Commentary

Preclinical randomized controlled multicenter trials in translational stroke research

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Natalizumab is a humanized monoclonal antibody against alpha-4 integrin (CD49d) that reduces the incursion of circulating leukocytes into the central nervous system across the endothelium (1). As widely shown in experimental models of brain ischemia and in clinical studies, the infiltration of leukocytes across the blood-brain barrier is a well-known mechanism implicated in the progression of ischemic damage. One of the endothelial receptors involved in the transmigration of leukocytes is the alpha-4 integrin (CD49d). The inhibition of this receptor may modulate the invasion of leukocytes into the ischemic brain and it has been considered as a promising therapeutic target for neuroprotection (2). However, a number of single center experimental studies have evaluated the neuroprotective efficacy of antagonizing that receptor with mixed results (1).

In this context, Llovera *et al*. designed a preclinical prospective, multicenter, randomized and controlled trial (pRCT) to test the neuroprotective efficacy of anti-CD49d antibodies (3). The predetermined primary endpoint of the trial was infarct volume measured in two models of experimental brain ischemia that included a transient middle cerebral artery occlusion (MCAO) model and a permanent MCAO model. The secondary endpoints were functional outcome and the invasion of leukocytes into the brain. The main finding of the study was that treatment with CD49d-specific antibodies was able to reduce significantly both leukocyte invasion and infarct volume after the permanent distal occlusion of the middle cerebral artery, a model characterized by the production of mainly small cortical infarcts. Contrarily, anti-CD49d treatment was not able to reduce infarct volume or leukocyte invasion into the brain in the model of transient proximal occlusion, a model that resulted in larger infarcts. The design of the study was clearly reported and included detailed information on the experimental, statistical, and analytical methods. Importantly, these information included details about the operational criteria used for selecting the study populations, as well as of the methods used for sample size calculations, randomization and blinding.

Besides the exploration of the effects of another potential neuroprotective drug, the major advance of the trial developed by Llovera and collaborators was the use for the first time in the preclinical setting of stroke research of an experimental design based in clinical trial quality standards. During the last decades, considerable efforts have been implemented in the field of human clinical trials. Those advances have been mainly focused in harmonizing the design and report of interventional studies. As a result, the Consolidated Standards of Reporting Trials (CONSORT) initiative has elaborated a number of guidelines aimed to improve the transparent reporting of human clinical trials, to reduce the influence of bias and false positive results and to aid to the interpretation of the results (4). Specifically, the CONSORT statement consists of a 25 items checklist and a flow diagram, which provide guidance to authors on how to report randomized clinical trials, with special emphasis on reporting how the trial was designed, analyzed, and interpreted. Overall, the adherence of the scientific community involved in clinical trials development to the CONSORT guidelines has resulted in consistent improvements of the quality and reliability of clinical trials (5). Following the impact of the CONSORT

statement in improving the standards of clinical interventional research and in order to overcome the limitations of preclinical *in vivo* studies, a number of initiatives have emerged to produce guidelines for reporting animal research. In this context, the Animals in Research: Reporting in vivo Experiments (ARRIVE) Guidelines were published in June 2010 (6). The ARRIVE guidelines consist of a checklist describing the minimum information that is needed to report in interventional animal studies, which include strict operational and statistical guidelines. The information to be reported includes several items such as the number and specific characteristics of animals used, the statistical and analytical methods employed and details on the randomization and blinding procedures used to reduce bias, among others. All of those requirements were fulfilled by the trial from Llovera *et al*. As a major advance in the field, the trial supported the feasibility of performing pRCTs in stroke research under conditions resembling multicentre controlled clinical trials.

Despite the conflicting results from preclinical studies of CD49 inhibition for neuroprotection in acute brain ischemia and before the publication of the study by Llovera *et al*., the ACTION trial (natalizumab versus placebo in acute ischemic stroke; ClinicalTrials.gov identifier: NCT01955707) was started. The ACTION trial evaluated the efficacy and safety of natalizumab administered within 9 hours from symptom onset in acute ischemic stroke patients. It was designed as a proof-of-concept, double blinded phase-2 study (7). A total of 159 patients were randomized to receive a single dose of 300 mg of natalizumab administered intravenously (n=77) or placebo (n=82). The primary endpoint of the study was the relative change in infarct growth volume from baseline evaluated at day 5. A number of clinical secondary endpoints were predefined and contained the evaluation of treatment effect in several disability scales, which included the modified Rankin Scale, the NIH Stroke Scale and the Barthel Index. The results from the ACTION trial were recently communicated (7). Overall, the study was negative, as natalizumab was not able to reduce infarct growth at days 5 or 30. Surprisingly, more patients who received natalizumab had better clinical recovery at days 30 and 90. Regarding safety, the incidence of death or serious adverse events was similar across treatment groups. In prespecified subgroup analyses signals of greater clinical benefit after natalizumab administration were found in patients with smaller infarcts at baseline. The signals of potential efficacy observed in the ACTION trial support further studies of natalizumab administration in acute ischemic stroke. The design of those future clinical trials may be improved using the results obtained in the preclinical trial. Indeed, the effects of natalizumab administration observed in the permanent MCAO model paralleled the signals of greater clinical benefit after natalizumab treatment in patients with smaller infarctions at baseline. Taken together, these results suggest that the benefits of immune-targeted approaches may depend on infarct severity and localization. Indeed, both the results of the preclinical and clinical trials indicate that the effect of the drug may be modified by baseline stroke severity. Thus, patients with small infarct core at stroke admission may represent a target population for the use of anti-CD49d therapies.

Multiple reasons may explain the repeated failures in the translation of potential cerebroprotective drugs from the bench to the bedside in acute ischemic stroke. These reasons include biased selection of substances for clinical testing, the choice of irrelevant therapeutic windows based in the physiopathology of the disease and deficiencies in the design of preclinical and clinical studies (8,9). Indeed, preclinical studies in the stroke field lack robustness and reliability. The study reported by Llovera *et al.* represents a major advance in the field of translational stroke research because it demonstrates the feasibility of improving the quality of preclinical studies by adapting the standards achieved in the setting of human clinical trials (10). Moreover, the neuroprotective effects observed in the pRCT paralleled those found in the ACTION trial, supporting the ability of pRCT in the detection of relevant therapeutic targets in humans. In the setting of translational stroke research, the new route provided by Llovera *et al.* should be followed in future studies in order to ensure the reliability of preclinical data before proceeding to the design of clinical trials involving novel neuroprotective drugs. Hopefully, pRCT may represent the missing link for the success of translational stroke research.

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Footnote

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