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The use of (nuclear and fluorescent) molecular imaging in early phase clinical trials

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Abstract

Nuclear imaging can play an important role in the development of new medicines. By labelling a drug with a radionuclide, its biodistribution throughout the body can be visualised. This provides visual and quantitative data on uptake within and outside the targeted disease area. The low radioactivity required for this makes it suitable for clinical trials with a low, subtherapeutic dose. Such trials, commonly referred to as Phase 0 or early Phase 1, adhere to a special regulatory framework: exploratory trials. This framework was introduced by the FDA and EMA about twenty years ago to improve and accelerate drug development by bridging the gap between preclinical and clinical research. The use of nuclear imaging allows harnessing the full potential of this first-in-human study.

In Phase 0 studies, the investigational new drug (IND) is administered directly to patients. A good manufacturing practice (GMP) produced IND, supported by data obtained through an extended single-dose toxicity study, often in rodents, is required. This contrasts sharply with the traditional –and current– drug development pathway, which relies heavily on extensive, costly, and time-consuming animal testing. Commonly, INDs only proceed to clinical (human) trials after positive results from animal testing. Once in the clinical phase, for most types of drugs and diseases, the IND is first tested on healthy volunteers (HV). Once a safe, optimal, or maximum tolerated dose has been found, efficacy is evaluated in patients. In this system, many years and resources are spent before –and if– the IND reaches the patient. It is estimated that 75% of the costs of drug development are due to failure in the early stages of development (1). Drug development is famous for its extremely high failure rate, which means that most animals sacrificed, and HVs exposed to risks have done so without any benefit.

Animal data does mitigate risks to some extent, but shows no proof for in-human targeting and efficacy upfront. The failure rate of INDs in clinical trials, around 90% (2), has remained very high for decades despite attempts to reduce this figure. This percentage is based on failures primarily in clinical trials, which means only for INDs that have already passed animal testing. The number of failed candidates in the preclinical phase is much higher, and this may also include candidates that were discontinued prematurely based on animal data that may not be relevant to humans at all. For example, common drugs such as penicillin, aspirin, and paracetamol would have never gotten market approval since they would not

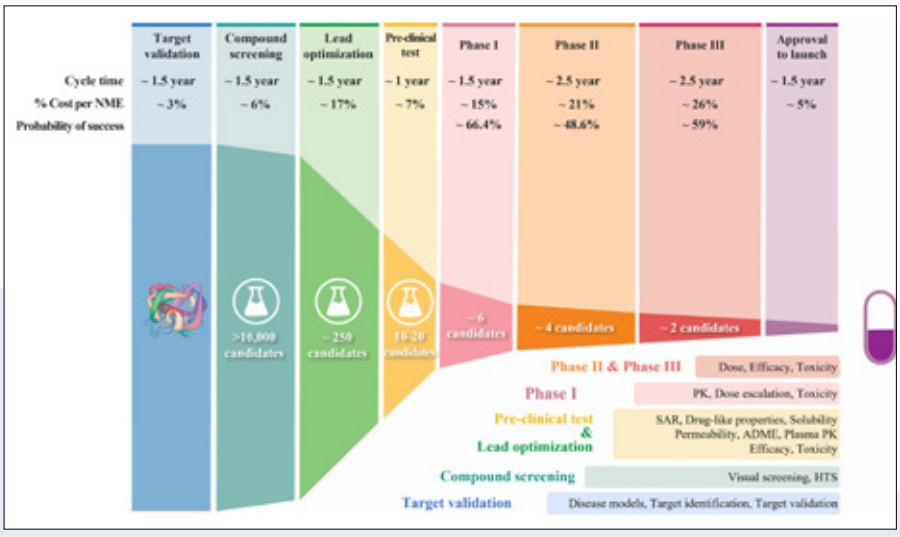

Figure 1. The process of drug discovery and development, and the failure rate at each step.

From: Sun, D., Gao, W., Hu, H., & Zhou, S. (2022). Why 90% of clinical drug development fails and how to improve it? Acta Pharmaceutica Sinica B, 12(7), 3049-3062. https://doi.org/10.1016/j.apsb.2022.02.002

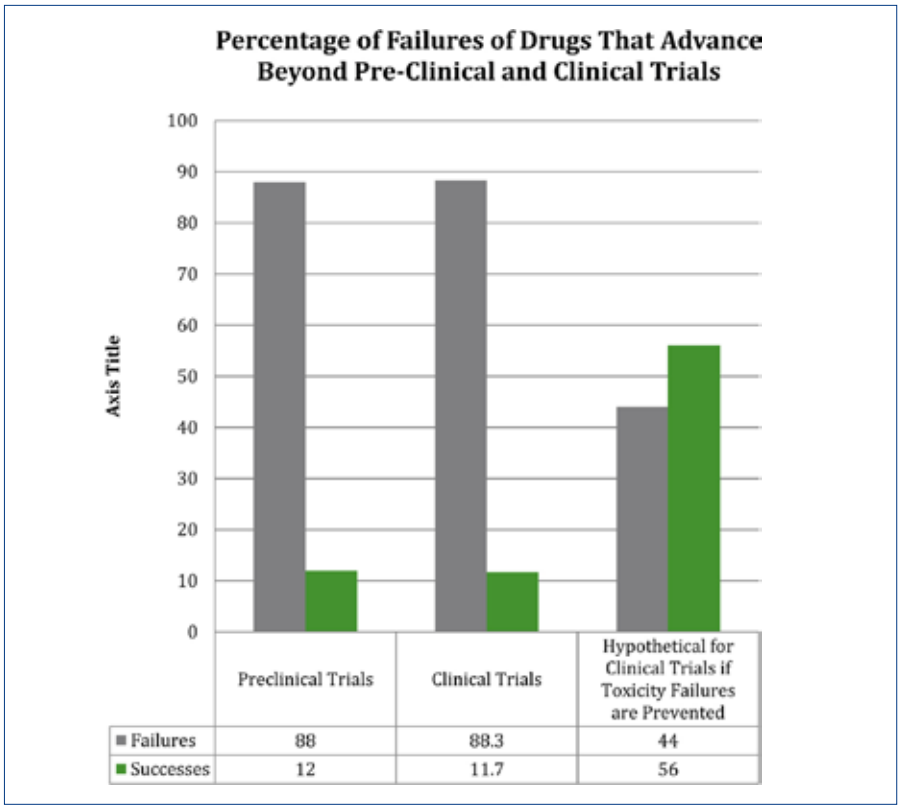

Figure 2. Failures in Translational Research: Preclinical and Clinical Trials. Percentages of drugs that fail in preclinical trials (due to drug toxicity or failure of efficacy in animal testing) and in clinical trials (due drug toxicity or failure of efficacy in human testing) are shown in columns 1 and 2. The third column demonstrates what would happen if animal and human toxicity were closely correlated and therefore drugs with human toxicity were eliminated at the preclinical testing stage by animal toxicity testing (one-half of all drug failures in clinical trials are due to toxicity issues despite safety in animals). Success rates of clinical trials increase from 11.7% overall to approximately 56%.

From: Norman, G. Limitations of Animal Studies for Predicting Toxicity in Clinical Trials: Is it Time to Rethink Our Current Approach?. J Am Coll Cardiol Basic Trans Science. 2019 Nov, 4 (7) 845–854. https://doi.org/10.1016/j.jacbts.2019.10.008

pass current animal testing (3).
A more patient-centric approach in drug development seems logical, where data in the patient population of interest are obtained first. But doing so, without extensive safety studies in animals and HVs, is only considered safe with a very low dose (4) of the IND. Nuclear molecular imaging, which can be realised with a low dose (i.e. a so-called microdose, but higher, still subtherapeutic doses are possible), perfectly matches this. The important go/no-go decision of which candidates are advanced to further animal testing and clinical trials can nowadays be based on human-relevant data and not solely on preclinical *in vitro* and *in vivo* data. Of note, current regulations do not preclude further studies involving animals or HVs after Phase 0. However, subsequent animal data can be validated against human data. The major difference is that only promising candidates based on human data would be advanced. Even lead candidate selection from multiple candidates found in drug discovery is possible. By early discontinuation of programs based on relevant, human data, more resources (time, funds, capacity in labs, hospitals, available participants, et cetera) are available, which can be spent on more highly promising candidates.

Prof. Go van Dam, former surgeon oncologist, CEO and founder of TRACER CRO explains. "Building on the Phase 0 framework, TRACER has developed a method that enables the investigation of INDs in humans using a (radio)labelled drug at subtherapeutic, often microdose (≤ 100µg), levels. While such doses are too low to produce a therapeutic effect, they are sufficient for sensitive nuclear imaging techniques to visualise and quantify on- and off-target distribution, pharmacokinetics, and dosimetry. Providing valuable data to drug developers to decide on a lead compound, prioritise promising compounds, and adjust or discontinue the development of INDs with insufficient target uptake (5)."

The reason for seeking alternatives to translational research, and animal testing in particular, is clear. Regardless of the role that animal testing has played in our medical and physiological knowledge, animal studies have proven to be poor predictors of both the toxicity and efficacy of a drug in humans (6). This is the reason why regulators introduced Phase 0 in the first place.

Phase 0 was introduced more than twenty years ago, with the FDA's "Exploratory IND studies (7)" (2006) and EMA's "Position paper on Non-Clinical Studies to Support Clinical Trials with a Single Microdose (8)" (2003). Regulations were later

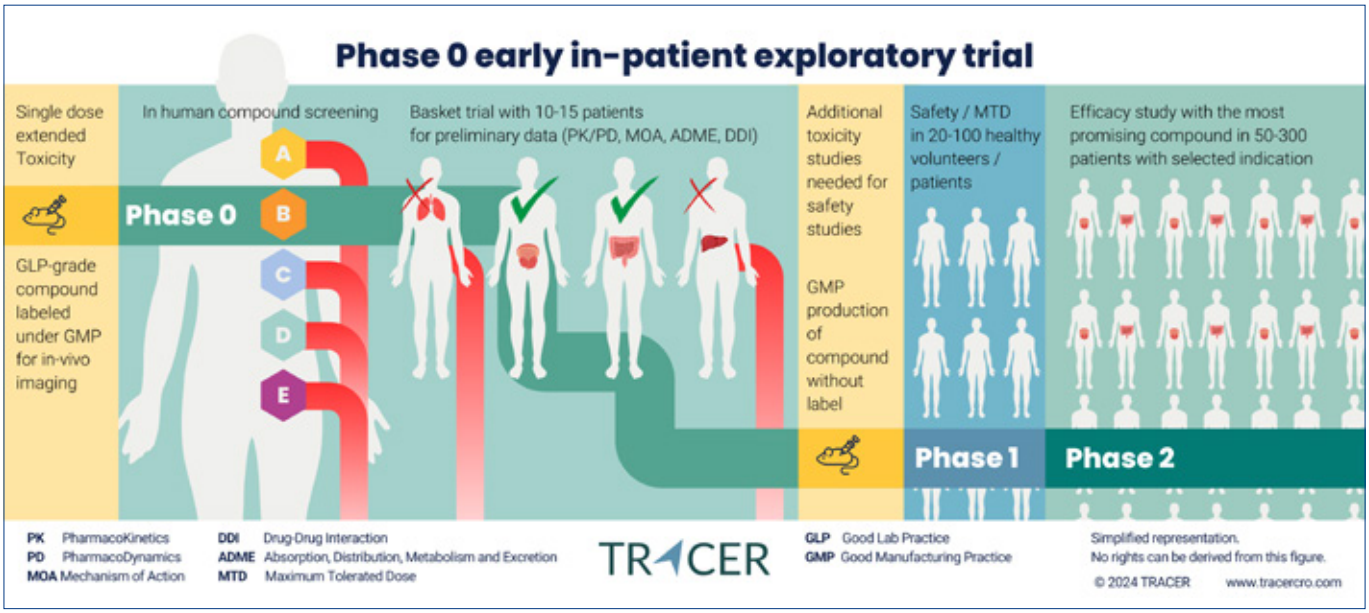

Figure 3. Phase 0 early in-patient exploratory trial

PK PharmacoKinetics
PD PharmacoDynamics
MOA Mechanism of Action
DDI Drug-Drug Interaction
ADME Absorption, Distribution, Metabolism and Excretion
MTD Maximum Tolerated Dose
GLP Good Lab Practice
GMP Good Manufacturing Practice
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harmonised under ICH M3(R2) in 2009 (4). The FDA again emphasises the need for more predictive and human-relevant alternatives in translational drug development in the recently published document Roadmap to Reducing Animal Testing in Preclinical Safety Studies (April 2025) (9). Mentioning exploratory trials alongside other new approach methods (NAMs) like organoids, organ-on-a-chip, artificial intelligence (AI), and machine learning (ML).

Why has Phase 0 not yet become the norm in drug development? Van Dam: "This is a question we at TRACER often get asked in conversations with drug developers. Although there are many studies and reviews that have investigated the usefulness of Phase 0 (10-14), actual data from such studies is still very limited. In addition, many Phase 0 studies have been conducted using accelerator mass spectrometry (AMS) or liquid chromatography with tandem mass spectrometry (LC-MS/MS) (15). This provides data on the amount of a drug in biofluids such as blood or urine. Providing information about concentration, time, and excretion in fluids or samples, but not for on- and off-target accumulation. The use of nuclear imaging provides data answering a whole different question, especially when total or whole body positron emission tomography (PET) is used. Measurements are not limited to body fluids or samples, as with AMS and LC-MS/MS, but can be performed in real time and non-invasively in all tissues and organs. PET can demonstrate biodistribution and target engagement. When dynamic PET is chosen, multiple imaging moments over time can be used to demonstrate how a drug accumulates in the body. This data can even be quantified to estimate therapeutic values and, thus, dosimetry (16).

Van Dam: "To fully harvest the power of PET for drug development, an IND must be labelled with a radionuclide. It must be estimated and measured in advance whether the labelling does not affect the drug's in-human behaviour. In addition, it is good to look at saturable processes that may be of influence when quantifying microdose data to therapeutic values (17). This applies in particular to oral and other non-parenteral methods of administration, where bioavailability will always be lower than with an intravenous (IV) administration. However, with state-of-the-art total and whole body PET scanners developed in recent years, oral PET is also possible. Radionuclides approved for this purpose are still limited, but half-life times and labelling options are sufficient to perform this type of research (18)."

In addition to nuclear imaging, there are a number of other methods to measure the presence of a drug in the body, but these options are somewhat more limited. Their applicability depends on their primary

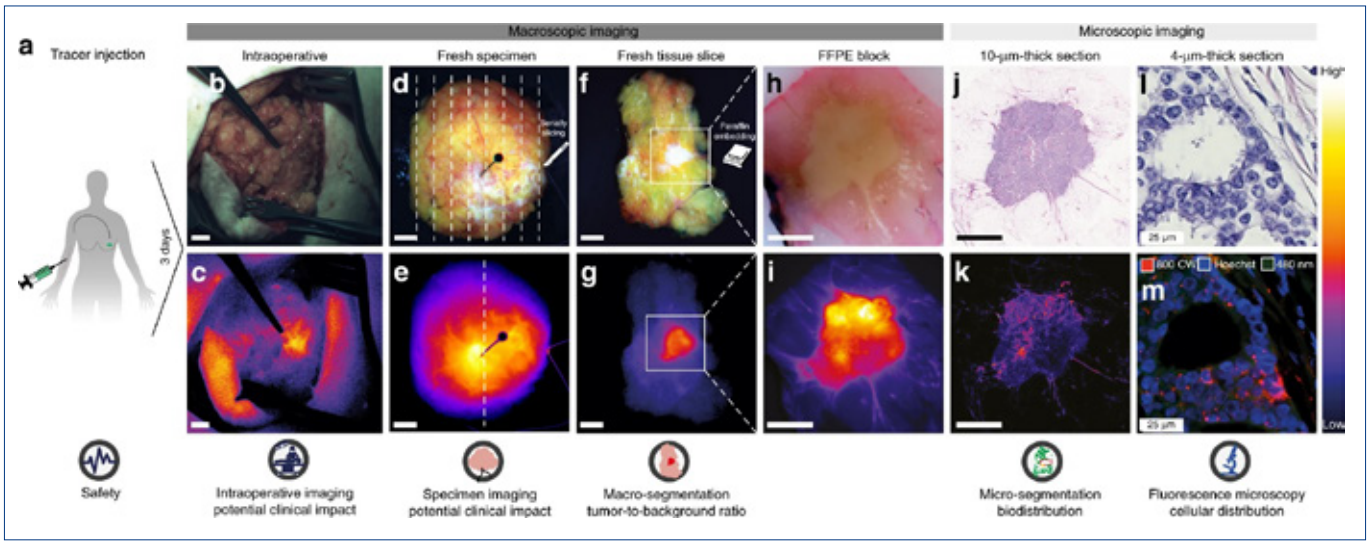

*Figure 4. T*he clinical analytical framework enabling correlation of intraoperative fluorescence signals with histopathology, from macroscopic to microscopic levels. **a** Intravenous administration of bevacizumab-800CW three days prior to surgery. **b**, **c** Color image and corresponding fluorescence image obtained in vivo during surgery to determine potential clinical value. **d**, **e** Imaging of the fresh surgical specimen, followed by serially slicing. **f**, **g** Imaging of the fresh tissue slices to determine tumor-to-background ratio based on macro-segmentation, followed by paraffin embedding. **h**, **i** Imaging of formalin-fixed paraffin-embedded (FFPE) blocks to determine heterogeneity of tracer uptake within a tumor. **j**, **k** Imaging of 10-µm-thick tissue sections for micro-segmentation to reveal microscopic biodistribution and correlation with fluorescence signals from the macroscopic to microscopic level. **l**, **m** Fluorescence microscopy to determine tracer distribution on a cellular level. Scale bars represent 1 cm, in l, m the scale bar represents 25 µm.

Koller, M., Qiu, S., Linssen, M. D., Jansen, L., Kelder, W., De Vries, J., Kruithof, I., Zhang, G., Robinson, D. J., Nagengast, W. B., & Van Dam, G. M. (2018). Implementation and benchmarking of a novel analytical framework to clinically evaluate tumor-specific fluorescent tracers. Nature Communications, 9(1), 3739. https://doi.org/10.1038/s41467-018-05727-y

objective and disease area of interest. Fluorescence molecular imaging is the most prominent alternative, but the depth at which fluorescent light can be measured is very shallow, less than 3cm. It can be used non-invasively *in vivo* for examination of shallow tissue like the skin, eye, lung (bronchoscopy), gastrointestinal tract (endoscopy), or for *ex vivo* validation/cross-correlation histopathology of biopsies. Another in vivo use case is more invasive during intraoperative surgery or pathology-assisted surgery. Fluorescence-guided surgery is how Van Dam got involved in molecular imaging.

In his clinical work as a surgeon oncologist, Van Dam sought a method to visualise tumours during surgery in the early 2000s. To achieve this, a multidisciplinary team of clinicians, pharmacologists, engineers, and pathologists was assembled. A preliminary setup –using duct tape and binding wire– was created with the aid of a fluorescent camera and folate-FITC as the fluorescent targeting agent. In 2011, the results of this research were published in Nature Medicine, reporting the first in-human fluorescent targeted imaging study *Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results* (20).

In the years that followed, Van Dam expanded his research by labelling other targeting and imaging agents for different receptors. The possibilities extended beyond making tumours visible during surgery. The most important question it answers is: Does the drug reach the right place in the right amount? This is an important question in clinical research to assess the safety and effectiveness of an IND. To quickly discover promising drugs and thereby accelerate drug development, TRACER was founded together with business developer Ari Aminetzah.

Van Dam: "Because it is a relatively new method, the emphasis in the early years of TRACER has been strongly on educating the target audience. Influencing the field with information about the possibilities, such as this article, but also blogs, LinkedIn posts, and speaking at conferences, remains an important part of TRACER's work. However, now that results from studies are available, discussions with drug developers –TRACER's current and new potential clients– are much easier than before. Data is simply convincing, as in the proverb "a picture is worth a thousand words."

Conclusion

Nuclear imaging makes it possible to collect useful data from the very low dose used in in-patient studies under the Phase 0 exploratory trial framework. The obtained visual and quantitative data can be used to compare and validate the data from animal studies and trials with HVs. Phase 0 does not replace animal studies or studies in HVs but is conducted before these take place. It serves, therefore, as a gatekeeper to advance only promising candidates based on patient-relevant data into further research. As a result, resources can be allocated to promising drug candidates based on in-human data, allowing them to advance faster and increasing the chance of success for a drug to be efficacious and obtain market approval. ♦

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