

## Drug Discovery and Development: An Overview from the Regulatory Prospective

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### Abstract

The improvement of a medication from an underlying plan to its entrance into the market is an extreme process which can take around 8-12 years and cost around \$1.7 billion. The thought for another advancement can originate from an assortment of sources which incorporate the flow necessities of the market, new rising infections, good clinical research, business part, and so on. Once an objective for revelation has been picked, the pharmaceutical businesses or the related scholastic focuses take a shot at the early procedures to recognize the substance atoms with reasonable qualities to make the focused on medications. This article will provide information on the key ideas of drug discovery, drug development and clinical phases of the drug discovery.

**Keywords:** Drug discovery; Drug development; Target identification; Target validation; Lead generation; Lead optimization; Chagas disease

### Introduction

It is a procedure which is planned for recognizing little engineered molecule or else substantial molecule to reaching assessment such as potent medication competitor. Comprehensively, cutting edge drug development method incorporates distinguishing proof as disease to dealt with & it is neglected medicinal neediness, determination of a drug-able sub-atomic object & approval, *in vivo* measure improvement taken after by HTS of molecule archives in contradiction of the objective of recognize hits & hit advancement to produce lead exacerbates that show sufficient power & selectivity towards the natural focus *in vitro* and which exhibit adequacy in animal models of disease. Consequently, the lead mixes are additionally enhanced to increase their adequacy and pharmacokinetics before they progress towards medical advancement. Drug advancement process can be differentiated into preclinical and clinical improvement stages. In preclinical advancement, toxicological and wellbeing pharmacology investigations of the hopeful are led so as to build up the greatest safe focuses in creatures and decide the antagonistic impact capability of the medication being developed. In the event that the applicant shows adequate viability and security in preclinical assessment, authorization is looked for from regulatory authorities to start its clinical trial wherein the wellbeing and viability of the RLD is evaluated in pilot and significant studies [1]. The discovery and improvement of new drug is time and cost concentrated and at present around twelve years and a normally around of \$1.7 billion is required to discover another new drug. Throughout the years, there is a diminishing pattern in the quantity of new drugs getting advertising endorsement. Further, the best administration of pharmaceutical industry needs to turn away the dangers related with disclosure and improvement. Besides, weight on national wellbeing administrations because of expenses related with pharmaceuticals adverse effects their estimating. Furthermore, patent lapses and their nonspecific substitutions have decreased the benefits and ensuing development of the pharmaceutical business, bringing about diminished interest in inventive research. Another worry is unfavorable natural effect of pharmaceuticals and there are clear orders from administrative organizations to guarantee ventures to lessen this effect. An immediate outcome of the dreary drip to efficiency is expanded unions & procurements found in these organizations, with the essential target of decreasing research & development prices and making cooperative energies. In any case, interests in creative research post-mergers and acquisitions and pipeline progressions have really diminished and henceforth industry solidification to build efficiency

is faulty. Regardless of the fall in research & development profitability because of the previously mentioned worries, there is as yet a high neglected need in the helpful zones of malignancy, Alzheimer's sickness, and diabetes, and there is a worldwide rise of Multi Drug Resistance (MDR) safe bacterial contaminations for which new medications are intensely required [2]. This article discusses with example of drug discovery process associated with Chagas Disease (CD) which is also known as American *trypanosomiasis*.

### Identification of Unmet Medical Need

To activate a medication revelation platform is a therapeutic situation which one's behavior isn't attractively tended to by right now accessible treatment modalities. This is alluded to as a neglected restorative requirement to that of situation. To detailed, novel medications required whichever to cure a malady for whom none other management exists or which provides extra preferences against current medicines such as prevalent remedial viability, decreased antagonistic impacts, enhanced consistence, less medication investigational study collaborations, and thus a general change in the personal satisfaction of a patient. Ways to deal with distinguish the neglected restorative need incorporate market investigation, contributions from enter supposition pioneers in a helpful territory, input from therapeutic experts and logical gatherings. Moreover, a careful comprehension of infection etiology, the study of disease transmission, accessible helpful alternatives and their deficiencies drive a reasonable hole investigation and in this way encourage shortlisting of restorative needs specifically malady condition. Chagas disease (CD), or American *trypanosomiasis*, is the result of human infection by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). More than a century after its first description by Carlos Chagas, the disease is endemic in 21 Latin American countries, with Bolivia currently experiencing the highest disease burden. It remains an important public health issue in Latin America. However, cases of CD are being increasingly detected in the United States, Canada,

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many European countries and some Western Pacific countries, owing to a rise in population movements between Latin America and other continents.

## Target Identification

Organically dynamic mixes, regardless of whether they be a little engineered particle or an expansive atomic weight immune response, inspire their action and in this way a quantifiable clinical impact by collaborating with a normally existing sub-atomic structure, which with regards to investigational study disclosure is alluded to as an “objective” [3]. These incorporate chemicals, cell mediated receptors, metabolites substrates, particle channels & mRNA. This objectives will extensively arranged into built up and novel targets [4]. The objectives which have been logically demonstrated to be very much characterized physical and patho-physiological parts comes under the previous classification, though newfound ones whose part is ending up being clearer with propelling examination constitute the last class [5]. The different ways to deal with target distinguishing proof are quickly talked about underneath [6]. In previous selection methodology, mixes are assessed in cell-created tests or creature structures of sickness with an expect to distinguish mixes which inspire a foreseen correction in that of screening methodology [7]. This may incorporate change in articulation of a solitary or numerous proteins *in vitro* or acquiring wanted pharmacological reaction *in vivo* [8]. Ensuing to the distinguishing proof of a functioning aggravate, its sub-atomic target is then controlled by hereditary methodologies like articulation cloning systems, in silico methodologies, or synthetic proteomic based methodologies like liking chromatography, action based protein profiling, and name free strategies [9]. Molecular and functional imaging techniques are also valuable tools for target identification [10]. Chagas disease is usually transmitted by the “kissing bug,” which is a blood-sucking reduviid bug of the subfamily Triatominae. The majority of patients remain undiagnosed until later stages.

## Target Validation

After finding the target, probable molecule requires to go for method of validation where its purpose in disease condition is determined. At hand there is number of methods for validation and few of them will be conferred here [11]. Usage of antisense method is a widely known way where small nucleic acid, in addition of particular area to mRNA to concentration, will be considered [12]. This collaboration of small single stranded nucleic acids with the target messenger RNA gives in disturbance of transformation and consequently obstructs combination of that structure. To demonstrate, breakdown of tetrodotoxins resilient Na<sup>+</sup> pathway Nav1.8 by small single stranded nucleic acids, destroyed intra-theal N-Methyl-D-Aspartate prompted automated hyper nociception to mice, thus significance of these Na<sup>+</sup> ion-channels are very high in pain pathway [13]. Utilizing hereditarily adjusted creatures for target approval is an engaging approach as it allows the investigation of the phenotypic outcomes of quality control [14]. Advancement of knockouts, thump ins, restrictive thump outs, and transgenic creatures are occurrences of hereditarily altered creatures [15]. An impediment of the hereditary way to deal with target approval is that qualities create different forms of the proteins who possesses somewhat extraordinary capacities, & varieties in that can likewise be a result in post structural changes [16,17]. Clinically, human CD has two phases: the acute and the chronic phase. The acute phase is most often asymptomatic, but in untreated acute cases, it may present fatality rates ranging from 2% to 8%. During this phase, the parasite load is at its highest and can be detected by direct examination of fresh blood using either microscopy or PCR; hemoculture and xenodiagnoses are also diagnostic methods

used at that stage. Following infection, the immune system kicks in and induces a reduction of the parasite load with subsequent control of the balance host/parasite.

## Hit Identification and Development of Assays

After the process of target validation, it is during the hit identification and lead discovery phase of the drug discovery process that compound screening assays are developed. A ‘hit’ molecule can vary in meaning to different researchers but in this in review we define a hit as being a compound which has the desired activity in a compound screen and whose activity is confirmed upon retesting. A variety of screening paradigms exist to identify hit molecules. High Throughput Screening (HTS) involves the screening of the entire compound library directly against the drug target or in a more complex assay system, such as a cell-based assay, whose activity is dependent upon the target but which would then also require secondary assays to confirm the site of action of compounds [18]. Additional strategy which involves creating notoriety is the part centered selection method in which databases of little atom sections were assessed with good focus & the targets were characterized as mixes whose albeit frail to movement indicate effective official. These sections were utilized as structure hinders to union of powerful & medication type of mixes [19]. In engaged selection methodology, constrained arrangements of the exacerbates, which are possessing exhibited movement towards a particular group of molecules (e.g., G-Protein Coupled Receptors) or basically comparative mixes were assessed to examine. This type of methodology likewise reaches out to computer-generated selection, in which computer-generated databases of previously available mixes might be curtailed to 3-D protein assemblies and their action towards objective might anticipated computationally [20]. To the vast majority of target recognizable proof methodologies it’s critical that creating natural measures for whom mixes were assessed by action. Organic measures might be sans cell utilized of assessment for mixes either for their proclivity as on account of receptors or inhibitory action for catalysts. Compartment-constructed measures in light hand are useful tests with particular information, to instance intra-cellular Ca<sup>+</sup> focus. Components are taken to the consideration that a choice of a measure organize incorporate significance of the test, its repeatability, test value, cost evaluation, impact of molecule or it is dissolvable in test, & the selection fixations.

Evolution during the past 10 years in CD has been threefold:

1. The appearance of new investigators on the scene
2. Technological developments that allowed testing of new strategies for Chagas research and development (R&D)
3. The efficacy assessment of two new chemical entities for the treatment of CD patients in the indeterminate stage of the disease, in the first clinical trials for the disease in 40 years.

## Confirmation of Hit Molecules

Herein stage finding distinguished hits were exposed to corroborative assessment utilizing a similar test conditions which were utilized amid hit recognizable proof. Further, learn that the movement is connected to the foreseen system and isn’t because of ancient rarities.

## HTS and Dose-Response Curves

Since the advent of the “classical” screening assay for *T. cruzi* based on absorbance readout, developed by Fred Buckner and collaborators and used by numerous laboratories on a small scale, advances have been made in terms of HTS format.

## Parasite Stage-Specific Assay

*T. cruzi* parasite exists in the human host in several forms according to its life cycle, including trypomastigotes, amastigotes, and intermediate forms thereof. Cell culture assays using trypomastigotes and blood trypomastigotes are available in low-throughput format and might be useful to profile identified hit compounds.

## Lead Generation

Friability is used to measure the tablet strength in combined manner. Lead age, likewise alluded by hit from the lead stage, includes improvement to distinguish it to a differing arrangement for creating target mixes. 3-5 synthetic arrangement were ordinarily decided to lead age & closely resembling mixes are assessed to set up QSAR to movement, target discrimination, physiological properties, ADME characteristics, PK & poisonous quality effects [21,22]. During this process, mixes union are started by therapeutic physicists by utilizing different methodologies such as regular natural science & combinational science. By that science method substantial quantities of sole mixes could be orchestrated in equivalent and could be utilized in amalgamation of equally little particles. Combinational science might characterized by deliberate & dreary, their association of an arrangement of various structural squares of different shapes to each other to harvest a substantial cluster of assorted sub-atomic elements [23]. The benefit of combinatorial science over the traditional methodology is quicker blend of, now and again, up to a million mixes at the same time, and thus it helps in fast and in addition proficient disclosure of lead mixes. If there should be an occurrence of the amalgamation of a blend of intensifies, the whole blend may exposed for assessment to movement pursued by ID by dynamic constituents. In an event that then again no actives are discovered, at that point no additional consideration given to blend [24]. Selection stream to target recognizable proof involves *in vivo* assessment to essential/sans cells tests and in addition specificity of the mixes for the objective. Further, the action of mixes is additionally assessed in referred to creature orthologs of the objective as the mixes must be assessed for their adequacy in creature models. In this manner, information for dynamic mixes is likewise created in-vitro useful or cell based tests [25]. What's more, physicochemical properties of agent mixes from the arrangement being investigated are additionally concentrated to affirm investigational study resemblance of the mixes. As the most favored course for organization of the medication is oral, the new concoction substance being developed ought to watch the Lipinski manage of 5 which declares that a compound will probably be film penetrable and consumed by the body on the off chance that it coordinates the accompanying parameters which are also known as Lipinski's rule of five [26]. Dissolvability evaluations were furthermore directed to heading of *in vitro* & *in vivo* examines and additionally its retention from the digestive system and the goal of the restorative scientific experts is to acquire mixes having a solvency of more noteworthy than sixty µg/ml [27]. Additionally, *in vitro* physicochemical characteristics of mixes were additionally summarized. This incorporate penetrability appraisal in colon cancer cell line as a ideal for abdominal ingestion, metabolism dependability assessment utilizing humanoid liver microtomes for deciding inherent freedom, CYP450 restraint & enlistment for survey if the molecule will can possibly impact digestion of correspondingly controlled medications and plasma protein restricting examine which has an orientation on medication appropriation and in general pharmacological activity [28]. It is likewise reasonable to evaluate the poisonous capability of mixes in the beginning period of medication revelation, also, a few *in vitro* examines utilizing humanoid cell lines has created to discourse this estimation. This incorporate cell toxicity

tests for research in impact of mixes on cell reasonability; human-ether-go hindrance measure utilizing human-ether-go over expressing cell appearances to foresee the QT interim continuation risk to mixes below scrutiny; hepatotoxicity test utilizing an assortment of frameworks like lever cell lines, segregated liver cells in suspensions, hepatic cuts, and intracellular portions to survey the gene toxicity such as lactogenic action [29]. Intense & particular mixes possessing alluring physiological & ADME characteristics are likewise summarized to PK in similar creature types for whom viability to mixes must be assessed [30]. Mixes composing fitting PK are then assessed in essential creature prototypes, which might likewise incorporate confirmation to system replica's that show lead commitment [31]. At long last, mixes are selected for creature replicas of humanoid ailment to viability [32].

## Lead Optimization

Objective of this advancement is that to produce pre-clinical improvement competitors to enhancing inadequacies in lead molecule by concoction changes. By and large, the point is improve the physiological characteristics & limit lethality responsibilities to a conceivably protected molecule having positive PK distinguished. It's critical that exhibit an immediate relationship between groupings of the molecule to serum along with PK impact, & this type of information may be used for anticipating treating schedule of the molecule. Furthermore, it's additionally advantageous for building up dosage straight introduction, as the mixes which don't show such conduct have constrained clinical utility, especially in the event that they have a tight remedial window. Recognizable proof and portrayal of the active components of the molecules are likewise led amid that phase as metabolites might impact molecule's adequacy. Therapeutic science does not finish up after the pre-clinical applicant is distinguished, after the scientists start exertion by hold-up system having a plan for recognize exacerbates which may substitute for no disappointments in pre-clinical & scientific improvement.

## Preclinical Drug Development

After the selection of molecule applicant selected, medication advancement procedure starts. Medication is advanced by different examinations intended to help its endorsement using administrative authorities by moving competitor in the human cell line using accommodation of an application known as Investigational New Drug application. This process comprises different exercises, counting security, pharmacology and toxicity considers on creatures & different exercises identified with science, assembling and control (CMC, for example, plan advancement and so forth and point by point proposed clinical conventions for starting clinical examinations.

## Parameters for Selecting a Clinical Candidate

Following critical parameters are necessary for selecting a clinical candidate:

1. Chemical characteristics: Required steady and stable molecule for conducting a study.
2. Physicochemical properties: Must follow the Lipinski's rule of 5.
3. Pharmacological characteristics: Should have high binding affinity to the target site. Should have good effect in in-vitro models.
4. PK characteristics: Should have good bioavailability, Good half-life metabolic pathway are required to be well described.

5. Safety & harmfulness effects: Molecule ought to be without cardiovascular poisonous quality (hERG official), & ought to show adequate outline of acceptance & hindrance of CYP450 chemicals. At last molecule ought to be without any genuine creature poisonous quality [33].

## Clinical Drug Development

In the event that the IND is endorsed, clinical medication advancement starts. The purpose of different periods of scientific trials were comparative despite of the fact that the outlines of these trials can be significantly extraordinary. When all is said in done, a stage I trial is led to evaluate wellbeing and mediocrity of a medication and is normally directed on ten to hundred good volunteers. Together PK & pharmacodynamics angles were checked. Maximum Toxicity Dose (MTD) is resolved. The trial is for the most part open-mark (nonblinded). Stage II trial is the primary examination that explores clinical viability of the medication and thus this is completed in patients. In this trial, around fifty to five hundred volunteers get the IND chiefly for survey viability of the medication in volunteers. Be that as it may, the trial can have various destinations like contemplating dosage reaction association & deciding treating regime (ideal measurements & recurrence of organization). The security evaluation proceeds as in Phase I. The trial is for the most part randomized and controlled and might be single or twofold visually impaired trial. The chief share of clinical competitor's bomb in this stage because of absence of viability or wellbeing issues. Stage III trials affirms the viability of investigational study in a bigger populace, normally a couple of 100 to 1000 members. Trial is a multi-centric (led to numerous locales) & contrasts IND investigational study and the best existing treatment or standard of consideration to specific sickness. Wellbeing of patients is additionally evaluated to bigger area with the goal of normal unfriendly occasions might be recognized. They are regularly randomized, controlled, twofold visually impaired trials with various examination arms, and are the most costly and complex trials. On the off chance that positive outcomes are acquired, all information by day is arranged for administrative endorsement to permit the medication. After medication is showcased, after-promoting observation or Phase 4 trials start as extra continuation concentrates to distinguish uncommon on a significantly bigger populace or impacts in certain exceptional populace, tranquilize medication or medication infection connections, and so on for the most part to test the medication in a genuine setting. Stage IV examines have colossal ramifications, including modifying the naming of the medication, contraindications, collaborations, and even removal of a promoted IND. CD drug discovery is entering a new and exciting era, and we are much better off today than a few years ago. One can look to the future with more confidence, even if there is still a long way to go in understanding the complexity of this disease and the host-parasite interactions.

## Conclusion

Pharmaceutical industry is right now under heavy burden of increasing costs, evaluating the hazards related with drug discovery and development. Be that as it may, because of high neglected new drug necessities, the disease treatment will keep on being controlled by advancement created by the business in a joint effort with scholarly establishments and different methods of open private associations. Licensed innovation insurance, be that as it may, is critical in helping medicinal research & development. Novel advancements in discipline & innovation & other creative & developing ways to deal with enhance research & development profitability should receive by an extended haul approach with the end goal to be really steady of novel medication explore.

## References

1. Paul MS, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, et al. (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat Rev Drug Discov* 9: 203.
2. LaMattina JL (2012) The impact of mergers on pharmaceutical R&D. *Nat Rev Drug Discov* 10: 559-560.
3. Imming P, Sinning C, Meyer A (2006) Drugs, their targets and the nature and number of drug targets. *Nat Rev Drug Discov* 5: 821-834.
4. Lee J, Bogoy M (2013) Target deconvolution techniques in modern phenotypic profiling. *Curr Opin Chem Biol* 17: 118-26.
5. Sandercock AM, Rust S, Guillard S, Sachsenmeier KF, Holowecyj N, et al. (2015) Identification of anti-tumour biologics using primary tumour models, 3-D phenotypic screening and image-based multiparametric profiling. *Mol Cancer* 14: 147.
6. Lee YH, Song GG (2015) Vascular endothelial growth factor gene polymorphisms and psoriasis susceptibility: a meta-analysis. *Genet Mol Res* 14: 14396-14405.
7. Cao C, Moutl J (2014) GWAS and Drug targets. *BMC Genomics* 15: S4.
8. Sanseau P, Agarwal P, Barnes MR, Pastinen T, Richards JB, et al. (2012) Use of genome-wide association studies for drug repositioning. *Nat Biotechnol* 30: 317-320.
9. Snaith MR (2002) The use of transgenic systems in pharmaceutical research. *Brief Funct Genomic Proteomic* 2002: 119-130.
10. Willmann JK, van Bruggen N, Dinkelborg LM, Gambhir SS (2008) Molecular imaging in drug development. *Nat Rev Drug Discov* 7: 591-607.
11. Parada CA, Vivancos GG, Tambeli CH, Cunha FQ, Ferreira SH, et al. (2003) Activation of presynaptic NMDA receptors coupled to NaV1.8-resistant sodium channel C-fibers causes retrograde mechanical nociceptor sensitization. *Proc Natl Acad Sci USA* 100: 2923-2928.
12. Leuschner F, Dutta P, Gorbato R, Novobrantseva TI, Donahoe JS, et al. Therapeutic siRNA silencing in inflammatory monocytes in mice. *Nat Biotechnol* 29: 1005-1010.
13. Stengel PW, Yamada M, Wess J, Cohen ML (2002) M(3)-receptor knockout mice: muscarinic receptor function in atria, stomach fundus, urinary bladder and trachea. *Am J Physiol Regul Integr Comp Physiol* 282: R1443-R1449.
14. Rakhade SN, Fitzgerald EF, Klein PM, Zhou C, Sun H, et al. (2012) Glutamate receptor 1 phosphorylation at serine 831 and 845 modulates seizure susceptibility and hippocampal hyperexcitability after early life seizures. *J Neurosci* December 32: 17800-17812.
15. Meirman AC, Rob'e A, de Kerchove d'Exaerde A, Kieffer BL (2016) GPR88 in A2AR neurons enhances anxiety-like behaviors. *J Neurosci* 3: 1-5.
16. Cannon MV, Sillj'e HH, Sijbesma JW, Khan MA, Steffensen KR, et al. (2016) LXR $\alpha$  improves myocardial glucose tolerance and reduces cardiac hypertrophy in a mouse model of obesity-induced type 2 diabetes. *Diabetologia* March 59: 634-643.
17. Smith C (2003) Drug target validation: hitting the target. *Nature* 422: 341-345.
18. Mashalidis EH, 'Sled'z P, Lang S, Abell C (2013) A three-stage biophysical screening cascade for fragmentbased drug discovery. *Nat Protoc* 2013: 2309-2324.
19. Swinney DC, Anthony J (2011) How were new medicines discovered? *Nat Rev Drug Discov* 10: 507-519.
20. Hughes JP, Rees S, Kalindjian SB, Philpott KL (2011) Principles of early drug discovery. *Br J Pharmacol* 162: 1239-1249.
21. Keseru GM, Makara GM (2006) Hit discovery and hit-to-lead approaches. *Drug Discov Today* 11: 741-748.
22. Pandeya SN, Thakkar D (2005) Combinatorial chemistry: A novel method in drug discovery and its application. *Indian J Chem* 36: 335-348.
23. Leeson P (2012) Drug discovery: Chemical beauty contest. *Nature* 481: 455-456.
24. Kerns EH, Di L, Carter GT (2008) In vitro solubility assays in Drug Discovery. *Curr Drug Metab* 9: 879-885.
25. van Breemen RB, Li Y (2005) Caco-2 cell permeability assays to measure drug absorption. *Expert Opin Drug Metab Toxicol* 1: 175-185.

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26. Baranczewski P, Stańczak A, Sundberg K, Svensson R, Wallin A, et al. (2006) Introduction to in vitro estimation of metabolic stability and drug interactions of new chemical entities in drug discovery and development. *Pharmacol Rep* 58: 453-472.
  27. Yan Z, Caldwell GW (2001) Metabolism profiling, and cytochrome P450 inhibition & induction in drug discovery. *Curr Top Med Chem* November1: 403-425.
  28. Lambrinidis G, Vallianatou T, Tsantili-Kakoulidou A (2015) In vitro, in silico and integrated strategies for the estimation of plasma protein binding. A review. *Adv Drug Deliv Rev* 86: 27-45.
  29. Riss TL, Moravec RA, Niles AL (2011) Cytotoxicity testing: measuring viable cells, dead cells, and detecting mechanism of cell death. *Methods Mol Biol* 740: 103-114.
  30. Pollard CE, Valentin JP, Hammond TG (2008) Strategies to reduce the risk of drug-induced QT interval prolongation: a pharmaceutical company perspective. *Br J Pharmacol* 154: 1538-1543.
  31. Go´mez-Lecho´n MJ, Castell JV, Donato MT (2010) The use of hepatocytes to investigate drug toxicity. *Methods Mol Biol* 640: 389-415.
  32. Kirsch-Volders M, Plas G, Elhajouji A, Lukamowicz M, Gonzalez L, et al. (2011) The in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. *Arch Toxicol* 85: 873-899.
  33. Hefti FF (2008) Requirements for a lead compound to become a clinical candidate. *BMC Neurosci* 9: S7.