Preclinical Drug Development: Translating Basic Research into Clinical Work

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**Introduction**

Recent advances in biomedical research and the expansion of preclinical laboratory investigation have resulted in improved knowledge of the biology and genesis of cancer, and have shed relevant insights on the complex relationship between tumor cells and their microenvironment. Several signaling pathways deregulated in cancer cells have been discovered and components of these pathways that are critical for tumor genesis and progression represent targets for the development of new agents. The identification of a large number of possible targets has resulted in an even larger number of new therapeutic agents that have entered clinical evaluation, giving hope that better treatment strategies could be developed to overcome resistance of cancer cells to standard treatments and to improve patient outcomes. Unfortunately, despite the efforts of the pharmaceutical industry and academic institutions, only few new agents have shown a significant impact on survival and most patients with advanced disease remain incurable.

There are several reasons that could explain why most of the new anticancer agents have failed to improve treatment results. Cancer is a complex disease and several genetic changes accumulate that affect components of
the same or different pathways, and render it difficult to identify a specific
target that would be uniquely susceptible to pharmacological inhibition.
On the other hand, many agents that have shown significant antitumor
activity in preclinical studies have failed to reproduce these results when
tested in the clinic. Preclinical studies are important because they repre-
sent the first step toward the development of a new therapeutic compound,
providing important information on the mechanism of action, antitumor
activity, pharmacology, and toxicology that can guide its subsequent clinical
development. The selection of appropriate preclinical experimental
models, most reflective of the complexities of different cancers, may help
to identify the most promising therapeutic agents that would sustain the
rigor of clinical evaluation. Moreover, it has now become evident that
elucidation of “driver” molecular changes can identify patients most likely
to benefit from specific targeted therapy. It is therefore expected that the
development of new anti-cancer agents should proceed together with
the identification of predictive biomarkers of response, which should be
evaluated early in the discovery phase.

In-vitro Evaluation of New Anti-cancer Agents

The development of a new anti-cancer agent begins with the evaluation of
its antitumor activity against a panel of malignant cell lines. These tests help
to identify compounds that deserve further evaluation in animal models.
Several methods exist to detect the antitumor effect of a new agent against
tumor cell lines, such as antiproliferative assays using incorporation of
radioactive nucleotides like [\(^3\)H]thymidine, direct cell counting, or colony
formation. Others assess viability or growth using colorimetric assessment.

A screening model that has been very useful for the evaluation of hundreds
of compounds is represented by the US National Cancer Institute screen-
ing model (NCI-60). It is composed of 60 different cell lines derived from
the major human tumors and provides a source for rapid evaluation of the
in-vitro antitumor activity of new compounds. Every new agent is tested
against each of the cell lines in order to evaluate its ability to inhibit growth
or cause cell death. Based on parameters such as the concentrations of the
drug that cause: growth inhibition of 50% of the cells (GI\(_{50}\)), or total growth
inhibition (TGI), or cytotoxic killing of 50% of the cells (LC\(_{50}\)), a specific

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fingerprint is produced for each compound that can be compared with the activity of others with the same or different mechanisms of action.

Although the empirical screening of natural products has discovered anticancer drugs such as paclitaxel and trabectedin, the current strategy of drug discovery favors approaches that are rationally and biologically driven to develop agents that inhibit specific molecular targets involved in tumor formation and progression. This can be achieved through high-throughput screening of small-molecule libraries or through a more sophisticated structure-guided discovery approach that leads to the identification of compounds that interfere with specific molecular targets. Lead compounds are subjected to specificity evaluations to test their ability to engage and interact with their putative molecular targets. Studies in vitro are therefore designed not only to show that a new agent has inhibitory or cytotoxic activity against cell lines, but also to demonstrate that it is able to produce target inhibition to support its underlying mechanism of action.

The assessment of target inhibition of a new agent in vitro (in cell-based and non-cell-based assays) is generally based on the concentration of the drug necessary to inhibit the activity of its target. For many new agents that target specific enzymes, this is assessed by measurement of the concentration of the drug needed to produce 50% of enzymatic inhibition ($IC_{50}$). Drugs that cause inhibition at low doses in vitro (i.e. $IC_{50}$ at low nanomolar range) are preferred, as they more likely result in a favorable therapeutic index in clinical studies. The $IC_{50}$ against other enzymes in the same family must also be determined in order to define the specificity of the agent against its target. If a drug inhibits several enzymes at low $IC_{50}$, it may act by modulating different targets. This finding must be taken into consideration when studying the mechanism of antitumor activity of a new drug, and it may also raise the possibility of undesirable side effects due to multiple target inhibition.

While in-vitro studies using tumor cell lines represent an initial step in the evaluation of antitumor activity and the elucidation of the mechanism of action of a new anti-cancer agent, they have limitations in predicting positive effects in animal models and, more importantly, in patients with cancer. In fact, cell lines present important biological differences from the tumors they derive from and they do not reflect the intricacy of human cancers and the complex interplay between cancer cells and their microenvironment.

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It has now become clear that important mechanisms of resistance to treatment depend on the relationships between cancer cells and the surrounding stromal cells and these conditions are not easy to reproduce in preclinical studies. The use of ex-vivo models (i.e. using cells or tissues taken directly from patients and tested in an external environment with minimal artificial alterations), co-culture of tumor cells together with stroma cells, and the development of in-vitro models with tumor spheroids or multilayered cells represent some of the possibilities to better reproduce in vitro the complexity of human tumors and the relationships with their microenvironment.

Recently, the discovery that specific genetic changes are responsible for the development of particular tumor types has permitted the antitumor activity of some new agents to be evaluated in tumor cell lines expressing these genetic changes. Furthermore, comparison of the behavior of these agents in wild-type cell lines of the same tumor types can be informative. An example is represented by the antitumor activity of PARP inhibitors in the context of BRCA1/2-deficient cell lines in comparison with lack of activity in cell lines with heterozygous or wild-type BRCA1/2. The evidence from such in-vitro studies was the basis for the subsequent clinical development of several PARP inhibitors for tumors bearing homozygous mutations in BRCA1/2 genes. The in-vitro evaluation of a new agent’s antitumor activity against tumors with specific genetic changes may therefore provide a strong rationale to support its clinical development in a genetically selected patient population.

**Studies in Animal Models**

Once studies in cell lines have shown that a new agent has antiproliferative properties and is able to inhibit its target, in-vivo studies in experimental animal models are undertaken to further define the antitumor activity and provide pharmacology and toxicology data needed for the subsequent clinical development. The antitumor effect of a new agent must be evaluated in vivo and, for those agents in which the target is known (or believed to be known), efforts should be made to show that the observed antitumor effect is related to target modulation and to establish if a dose-dependent relationship exists between target inhibition and the observed antitumor effect.
Pharmacodynamic endpoints used to define target inhibition in vivo may vary based on the target and the mechanism of action of the drug (e.g. measurement of substrate phosphorylation for kinase inhibitors, measurement of mRNA and protein levels for small oligonucleotides, etc). More recently, imaging techniques have also been used to detect the effect of some new agents in animal models (e.g. detection of angiogenesis inhibition using dynamic contrast-enhanced magnetic resonance imaging).

In addition to pharmacodynamic measurements, pharmacokinetic studies provide information about drug absorption, metabolism, excretion, and plasma-protein binding. Safety pharmacology and toxicology studies are also performed in animals. The objective is to estimate a safe starting dose for first-in-human phase I studies, assess toxic effects with respect to target organs, and help to select different dosing regimens and dose-escalation schemes for clinical studies.

The choice of starting dose for first-in-human studies is usually based on toxicology evaluation in both rodent and non-rodent (dog or monkey) species and the most sensitive species is chosen for safe starting dose determination. The US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) recommend that new anti-cancer agents be evaluated in both rodent and non-rodent species before undergoing human phase I evaluation. One tenth of the lethal dose to 10% of mice (LD10) and one sixth to one third of the lowest dose that results in no toxicity (TDL) in non-rodent species are some of the parameters that have been most frequently used to select a starting dose for many anti-cancer agents. While these methods have been widely used for cytotoxic agents, their ability to predict a safe starting dose for molecularly targeted agents is debated, and thus far it is not clear which animal models could better predict a safe starting dose. In addition, no standard parameter exists and for most new agents a multitude of parameters has been used to determine a safe starting dose. In our recent review of first-in-human studies of molecularly targeted agents, only 3.7% of phase I trials had a starting dose that exceeded the maximum tolerated dose, providing evidence that, with the exception of a very small proportion of new agents, the choice of the starting dose has been generally safe. A better understanding of the target and mechanism of action of a new agent can help to select the animal models that would best predict for toxicities in humans.
The most important question regarding the in-vivo study of a new anti-cancer agent, however, is represented by the likelihood that the antitumor activity observed in animals may translate into a clinically significant efficacy. Substantial controversy exists as to the best animal model that would positively predict for antitumor activity in humans. Generally, there is no single system that is considered the best positive predictor of antitumor activity for human tumors. Xenograft tumors implanted in immunodeficient mice by subcutaneous or orthotopic inoculation of tumors (grown in vitro or obtained from patients’ tumor biopsies) have served as a model for the evaluation of a large number of anti-cancer agents. Xenografts have several limitations (e.g., a low tumor establishment rate for many human tumors, low reproducibility of “real” cancer with respect to surrounding tumor environment, and growth rates that do not mimic the ones in human cancer, among others), but they represent a valid model and they have contributed to the identification and development of many new agents. More recently, the possibility to obtain genetically engineered mice that recapitulate a specific cancer genotype has opened new horizons in the preclinical evaluation of new compounds. The discovery that genes with either oncogenic or tumor-suppressor activity may be altered in human cancer, and the possibility to introduce these changes by various techniques into mice, raise the possibility to study the antitumor activity of a new agent against tumors that more closely recapitulate the biology of human cancer.

**Companion Diagnostics Development**

The high failure rate observed in late clinical trials of many “promising” new anti-cancer agents has motivated efforts to define alternative strategies of drug development and evaluation of antitumor activity of new agents, both in the clinical as well as in the preclinical settings. Up to now, only few predictive biomarkers are used in routine clinical practice and most have been established retrospectively. The emergence of the so-called companion diagnostics would potentially help expedite the drug development process by identifying predictive biomarkers early in the preclinical setting and carrying out analytical and clinical validation during drug development. Companion diagnostics are assays performed starting from the preclinical stage to help elucidate the efficacy and/or safety of a new
drug for a target patient population, based on specific genotype and biological characteristics of the tumor.

Spigel and colleagues reported recently the results of a randomized phase II trial of erlotinib in combination with MetMab (a monoclonal antibody targeting MET) versus erlotinib plus placebo in patients with previously treated non-small cell lung cancer. The study included evaluation of the expression of cMET in tumoral tissue through both fluorescence in-situ hybridization (FISH) and immunohistochemistry (IHC). They showed that the addition of MetMab to erlotinib resulted in significant improvement in progression-free and overall survival only in patients who had high expression levels of cMET and that expression by IHC was the most sensitive predictor of benefit from MetMab. Conversely, patients with low cMET expression did not benefit from MetMab. In fact, those treated with the combination had significantly worse outcomes that those treated with erlotinib alone. This study represents an example of prospective evaluation of a predictive biomarker and underlines the importance of companion diagnostics in the evaluation of experimental treatments.

To support drug development toward a tumor-specific focus after early clinical trials, preclinical studies should be performed to allow the discovery of biomarkers and the development of assays to evaluate them. This will lead to the development of a diagnostic predictive signature to be clinically evaluated in early clinical trials and prospectively validated in randomized phase II and III studies.

Summary
Modern drug development in oncology relies on the identification of molecular changes that drive the malignant transformation and are responsible for the development and progression of cancer. This is now possible through improvements in our knowledge of the biology of cancer. The development of new cancer therapeutics has been, however, slow and inefficient; as such, alternative strategies are needed. Preclinical studies are important by providing information that is necessary for the subsequent clinical development of any new anti-cancer agent. Data generated from relevant cell lines and xenograft models are crucial to
facilitate a better understanding of the agent’s target, mechanism of action, antitumor activity against different tumor types, pharmacology, and toxicology, before entering human clinical testing. The use of preclinical models that more closely reflect the biology of human cancer will help to improve the success rate of new anti-cancer compounds. Finally, the discovery of predictive biomarkers of response, their development in preclinical studies, and their subsequent validation in clinical studies will help to define patient populations most likely to benefit from future treatments.

Further Reading