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Safety evaluation of intra-arterial cell delivery in stroke patients— a framework for future trials

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Effective treatments are not yet available for the majority of stroke patients despite the significant advances in acute stroke management and care evident since the advent of therapeutic recanalization in 2015. Unfortunately, even patients who are eligible for recanalization treatment often suffer from residual functional deficits. Hence, there is an unmet demand for additional stroke therapies promoting functional recovery, not only those that can be considered as complementary approaches to recanalization but also for treatments that can be provided beyond its narrow time window. Cell therapies are an emerging paradigm in translational neuroscience and have been widely investigated in experimental stroke models (1). Preclinical evidence collected over the past two decades has revealed that administration of cells can exert robust effects in improving functional outcome when delivered in subacute (2,3) and even in chronic stroke stages (4). These promising findings have promoted small, early phase clinical studies intended to assess the feasibility, safety and efficacy of cell therapy approaches (5).

Primary versus secondary endpoints in early-stage clinical trials

The most important efficacy endpoints in clinical trials are functional outcome and a reduction in the size of the ischemic lesion, but both of these parameters typically

exhibit considerable inter-individual variations. Although being invaluable to move the field forward, many of these early-stage clinical trials have been uncontrolled or simply underpowered to assess efficacy endpoints in a reliable manner. Budgetary and time constraints nevertheless force industrial sponsors to include some kinds of efficacy endpoints despite power limits, even though it is evident that the trials may potentially miss all but the largest sized effects. In the worst case scenario, the progress of a potentially effective and clinically relevant experimental therapy may be discontinued due to the failure to achieve some pre-determined level of statistical significance in an early-stage trial.

The RECOVER-Stroke study: novel design features

The recent randomized, sham-controlled, multi-center clinical RECOVER-Stroke study conducted by Savitz *et al.* (6) successfully avoids these risks by strictly defining and following primary and secondary safety endpoints. The study featured an impressive array of safety endpoints and stratified patients according to NIHSS scores (≤ 15 versus ≥ 16) and whether the patients suffered from a lacunar versus a cortical stroke. Importantly, this study also represents the first serious attempt to assess the safety of cell delivery by the intra-arterial route in stroke patients. Altogether 48

patients were recruited. Initially recruitment of up to 100 patients had been planned, but the trial was stopped after an interim repowering analysis revealed that the number of enrolled patients was already sufficient to prove safety. This again highlights the clever design of this clinical investigation, being different from many other studies which have often defined efficacy endpoints as secondary but subsequently re-defined them as primary endpoints.

Characterization of the used cell product

A special subpopulation of bone marrow cells that express high levels of aldehyde dehydrogenase (ALDH) was administered in the trial. These cells, which were available as the commercial ALD-401 cell product, were selected for CD34⁺ and CD133⁺ stem and progenitor cell surface markers but are depleted of stem and progenitor cells which are present in unselected populations such as umbilical cord blood or bone marrow mononuclear cells. Nevertheless, ALDH cells still represent a heterogeneous population containing hematopoietic, endothelial, mesenchymal and potentially neural progenitor cells (7). Previously, ALDH cells have been reported to promote repair processes in experimental models of cardiovascular disease and limb ischemia (7). However, the exact therapeutic mechanisms are not entirely clear, which has also been the case for many other cell products. Cells expressing high levels of ALDH may be more resilient to insults, increasing their survival in hostile tissue environments (8) such as are present in the brain after a stroke. In fact, it might be advantageous that ALDH cells contain diverse cell types, as the different cells may be able to exert beneficial effects after experimental stroke by distinct mechanisms. These mechanisms could include paracrine neuroprotective effects, angiogenesis, and immunomodulation although tissue replacement seems to be less likely (9). Even if many of these mechanisms are presumably mediated by ALDH cells, there is no direct evidence that they would exert any 'cumulative' effects or superior properties over the other, more homogenous, cell products described in the literature. It is also not clear whether any individual effects might be of particular importance in mediating the beneficial effects of ALDH cells. Although not being absolutely essential for inclusion in an early-stage clinical trial at the moment, incomplete knowledge of therapeutic mechanisms is increasingly viewed as a short-coming by some regulatory authorities such as the European Medical Agency. Hence, there is increasing regulatory pressure on investigators and sponsors

to provide specific knowledge clarifying the therapeutic intervention's modes of action. This is indeed an important aspect as such knowledge forms the basis for the design of meaningful potency assays. Those would go beyond simple phenotypical profiling and a general assessment of basic biological properties of the cell products since this information may not be sufficient to indicate the presence or lack of efficacy for a specific therapeutic application (10).

Route of ALDH cell administration

Intravenous cell infusion is the most noninvasive delivery route for cell products and hence has been predominantly chosen for many early-stage stroke trials (5). However, a major disadvantage of this form of delivery is that most intravenously administered cells become entrapped in the capillary networks of the lungs and internal organs, dramatically reducing the number of cells reaching the target tissue (11). Although the peripheral effects of cells are believed to contribute to the beneficial effects evident in models of experimental stroke, the presumed lack of therapeutic cells in the target tissue (i.e., the brain) is generally seen as a drawback in clinical scenarios. Intra-arterial cell delivery offers a way to circumvent the pulmonary circulation at least during the first passage (12), but it can be associated with severe complications such as cerebrovascular micro-occlusions, particularly when larger cell populations are infused (13,14).

Result overview and safety considerations

The results of the RECOVER-Stroke trial are clear-cut and indicate a favorable safety profile of ALDH cells when administered intra-arterially after a stroke. The average size of ALDH cells is smaller than that of mesenchymal stem cells, in general supporting the favorable safety profile. However, four patients from the therapy group exhibited multiple small, scattered hypointensities in follow-up magnetic resonance imaging sessions, potentially representing secondary microinfarcts. A more detailed inspection of their distribution pattern and bilateral location indicated that in three out of four patients the lesions were more likely related to the angiographic procedure itself rather than ALDH cell administration. These lesions were also clinically asymptomatic and did not require any additional therapeutic interventions. However, the ALDH population contains some mesenchymal stem and progenitor cells (7), and therefore, one cannot exclude

the possibility that these cells might be at least partly responsible for these microinfarcts. Micro-occlusions have also been claimed to be related to cell dose (15). Careful dose-escalation studies are necessary before embarking on efficacy studies not only to detect possible complications but also to define the optimal dose for achieving therapeutic efficacy. Indeed, a higher dose of intra-arterial autologous bone marrow mononuclear cells was related to a better outcome in stroke patients, especially when more than 310×10^6 cells were injected (16).

The frequency of seizures was much higher in the treatment group, raising some safety concerns. Increased seizure activity has also been reported after systemic cell infusion (17) and may be related to the initial hyperexcitability of the ischemic brain (18). It could be speculated that the increased frequency of seizures is evidence that the transplanted cells reached their intended target to activate brain repair and reorganization processes. It is possible that by adjusting either the cell dose or timing of cell delivery, one might be able to avoid this serious complication. In most instances, control of seizures by antiepileptic medication must be carefully considered as these drugs may impair functional recovery (19).

Potential reasons for the overall outcome of the RECOVER-Stroke trial

The RECOVER-Stroke trial showed neutral results on secondary efficacy endpoints addressing the neurological outcome. There was a mean difference favoring the treatment group which, however, did not translate into formal statistical significance due to the relatively large standard deviation in both groups and the fact that the trial was terminated when it reached the safety endpoint. Hence, the secondary neutral outcome does not indicate that ALDH cells lack efficacy after a stroke, as other design features may have contributed to the neutral secondary outcome. One example is the time point of cell administration. A major advantage of ALDH cells is that they can be isolated from the patient's own bone marrow without requiring a long processing time, in theory allowing swift transplantation. The cell processing time in the RECOVER-Stroke trial was 2 days, with a number of logistical challenges being encountered. Nevertheless, it is not completely clear why cell delivery was scheduled at 13–19 days after the ischemic event, in particular since much shorter therapeutic time windows have been utilized in most adult stem populations in preclinical trials. Although

not known at the time of initiation of the RECOVER-Stroke trial, there is recent clinical evidence also suggesting that adult cell therapies have a therapeutic time window that is substantially longer than that of established recanalization therapies, but is still within the range of hours. For instance, the recent MASTERS trial using a mesenchymal-like stem cell population from bone marrow only achieved its efficacy endpoints when transplantation took place within a much earlier time window after stroke (<36 h) (20). Interestingly, this time window had already been identified in related preclinical datasets. Another aspect which may explain the failure to detect efficacy in the RECOVER-Stroke trial is the relative heterogeneity of its study population that included patients with lacunar and territorial infarcts. This heterogeneity might be problematic in efficacy-oriented clinical studies although strict inclusion and exclusion criteria were applied, as were appropriate patient stratification strategies. For instance, different locations of a lacunar infarcts as well as differences in both location and size of a cortical infarcts can result in very different functional deficits. Recent recommendations for translational research therefore suggest that there should be strictly selected patient subpopulations that are as homogenous as possible with respect to stroke type, location and functional defects, and ideally reflecting the preclinical stroke model in which efficacy has been demonstrated (21). Obviously, this kind of selection process will slow down recruitment and hence demand a longer study duration and more substantial financial resources, but might increase the chances for a positive outcome. A careful harmonization of preclinical and clinical approaches and the selection of the study population becomes even more relevant when considering potentially confounding aspects such as comorbidities and age (22). Those cannot be holistically investigated in preclinical experiments but are a major source of effect-masking inter-subject heterogeneity in clinical trials.

Summary and outlook

Undoubtedly, the RECOVER-Stroke study is an outstanding example due to its well-defined scope and methodological rigor. A number of questions remain open, but these may well be robustly addressed in future downstream phase III investigations. Importantly, all sham procedures were carefully planned and conducted ensuring the blinding of patients and investigators e.g., numerous mock procedures were conducted in the control population.

Both the practical and logistic challenges including trial design, randomization, blinding and power analysis have been described very transparently. The sharing of this information and experience will be invaluable for investigators planning similar studies. Another valuable aspect of the study is that it specifically describes how to tackle many of the practical and logistic challenges inherent in most cell therapies. Hence, the RECOVER-Stroke trial conducted by Savitz *et al.* may well serve as a role model for future early-stage cell therapy clinical trials in stroke.

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Footnote

Conflict of Interest: The authors have no conflict of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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