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Review Article

Heterotransplant mouse model cohorts of human malignancies: A novel platform for Systematic Preclinical Efficacy Evaluation of Drugs (SPEED)

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Abstract: Advances in molecular biology demonstrate that cancer is heterogeneous disease necessitating a personalized management approach. This is introducing a paradigm shift in clinical trial designs where molecular characterization of cancers is assuming importance equal to (or even more than) the traditional histologic diagnosis as the eligibility criterion for randomized clinical trials of new therapies. Recommendations have been made to gather the molecular information from clinical phase II trials distinguishing responding from non responding tumors for subsequent planning of large scale phase III trials. However by the time we reach phase II level, more than a billion dollars apart from years of research have been invested. It would be therefore prudent to conceptualize laboratory based platforms to obtain the proof of concept as early as possible, even before embarking upon the pivotal clinical trials. In this regard, we hereby propose and detail a novel preclinical platform incorporating the existing mouse models to address the issue of tumor heterogeneity in a systematic manner through creation of a setting similar to phase II trials in human patients. By providing critical information about a drug's efficacy and the molecular determinants of response early on, this platform would potentially provide a solid foundation to build avant-garde clinical trials integrating recent advances in molecular medicine.

Key Words: Cancer, heterotransplant, animal model, therapeutics, drug development

Introduction

The approval rates for the Investigational New Drugs (INDs) have been decreasing whereas the developmental cost and duration are increasing exponentially [1,2]. It is estimated that approximately 90% of the INDs fail to get US FDA approval and this situation is even worse in the oncology field, with over 95% failure rate 3. What is more worrisome is more than 70% of the anti-cancer drug candidates fail in Phase II and approximately 60% of those entered into Phase III fail, causing enormous loss of time and resources [3,4]. The major cause of Phase II / III failures is lack of efficacy of the testing agents 4 with 80% of phase III failures in oncology attributed to lack of efficacy [5].

While lack of efficacy has been traditionally attributed to ineffective drugs, in the post genomic era it has been realized that many potentially effective agents fail because we fail to address the molecular subtypes and signatures accounting for variations in response to treatment and survival among patients [6,7]. This results in erroneous clinical trial design/ analysis, enormous wastage of patient and monetary resources, addition of another failed therapy to the graveyard, and demise of potentially effective therapies as exemplified by the failure of Gefitinib to improve outcome in non–small-cell lung cancer when added to chemotherapy. On the other hand, brilliant successes of Trastuzumab and Imatinib suggest that integration of

molecular determinants of response in clinical trials is a promising strategy to test the intrinsic efficacy of an IND against a specific patient subgroup. Recommendations have therefore been made for enrichment trial design by limiting enrollment to patients (over)expressing a molecular target or putative predictors of response [7,8]. However, at the time of clinical studies, such predictors are either hypothetical or we need another clinical trials of other drugs affecting the same target to reach a validated predictor.

To address these issues, experts have recommended utilizing phase II trial analysis to identify the molecular markers distinguishing responders and non responders for subsequent planning of randomized trials evaluating INDs [9,10]. It should however be remembered that by the time the we reach phase II level, approximately billion dollars and years of research has been invested [11]. Considering the slow pace of clinical trials, the high rates of compound attrition late in clinical phase, the relatively small number of patients available for trials and the finite R&D budgets of the biopharmaceutical industry, solving the challenges of personalized therapies requires low-cost, preferably laboratory based model systems to test an IND's efficacy and identify and validate molecular predictors of response even before we embark upon the clinical trials. Fortified with such a data, we would be able to design our clinical trials with greater confidence and save enormous amount of time, money and patient resources. We believe that a potential solution lies in the innovative use of mouse models of human cancer with an aim to facilitate the identification of the efficacious drugs, as well as predictors of response. However, to ferret out the most appropriate mouse model from amongst the existing ones, it is imperative to understand the advantages and pitfalls of available mouse models.

The recent unraveling of complete mouse genome sequence has strengthened our capability to study the parallels and contrasts between the pharmacology of drug stability, metabolism and action between mouse and human studies and have thus reinvigorated our interests in mice models [12]. While the development of genetically engineered mouse models has contributed greatly towards understanding the process of carcinogenesis and target selection, the xenograft models, established by injecting 0.5–1.0 million cultured cancer cells subcutaneously in a nude / SCID mice, have been more popular for drug screening purposes due to its ease, low cost, and faster establishment. Almost every successful cancer therapy developed in the modern era has undergone xenograft testing, however many agents that show consistent and potent anticancer efficacy in xenografts, fail in the clinical trials due to lack of efficacy. This might be due to reliance of xenografts on small numbers of homogeneous cell lines adapted to the artificial culture conditions and acquisition of biological characteristics significantly different from the original natural clone over serial passages in culture.

In an attempt to circumvent this flaw, investigators have tried transplanting fresh cells or tissues obtained directly from the cancer patients into the nude / SCID mice, called heterotransplants. This approach has demonstrated superior correlation of chemosensitivity and specificity data for individualized therapy [13-18]. Amongst these approaches, studies where intact tissue from the patient was transplanted into the mice have shown the excellent patient response prediction rates of 90% and 97% for chemosensitivity and chemoresistance, respectively [19]. We have previously reported the response rate of 21% (95% CI, 9–38%) to paclitaxel in a series of 34 NSCLC heterotransplants, comparable to the response rates observed in chemotherapy-naïve NSCLC patients [20]. As the original microenvironment of the human tumor is retained, the crucial interplay of human stroma - neoplastic components is recapitulated to a greater extent in heterotransplants. This contributes to better replication of pharmacogenomic profiles, histology, chromosome complement, antigen expression, and gene expression of human tumors [19-21], accounting for their better preclinical predictive value as compared to cell line based xenografts. Therefore out of the available models, heterotransplants seem closest to an 'ideal' model to establish a novel platform for Systematic Preclinical Efficacy Evaluation of Drugs (SPEED).

The SPEED approach is designed to address the fundamental uncoupling or disconnection between the bench scientist, who may not understand the intricacies of clinical efficacy trials, and the clinical scientist, who may find it

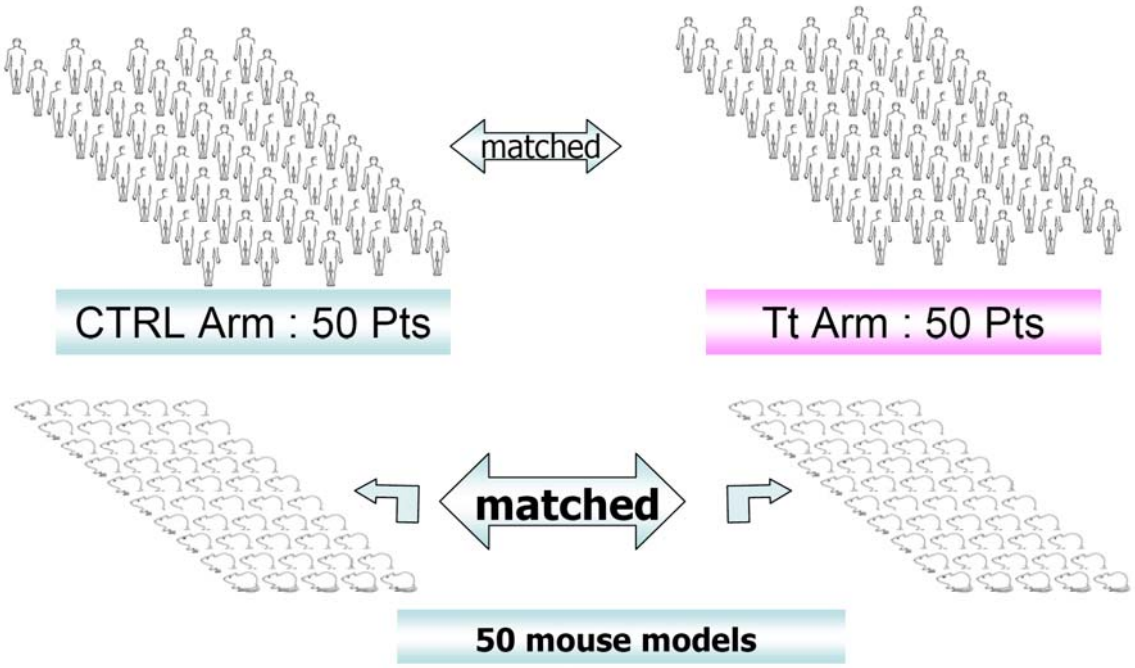

Figure 1: A mouse cohort prepared from 50 models for a specific malignancy would be equivalent to 100 patients enrolled in a clinical trial. Both test (Tt) and controls (CTRL) arms of the mouse cohort can be generated readily at a fraction of cost and time compared to clinical trials. The two arms of mouse cohort are adequately matched with regard to various biological properties.

difficult to make calculated translation of bench side discoveries. For SPEED, a reasonable number (close to the number needed for clinical phase II studies) of well characterized and validated heterotransplant mouse models would be established to make perpetual cohorts comprising of various histologies (**Figure 1**). These cohorts would be used to carry out preclinical IND efficacy studies in a systematic manner (replicating clinical phase II trials) to gauge an agent's possible clinical efficacy and identify the molecular markers of response.

We must understand that as of now, the efficacy criteria used to advance an agent in preclinical studies are different from those in the clinical setting. For example, the NCI criterion for assessing a drug response in mice models is 58% inhibition of tumor growth. In a clinical trial, however, this would define a Progressive Disease. Alternatively, animals are treated immediately after transplantation, before the development of overt tumors; in essence, studying a form of chemoprevention which is of limited therapeutic relevance. Therefore the efficacy results of mice studies frequently do not translate into standard clinical results.

Moreover, these preclinical efficacy results, usually obtained in a limited number of mice, are often considered exciting enough to initiate pivotal clinical trials, not surprisingly leading to more than 90% failure rates. Until we systematically test the drugs at preclinical stage within a framework of convincing statistical power/ design, we might not emerge of the current situation where success in drug development depends more upon chance rather than scientific and robust foundation. In this regard, The SPEED platform provides us an opportunity to apply the principles of biostatistics to meticulously design the preclinical studies in a systematic manner. The required number of models according to the power of study can be created and not only the clinical criteria of 'response' but also the 'response rates' can be measured in the mouse cohort to gauge the clinical efficacy of the INDs. A careful sample size calculation should help us achieve the scientific objectives

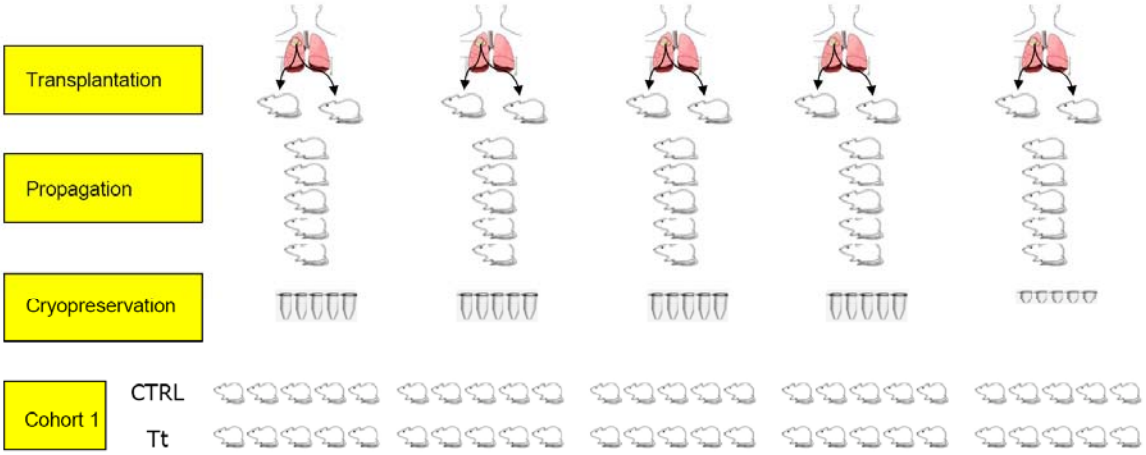

Figure 2: A sample of fresh tumors are obtained from lung cancer patients at the time of surgery and transplanted subcutaneously into 4- 6 weeks old female nu+/nu+ mice under anesthesia by a small incision in bilateral axillary area. On reaching a size of 1,500 mm3, the tumors are harvested and propagated further in 2nd batch of mice. After these tumors are established, tumor tissue is harvested, cryopreserved, and can be used to prepare mouse cohorts as and when required. Multiple cohorts may be generated simultaneously to test various drugs, doses, or combinations.

and extract information from the preclinical data to translate into clinical setting.

As a first step in this regard, we have initiated to establish a cohort of heterotransplant models from primary non small cell lung cancer (NSCLC) tumors of unrelated patients. The tumors are propagated in mice for limited number of passages to retain the human components of the tumor as much as possible. Because tumor pieces can be cryopreserved and re-implanted with high success rates as and when required [21], each model will allow expansion of early passage materials for hundreds of mice tests (**Figure 2**). By enabling us to collect data on modulations in biology, molecular profiles, and response to various interventions in these close- to- human mouse models, SPEED approach has significant potential for anticancer research.

SPEED approach can be utilized to discover molecular markers of response and evaluate targeted agents for suitability to modern personalized clinical trials. In numerous cases the excellent preclinical results with INDs do not translate in the clinical practice because we do not have guiding examples of where a class of agents would be most effective. Most trials of targeted agents are still conducted empirically and 'fail' because many participants did not express the target or the molecular predictor(s) of response [6,22]. In this respect, SPEED approach can guide us by providing preclinical information to enrich trial cohorts by selecting appropriate patients expressing molecular signatures predictive of response and bring on a paradigm shift from the current template approach to the cutting edge personalized approach in clinical trials.

SPEED approach can address many other clinical questions. For, example, there are questions about the prognostic value of costly or invasive post-therapy analysis of residual tumors, which can be addressed using comparable mouse models exposed to the same therapy. We can establish a mouse cohort from patients with residual or progressive/ metastatic tumors to define the molecular/ genetic determinants of response or resistance. Yet another key potential role of these cohorts is to evaluate for differences in the molecular signatures of tumor cells and stroma cells to delineate the patterns of host-tumor microenvironment that occur during cancer regression or progression. These cohorts can be used to test new delivery systems, including those enabled by recent advances in nanotechnology. In addition,

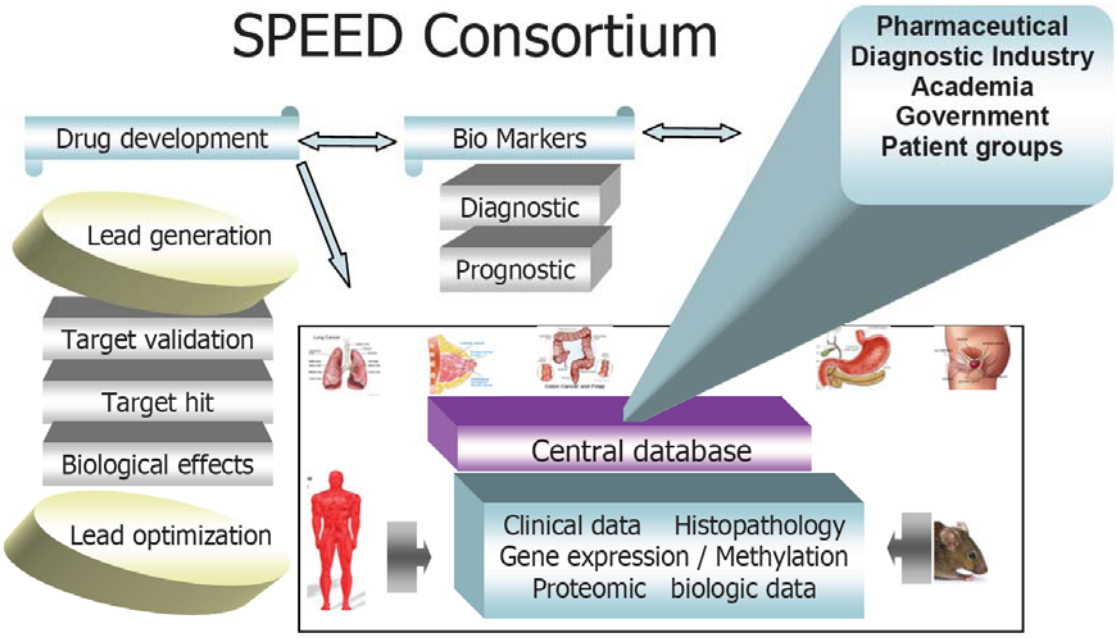

Figure 3: SPEED Consortium proposes the key stakeholders including academia, industry, government bodies, and philanthropic groups to join hands and leverage all resources (financial, data and information, scientific expertise) to expedite anticancer drug development process.

these cohorts could aid in development of novel imaging agents, mathematical modeling, and data reconstruction and visualization to address the questions about human cancers.

A core constituent of SPEED would be the central database housing histopathologic, genetic, expression profiling, methylation profiling, proteomic and other biologic data about heterotransplant tumors (in early, intermediate, and later passages) and their human counterparts as an integrated source of information. Its later version would include information about the SPEED results testing therapeutic agents and experimental protocols and may have free public access for widespread utilization.

There are some caveats and biases of heterotransplant models to be considered during the drug development approaches. Heterotransplants usually involve non-orthotopic tumors and might select out angiogenic clones capable of sustained tumor growth after transplantation. It may not fully capture the genetic diversity of metastatic disease, a major biological issue challenging the efficacy of targeted therapeutics in the deadly solid tumors. Also, in our own experience only approximately 50% heterotransplanted human tumors actually achieve engraftment in mice. Therefore these models may provide efficacy data only against those tumors growing in mice. As the host (SCID and nude) mice have profound defects in their immune response, it would preclude the testing of immunomodulatory agents in the system. Also because SCID mice show defects in DNA repair (which could limit testing of some cytotoxics) and nude mice show an overall frailty, it can limit their capacity to tolerate novel treatments. There may be further limitations of heterotransplant models but we should remember that in view of more than 90% drug failure rates, it is imperative to explore novel approach to use existing models while continuing our search for the better models. Till the time we find one, the SPEED approach may well be based on heterotransplant mouse models. Future clinical trials based on this approach would guide us to refine this preclinical strategy

further and the knowledge build up would help in the evolution of a more scientific and robust foundation of IND development process.

Future Directions

The scientific scope of SPEED may be expanded to include other malignancies through concerted efforts of interested groups and stakeholders to institute a consortium (**Figure 3**). The primary goal of the SPEED consortium would be to provide accurate, faithful, and reproducible mouse model cohorts to the research community for further investigation and explorations. SPEED consortium through development of promising agents and biomarkers could also guide federal regulatory bodies on their use and quality control for cancer treatment. Moreover, the consortium illustrates the exceptional potential for development of evidence to improve understanding of the biology of cancer.

In conclusion, the high attrition rates compel us to revisit the science, strategy and processes currently used in drug development. There is a need for scientific and technological innovations to obtain early readouts for proof of concept to decrease drug attrition rates at a later stage. In this regard SPEED paradigm, fostering meticulous statistical modus operandi, provides a perfect launch pad to the mouse models to leap into an exciting new era of drug development and provides a robust keystone to decrease current drug attrition rates in clinical trials.

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