaluation of drugs and targets. Similarly, it may be difficult to establish with a reasonable degree of certainty, without recourse to significant basic research, that the assay method(s) used to assess the levels of the drug target directly measures the amounts of the active/susceptible form of the target molecule.

In summary, it is clear that targeted treatment is likely to become the norm going forward. In terms of medical practice this can be expected to lead to better patient management, as well as more cost-effective treatment. Pharmaceutical companies can look forward to using the information gained through the use of

molecular diagnostics through a more rapid, and perhaps mandated, path to drug approval requiring detailed information as to the particular biological features of tumors, and other pathological, tissues. Ultimately, what are now termed companion diagnostics may be the tip of the molecular iceberg for the coordination of translational research, assay development and drug design, clinical testing, regulatory approval, and patient management and treatment.

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