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Animal Models: Value and Translational Potency

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Abstract

Modern biomedical research with the aim of translating research findings into novel therapies to benefit patients relies to a large extent on animal models of human pathophysiology. However, success stories of translational research – i.e. preclinical research that successfully predicts positive outcome of a clinical trial – are scarce. Here, we therefore address the current state of preclinical disease modeling as well as actions that have been taken to improve the translational value of animal models.

Keywords

Animal models, pathophysiology, disease mechanisms, preclinical randomized controlled trials, modeling of care

3.1.6.1. What is the value of animal models? – Pathophysiological concepts

The majority of translational research relies on preclinical animal models. However, given an incredible number of examples of failed translation, i.e. phase II or phase III clinical trials, which were not able to reproduce the beneficial effect of preclinical findings (O'Collins *et al*, 2006; Perrin, 2014; Prinz *et al*, 2011), the translational value of animal models has been questioned. In particular rodent models have been accused of falsely modeling human disease conditions.

Nonetheless, many animal models are geared to replicate pathophysiological conditions found in patients. An ideal animal model of a human disease is characterized by similarities between both in terms of 1) pathophysiology, 2) phenotypical and histopathological characteristics, 3) predictive biomarkers for course or prognosis, 4) response to therapies and 5) drug safety or toxicity (Perrin, 2014; Prabhakar, 2012).

Four types of animal models are used in preclinical research: 1) disease induction models, 2) xenograft animal models, 3) inbred strains, and 4) transgenic models (Prabhakar, 2012). The rodent stroke model of middle cerebral artery occlusion is a typical disease induction model. Xenografting or transplantation of organs or tissues from one species into another is often used in cancer research. “Humanized” mice are another example of xenograft models (see below). Inbred animals are genetically homogenous allowing investigation of pathobiology with small sample sizes (Prabhakar, 2012). Using methods of molecular biology specific genes are either deleted (knock-out), mutated or overexpressed in transgenic animals, mainly mice. Often these models are combined, e.g. disease induction models in transgenic mice are often used to investigate the contribution of specific genes in diseases.

Rodent models of cerebral ischemia are good examples of animal models that replicate human pathophysiology well (Astrup *et al*, 1977; Heiss, 2011). The ischemic penumbra is defined as the area surrounding the core of the ischemic lesion. While physiological cascades are compromised, this area of brain tissue can potentially be rescued by medical intervention. This concept was first described in animal models (Astrup *et al.*,

1977; Heiss, 2011) and has since been found to be relevant for human stroke pathophysiology (Dirnagl *et al*, 1999; Donnan *et al*, 2008; Mergenthaler *et al*, 2004; Mergenthaler & Meisel, 2012). The same has been found true for the concept of stroke-induced immunodepression. While the pathophysiological concept has initially been described in animal models (Chamorro *et al*, 2012; Prass *et al*, 2003), clinical trials have been able to replicate this concept in human stroke pathophysiology (Chamorro *et al.*, 2012; Harms *et al*, 2008; Mergenthaler & Meisel, 2012), albeit therapeutic protocols making use of this concept are still under development (Mergenthaler & Meisel, 2012).

Likewise, animal models of cancer, and in particular genetically engineered mouse models, have significantly contributed to the understanding of tumor biology and cancer pathophysiology. In particular advances in genetic engineering have allowed modeling the manifold genetic defects underlying many forms of cancer (Cheon & Orsulic, 2011). Likewise, the concept that several mutations in the genome might be required for tumor development as well as prototypic oncogenes have been established by the use of mouse models (Cheon & Orsulic, 2011). However, similar to the situation in stroke (Dirnagl & Fisher, 2012) mouse models in preclinical cancer research have yet to prove their translational capacity (Cheon & Orsulic, 2011).

3.1.6.2. What is a good animal model for translational research?

It is clear that there is no single ideal animal model of human disease conditions. Likewise, the design of preclinical experimental studies at present offers substantial room for improvement. While this topic has recently received significant attention, many of the proposed remedies for the “translational roadblock” have yet to prove themselves in translational studies and the design of clinical trials. Among others, considering the complex characteristics of the animal models as well as of the human disease state is essential when selecting an appropriate model for preclinical studies. Three aspects are often not

considered in preclinical studies: the heterogeneous nature of disease, the presence of comorbidities, and appropriate outcome measures (Mergenthaler & Meisel, 2012).

Several approaches to improve translation from animals to the clinic have been suggested. Before starting clinical trials preclinical investigations should be performed in multiple experimental setting involving different small and large animals modeling different disease states including the characterization of the optimal therapeutic window, optimal administration routes and schemes as well as dose-response curves (Xiong *et al*, 2013). Furthermore, preclinical studies need to reflect the clinical scenarios. Importantly, these include relevant treatment windows and outcome parameters. For example, drug administration at onset or even before injury, as performed in many preclinical studies investigating disease mechanisms is of minor relevance for therapy.

Most preclinical research in stroke or traumatic brain injury (TBI) suffers from short-term studies demonstrating treatment effects 1 to 7 days after the event (Xiong *et al.*, 2013). Investigations on long-term outcome weeks to months after injury are still scarce. On the contrary, primary endpoints of clinical phase III trials have to focus on relevant long-term outcome measures.

Disease modeling focused on pathophysiological research is invariable an oversimplification of the clinical situation. For example, stroke patients often suffer from a variety of other diseases such as hypertension, diabetes mellitus or chronic obstructive pulmonary disease, which are commonly not modeled. Beyond the comorbidities patients have before stroke onset, patients are often affected by several post-stroke complications, such as infection or depression, which are also usually either not modeled or not considered. The same holds true for other disease models such as TBI. Moreover, stroke patients receive a myriad of treatments including medication and general care such as nursing and physiotherapy, among others. Although stroke unit care is efficient without any doubt we do not know, which single pieces of treatment are of relevance. Nevertheless, modeling of care

is probably one prerequisite in successful translation of treatment strategies of complex disorders such as stroke (Mergenthaler & Meisel, 2012).

3.1.6.2.1. Modeling comorbidities

Most investigators disregard the fact that most patients are not young or middle-aged males without any comorbidities (Howells *et al*, 2010; Sena *et al*, 2010). One fundamental criticism of animal research is that most models do not consider age (Howells *et al.*, 2010), which is one of the most relevant cofactors of outcome for most non-communicable disorders (Howells *et al.*, 2010; Lozano *et al*, 2012). However, young to middle-aged inbred rodents of one gender and of homogeneous genetic backgrounds are typically used for preclinical animal studies. Ideally, preclinical animal studies should use animal populations of mixed gender, advanced age and with various comorbidities, such as diabetes mellitus, hyperlipidemia, hypertension, obesity or other risk factors which are relevant for the respective human disease. Such an approach would model the human etiology of most diseases more closely. In many cases, such models are readily available (Howells et al., 2010). In addition, experimental animal populations should be increasingly complex as a therapeutic intervention advances in the translational pipeline (Figure 1). The concept of establishing a framework as well as funding schemes to enable such preclinical randomized controlled trials (pRCTs) has been suggested in many medical disciplines including cancer (Cheon & Orsulic, 2011) and stroke (Bath *et al*, 2009; Dirnagl & Fisher, 2012; Mergenthaler & Meisel, 2012).

3.1.6.2.2. Modeling care of patients

Many successful therapeutic strategies rely on ‘intensified care’ of (critically ill) patients in the acute phase of the disease on dedicated and highly specialized hospital wards. Acute care is usually complex and committed to optimize physiological parameters.

Including such a strategy in preclinical modeling would aid to better model clinical care of patients as well as its associated complications.

In cerebral ischemia, stroke units are prepared to treat the clinical condition as well as potential complications (Donnan *et al.*, 2008). Infections have largely been neglected in preclinical stroke research (Meisel & Meisel, 2011; Meisel *et al*, 2005), although they heavily influence stroke outcome (Mergenthaler & Meisel, 2012; Westendorp *et al*, 2011). While preventive antibacterial treatment not only prevents infections, it also improves survival and neurological outcome after experimental stroke compared with placebo treatment (Meisel *et al*, 2004); recent phase IIb trials have successfully proven this experimental concept (Chamorro *et al*, 2005; Harms *et al.*, 2008; Schwarz *et al*, 2008) by demonstrating that prevention of infection is effective in stroke patients (van de Beek *et al*, 2009). Thus, basic research findings and preclinical modeling preceded the development of this new treatment approach (Mergenthaler & Meisel, 2012).

A novel approach to preclinical research would include modeling the acute, subacute and chronic phase of disease. Clinical and empirical evidence indicate that intensified and specialized treatments are beneficial for long-term outcome. Thus, taking “care” of patients should be reflected in future preclinical trials. In summary, preclinical trials as the foundation for future clinical trials should include large and complex cohorts of animals, and include gender-mixed, aged animals from different strains, ideally with different comorbidities, and model care of (hospitalized) patients. Furthermore, complex long-term outcome analyses should be performed to evaluate the success of a novel therapeutic concept or pharmacological agent (Figure 1).

3.1.6.3. What is the translational value of animal models?

Recurrent failure to translate promising treatment strategies in animal models into the clinic has challenged the value of animal research for predicting the effectiveness of treatment strategies in humans. Thus, animal models of human disorders are more and more

condemned, have been considered meaningless or at best as imprecise for the human setting, all medical areas employ models that have advantages or limitations. At least, animal models are used successfully to define basic pharmacokinetic properties as well as to investigate safety and toxicity issues (McGonigle & Ruggeri, 2014).

One example for this approach is the following. The devastating neurodegenerative disorder amyotrophic lateral sclerosis (ALS) is characterized by a progressive degeneration of motor neurons leading to a generalized paralysis, respiratory insufficiency and death usually within 3 to 5 years. Stem cell transplantation has emerged as a promising approach for ALS patients. Rather than motor neuron replacement current approaches consider mesenchymal or neural stem cells as supporters for motor neurons delaying neurodegeneration. Although some ALS models suggest that stem cell-based approaches might delay motor neuron degeneration current strategy in the field is rather not proving efficacy than demonstrating safety in preclinical models aiming at quick “translation” to the clinical setting investigating efficacy in patients. The main argument for this approach is the rather poor understanding of the ALS pathobiology (Thomsen *et al*, 2014). However, whether or not this safety focused approach in translation is successful or not remains to be demonstrated.

Even preclinical studies aiming at toxicity analysis might fail in predicting safety for humans. For example, the immunomodulatory humanized agonistic anti-CD28 monoclonal antibody TGN1412, which was developed for autoimmune disorders such as multiple sclerosis or rheumatoid arthritis was tested successfully for safety in various animal models including mice. However, in the first in man (phase I) trial TGN1412 caused a severe systemic inflammatory response syndrome due to a “cytokine storm” resulting in a disastrous outcome with a multi organ failure for the study participants, despite the fact that the dose used was 500 times lower than the dose found to be safe in animal studies (Suntharalingam *et al*, 2006).

Drug discovery begins with target identification and validation, proceeds with identification and development of candidate therapeutic agents. At each step of this process, which often requires more than 12 years, animal models are needed (Whiteside *et al*, 2013). However, only 15% of novel drugs successfully tested in animal models pass early clinical trials and approximately half of them surviving phase III becomes finally approved by the regulatory authorities for clinical practice (Ledford, 2011).

Extrapolation of preclinical findings into the clinical settings might also depend on the substances under investigation. For example, animal models mimicking airway susceptibility in different lung disorders have been demonstrated to be predictive for the human situation for anesthetic drugs like halothane, isoflurane, propofol and ketamine but not lidocaine, morphine or muscle relaxants. Among others, variability between species in different receptor distributions and drug affinities might account for the different predictability of the preclinical models (Habre & Petak, 2013).

Animal models of human tumors are considered as indispensable for drug discovery and development. The commonly used ectopic and orthotopic xenografts models, primary human tumorgraft models, genetically engineered models, or various multi-stage carcinogen-induced models all have different strengths and weaknesses (Cheon & Orsulic, 2011; Heyer *et al*, 2010). These models should be used as sophisticated biological tools at specific stages of drug development in a hierarchical manner of increasingly complex modelling (Figure 1) of the diversity of human cancers (Ruggeri *et al*, 2014).

One approach to test the predictive power of animal models is conducting reverse-translational studies investigating known effective treatment strategies of human disorders in appropriate animal models. Temozolamide is a good example of a successful forward and reverse translational approach for the treatment of glioblastoma. A systematic review and meta-analysis of temozolomide in animal models of glioblastoma predicted clinical efficacy. This treatment is effective in reducing tumor volume and improving survival clinically as well as in experimental models of malignant glioma. The reported efficacy for treatment has not

significantly changed after publication (Hirst *et al*, 2013) of the seminal phase III temozolomide trial demonstrating efficacy in glioblastoma patients (Stupp *et al*, 2005), although evidence suggests a publication bias overemphasizing its therapeutic efficacy (Hirst *et al.*, 2013).

Genetic mouse models of Huntington's disease (HD) should help to identify and prioritize the most promising treatment strategies to be tested in clinical trials (Menalled & Brunner, 2014). Many neural circuits affected by Huntington disease are evolutionary conserved. More than a dozen genetic mouse models express a mutation similar to that responsible in HD with many variations in CAG length of the Huntington gene. These models mimic the human genetic insult with different phenotypic aspects of HD (Menalled & Brunner, 2014)

Numerous transgenic or surgically induced pig models of neurodegenerative disorders have been established in order to develop cell-replacement strategies. Defining the optimal cell dose, immunosuppression protocols and testing new cell delivery devices were a prerequisite for designing human clinical trial protocols in neurodegenerative disorders such as ALS, stroke, spinal cord and traumatic brain injury, Huntington's disease, Alzheimer's disease and Parkinson's disease. In contrast to other animal models, fully or partially MHC-matched pig strains model the human situation, thereby better modelling host versus graft and graft versus host reactions of cell and tissue replacement strategies (Dolezalova *et al*, 2014).

In neuropathic pain research, the effect size of successful pain treatment is almost twice in animal models as in clinical trials. Correspondingly, the number needed to treat (NNT), which reflects the number of individuals that must be treated in order to see one successful treatment outcome, is almost half in animal compared to clinical pain trials. Among others, placebo effects in clinical trials, which are absent in animal research, are significant confounders. Effect sizes of at least 60% pain relief in animal models are required to predict clinical efficacy (Whiteside *et al.*, 2013).

Psychiatric disease is not directly translatable to animal models. For example, even transgenic mouse models of neuropsychiatric disorders cannot fully represent the broad spectrum of symptoms, including confusion or suicidal thoughts. However, these models serve to explore psychiatric disorders by unravelling disturbances of neural circuits underlying disease relevant phenotypes, in particular how environmental and (epi-)genetic factors interact to shape behavioral phenotype and predispositions to psychiatric disorders (Donaldson & Hen, 2014). Traditionally in psychiatric animal models abnormal animal behavior was created, phenotypically resembling the aspects of mental disorders. Reverse translation using knowledge about the mechanisms of human disorders has been used to identify and develop animals that have the molecular and cellular abnormalities found in these diseases (Malkesman *et al*, 2009). For example, depression has been modeled in mice having point mutations in the mitochondrial DNA polymerase (Kasahara *et al*, 2006) and glutamate receptor 6 knock mice have a high face and predictive validity for mania (Shaltiel *et al*, 2008).

Lost in translation has become a very popular paraphrase for the obstacles encountered in translational research. Three reasons for the “Lost In Translation Problem” have been suggested. First, small differences in the models might lead to vast differences in the results, which has been attributed to the chaotic behavior of the models and termed the “butterfly effect”. Second, the effect size is decreasing from biochemical models over cell and tissue cultures to animal experiments to human studies, which seems to be unexpected according to the “princess and the pea” story. Finally, the “two cultures” of preclinical and clinical research are different (Ergorul & Levin, 2013; Mergenthaler & Meisel, 2012).

3.1.6.4. Remedies for failed translation. – Improving preclinical research

3.1.6.4.1. Improving models

In order to improve the quality of translational biomedicine it has been suggested to make the process of preclinical research more like clinical research. Among them, applying

similar rules used by regulatory agencies for clinical trial has been suggested also for preclinical studies. Using methods such as systematic reviews and meta-analyses have become more and more popular in animal research to identify robust treatment effects. Commonly accepted “futility” and “stopping” rules in clinical research become increasingly accepted in preclinical research. These approaches have been demonstrated to improve the predictive value of animal research (Perel *et al*, 2007).

An ideal animal model will meet all of the following 3 criteria: face validity, predictive validity and construct validity. Face validity refers to the phenomenological similarity between the model and its corresponding disorder. Predictive validity refers to the ability of the model to have comparable biomarkers and treatment responses as the human disorder. Construct validity reflects the degree to which a model measures what it claims to be measuring (Willner, 1986).

In order to improve construct validity it has been proposed that therapeutic interventions should be tested in animal models of CNS disorders under conditions of greater environmental enrichment. One limitation of current research is that most animal studies are performed under caging conditions with sedentary, unstimulated animals having unlimited access to food. Enriched environment stimulating sensory system, cognition and physical exercise have been demonstrated to affect outcome significantly (McOmish *et al*, 2014).

In order to improve translational power, the use of more humanized models has been suggested (Ergorul & Levin, 2013). Immunodeficient mice that have been engrafted with human primary haematopoietic cells and tissues generating a functional human immune system in these mice are a well-established example of humanized mice. These models have been successfully used investigating infectious diseases, autoimmune disorders and tumors (Shultz *et al*, 2012).

Recent exciting findings in stem cell biology open the door to novel approaches in disease modeling using human model systems. Terminally differentiated human somatic

cells may be reprogrammed to an induced pluripotent stem cell (hiPSC) state in order to then differentiate these cells into any cell type of interest (Lee & Studer, 2010). These developments might revolutionize investigations of human disorders, in particular those affecting the CNS (Philips *et al*, 2014). Patient-derived hiPSCs can be differentiated in specific neuronal subpopulations, e.g. cortical neurons (Zhang *et al*, 2013) or striatal medium spiny neurons (Philips *et al.*, 2014), which are affected in brains of patients suffering from Huntington's disease. Obviously, brain cells are usually not directly accessible as primary material, neither for study disease mechanisms nor for specific treatment. Furthermore, in recent years, organoid technologies have revolutionized experimental biomedical research (Schutgens & Clevers, 2020). In the context of brain research, human brain organoids (Lancaster, 2020; Lancaster *et al*, 2013) provide for the first time a human model system with the prospect of studying developmental aspects and disease mechanisms in a brain-like model system and have thus far mostly been used for studying brain development and developmental disorders (Marton & Pasca, 2020). Using hiPSC technology, specific cell differentiation, organoid technologies and refined genomic editing tools (Hendriks *et al*, 2020), correction of mutations are feasible and specific treatment is conceivable (Kaye & Finkbeiner, 2013). Although it has been suggested that failure in clinical trial could have been predicted at least in some cases using human pluripotent stem cell-based model systems (Antonic *et al*, 2018), translational success using these models has yet to be established.

Finally, cell based models cannot reflect the complexity of an organism. For example, investigating systemic effects of local disease, such as post-stroke pneumonia, requires animal models (Prass *et al.*, 2003) to complement mechanistic cellular modeling. Another example is the blood brain barrier (BBB), a highly selective permeability barrier separating the blood from the brain extracellular fluid. Although sophisticated in vitro models of BBB have been developed in the last decade, drug transport across the BBB and brain specific drug delivery strategies remain challenging for developing of successful treatment strategies (Bicker *et al*, 2014). Enzymes usually cannot pass the BBB. However, local enzyme replacement therapy in the brain by intrathecal application is a promising strategy for the

treatment of patients with metabolic disorders caused by the absence or malfunction enzymes involved in cerebral metabolism. For example, repeated injections of a recombinant enzyme into the spinal fluid (intrathecal) corrects enzyme deficiency and normalizes lysosomal storage in a canine model of mucopolysaccharidosis (Dickson & Chen, 2011).

3.1.6.4.2. Improve rigor of preclinical studies

The lack of reproducibility of preclinical studies and the failure of translation to the clinic have attracted attention in the last years (Howells *et al*, 2014; Ioannidis, 2005; Macleod *et al*, 2014; Perrin, 2014; Prinz *et al.*, 2011). One important reason is the publication bias toward reporting positive results due to difficulties or missing incentives in publishing negative results (Dirnagl & Lauritzen, 2010; Dwan *et al*, 2013). Moreover, experimental design (Ioannidis *et al*, 2014; Neumann *et al*, 2017), including statistics (Schlattmann & Dirnagl, 2010), has been challenged as a quality problem in preclinical trials. For example, definition and declaration of statistical approaches and endpoint measures needs to be performed before preclinical trials are finally analyzed or even started (Dirnagl & Lauritzen, 2011). Whereas clinical trial registries are widely accepted as good clinical research practice, preclinical trial registries are rather uncommon and may need to be established (Dirnagl, 2020). Thereby, post-hoc analyses generating hypotheses in an exploratory manner can be clearly distinguished from a primary hypothesis that has been tested in a confirmatory approach. A priori power calculations and sample size considerations, randomized assignment to groups and blinding for treatment groups are further important issues well established in clinical but rather not preclinical research (Button *et al*, 2013).

Finally, it has been suggested that bringing the rigor and quality of study design expected in clinical trials to preclinical trials will improve translational success (Dirnagl & Fisher, 2012; Ioannidis *et al.*, 2014; Macleod *et al.*, 2014). This includes better knowledge about the drug and thorough target assessment before starting a preclinical trial (Emmerich *et al*, 2020). For example, pharmacokinetics might be different between mutants and wild-

type mice (Menalled *et al*, 2010). Confirmation of research findings includes replication of preclinical research in independent laboratories (Figure 2). Using different models will increase robustness of the observed findings in treatment effects (Menalled & Brunner, 2014).

Endpoint measures are of great importance in preclinical research as well as in clinical research and should therefore follow endpoints used in clinical research as close as possible. For example, Huntington's disease is not only characterized by motor symptoms but also by cognitive and psychiatric symptoms appearing years before the loss of motor control. These complaints often have a large impact on the quality of life. Although survival is an important outcome measure also in clinical trials, caution is required when translating preclinical into clinical findings. In contrast to animals even in preclinical research, survival in patients does not only depend on the specific intervention under investigation but also on general care as well as ethical and religious issues leading to end-of-life decisions.

Specific suggestions for improving the predictiveness of preclinical stroke research have been oriented on accepted standards of clinical research (Bath *et al.*, 2009; Dirnagl, 2020; Macleod *et al*, 2009; Mergenthaler & Meisel, 2012). In order to improve internal validity, good clinical research avoids any kind of bias, in particular selection bias (biased allocation to treatment groups), performance (biased care of treatment groups apart from intervention under study), assessment (biased rating due to knowledge of treatment assignment) and attrition (biased handling of protocol violation and loss in follow up).

Preclinical research in the final stages of translation into clinical trials should follow the guidelines of clinical research by: 1) improving internal validity by predefined inclusion/exclusion criteria and primary endpoint(s), randomization, blinding for treatment allocation and outcome assessment intention-to-treat analysis; 2) improving external validity by studying pathophysiology and treatment strategies in animals of both sexes, old age and with co-morbidities, disease-related appropriate dosing and treatment windows for the drug under investigation; 3) replicating pivotal findings; 4) publishing negative as well as positive

results; focus on long-term functional outcome; use meta-analyses of pre-clinical studies; 5) establishing registries of preclinical studies and 6) international multicenter phase III preclinical trials (Dirnagl & Endres, 2014; van der Worp *et al*, 2010). Moreover, preclinical trials need a standardized and “humanized” modelling of general as well as disease specific patient care (Mergenthaler & Meisel, 2012).

3.1.6.5. Summary

In summary, many well-defined animal models for human disease are employed in modern preclinical and pathophysiology-driven research. However, the scientific community across all fields of modern biomedicine has become aware of weaknesses in current preclinical animal modeling. Here, we have outlined several strategies that have already been set into action to overcome the translational gap that is common to all current preclinical modeling of human disease.

3.1.6.6. References

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Figures

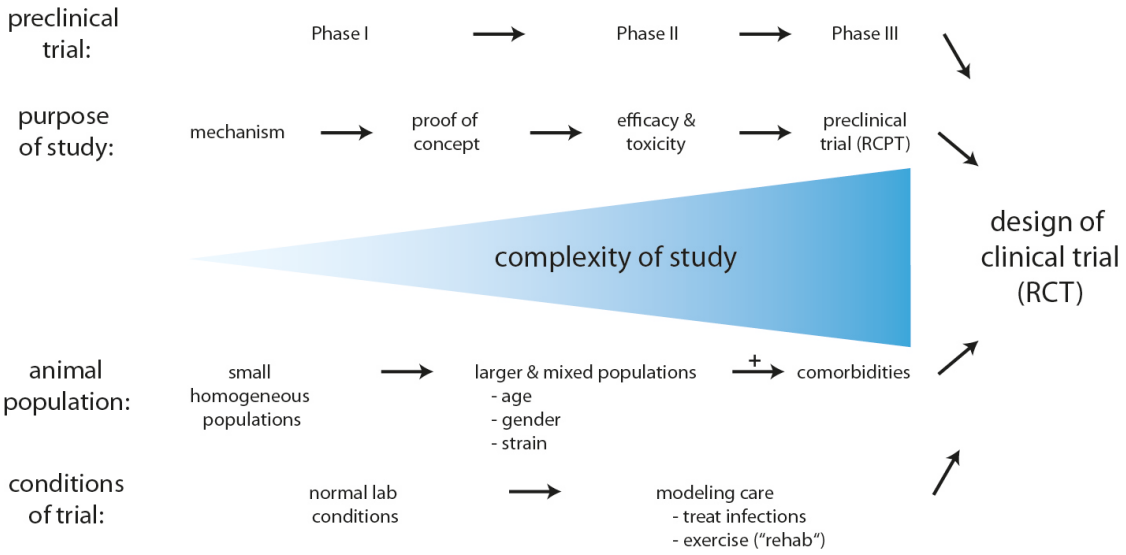

Figure 1. The preclinical trial phases of translational research. As therapeutic agents or concepts advance in development, the experimental setting increases in complexity. It ranges from small cohorts to investigate novel mechanisms to large mixed populations with (multiple) comorbidities and additional modeling of stroke care. The final stage of preclinical development is the conduct of a randomized-controlled preclinical trial (RCPT), ideally in a stroke unit setting. Randomized clinical trials (RCT) commence after this process has been completed and is based on evidence gained in preclinical testing. *Reproduced with permission from: Mergenthaler P & Meisel A. (2012). Do stroke models model stroke? Dis Model Mech. 5, 718-725.*

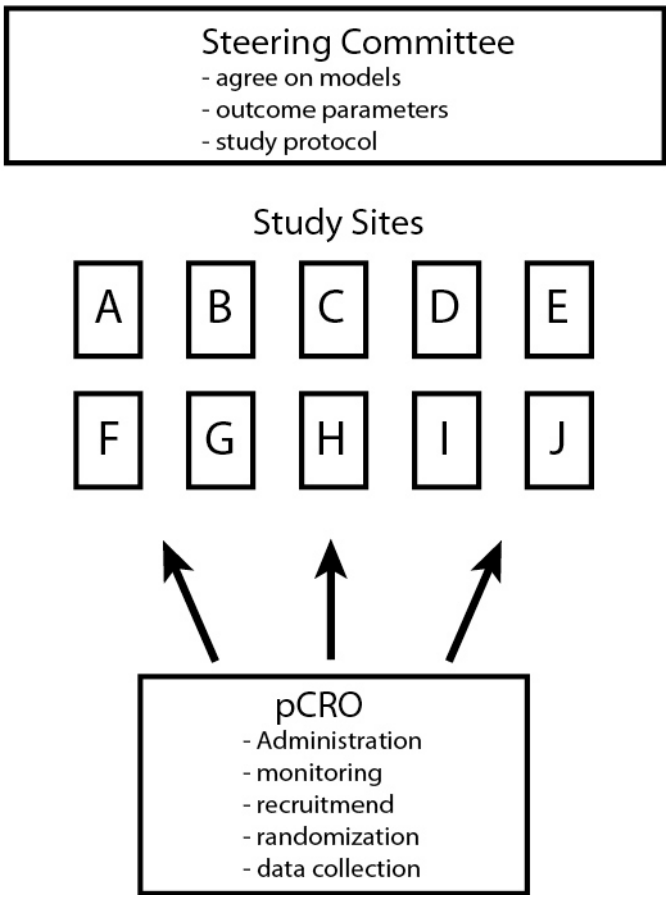

Figure 2. Modeled after randomized controlled clinical trials (RCT), the final stage of preclinical testing is to conduct a randomized controlled preclinical trial (RCPT). A steering committee agrees on the intervention to be tested and all related aspects (e.g. models, outcome parameters, etc.). All administrative matters are centrally organized by a preclinical research organization (pCRO) and include objective criteria for the recruitment of study sites, the modes of randomization, collection of the data from the study sites and central monitoring of all aspects of the trial. Ideally, all study sites are capable of performing the same experiments (i.e. they have access to the same models and equipment). All aspects of the RCPT are monitored by an independent organization. *Reproduced with permission from: Mergenthaler P & Meisel A. (2012). Do stroke models model stroke? Dis Model Mech. 5, 718-725.*