The pharmaceutical industry today is highly concerned by the fact that research and development budgets have increased dramatically over the last decade; whereas the number of new chemical entities being approved each year has been stable or even decreasing. In other words, there appears to be a diminishing return on investment despite the introduction of new technologies. The increase in spending is in part due to the rising cost of clinical trials.

Thus, there is considerable amount of investments behind each agent that fails at the clinical trial stage because the compound has lower efficacy or higher toxicity in humans than was expected from the preclinical development stages. If the selection process of compounds chosen to go into trials can be improved, then the cost per new drug can be decreased. Most preclinical testing has been performed on animal systems such as mice, rats and dogs, which can differ considerably in their response from humans. The use of human tissues in early stages of preclinical drug discovery and development can improve the success rate in patients. It does so by defining specific molecular targets for drug discovery and demonstrating efficacy as well as toxicity in human systems. However, anyone who has done research on human tissues knows that it takes a great deal of time and effort to identify sources of the tissue and to obtain the necessary ethical approvals.

Asterand is a focused research service company in the area of human tissue research. We have developed a large network of hospitals, clinics and institutes to provide tissues and biofluids, all of which have been properly consented for a wide range of research approaches and for storage in our biorepository. We have developed standard operating procedures for tissue collection, storage and quality control assessment. Our network also provides us with fresh tissues that are necessary for functional testing of test substances. Recently, through our merger with the UK-based human tissue company, Pharmagene, we have broadened our range of research services using fresh human tissues to include many non-oncology therapeutic areas.

The completion of the sequencing of the human genome, combined with the advent of high throughput gene array technologies has revolutionized the approach to identify differences in gene expression between diseased and normal tissues by genomics analyzes. More recently, advances in protein analysis have allowed similar studies on the protein level by proteomic analyzes. The methods generally require fresh or freshly frozen diseased and normal tissues. However, we are exploring new methods that allow us to obtain similar types of data from tissues that have been fixed in formalin and embedded in wax blocks as is the practice in clinical pathology laboratories for diagnosis. The genes products identified as being associated with the diseased tissue are called “biomarkers” and may be targets for therapy or diagnostics.
The next critical step in the biomarker discovery process is called “target validation”. This is to determine the spectrum of tissues in which the gene of interest is expressed, diseased and normal. Human tissues are essential for this task. Profiling gene expression can be performed at the RNA level by RT-PCR or in situ hybridization or at the protein level by immunohistochemistry if a specific antibody is available. We have build up a database with approximately 2000 genes profiled over 72 normal tissues from at least 3 donors by RT-PCR that can show at a glance in which tissues a gene may be expressed. However, this type of analysis does not provide information about the cells that express the gene. A rapid approach to look at many tissues, diseased or normal, in one experiment is through the use of tissue microarrays. To make these arrays, small cores are taken from frozen or fixed pieces of tissue and the cores are aligned in a wax block. Our standard high density array consists of 360 cores representing triplicate cores from 120 tissue samples. For instance, a breast cancer array could have cores from 120 different patient tumors, or 60 if they are matched with the adjacent normal tissue that is also routinely removed at the time of surgery. Thus, many cases can be examined at the cellular level in a single staining procedure. After these approaches have identified genes that fit the desired expression profile, the results are usually confirmed using full sections of frozen or fixed samples.

Once the correct target for a disease has been identified, the process moves to finding an agonist or antagonist of the target activity. This process usually starts with high speed screening assays using purified targets in assays that measure enzyme activity or ligand binding. The leads from these assays are then examined through a series of cell based assays, followed by animal assays.

Animal assays, even with transgenic animals expressing human genes or human cancers transplanted in congenitally immune suppressed mice, can be misleading. A case in point is development of the anticancer drug capecitabine, which is a pre-pro-drug of 5-fluorouracil. The pro-drug 5’-deoxy-5-fluorouridine (FurtulonTM) is an orally available compound that is converted to 5-fluorouracil by an enzyme (thymidine phosphorylase), which is highly expressed in many human tumors. This drug is widely used in Japan and other countries in Asia. Unfortunately, the activating enzyme is also present in the intestines of a quarter of the patients who take the drug, causing severe diarrhea. Thus, a pre-pro-drug approach was taken to design a molecule that would be activated to the pro-drug only in the liver. The compounds were screened for activity in mice with human xenograft tumors and one was selected for further development. This compound entered clinical trials in Japan but proved to have poor bioavailability in humans, only about 40% of the compound was converted to the active form because the activity of the enzyme required for the first activation step was much lower that that in the mouse. A second round of screening was devised using human enzymes and eventually two compounds with good pharmacokinetic properties in monkeys were compared for their pharmacokinetics in humans. This demonstrates the importance of optimizing drugs for human metabolism and why we offer a range of services to test compounds on human tissues.
Many similar examples of animal models not predicting human efficacy exist in other therapeutic areas. The same is true for toxicity testing of drugs. A classic example is the anticancer drug cisplatin. Cisplatin has nephrotoxicity that is not evident in rodents. A new model for developing new analogs that would not have the same toxicity was eventually found, the dog, which better represented the human toxicity. However, because the overall gene expression in dog kidneys is not the same as in human kidney cells, this model cannot accurately predict toxicity that will be seen in the clinic.

The impediment to doing functional studies with fresh human tissues rather than animal tissues in most laboratory situations is that acquisition of the tissue cannot be planned ahead, so researchers must ready to perform the experiments as soon as the tissue is received. Asterand has more than 10 years experience in this area and our laboratories are set up to work 24 hours a day, seven days a week whenever the tissues are available.

In summary, the use of human systems at a variety of stages in the drug discovery and development process should decrease the number of drugs that fail in clinical trials. Because clinical trials represent the largest proportion of the R & D budget for pharmaceutical companies, this can significantly decrease the R & D investment per drug that reaches the market.

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