Sigrid Nikol is an angiologist at the Asklepios Klinik St Georg (Hamburg, Germany), whose current research centres upon the development of angiogenic therapies for the treatment of critical limb ischemia (CLI). Joining others in a session focussed upon patient-oriented approaches in severe CLI renal failure and beyond, she discussed cell therapy in peripheral artery disease (PAD).

Angiogenesis may be a promising solution for CLI patients who are at risk of major amputation – those who are deemed unsuitable for revascularisation, or whose revascularisation procedure has failed. While recanalisation techniques continue to improve, no medical therapy is available yet.

Angiogenesis offers an alternative strategy to increasing perfusion in areas of the leg and foot that require it, by creating new vascular structures. Gene therapy study has revolved around the delivery of angiogenic growth factor genes, including vascular endothelial growth factor (VEGF), fibroblast growth factor-1 (FGF-1), hepatocyte growth factor (HGF), amongst others. Cell therapy, on the other hand, seeks to bring a cocktail of growth factors into the vascular milieu by the administration of various types of stem cells. Both of these therapies seek to promote angiogenesis (and hence tissue survival) in an environment where endogenous remodelling is reduced leading to ischaemia.

Both gene and cell therapy have evolved immensely over the past decades, and Professor Nikol’s research focus is currently on the latter of the two approaches. “The first cells that were used were autologous cells from the bone marrow,” she explained, in conversation with LINC Review. “There were a large number of small trials, usually uncontrolled case reports or short series of five to ten patients, that all reported positive results. “But if you look at data from the few existing randomised controlled trials (RCT), and the meta-analysis of those RCTs, they don’t look as good. This is especially the case for the placebo-controlled RCTs. Meta-analyses did not show any benefit for treatment with autologous cells.”

Data are indeed controversial. A recent metaanalysis of placebo-controlled RCTs by Rigato et al. found that efficacy of cell therapy was insignificant for endpoints including major amputation-free survival and wound healing. The first prospective, randomised, double-blind, placebo-controlled multicentre trial was carried out by Powell et al., who in 2011 reported the results of RESTORE-CLI – which enrolled 86 patients, 46 of whom completed a six-month follow-up. While amputation-free survival and time to treatment failure were different, no relevant difference in rates of major amputation were found.

More recently, the JUVENTAS study, results of which were published in 2015, found no significant reduction in rates of major amputation following the intra-arterial infusion of mononuclear cells from autologous bone marrow into the common femoral artery of patients with non-revascularisable CLI. Although secondary outcomes, including quality of life, rest pain and ankle-brachial index all improved during follow-up, no significant differences were found between treatment or placebo groups.

Because of the negative findings for autologous cells, Professor Nikol became interested in studying cells from other sources: “Autologous cells are derived from the same sick PAD patient you are going to treat,” she said. “Not only are the number of stem cells in these patients (being sick with cardiovascular disease...
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PAD) lower than in the normal population, but also the quality of these cells is different.”

This was demonstrated by the JUVENTAS investigators in 2013, in a study that found circulating endothelial progenitor cells reduced in CLI patients compared to controls. Moreover, they identified reduced matrix metalloproteinase-9 levels, and lower bone marrow CD34(+)-cell levels, indicating local inflammatory activity coupled with reduced progenitor cell recruitment.

“We had to look for other cell sources,” continued Professor Nikol. “Therefore, trials using placenta-derived stem cells as an alternative to autologous cells are promising. They are derived from placenta of young, healthy women who had just given birth. These cells are multiplied in reactors using advanced technology, controlled and checked, deep-frozen in liquid nitrogen, and then transported to us for use in the patients.”

At present, only phase I studies have been completed using placenta-derived mesenchymal-like stem cells in CLI. Beyond that, one multinational phase II study in intermittent claudication has just completed its recruitment of 172 patients, with results expected in early 2018. One pivotal study in CLI involving 40 sites in Europe and the US will start in only few months, and an additional pre-marketing trial in CLI is planned to be initiated in Japan.

Commenting on evidence available today on this therapy, Professor Nikol said: “The efficacy data are still limited since only data from the phase I trials are available to date. But the safety profile has been good so far.”

As well as being derived from healthy individuals, allogeneic cells such as placenta-derived stem cells confer considerable advantages over autologous cells. Allogeneic placenta cells are immediately available in unlimited quantity, with standardisation yielding consistent cell quality. Furthermore, the collection of the placental stem cells is non-invasive.

Asked what negative side-effects have been observed in patients treated with those cells in clinical trials, Professor Nikol replied: “Cell therapy with placenta-derived cells so far did not increase cancer, proliferative retinopathy or significant worsening of other biological functions, such as renal function. Thus, we have a good safety profile for these placenta-derived stem cells except for some inflammatory/allergic reactions, mostly at the site of injections.

“There were approximately 170 patients in the earlier trials. The side effects we saw were mostly transient local reactions at injection site; in a few cases there were signs of systemic allergy (mostly mild and transient); and halitosis related to the excipient DMSO. So if there was a reaction it was local in most of the cases.

“In this regard, we are careful about patients who have comorbidities, as their situation may worsen of the situation (although this is just a suspicion).”

Interestingly, Professor Nikol and colleagues are not restricting their studies to the most severe CLI patients. Whereas pilot studies of placenta-derived stem cells were carried out in patients with ulcers, patients recruited to the phase II study have experienced intermittent claudication only – that is, they have walking difficulties, but no rest pain or ulcers. Some of these patients may be eligible for endovascular or surgical revascularisation procedures, explained Professor Nikol, but they chose to opt for this novel strategy. “Most claudication patients usually still have these endovascular or surgical options,” she said, adding: “Some of them had very long occlusions that could have been treated with an intervention, but with a high re-occlusion rate. They chose to go for a less invasive approach using these cells.”

As such, does she foresee that the therapy could apply to more than just ‘no-option’ patients, i.e. as something to be offered as a pre-emptive measure? “I can imagine three scenarios,” she replied. “One is actually that the patient is no-option or poor-option (i.e. endovascular or operation are high risk options). High risk can mean either that the operation has a high risk of itself, or that it has a high risk of failure. The second scenario would be to use these cells in combination with endovascular or surgical therapy to improve their outcome. The third option could be a stand-alone therapy in earlier stages of PAD.

“Yet, the ideal stage for the treatment with angiogenic therapies needs to be defined – for some of the CLI patients it may be already too late. We just finished recruitment of claudication patients. During this year, 2017, we will start the CLI trial; we will have the study investigator meeting in March.

We will include CLI patients with poor option, both endovascular or surgical. We need centres referring those CLI patients to the sites participating to the study and may need more active study centres.”

This latest phase III CLI trial is a randomised, double-blind, multi-centre, placebo-controlled, parallel-group study to evaluate the efficacy, tolerability and safety of intramuscular injections of placental-derived stem cells for the treatment of subjects with CLI with minor tissue loss who are unsuitable for revascularisation. Forty participating sites
We had to look for other cell sources; therefore, trials using placenta-derived stem cells as an alternative to autologous cells are promising.” Sigrid Nikol

are located in Germany, UK, US, Poland, Hungary, Czech Republic and Austria, and total recruitment is expected to be around 250. The intervention arm will receive doses in the affected leg intramuscularly, twice, at an eight-week interval. Follow-up will continue for 12 to 36 months, with a primary endpoint of time to occurrence of major amputation of the index leg or death and secondary endpoints of pain, wound healing, quality-of-life; and perfusion parameters (TcPO2).

One issue that pervades cell therapy is the survival time of the administered cells themselves, after finding themselves within the ischaemic environment. “Of course, these cells can only secrete growth factors as long as they remain alive,” noted Professor Nikol. “They don’t stay there forever. The less reaction you have against these cells, the longer they stay alive. But of course it is a matter of weeks and then they are probably faded. Some researchers have started to encapsulate cells for therapy in order to enhance their lifespan in the body. Apparently that does work. It may enhance the biological effect and extend the time interval between those intramuscular cell injections.”

In previous decades, Professor Nikol was involved with gene therapy research for CLI – work that culminated in 2011 in the TAMARIS trial, the largest clinical trial of gene therapy for CLI patients with ischaemic skin lesions. “I was involved with gene therapy for more than 20 years,” she recounted. “I started with animal experiments and then moved on to clinical trials. We really thought we might be successful with angiogenic gene therapy. Some of the animal experiments really looked good, and even some of the phase II trials confirmed those results, until we did the phase III TAMARIS trial. There, the good result we had in the phase II trial – of a significantly reduced risk for major amputation and death in 125 patients – could not be translated into phase III with 525 patients. This was extremely disappointing.”

As a result, pharma companies pulled out of gene therapy altogether. Yet, it did provide valuable lessons in how to design those angiogenesis trials as well as the characterisation of no-option and poor option CLI patients. Deciphering why gene therapy angiogenesis might not have worked, Professor Nikol suggested: “Just one single growth factor was enhanced with gene therapy. But we know that angiogenesis is multifactorial process, a cascade of many factors. So maybe it is simply not enough to enhance one single growth factor. In contrast, cells tested here secrete a cocktail of growth factors, which is more likely to work.”

Concluding with her thoughts on future directions, Professor Nikol noted that a broader range of cell sources needs to be investigated. “Neuronal stem cells, or cells derived from adipose tissue are among others that are being investigated. Some may be genetically modified, such as neuronal stem cells genetically modified to overexpress certain growth factors. Embryonic stem cells have also been considered. They are not used for ethical reasons, but we do have other stem cells with similar characteristics, such as germ line stem cells derived from ovaries and testes.” Regardless of the source, allogeneic cell therapies could be a central treatment option for large numbers of patients with PAD.