THE CANCER CONUNDRUM

increasing clinical success by implementing improved methodologies in early phase drug discovery

Using primary cells and 3D culture models to enhance cancer drug discovery

The high stakes of drug discovery and development lead pharmaceutical companies to be meticulous in ensuring a target is validated and that compounds are effective against this target in order to have the best chance of developing a successful drug. Taking into account the long-term investment of both time and money to find, test and develop a therapy for a disease like cancer, pharmaceutical companies need to better recognise the importance of using more robust methods, such as primary cells and 3D culture models, in early phase drug discovery to decrease the risk of late-stage failures.

By Lubna Hussain

It is common knowledge that the drug discovery and development process endures a long pipeline and involves massive spend by pharmaceutical companies. One drug can take 12-15 years from idea to launch, bringing the total cost of a single drug to more than $2 billion[1-3]. However, despite an investment of more than $600 million in R&D alone, only a small number of drug candidates make it through to validation and development. With data showing that only one out of every 5,000-10,000 screened compounds make it to FDA approval[4], it is no wonder that companies experience low success rates, equating to lost expenditures and time.

Drugs tend to fail due to either efficacy or safety concerns. While focus tends to be directed towards late-stage drug development and clinical trials to evaluate the efficacy of a compound, the early stages of drug discovery are equally important in determining the success of the drug. The efficiency of each phase, from target discovery and validation to assay development and drug screening, are all crucial for deciding which drug target(s) pass into clinical development. Consequently, re-evaluating the steps needed to improve the early stages of drug discovery is an emerging trend among pharmaceutical companies, emphasising the creation of more accurate disease models as well as more consistent methodologies. The effort is undertaken to minimise the risk of late-stage failures, save time, reduce costs and reduce the use of animal testing.

In an effort to improve the early phases of drug discovery where costly infrastructure or appropriate expertise may not be available in-house, pharmaceutical companies are turning to outsourcing. However, academic laboratories, pharmaceutical companies, biotechs and CROs have their own approaches to cancer research. As a result, data from these various sources will have originated from different research methods and need standardisation. For collaborations between groups to be successful, data must be generated consistently

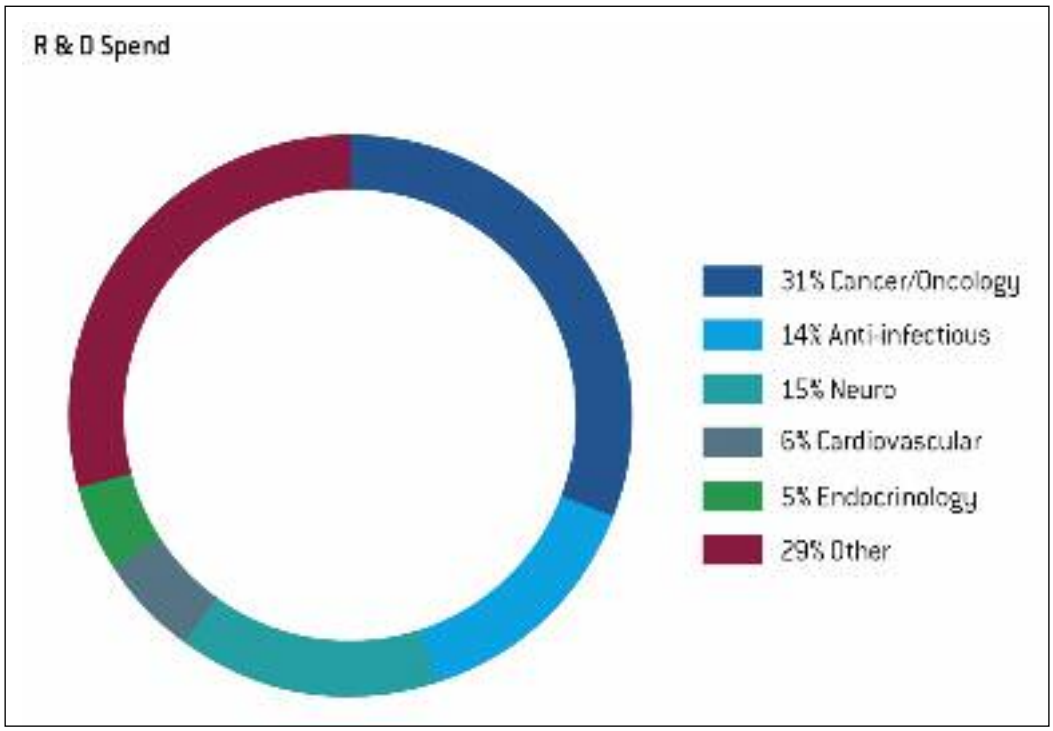

Figure 1
R&D spend by disease area. Data courtesy of AT Kearney (2013)[6]

by all sources. In this way, implementing standard methodologies could result in higher quality and more reliable data for drug discovery.

Improvements in the early phases of drug discovery are imperative across all disease areas but are especially needed in cancer research. Cancer is one of the most funded research areas for pharmaceutical companies, but there are challenges associated with bringing cancer drugs to market.

The challenges with preclinical data in cancer

With an estimated 14 million new cancer diagnoses worldwide in 2012 and high mortality rates attributed to the disease[5], it is not surprising that cancer continues to be a prevalent research area for academia, pharma and biotech. According to a report by AT Kearney, cancer remains the most funded disease in R&D over neurology, infectious disease, and cardiology (**Figure 1**)[6]. Target discovery and target validation in cancer drug discovery stand out as obvious areas for improvement, primarily because these phases are important to discovering targets which feed into late-stage success. If the results from these early phases are inaccurate or unreliable, then the entire pipeline could fail.

An issue that has been more recently highlighted within the industry is the need to improve preclinical models in cancer. A fitting move towards tackling this challenge would be to reduce or eliminate the use of data from mischaracterised cell lines within the early target discovery and target validation phase. Interestingly, a recent study indicates that oncology, by far, has the highest share of literature based on contaminated cell lines (**Figure 2**)[7]. This published literature typically uses data obtained from widely used and shared cell lines that have historically become the standard tool for use in cancer research. Unfortunately, until recently, not much emphasis has been put on authentication or characterisation of these cell lines or even questioning their biological relevance. As a result, mischaracterised, contaminated cell lines have made their way into publications over the years as a standard tool for cancer studies. Not only does this lead to inaccurate data being published, but ultimately becomes the basis for more incorrect data in subsequent research.

There are implications with using data from such misidentified or contaminated cell lines, ranging from costly drug compound testing in the discovery phase based on faulty data, or missed drug targets never tested or reported on relevant cells. Such issues are a key indicator for the need to improve methodologies in the early phases of cancer drug discovery. If the industry acknowledges the criticality of sound preclinical research feeding into the success of a clinical phase, it is equally important to accept and be cognisant of the use of reliable building blocks (ie

reagents, cells) that are the foundation for developing good preclinical models.

How can pharmaceutical companies improve the drug discovery process for better outcomes? By using more accurate and physiologically-relevant models, pharmaceutical companies can help reduce instances of drug failures further down the line by selecting better drug targets in the early stages.

The necessity of primary cells: study human characteristics not 'just cells'

Physiologically relevant cell culture models are the mainstay for better understanding and exploring cancer biology. The cell type used, the model created and the cellular interactions studied all influence the efficiency of early phase drug discovery. While immortalised cell lines are widely used in cancer research, they are overly propagated causing the cells to transform over time and leading to incomplete or inaccurate data. Conversely, primary cells are cells isolated directly from human or animal tissue and are becoming increasingly important for studying cancer progression as they better mimic *in vivo* conditions (**Figure 3**).

Distinguishing between primary cells and generated cell lines becomes especially relevant when considering a cancer model. Cell lines are typically a cost-effective tool for basic research that can be easily handled and continuously propagated. However, serial passaging is known to cause genotypic and phenotypic variation in cell lines. Variation can often be so far from that of the original tissue to where they do not mimic the *in vivo* environment very closely. Cells that do not represent the original tissue could result in false negative or false positive findings, leading to increased costs by continuing with ineffective drug substrates or overlooking key targets during the discovery phases. In either case,

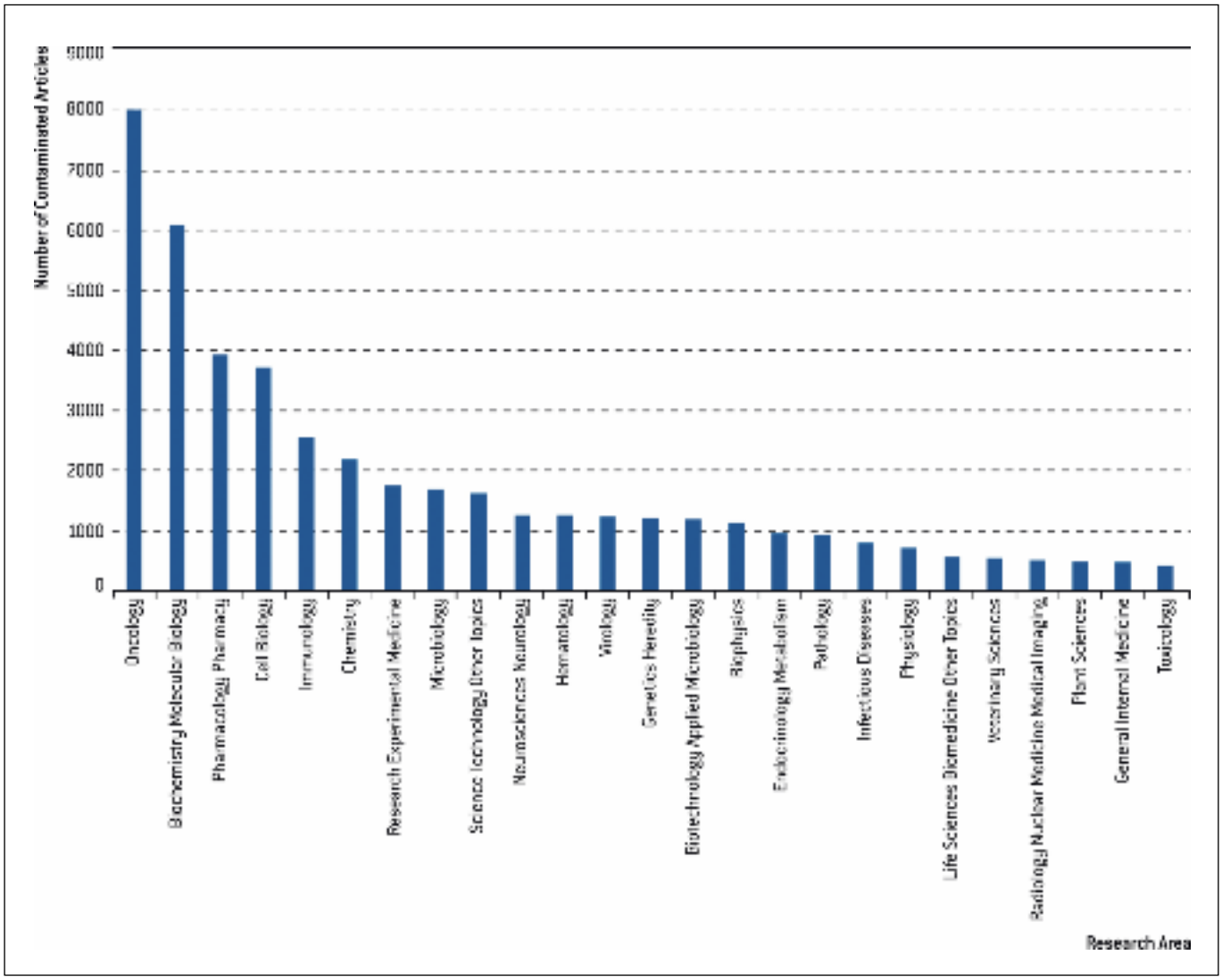

Figure 2
Number of papers published using contaminated cell lines by research area. Data courtesy of SPJM Horbach and W Halffman (2017)[7]

Figure 3
Characteristics of primary cells versus immortalised cell lines

Characteristics	Primary Cells	Immortalized Cell Lines
Lifespan	Limited, resembles tissue characterics	Infinite, loses tissue characteristics
Closer to an *in vivo* model?	Yes, isolated directly from the tissue	No, clonally selected over time
Reduces animal testing costs?	Yes, used in advanced cell culture models to refine experiments	Limited ability to develop biologically relevant complex *in vitro* models
Mutations/ Modifications?	Low	High
Authentication required before use?	No, if bought commercially	Yes, mandated by many government institutes and scientific journals
Donor characteristics available?	Yes	No

this can have costly implications for pharmaceutical companies.

In fact, 18-36% of cell lines used in published research are misidentified or cross-contaminated[8]. Furthermore, the results of assays using cell lines cannot easily be replicated *in vivo* due to cell line variability and disparity from the *in vivo* environment. The use of uncharacterised cell lines has become such an issue that the NIH requires cell line authentication in funded publications.

Unlike continuous cell lines, human primary cells provide researchers with an *in vitro* model closest to the tissue type that they are isolated from. Primary cells have limited lifespan but also have a low mutation rate, resulting in less variation from the original tissue. When studying cancer progression or cancer development *in vitro*, it is typically recommended that such studies be conducted within normal primary cells, which then can be exposed to cancer biomarkers. More importantly, when studying primary cells, researchers are also acquiring an added opportunity to study donors. Several factors such as age, medical history, race and sex can be considered when building a comprehensive profile of drug substrates within these *in vitro* models.

When evaluated in comparison studies, cell lines and primary cells have demonstrated differences in behaviour and dose responses. With a growing trend towards personalised medicine and a clear need to improve reagents used across preclinical research, primary cells offer a natural and more robust solution for target discovery and validation. Such donor variability and tissue complexity cannot be achieved as easily with cell lines that are very systematic and uniform in nature.

In order to standardise cancer data against a normal state, the most sensible factor to incorporate would be 'normal' primary human cells as an optimal control. This allows researchers to understand the progression from a normal tissue-like state to a cancerous one. More importantly, it allows taking the donor characteristics into consideration and, as such, a comprehensive cancer profile is built. As a result, pharmaceutical companies and research laboratories are now seeing the value of using 'normal' human primary cells as necessary side-by-side controls to establish a more accurate baseline for cancer development. Such studies have traditionally been performed on immortalised cell lines which provide the uniformity in an experiment but do not capture the true diversity of a living tissue. As more and more researchers incorporate 'normal' primary cells as side-by-side controls in cancer experiments, basic research and drug discovery can move closer to generating better data and better outcomes.

Primary cells – what's next?

Successful targets progress through the initial phases of drug discovery to assay development, where a need for higher throughput approaches calls for new technologies, such as 3D cell culture models. These specialised models can better mimic the natural cellular *in vivo* environment, resulting in a more biologically relevant approach and providing more reliable data. A clear need has emerged recently within the pharmaceutical drug discovery pipeline for 3D culture technologies that can provide throughput in a 96-well, 384-well and 1536-well format, allowing researchers to generate large numbers of tissue-like models for drug screening.

A combination of using human primary cells in 3D culture models is currently considered the gold

standard to mimic tumour microenvironments more closely *in vitro*. This can potentially reduce the number of animal studies, and their associated costs, if targets are preliminarily screened in a more biologically-relevant human *in vitro* culture. The approach also helps realise differences and variances seen in cell performance as researchers transition from a 3D *in vitro* human model into *in vivo* animal studies.

Not only are primary cells in 3D culture preferred from a cost benefit standpoint to help reduce animal testing costs, this approach also alleviates many of the ethical concerns associated with animal use expressed across many regions of the world. In addition, the ability to use more physiologically-relevant *in vitro* 3D tumour models created with patient-derived primary cells could accelerate the discovery and development of the next generation of personalised cancer treatments.

Conclusion

Cancer is one of the most highly-funded disease areas; however, the failure rate of cancer drugs remains high. To address this challenge, there has been growing recognition within the pharmaceutical industry that human primary cells and 3D culture methods are likely to offer a promising solution for improving the early phases of cancer drug discovery which have traditionally been based on less effective preclinical models. Implementing more biologically-relevant cancer models in target discovery and validation could help to reduce the risk of drugs failing in the later stages, saving pharmaceutical companies considerable amounts of time and money. Moreover, new 3D technologies are aiming towards a higher-throughput approach for drug screening applications and could be a promising pre-screening *in vitro* tool and an alternative to reduce animal models. However, in addition to pharmaceutical companies using better *in vitro* cancer models, there is a need for consistency in methodologies and reagents used across academia, pharma and biotechs. Method standardisation would ensure data published, which will ultimately form the basis of subsequent studies, is more accurate and reliable, helping to advance cancer drug discovery. DDW

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