Pregledni članak / Review article

Almanah 2012.: liječenje stanicama u srčanožilnim bolestima. Časopisi nacionalnih društava predstavljaju odabrana istraživanja koja donose napredak u kliničkoj kardiologiji

Almanac 2012: cell therapy in cardiovascular disease. The national society journals present selected research that has driven recent advances in clinical cardiology

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SAŽETAK: Brz prijelaz iz laboratorija do bolesničke postelje koji se dogodio kod primjene regenerativne medicine u kardiologiji doveo je do uzbudljivih novih napredaka u našem razumijevanju nekih od osnovnih mehanizama ljudske biologije. Prva generacija stanica, koja je korištena u prvoj i drugoj fazi kliničkih ispitivanja (uglavnom mononuklearne matične stanice koštane srži), sada ulazi u treću fazu kliničkih ispitivanja, čiji je cilj proizvesti terapeutik koji se temelji na terapiji stanicama te bi mogao utjecati na ishod liječenja bolesti srca. Terapija stanicama prve generacije je bila usmjerena na praćenje sigurnosti primjene, kao i prikaz djelovanja te terapije, što je objavljeno u brojnim meta-analizama. Zahvaljujući dosadašnjem stečenom znanju, napredujemo prema sljedećoj generaciji stanica — stanice dobivene inženjeringom — koje su razvijene tako da daju fenotip koji će još više poboljšati proces obnavljanja/spašavanja miokarda. Ovaj pregledni članak donosi pregled najnovijih temeljnih znanstvenih istraživanja koja bi uskoro mogla biti primijenjena na ljudima te rezultata najnovijih kliničkih studija.

SUMMARY: The rapid translation from bench to bedside that has been seen in the application of regenerative medicine to cardiology has led to exciting new advances in our understanding of some of the fundamental mechanisms related to human biology. The first generation of cells used in phase I-II trials (mainly bone marrow mononuclear cells) are now entering phase III clinical trials with the goal of producing a cell based therapeutic that can change the outcome of cardiac disease. First generation cell therapy appears to have addressed safety concerns as well as showing 'activity' in numerous published meta-analyses. With the knowledge gained to date, the field is moving towards the next generation of cells-the 'engineered' cell-that have been developed to display a phenotype that will further enhance the myocardial repair/salvage process. This almanac review covers the latest basic research that may soon have application to humans as well as the results of the latest clinical trials.

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Novosti u liječenju srčanožilnih bolesti stanicama

Liječenje stanicama jedan je od najvažnijih "novih horizonta" u liječenju srčanožilnih bolesti. Nude se nove mogućnosti razvoja terapeutika koji bi mogli potpuno promijeniti način liječenja bolesnika te predstavlja područje istraživanja koje objedinjuje sve bolje poznavanje patofiziologije srčanožilnih bolesti i neke od najosnovnijih bioloških pojmova iz područja embriologije. Rast predkliničkih istraživanja srčanožilnog sustava koji iz toga proizlazi i brza translacija primjene kod ljudi od koristi su za cjelokupnu humanu biologiju. Ovo područje razvija se vrlo brzo; ovdje predstavljamo ključna dostignuća u posljednje 2 godine. Ovaj pregledni ćlanak podijeljen je na dva dijela kako bi se prikazala sinergija između temeljnih znanstvenih i translacijskih istraživanja.

Novija temeljna znanstvena istraživanja liječenja stanicama srčanožilnih bolesti

Novi modeli unapređuju razumijevanje regeneracije

Tropska riba - zebrica (eng. *zebrafish*, lat. *Danio rerio*)

Dugi niz godina rade se istraživanja regeneracije amfibijskog srca na najkorištenijem modelu zebrici, zahvaljujući njenoj velikoj regenerativnoj sposobnosti i prikladnosti za gensku manipulaciju. Srce zebrice se u potpunosti regenerira nakon kirurškog odstranjivanja vrška srca, što je oštećenje u kojem se gubi oko 20% ukupne mase klijetke.[1] Prvi eksperimenti ukazali su da su nediferencirane progenitorne stanice glavni izvor regeneriranih kardiomiocita kod zebrice; međutim dva nova istraživanja mapiranja gena jasno pokazuju da su glavni izvor ipak već od prije postojeći usmjereni kardiomiociti.[2,3] Ove dvije skupine istraživača neovisno su stvorile transgensku ribu kod koje promotor specifičan za kardiomiocite, *cmlc2* (prema eng. *Cardiac Myosine Light Chain 2*, srčani miozinski laki lanac; poznat i kao *myl7*) određuje izražaj rekombinaze Cre uzrokovane tamoksifenom. Ove životinje su križane s reporter linijom u kojoj odstranjivanje pomoću Cre zaustavnog slijeda omeđenog *loxP* regijama potiče konstitutivni izražaj zelenog fluorescentnog proteina (GFP, prema eng. *green flourescent protein*). Kod potomaka ovakvog križanja, primjenom tamoksifena potaknulo se sve stanice srčanog mišića koje su postojale prije i njihove potomke da izraze GFP. Ako je stoga regenerirani miokard dobiven iz nediferenciranih progenitornih stanica, novi srčani vršak bi trebao biti GFP^-. Međutim, u obje skupine velika većina novoregeneriranih stanica srčanog mišića je bilo GFP^+, što navodi na zaključak da se regeneracija srca kod zebrice odvija uglavnom proliferacijom od prije postojećih kardiomiocita. To je suprotno ranijem mišljenju da novi kardiomiociti nastaju iz matičnih stanica.

Usporedba miša i zebrice

Kardiomiociti sisavaca također se u određenom stupnju obnavljaju tijekom starenja i bolesti, iako nemaju regenerativnu sposobnost srca zebrice. Nedavno istraživanje[4] pokazalo je da se razlike u razvoju srca sisavaca i riba ne manifestiraju u ranim fazama razvoja. Koristeći metode istraživanja zebrice, autori su odstranili vršak lijeve klijetke kod miša starog jedan dan i primijetili su da je regenerativna reakcija bila brza, kao kod odrasle zebrice. Tri tjedna nakon zahvata odstranjeni dio zamijenilo je normalno miokardno tkivo koje je u roku od 8 tjedana imalo normalnu kontraktilnu funkciju. Istraživanja mapiranja gena pokazala su da je regeneracija

Update on cell therapy for the treatment of cardiovascular diseases

Cell therapy is one of the most important 'new horizons' in cardiovascular disease. It offers new opportunities to develop therapeutics that could revolutionise the way we treat patients and a field of research that combines an increased understanding of the pathophysiology of the cardiovascular disease with some of the most basic biological concepts involved in embryology. The resultant growth of preclinical research in the cardiovascular system and the rapid translation into humans have led to benefits for human biology as a whole. The field is rapidly advancing; here, we present key developments in the last 2 years. In order to reflect the synergy between basic and translational research, this review is therefore divided into two sections.

Basic science update on cell therapy in cardiovascular disease

New models enhancing our understanding of regeneration

Zebrafish

There is a long history of research on amphibian heart regeneration with the most adopted model the zebrafish given its substantial regenerative capacity and amenability to genetic manipulation. The zebrafish heart fully regenerates after the surgical amputation of the cardiac apex: an injury that corresponds to a loss of approximately 20% of the total ventricular mass.[1] Initial experiments suggested that undifferentiated progenitor cells were the principal source of regenerating cardiomyocytes in zebrafish; however, two recent gene mapping studies clearly demonstrate that preexisting committed cardiomyocytes are instead the main source.[2,3] These two groups independently generated transgenic zebrafish in which the cardiomyocyte-specific *cmlc2* (also known as *myl7*) promoter drives the expression of tamoxifen inducible Cre recombinase. These animals were crossed with a reporter line in which Cre-mediated excision of a *loxP*-flanked stop sequence induces constitutive expression of green fluorescent protein (GFP). In the offspring of this cross, all pre-existing cardiomyocytes and their progeny were induced to express GFP by tamoxifen treatment. Therefore, if the regenerated myocardium was derived from undifferentiated progenitor cells, the new ventricular apex should be GFP^-. Instead, both groups found that the vast majority of the newly regenerated cardiomyocytes were GFP^+, suggesting that the heart regeneration in zebrafish is principally mediated by the proliferation of pre-existing cardiomyocytes. This is contrary to the previously held belief that the generation of new cardiomyocytes from stem cells was the underlying aetiology.

Mice versus zebrafish

Although they lack the regenerative capacity of the zebrafish heart, postnatal mammalian hearts also undergo a degree of cardiomyocyte renewal during normal ageing and disease. Recently, a study[4] showed that the differences between mammalian and fish hearts may not necessarily apply early in development. Using approaches from the zebrafish model, the authors resected the left ventricular (LV) apex of 1-day-old neonatal mice and observed a brisk regenerative response similar to that in the adult zebrafish. By 3 weeks after injury, the defect had been replaced by normal myocardial tissue, which showed normal contractile function by 8

postignuta proliferacijom kardiomiocita koji su postojali prije, kao što je slučaj i kod zebrice. Naime, ova regenerativna sposobnost nije primijećena kod miševa starih 7 dana, što navodi na zaključak se taj gubitak podudara s binukleacijom kardiomiocita i skraćenjem trajanja staničnog ciklusa. Ipak, ovo istraživanje ukazalo je da su regenerativni mehanizmi nalik onima kod zebrice u srcu sisavaca pritajeni. Isto tako smo dobili genetski lako obradiv model za seciranje dijelova ispitivanih mehanizama i kod odraslih sisavaca.

Alternativni izvori stanica srčanog mišića: nova poimanja i unaprijeđeno razumijevanje

Fibroblasti kao izvor stanica srčanog mišića

U novije vrijeme je dokazano da fibroblasti kod infarkta mogu potencijalno biti direktno reprogramirani u kardiomiocite. Znanstvenici su prije petnaest godina pokazali da se fibroblasti mogu diferencirati u skeletne mišiće in vitro ili u ozlijeđenom srcu uslijed pojačane ekspresije gena koji nosi kod za mišićni transkripcijski faktor, MyoD. Međutim, unatoč opsežnim ispitivanjima, nije pronađen sličan glavni gen za srčani mišić, pa se interes za reprogramiranje smanjio. Potaknuti otkrićem induciranih pluripotentnih matičnih stanica (iPSC, prema eng. *pluripotent stem cell*), znanstvenici ponovno proučavaju to područje, koristeći kombinacije transkripcijskih faktora kako bi reaktivirali jezgrenu transkripcijsku mrežu željenih vrsta stanica. U posljednje 2 godine dvije istraživačke skupine su postigle napredak k tome cilju. Prva skupina[5] pregledala je ukupno 14 transkripcijskih faktora srca te je ustanovila da je specifična kombinacija triju transkripcijskih faktora, Gata4, Mef2c i Tbx5, dovoljna za stvaranje funkcionalnih kucajućih kardiomiocita direktno iz mišjih postnatalnih srčanih ili kožnih fibroblasta te da su ti inducirani kardiomiociti bili globalno reprogramirani da poprime ekspresiju kardiomiocitnog genetskog profila. Ovi faktori aktivirali su transgen u 20% fibroblasta, a kod otprilike 4% stanica došlo je do izražavanja endogenih sarkomerskih proteina kao primjerice troponina T, od kojih je ~1% imalo funkcionalne karakteristike poput spontanog kucanja. Dakle, većina stanica je samo djelomično reprogramirana, iako su se njihov cjeloukupni genetski izražaj očito pomaknuo od fibroblasta prema kardiomiocitima.

Druga skupina istraživača[6] primijenila je drugačiju metodu reprogramiranja mišjih embrionalnih fibroblasta u kardiomiocite. Ona je koristila "faktore Yamanaka" - OCT4 (poznati i kao POU5F1), SOX2, KLF4 i c-MYC - za pokretanje reprogramiranja, no tada su blokirali signalni put JAK-STAT, koji je nužan za pluripotenciju kod miša te dodali kardiogeni faktor BMP4. Te promjene donijele su minimalno stvaranje iPSC, no zato su aktivirale proces stvaranja srčanih progenitornih stanica, pa je u 2 tjedna nastao znatan broj kolonija kucajućih stanica. Osamnaest dana nakon indukcije, oko 40% stanica izražavalo je srčani troponin T. Treba napomenuti da su u ovom istraživanju korišteni embrionalni fibroblasti miša, dok su *Leda i sur.*[5] koristili uglavnom postnatalne srčane fibroblaste miša. Reprogramiranje fibroblasta, koji inače stvara ožiljak, u kardiomiocite je privlačno, osobito ako se može izvesti izravno u infarciranom području. Za uspješnu kliničku primjenu moramo znati kakvi su zapravo ti reprogramirani kardiomiociti, a sam proces mora biti puno učinkovitiji i bez transgena.

Inducirane pluripotentne matične stanice

Nedavno objavljen rad u ovom časopisu svratio je pažnju na veliko očekivanje da će iPSC (reprogramirane somatske

weeks. Genetic fate-mapping studies indicated that this regeneration was mediated by the proliferation of pre-existing cardiomyocytes, again as in the zebrafish. Notably, this regenerative capacity was not observed in 7-day-old mice, suggesting that its loss may coincide with cardiomyocyte binu- cleation and reduced cell-cycle activity. Nonetheless, this study indicates that zebrafish-like regenerative mechanisms are latent in mammalian hearts. It also provides a genetically tractable model for dissecting the blocks to these mechanisms in the mammalian adult.

Alternative sources of cardiomyocytes: new concepts and advanced understanding

Fibroblasts as source of cardiomyocytes

It has recently been demonstrated that fibroblasts in infarcts could potentially be reprogrammed directly to cardiomyocytes. Fifteen years ago, researchers showed that fibroblasts could be differentiated into skeletal muscle in vitro or in the injured heart by overexpressing the gene encoding the myogenic transcription factor, MyoD. However, despite extensive work, no comparable master gene for cardiac muscle was found, and interest in reprogramming waned. Spurred by the discovery of induced pluripotent stem cells (iPSCs), scientists have now returned to this field, using combinations of transcription factors to reactivate core transcriptional networks of desired cell types. In the last 2 years, two groups have made progress to this goal. The first group[5] screened a total of 14 cardiac transcription factors finding that a specific combination of three transcription factors, Gata4, Mef2c and Tbx5, was sufficient to generate functional beating cardiomyocytes directly from mouse postnatal cardiac or dermal fibroblasts and that the induced cardiomyocytes were globally reprogrammed to adopt a cardiomyocyte-like gene expression profile. These factors activated the transgene in 20% of fibroblasts of which approximately 4% of the cells expressed endogenous sarcomeric proteins such as cardiac troponin T, with ~1% showing functional properties such as spontaneous beating. Thus, most of the cells were only partially reprogrammed, although their global gene expression patterns had shifted markedly from fibroblast to cardiomyocyte.

The second group[6] used a different method of reprogramming mouse embryonic fibroblasts to cardiomyocytes. They used the 'Yamanaka factors'-OCT4 (also known as POU5F1), SOX2, KLF4 and c-MYC-to initiate reprogramming, but then blocked signalling through the JAK-STAT pathway, which is required for pluripotency in the mouse, and added the cardiogenic factor BMP4. These modifications yielded minimal generation of iPSCs, but instead activated the cardiac progenitor programme and, within 2 weeks, generated substantial numbers of beating colonies. By 18 days after induction, approximately 40% of the cells expressed cardiac troponin T. It should be noted that this study used mouse embryonic fibroblasts, whereas *Leda et al*[5] principally used postnatal mouse cardiac fibroblasts. Reprogramming the scar-forming fibroblast to a cardiomyocyte is appealing, particularly if it can be done directly in the infarct. To succeed clinically, we need to know how normal these reprogrammed cardiomyocytes are, and the process will have to be much more efficient and transgene-free.

Induced pluripotent stem cells

A recent report in this journal drew attention to the great promise of iPSC (reprogrammed somatic cells) as a renew-

stanice) biti obnovljiv izvor autolognih stanica.[7] Te stanice su prije 5 godina otkrili Takahashi i Yamanaka[8] nakon uvođenja gena u stanice odraslog miša, koje su reprogramirali tako da su bile slične embrionalnim matičnim (ES, prema eng. *embryonic stem*) stanicama. S obzirom da je DNA takvih stanica identičan DNA kod bolesnika, pretpostavljalo se da ih imunološki sustav neće napasti, iako njihova imunogenost nije aktivno ispitivana. Međutim, rad[9] objavljen 2011. u časopisu *Nature* pokazao je na transplantacijskom modelu miša da su neke iPS stanice zaista bile imunogene, što je izazvalo zabrinutost zbog njihove primjene u liječenju. Ovo istraživanje ispitivalo je imunogenost iPS stanica kod miša na temelju testa nastanka teratoma. U miševe, koji su bili ili imunološki kompromitirani ili su bili genetski podudarni sa stanicama davatelja, ubrizgane su iPS stanice. Ubrizgavanje matičnih stanica uobičajeno uzrokuje stvaranje dobroćudnih tumora koji se nazivaju teratomi, koji se sastoje od više vrsta diferenciranih stanica. Postupak je potvrđen i primjenom genetski identičnih (autolognih) embrionalnih matičnih stanica koje su proizvele teratome, dok je linija genetski nepodudarnih embrionalnih matičnih stanica odbačena prije stvaranja teratoma. Ishod transplantacije autolognih iPS stanica porijeklom iz fetalnih fibroblasta kod podudarnih miševa bio je odbacivanje teratoma bez obzira na način stvaranja iPS stanica, što upućuje na činjenicu da su u ovom testu podudarne iPS stanice imunogenije od podudarnih embrionalnih matičnih stanica.

Istraživanje je također otkrilo antigene koji su možda doveli do imunološkog odbacivanja iPS stanica, s obzirom da je pronađena skupina od devet gena koji su bili izraženi u abnormalno visokim razinama. Poticanje izražaja tri od četiri gena (*Hormad1, Zg16* i *Cyp3a11*) u neimunogenim embrionalnim matičnim stanicama znatno je smanjilo sposobnost stanica da stvaraju teratome u transplantacijama kod genetski identičnih miševa. Ovo istraživanje daje više pitanja nego odgovora, a što se kliničkih istraživanja tiče postoji puno ograničenja; međutim, naglašava se da trebamo još puno toga naučiti o mehanizmima reprogramiranja stanica i o svojstvenim sličnostima i razlikama između embrionalnih matičnih stanica i iPS stanica.

Dodatne terapije za poboljšanje diferencijacije matičnih stanica

Uz terapiju samim matičnim stanicama dodatno su se pojavila još dva nova moguća postupka pri obnovi miokarda.

Timozin β4

Jedno od najzanimljivijih napredaka u regenerativnoj medicini u posljednje 2 godine bilo je utvrđivanje "pravog izvora progenitornih stanica miokarda" (stanice porijeklom iz epikarda)[10] koje timozin β4 može potaknuti da se diferenciraju u kardiomiocite. Ovo istaknuto istraživanje *Smarta i sur.*[11] predstavlja veliki iskorak u utvrđivanju održivog izvora matičnih stanica/progenitornih stanica koje bi mogle regenerirati novi mišić nakon ishemijske bolesti srca i akutnog infarkta miokarda. Oni su pokazali na uzorku miša da odraslo srce ima ostatnu populaciju progenitornih stanica koje imaju potencijal da se nakon infarkta miokarda diferenciraju u kardiomiocite. Progenitorne stanice su bile tretirane sa peptidom zvanim timozin β4 koji je potaknuo embrionalno reprogramiranje koje je dovelo do mobilizacije ove populacije i do kasnije diferencijacije koja je proizvela de novo kardiomiocite. Nakon eksperimentalno izazvanog infarkta miokarda, pokazalo se da su ove stanice migrirale na mjesto ozljede, gdje su se onda bez ikakvog traga stanične fuzije diferencirale u strukturno i funkcionalno aktivne kardiomiocite. Kod tih kardiomiocita viđeno je stvaranje "gap junction" sa susjednim

able source of autologous cells.[7] These cells were first discovered only 5 years ago by Takahashi and Yamanaka[8] following the introduction of genes into adult mouse cells reprogramming them to resemble embryonic stem (ES) cells. Given that the DNA of such cells is identical to that of the patient, it has been assumed that they would not be attacked by the immune system although their immunogenicity has not been vigorously examined. However, a study[9] published in Nature in 2011 showed that in a mouse transplantation model, some iPS cells are indeed immunogenic, raising concerns about their therapeutic use. This study examined the immunogenicity of mouse iPS cells, using a teratoma-formation assay. They injected iPS cells into mice that were either immune-compromised or genetically matched with the donor cells. This normally results in the formation of benign tumours called teratomas, which consist of many types of differentiated cells. The approach was validated using a line of genetically matched (autologous) ES cells which gave rise to teratomas, whereas a line of unmatched ES cells was rejected before teratomas were produced. The transplantation of autologous iPS cells derived from fetal fibroblasts into matched mice resulted in the rejection of teratomas, irrespective of the approach used to generate the IPS cells, indicating that, in this assay, matched iPS cells are more immunogenic than matched ES cells.

The study also identified the antigens that may have caused immune rejection of the iPS cells, discovering a group of nine genes that were expressed at abnormally high levels. Inducing the expression of three of these genes (*Hormad1, Zg16* and *Cyp3a11*) in the non-immunogenic ES cells significantly impaired the cells' ability to form teratomas on transplantation into genetically matched mice. This study provides more questions than answers with many limitations in relation in clinical studies; however, it highlights that a great deal needs to be understood about the mechanisms underlying cellular reprogramming and the inherent similarities and differences between ES cells and iPS cells.

Adjunctive therapies to improve stem cell differentiation

As a related spin-off to cell therapy, two new approaches to cardiac repair have been reported.

Thymosin β4

One of the most exciting developments in regenerative medicine over the past 2 years has been the identification of 'bona fide source of myocardial progenitors' (epicardial derived cells)[10] which can be induced by thymosin β4 to differentiate into cardiomyocytes. This landmark study by *Smart et al*[11] provides a major step forward in identifying a viable source of stem/ progenitor cells that could contribute to new muscle after ischaemic heart disease and acute myocardial infarction (AMI). They demonstrated that in a mouse model the adult heart contains a resident progenitor cell population, which has the potential to become terminally differentiated cardiomyocytes after MI. Progenitor cells were primed with a peptide called thymosin β4 which induced embryonic reprogramming resulting in the mobilisation of this population and subsequent differentiation to give rise to de novo cardiomyocytes. Following experimentally induced MI, these cells were shown to migrate to the site of injury and then differentiate without any evidence of cellular fusion into structurally and functionally active cardiomyocytes. These cardiomyocytes showed evidence of gap junction formation

stanicama, usklađen transport kalcijevih iona i stvaranje funkcionalnog kontraktilnog aparata. Unatoč niskom ukupnom broju stanica koje se nalaze na mjestu ozljede i relativno ukupnom nižem stupnju stupnju diferencijacije, serijska snimanja magnetskom rezonancijom pokazala su značajna poboljšanja u ejekcijskoj frakciji, volumenu srca i veličini ožiljka u usporedbi s kontrolnom skupinom životinja. Korištenje timozina β4 u tom postupku bilo je ključno za te rezultate i možda nagovijesti i novu strategiju unaprijeđenja liječenja miokarda kod ljudi.

Mikro RNA (prema eng. *ribonucleic acid*, ribonukleinska kiselina)

Mikro RNA (mala nekodirajuća RNA) imaju ključnu ulogu u diferencijaciji i samoobnavljanju pluripotentnih matičnih stanica, kao i u diferencijaciji stanica srčanožilnog porijekla. Stoga su se mikro RNA pokazali kao potencijalni modulatori diferencijacije matičnih stanica; osobito se miR-1 pokazao ključnim u reguliranju diferencijacije progenitornih stanica srčanog mišića. Istraživanje objavljeno 2011. godine[12] nastojalo je to unaprijediti i ocijenilo je povećava li povećani izražaj miR-1 u matičnim stanicama (miR-1-ES stanice) diferencijaciju u kardiomiocite nakon transplantacije u infarcirani miokard. U tom istraživanju, kod miševa s infarktom miokarda u rubno područje infarciranog miokarda transplantirane su tri vrste stanica: miR-1-ES stanice, matične stanice (ES) stanice ili kontrolna podloga za uzgoj. Povećani izražaj miR-1 u transplantiranim matičnim stanicama zaštitio je miokard domaćina od apoptoze izazvane infarktom miokarda zahvaljujući aktivaciji p-AKT; inhibiciji kaspaze 3, fosfataze i tenzin homologa; i proizvodnji superoksida. Znatno smanjenje intersticijalne i vaskularne fibroze primijećeno je kod miR-1-ES stanica u usporedbi s kontrolnim skupinama. I na kraju, kod miševa kojima su transplantirane miR-1-ES stanice funkcija srca se znatno poboljšala u usporedbi s kontrolnom skupinom. To dovodi do zaključka da miR-1 potiče diferencijaciju kardiomiocita iz transplantiranih ES stanica i sprječava apoptozu nakon infarkta miokarda; međutim, ono što je važno vezano za fibrozu jest da nije primijećena nikakva statistička razlika između skupine s miR-1-ES stanicama i one s ES stanicama, što znači da su potrebna daljnja istraživanja na tom području. Pregledna studija[13] postojećih dokaza o utjecaju mikro RNA na matične stanice/progenitorne stanice i na srčanožilno liječenje objavljena je nedavno.

Kliničke novosti u liječenju stanicama srčanožilnih bolesti

Prijelaz od predkliničkog opažanja do razvoja novih metoda liječenja može trajati više godina, pa čak i desetljeća. Deset godina nakon prve kliničke primjene matičnih stanica kod bolesti srca[14] raspravlja se o izboru vrsta stanica i njihovoj primjeni, a znanstvenici sve bolje razumiju to područje istraživanja i izazove translacijske medicine.

Iako postoji mnogo vrsta stanica koje se mogu koristiti u liječenju miokarda, pragmatički se pristupilo kliničkim studijama koje su koristile autologne mononuklearne matične stanice koštane srži (BMMNC, prema eng. *bone marrow mononuclear cell*) i neke vrste stanica koje se među njima nalaze (hematopoetske matične stanice, mezenhimske matične stanice (MSC, prema eng. *mesenchymal stem cell*) i endotelijske progenitorne stanice) u prvim uvođenjima u kliničku primjenu.[15] U novije vrijeme prati se nekoliko kliničkih studija transplantacije BMMNC stanica kod bolesti srca u prvoj i drugoj fazi istraživanja, koje su pokazale da su sigurne i izvedive, dok su izvješća o učinkovitosti, usprkos manjoj usklađenosti rezultata, postavili temelj budućim ispitivanjima.

with adjacent cells, synchronous calcium transients and the formation of operational contractile apparatus. Despite a low overall fraction of these cells being present at the site of injury and a relatively poor overall efficiency of differentiation, serial MRI scans revealed significant improvements in ejection fraction, cardiac volumes and scar size in comparison with sham treated animals. The pretreatment with thymosin β4 was crucial to these effects and may suggest a new strategy for promoting myocardial repair in humans.

MicroRNAs

MicroRNAs (small non-coding RNAs) play a critical role in differentiation and self-renewal of pluripotent stem cells, as well as in the differentiation of cardiovascular lineage cells. As a result, microRNAs have emerged as potential modulators of stem cell differentiation; specifically, miR-1 has been reported to play an integral role in the regulation of cardiac muscle progenitor cell differentiation. A study published in 2011[12] looked to take this one step further and assessed whether the overexpression of miR-1 in ES cells (miR-1-ES cells) enhances cardiac myocyte differentiation following transplantation into the infarcted myocardium. In this study, mice models of MI had miR-1-ES cells, ES cells or culture medium (control) transplanted into the border zone of the infarcted heart. Overexpression of miR-1 in transplanted ES cells protected host myocardium from MI-induced apoptosis through activation of p-AKTand inhibition of caspase-3, phosphatase and tensin homologue, and superoxide production. A significant reduction in interstitial and vascular fibrosis was quantified in miR-1-ES cells compared with control MI. Finally, mice receiving miR-1-ES cells had significantly improved heart function compared with respective controls. This would suggest that miR- 1 drives cardiac myocyte differentiation from transplanted ES cells and inhibits apoptosis post-MI; however, importantly with respect to fibrosis no statistical significance between miR-1-ES cell and ES cell groups was observed suggesting further study in this area is needed. A review[13] of the current evidence for the role of microRNAs in stem/progenitor cells and cardiovascular repair has recently been published.

Clinical update on cell therapy in cardiovascular disease

The translational path from preclinical observation to new treatment development can take many years, even decades. Ten years after the first clinical application of stem cells in cardiac disease,[14] many questions regarding cell types and their administration have been addressed and researchers are better understanding this area of research and the challenges of translational medicine.

Although many candidate cell types for myocardial repair exist, a pragmatic approach has been used in clinical trials which have utilised autologous bone marrow mononuclear cells (BMMNCs) and some of the component cell types found therein (haematopoeitic stem cells, mesenchymal stem cells (MSCs) and endothelial progenitor cells) in the first steps into the clinical setting.[15] Recent years have seen several phase I-II clinical trials of BMMNC transplantation in cardiac disease which have demonstrated safety and feasibility while reports of efficacy, although less consistent, have provided grounds for further investigation.

Novija dostignuća u primjeni autolognih matičnih stanica koštane srži (BMMNC)

U posljednje 2 godine neke od većih studija primjene matičnih stanica (BMMNC) kod akutnog infarkta miokarda navode da dugoročni rezultati potvrđuju njihovu sigurnost u razdoblju od 3 do 5 godina. Ohrabruju novije meta-analize koje su se bavile tim istraživanjima i koje su ponovno potvrdile skromno, ali važno "djelovanje" liječenja stanicama u poboljšanju raznih odabranih pokazatelja funkcije srca.[16,17]

Prva randomizirana kontrolirana studija liječenja matičnim stanicama kod akutnog infarkta bila je studija BOOST (prema eng. *Bone marrOw transfer to enhance ST-elevation infarct regeneration*, presađivanje koštane srži radi poboljšanja regeneracije nakon infarkta s elevacijom ST-segmenta) koja je zabilježila povećanje od 6,7% ukupne ejekcijske frakcije lijeve klijetke (EF) u liječenoj skupini, u usporedbi s povećanjem od 0,7% u kontrolnoj skupini tijekom praćenja od 6 mjeseci; to se pripisalo boljoj regionalnoj pokretljivosti infarcirane stijenke u sistoli.[18] Podaci iz petogodišnjeg praćenja[19] pokazali su smanjenje EF lijeve klijetke i povećanje volumena lijeve klijetke u obje skupine, a nije se utvrdila značajna razlika u smrtnosti niti krajnjem kliničkom ishodu. Zanimljivo je da je analiza podskupina ukazala na zaključak da je kod težih infarkta, koji su karakterizirani transmuralnim zahvaćanjem stjenke, liječenje stanicama bilo od znatne koristi u poboljšanju EF i dimenzije lijeve klijetke u usporedbi s kontrolnom skupinom.

Studija REPAIR-AMI (prema eng. *Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction*, reinfuzija obogaćenih progenitornih stanica i remodeliranje nakon akutnog infarkta miokarda) je dosad najveća randomizirana kontrolirana studija liječenja srca matičnim stanicama. Prvobitno istraživanje obuhvatilo je 204 bolesnika s akutnim infarktom i pokazalo je tijekom 4 mjeseca znatno bolju apsolutnu EF lijeve klijetke kod bolesnika koji su primili BMMNC stanice u usporedbi s kontrolnom skupinom. Kao što se vidjelo u BOOST studiji, bolesnici s većim infarktima imali su najviše koristi. Iako nije bila dovoljno statistički snažna za tu svrhu, bila je to prva velika baza podataka krajnjih kliničkih ishoda koji su prikazali smanjenje smrtnosti i pobola nakon intrakoronarne primjene matičnih stanica.[20] Ti nalazi su potvrđeni nakon 2 godine znatnim smanjenjem kombiniranih krajnjih kliničkih ishoda i povećanjem pokretljivosti stijenke lijeve klijetke prema nalazu MRI kod bolesnika koji su primili BMMNC.[21] Podaci iz petogodišnjeg praćenja koji su predstavljeni 2011. godine na znanstvenim skupovima Američkog kardiološkog udruženja (AHA, prema eng. American Heart Association)[22] obuhvaćali su 100 bolesnika u svakoj liječenoj skupini. Uz trend smanjenja stope smrtnosti, znatno su se smanjili i zajednični ishodišni cilj od smrti, ponovnog infarkta miokarda i revaskularizacije, a postupak je proveden jednokratnom intrakoronarnom infuzijom matičnih stanica.

Podaci koji se temelje na dugoročnom praćenju 100 bolesnika uključenih u studiju ASTAMI (prema eng. *Autologous Stem-cell Transplantation in Acute Myocardial Infarction*, transplantacija autolognih matičnih stanica kod akutnog infarkta miokarda) pokazali su tijekom trogodišnjeg razdoblja znatno bolje podnošenje tjelesnog opterećenja u liječenoj skupini, iako nije bilo znatne razlike u EF lijeve klijetke između liječene i skupine na placebu.[23] Petogodišnje praćenje provedeno u okviru istraživanja BALANCE (prema eng. *Clinical Benefit and Long-Term Outcome After Intracoronary Autologous Bone Marrow Cell Transplantation in Patients With Acute Myocardial Infarction*, klinička korist i dugoročni ishod nakon intrakoronarne transplantacije autolognih stanica koštane srži kod bolesnika s akutnim infarktom miokarda) pokazalo je znatno i kontinuirano poboljšanje funkcije lijeve

Recent developments in the use of autologous BMMNCS

The last 2 years has seen the some of the larger trials examining BMMNCs in the setting of AMI report long-term results confirming safety to 3-5 years. Reassuringly, recent meta-analyses to look at these studies have again confirmed a small but important 'activity' of cell therapy in improving various surrogate parameters of cardiac function.[16,17]

The first randomised controlled trial of stem cell therapy in AMI was the BOOST trial (*BOne marrOw transfer to enhance ST-elevation infarct regeneration*) reporting a 6.7% increase in global left ventricular ejection fraction (LVEF) in the treatment group compared with a 0.7% increase in the control group at 6 months; this was attributed to improved regional systolic wall motion in the infarct zone.[18] The 5-year follow-up data[19] showed a decline in LVEF and increase in LV volumes in both groups with no significant difference in mortality or clinical end points between the groups. Interestingly, subgroup analyses suggested that in more severe infarction, defined as greater transmurality, cell therapy conferred a significant benefit in LVEF and LV dimension compared with control.

The Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial is the largest randomised controlled trial in stem cell therapy for cardiac repair to date. The original study that enrolled 204 patients with AMI demonstrated a significantly greater improvement in absolute LVEF in patients treated with BMMNCs compared with control at 4 months. As seen in BOOST, the patients with larger infarcts derived the most benefit. Although not sufficiently powered for the purpose, this was the first large scale clinical endpoint data showing mortality and morbidity benefit conferred by intracoronary administration of stem cells.[20] This was borne out at 2 years with significant reductions in combined clinical end point and increases in LV wall motion when assessed on MRI in the patients who received BMMNCs.[21] The 5-year follow-up data, presented at the American Heart Association (AHA) Scientific Sessions 2011,[22] included 100 patients in each treatment arm. While there was only a trend towards improvement in mortality, there was a significant reduction of the combined end point of death, recurrence of MI and revascularisation conferred by a single intracoronary infusion of cells.

Long-term follow-up data from 100 patients enrolled in the Autologous Stem-cell Transplantation in Acute Myocardial Infarction (ASTAMI) trial showed a significant improvement in exercise capacity in the treated cohort at 3 years, although there was no significant difference in LVEF between treatment and placebo arms.[23] The 5-year follow-up for the 'BALANCE' study (*Clinical Benefit and Long-Term Outcome After Intracoronary Autologous Bone Marrow Cell Transplantation in Patients With Acute Myocardial Infarction*) showed significant and sustained improvement in LV function and reduction in mortality in 62 treated patients compared with 62 control patients. Although this suggests a significant mortality benefit, it is noted that this study was non-randomised.[24] Another large trial (HEBE) consisting of 200 patients has also been published recently[25] showing no significant improvement in LV function in BMMNC treated

klijetke i manju stopu smrtnosti kod 62 liječena bolesnika u usporedbi sa 62 bolesnika iz kontrolne skupine. Iako ovo upućuje na znatno smanjenje stope smrtnosti, treba naglasiti da ovo istraživanje nije bilo randomizirano.[24] Nedavno je objavljena druga velika studija (HEBE) provedena kod 200 bolesnika[25], a tijekom četveromjesečnog razdoblja nije pokayala znatno poboljšanje funkcije lijeve klijetke kod bolesnika podvrgnutih liječenju BMMNC u usporedbi s placebom, dugoročni učinci liječenja stanicama u ovom istraživanju još nisu objavljeni.

Većina ovih istraživanja provodi se u sklopu primjene stanica 5-8 dana nakon akutnog infarkta miokarda. Postoji potreba određivanja optimalnog vremena presađivanje stanica kod ishemijskog događaja. Moguće je da različiti rezultati poboljšanja funkcije lijeve klijetke i ishoda između studija ovisi o vremenu presađivanja stanica sa obzirom da se miokard nakon infarkta nalazi u promjenjivom upalnom okruženju. Novije istraživanje LateTIME bavilo se kasnijim vremenom, koje je bilo 2-3 tjedna nakon akutnog infarkta miokarda.[26] U njemu su autori utvrdili da kod 87 bolesnika randomiziranih ili za liječenje BMMNC, ili za kontrolnu skupinu, liječenje BMMNC u određenom trenutku nije poboljšalo ni ukupnu EF lijeve klijetke niti regionalnu pokretljivost stijenke u razdoblju od 6 mjeseci. Iako je 5-7 dana nakon akutnog infarkta miokarda vjerojatno optimalno vrijeme za primjenu liječenja stanicama, nisu ispitana ostala vremenska razdoblja. Cilj studija koje su u tijeku, TIME[27] i SWISS-AMI,[28] je dodatno istražiti vrijeme ubrizgavanja. Trenutno je jedino vrijeme koje nije ispitivano vrlo rana faza (<12 h nakon revaskularizacije). Klinička studija REGENERATE-AMI (EUDRACT 2007.-002144-16), u kojem su BMMNC presađene otprilike 6 sati nakon PCI je uključila malo više od polovice planiranih ispitanika, a rezultati će biti objavljeni 2013.

Sada je potrebno raditi na boljem definiranju bolesnika koji će imati koristi od liječenja stanicama. Rezultati petogodišnjeg praćenja tijekom studija BOOST i REPAIR-AMI ukazuju da ako se kao mjerilo krajnjeg ishoda uzme EF lijeve klijetke, podskupine s velikim funkcionalnim deficitom kao početnom vrijednošću imaju klinički značajna povećanja EF, dok je ukupni učinak možda skromniji kod svih sudionika. To je dalje potvrdilo podistraživanje FINCELL,[29] u kojem je 78 bolesnika primilo BMMNC ili placebo poslije trombolize ili PCI kod akutnog infarkta miokarda. Utvrđeno je znatno veće poboljšanje funkcije lijeve klijetke nakon primjene BMMNC kod bolesnika kod kojih je početna vrijednost EF lijeve klijetke bila ispod vrijednosti medijana skupine.

Unatoč raznolikosti opisanih rezultata studija, najveća dosadašnja meta-analiza koja obuhvaća 1.765 bolesnika i 33 randomizirane kontrolirane studije, pokazuje skromno, ali značajno poboljšanje EF lijeve klijetke od 2,87% tijekom kratkoročnog praćenja te kontinuirano poboljšanje od 3,75% nakon jednogodišnjeg praćenja,[16] što dovodi do zaključka da dodatno liječenje matičnim stanicama kod akutnog infarkta daje bolje rezultate od konvencionalnog liječenja. Iako su skromni, ti učinci mogu se usporediti s važnim istraživanja iz područja primarne angioplastike, ACE inhibitora i β-blokatora,[30] i dovode do zaključka da se može dodatno smanjiti smrtnost. Većina dosadašnjih studija u ovom području koristi EF lijeve klijetke kao pokazatelj kliničkog krajnjeg ishoda, bez razumijevanja kako su ti pokazatelji povezani s ishodom.

Nedavno su objavljene dvije studije primjene BMMNC stanica kod akutnog infarkta koje su pokušale ispitati alternativne zamjenske krajnje ishode praćenja. Cilj studije BONAMI (prema eng. *Bone Marrow in Acute Myocardial Infarction*, koštana srž kod akutnog infarkta miokarda) bio je ocijeniti učinak na vijabilnost miokarda unutar 3 mjeseca na uzorku

patients compared with placebo up to 4 months; however, the long-term effects of cell therapy in this study are yet to be reported.

The majority of these studies are in the context of cell administration 5-8 days following AMI. There is still a need to define the optimal time point for cell transfer relative to ischaemic insult. It is conceivable that the improvement in LV function and outcome seen inconsistently between trials may be dependent on the timing of cell transfer as the postinfarct myocardium will have a changing inflammatory milieu. The later time point of 2-3 weeks post-AMI is addressed by the recent LateTIME study.[26] Here, the authors found that in 87 patients randomised to either BMMNCs or control, BMMNC treatment at the given time point did not improve either global LVEF or regional wall motion at 6 months. Although the likelihood is that day 5-7 is the optimal time for delivery of cell therapy post-AMI, not all time points have been investigated. The ongoing trials TIME[27] and SWISS-AMI[28] aim to evaluate the timing of injection further. As yet, the only time point that has not been considered is the very early phase (<12 h postrevascularisation). The REGENERATE-AMI clinical trial (EUDRACT 2007-002144-16) in which BMMNCs are transferred approximately 6 h post-PCI is over halfway through recruitment and will report in 2013.

There is now a need to better define those patients who will benefit from cell therapy. The results of the 5-year follow-up from the BOOST and REPAIR-AMI trials suggest that if ejection fraction is used as a surrogate end point, while the overall effect may be modest for all-comers, subgroups with a large functional deficit at baseline do experience clinically meaningful increments in LVEF. This is further substantiated by the FINCELL substudy[29] in which 78 patients received either BMMNCs or placebo post-thrombolysis and PCI for AMI. Here, a significantly greater BMMNC associated improvement in LV function was observed in patients with baseline LVEF below the median for the group.

Despite the heterogeneity of trial results described, the largest meta-analysis to date comprising 1,765 patients and 33 randomised controlled trials demonstrates a modest but significant improvement in LVEF of 2.87% in short-term follow-up, with sustained LVEF improvement of 3.75% after follow-up over 1 year[16] suggesting that adjunctive stem cell treatment in AMI offers an improvement over conventional therapy. These effects while modest are comparable with those seen in landmark studies of primary angioplasty, ACE inhibitors and β-blockers[30] and suggest that a similar additional mortality benefit may be achieved. The majority of trials in this field to date use LVEF as a surrogate clinical end point with little understanding of how this parameter relates to outcome.

Recently, two trials of BMMNCs in AMI have been published attempting to explore alternative surrogate end points. The aim of the '*Bone Marrow in Acute Myocardial Infarction* (BONAMI)' was to assess the effect on myocardial variability at 3 months recruiting 101 patients with poor LV function post- AMI to receive BMMNCs or placebo. Myocardial viability was significantly improved in the treated group compared with control.[31] In another trial,[32] LVEF was assessed alongside myocardial perfusion in a similar patient cohort up to 12 months. A small improvement in myocardial perfusion was observed in the BMMNC group compared with control; there was however a significantly lower incidence of combined major adverse cardiac events in the treatment group, high-

od 101 bolesnika s lošom funkcijom lijeve klijetke, koji su nakon akutnog infarkta primili BMMNC ili placebo. Vijabilnost miokarda se znatno poboljšala u liječenoj skupini u usporedbi s kontrolnom skupinom.[31] U drugoj studiji,[32] uz perfuziju miokarda ocjenjivana je EF lijeve klijetke kod slične skupine bolesnika tijekom razdoblja od 12 mjeseci. Malo poboljšanje u perfuziji miokarda primijećeno je u skupini koja je primila BMMNC u usporedbi s kontrolnom skupinom; međutim, učestalost zajedničkih teških neželjenih srčanih događaja u liječenoj skupini je bio znatno niži, što je ponovno istaknulo loše definiran odnos između potencijalnih nadomjesnih parametara i mjerenja kobnih kliničkih ishoda.

Jedno od najvažnijih dosadašnjih dostignuća je pomak s druge na treću fazu kliničkih istraživanja. Većina postojećih kliničkih studija osmišljena je upravo radi ocjenjivanja sigurnosti i izvedivosti, a s obzirom da ne mogu ocijeniti učinkovitost upotrebe tehnologije, zamjenski parametri, kao na primjer EF lijeve klijetke, ocjenjuju djelovanje. Nedavno je iz EU fondova za bavljenje ovim pitanjima dodijeljeno udruženju 17 kliničkih centara diljem Europe 6 milijuna eura za osmišljavanje i provođenje najpotpunijeg istraživanja o ishodima primjene BMMNC kod akutnog infarkta (BAMI; http://www.bami-fp7.eu). BAMI će obuhvatiti 3.000 bolesnika s ukupnom smrtnošću kao primarnom krajnjim ishodom, što će biti jedno od najuzbudljivijih saznanja u tom području u posljednjih nekoliko godina. Rezultati istraživanja bit će objavljeni za 5 godina.

Liječenje stanicama kod kronične bolesti lijeve klijetke

Istraživanje STAR-heart najveće je izvješće o primjeni BMMNC kod ishemijskog zatajivanja srca, a njegovi podaci, koji se temelje na petogodišnjem praćenju, objavljeni su 2010. godine.[33] Nerandomizirano istraživanje prvotno je obuhvaćalo 391 bolesnika kod kojih je EF lijeve klijetke iznosila 35% ili manje, a kojima je predložena intrakoronarna primjena autolognih BMMNC. 191 bolesnik je liječen stanicama, a 200 bolesnika je podvrgnuto samo najbolje priznatom farmakološkom liječenju. Tijekom petogodišnjeg praćenja došlo je do znatnog poboljšanja EF, kontraktilnosti, potrošnje kisika i tolerancije tjelesnog opterećenja kod bolesnika liječenih BMMNC, a što je povezano s možda još zanimljivijim podatkom da je učestalost smrtnosti bila znatno niža nego kod kontrolne skupine. To još treba potvrditi dvostruko slijepo randomizirano istraživanje. Studija FOCUS-HF[34] bila je randomizirana kontrolirana studija 30 bolesnika čiji je cilj ocijeniti učinke transendokardne primjene BMMNC kod bolesnika s kroničnim ishemijskim zatajivanjem srca kod kojih ne postoji mogućnost daljnje revaskularizacije. Iako nije bilo razlike u EF lijeve klijetke između liječene i placebo skupine, nakon 6 mjeseci liječenje matičnim stanicama je znatno ublažilo simptome te poboljšalo kvalitetu života, kao i potrošnju kisika kod analize podskupine bolesnika u dobi do 60 godina. Još jedno novije istraživanje[35] ocijenilo je učinak liječenja stanicama kao dopune operaciji premoštenja (premoštenje koronarne arterije pomoću presatka; CABG, prema eng. *coronary artery bypass graft)* kod bolesnika s ishemijskim zatajivanjem srca koji su kirurški revaskularizirani. Nakon šestomjesečnog praćenja zabilježeno je impresivno poboljšanje EF i smanjenje volumena lijeve klijetke u skupini koja je primila BMMNC.

Dugoročni podaci iz prve randomizirane kontrolirane studije primjene BMMNC kod dilatativne kardiomiopatije (studija ABCD, prema eng. *Autologous Bone marrow Cells in Dilated cardiomyopathy*, autologne stanice koštane srži kod dilatacijske kardiomiopatije) objavljeni su 2010.[36] Kod 41 bolesnika praćenog tijekom 3 godine, EF je bila znatno bolja u liječenoj skupini, s tim da su rezultati bili bolji kod bolesnika sa simptomima 3. stupnja prema NYHA klasifikaciji nego lighting again an ill-defined relationship between potential surrogate markers and hard clinical outcome measures.

One of the most important developments to date is the move from phase II to phase III clinical trials. The majority of the current clinical trials have been designed to assess safety and feasibility only, and being underpowered to assess efficacy of the technology use surrogate markers such as LVEF to assess activity. In order to address this issue, the EU funding programme recently awarded a consortium composed of 17 clinical centres across Europe 6 million euros to design and conduct the definitive outcome study of BMMNC in AMI (BAMI; http://www.bami-fp7.eu). BAMI will enrol 3,000 patients with the primary end point as all-cause mortality making it one of the most exciting developments in the field for several years. The study will be reported in 5 years.

Cell therapy for chronic LV disease

The STAR-heart study is the largest reported experience of BMMNCs in ischaemic heart failure and reported its 5-year follow-up data in 2010.[33] The non-randomised study originally recruited 391 patients with an LVEF of 35% or less who were offered intracoronary administration of autologous BMMNCs. In all, 191 patients received cell therapy and 200 patients received best medical treatment alone. At 5-year follow-up, there were significant improvements in LVEF, contractility, oxygen uptake and exercise tolerance in patients treated with BMMNCs associated with perhaps more interestingly a significantly lower death rate than the control group. This requires confirmation in a double-blinded randomised study. The FOCUS-HF trial[34] is a randomised controlled trial of 30 patients designed to evaluate the effects of transendocardial delivery of BMMNCs in patients with chronic ischaemic heart failure with no option for further revascularisation. At 6 months, although there was no difference in LVEF between the treated and placebo groups, cell therapy was found to significantly improve symptoms and quality of life scores and in subgroup analysis oxygen uptake in patients who were 60 years and younger. Another recent study[35] assessed the effect of cell therapy as an adjunct to bypass surgery (coronary artery bypass graft (CABG)) in patients with ischaemic heart failure undergoing CABG. An impressive increase in LVEF and reduction in LV dimensions in the BMMNC group were reported at 6-month follow-up.

Long-term data from the first randomised controlled trial of BMMNCs in dilated cardiomyopathy (Autologous Bone marrow Cells in Dilated cardiomyopathy (ABCD) trial) were reported in 2010.[36] In the 41 patients followed to 3 years, there was a significant improvement in LVEF in the treatment group, greater in patients with the New York Heart Association (NYHA) class 3 symptoms compared with NYHA class 4 suggesting improvement in patients was greater in those with less severely damaged myocardium. There was also an associated symptomatic improvement but no mortality benefit was shown. Trials of BMMNCs in non-ischaemic cardiomyopathy are ongoing.

kod bolesnika sa simptomima NYHA 4. stupnja, što dovodi do zaključka da su rezultati bili bolji kod bolesnika s manjim oštećenjem miokarda. Simptomi su također bili blaži, no nije dokazano smanjenje smrtnosti. Studije primjene BMMNC kod neishemijske kardiomiopatije su u tijeku.

Translacija drugih vrsta stanica u kliničku primjenu

Još jedno veliko dostignuće u posljednje 2 godine bio je pomak ka kliničkoj translaciji različitih populacija stanica i potraga za optimalnom vrstom stanice za liječenje srca u nizu studija koje se po prvi put provode na ljudima.

Cirkulirajuće/mobilizirane hematopoetske matične stanice, koje se najčešće identificiraju biljezima CD34 i CD133, ispitivane su kao populacije koje su potencijalni kandidati za liječenje srca. Te populacije stanica mogu se odvojiti od BMMNC ili mobilizirati u krvotok pomoću farmakološkog sredstva kao na primjer čimbenika poticanja rasta granulocitnih kolonija (G-CSF, prema eng. *granulocyte colony stimulating factor*). Stanice CD34 sadrže više linija stanica endotelijskog porijekla, a prethodno su ispitivane kod akutnog infarkta i kod refraktorne angine pektoris. Znanstvenici koji su proučavali autologno liječenje stanicama CD34 kod kroničnе ishemije miokarda (ACT-CMI, prema eng. *Autologous Cellular Therapy CD34 in Chronic Myocardial Ischemia*) nedavno su objavili rezultate druge faze velike studije u kojoj je uspoređivano liječenje stanicama provedeno ubrizgavanjem u miokard malih i velikih doza autolognih stanica CD34 prikupljenih iz periferne krvi i liječenje placebom kod 167 bolesnika koji pate od refraktorne angine pektoris. Nakon 6 i 12 mjeseci zabilježena su znatno manja učestalost angine pektoris i bolja tolerancija tjelesnog opterećenja u skupini koja je dobila malu dozu u usporedbi s placebo skupinom. U placebo skupini smrtnost je također bila veća.[37] S druge pak strane, *Chih i sur.* navode da unatoč mobilizaciji stanica CD34 i CD34/CD133 pomoću G-CSF kod bolesnika s kroničnom ishemijskom bolesti srca nije opaženo nikakvo poboljšanje angine pektoris ili perfuzije miokarda.[38] Ovaj nesklad između rezultata tih istraživanja ponovo ukazuje na činjenicu da treba pažljivo razmisliti o načinu primjene i da je ubrizgavanje stanica u miokard možda učinkovitije kod ovakvih bolesnika.

Mezenhimalne matične stanice (MSC — *mesenchial stem cell*) mogu otpustiti cijeli niz kardioprotektivnih parakrinih čimbenika te se mogu diferencirati u više vrsta stanica koje sudjeluju u obnavljanju srca, pa se stoga sve više koriste u kliničkim studijama koje su dale obećavajuće rezultate. Još jedna prednost MSC je njihova logistička dostupnost iz koštane srži i adipoznog tkiva.

Nedavno su objavljeni rezultati dobiveni nakon šestomjesečne randomizirane kontrolirane studije prvi put provedene kod ljudi, a koja je obuhvatila 14 bolesnika i bavila se primjenom autolognih matičnih i regenerativnih stanica porijeklom iz adipoznog tkiva (ADRC, prema eng. *autologous adipose tissue derived stem and regenerative cell*) kod akutnog infarkta (istraživanje APOLLO, prema eng. *adipose-derived stem cells in the treatment of patients with ST-elevation myocardial infarction*, matične stanice porijeklom iz adipoznog tkiva u liječenju bolesnika s infarktom miokarda s elevacijom S-segmenta).[39] Svi bolesnici su podvrgnuti liječenju ili stanicama ili placebom unutar 24 sata od primarne PCI. Uključeni su bili bolesnici sa prvim infarktom miokarda u životu i kod kojih je EF lijeve klijetke bila između 35% i 50%. Nakon 6 mjeseci došlo je do znatnog poboljšanja stvaranja ožiljka miokarda i perfuzijskog defekta, do gotovo znatnog smanjenja veličine infarciranog područja i poboljšanja EF u skupini liječenoj stanicama u usporedbi s kontrolnom skupinom, a liječenje se pokazalo sigurnim. Podaci dobiveni nakon 18 mjeseci predstavljeni su 2011. na Međunarodnom simpoziju o liječenju matičnim stanicama i kardiovaskular-

Translation of other cell types into the clinical setting

Another major development in the last 2 years has been the move towards clinical translation of different cell populations and a search for the optimal cell type for cardiac repair with a number of first-in-human trials.

Circulating/mobilised haemopoietic stem cells identified most commonly by markers CD34 and CD133 have been investigated as potential candidate populations in cardiac repair. These cell populations can either be fractionated from BMMNC or mobilised into the circulation using pharmacological agents such as Granulocyte colony stimulating factor (G-CSF). CD34 cells contain more endothelial lineage determined cells and have been previously evaluated in both AMI and refractory angina. The Autologous Cellular Therapy CD34 in Chronic Myocardial Ischemia (ACT-CMI) investigators have recently reported on a large phase II trial evaluating intramyocardial injection of low and high dose autologous peripherally mobilised CD34 cell therapy against placebo in 167 patients with refractory angina. There was found to be a significant improvement in angina frequency and exercise tolerance in the low dose group compared with placebo at 6 and 12 months. There was also an increased mortality in the placebo arm.[37] In contrast, *Chih et al* report that despite mobilisation of CD34 and CD34/CD133 cells using G-CSF, no improvement in angina or myocardial perfusion was observed in patients with chronic Ischaemic heart disease (IHD).[38] Again, this discrepancy in the findings from these studies suggests that careful consideration to the method of delivery should be given and that intramyocardial delivery may be more effective in this type of patients.

MSCs are able to release a large range of cardioprotective paracrine factors and transdifferentiate into a number of cell types that are involved in cardiac repair and are therefore increasingly being used in clinical trials which have shown promising results. Another advantage of MSCs is their logistical ease of access via bone marrow and adipose tissue.

The 6 month results of the first-in-human randomised controlled 14 patient trial of autologous adipose tissue derived stem and regenerative cells (ADRCs) for AMI (the Adipose-derived stem cells in the treatment of patients with ST-elevation myocardial Infarction (APOLLO) trial) have recently been reported.[39] All patients received either cell therapy or placebo within 24 h of primary PCI. These were first MI patients with an LVEF between 35% and 50%. At 6 months, there was a significant improvement in myocardial scar formation and perfusion defect, near significant reduction in infarct size and improvement in estimated ejection fraction with cell therapy compared with control, and the treatment proved safe. The 18 month data were presented at the 2011 International Symposium on Stem Cell Therapy & Cardiovascular Innovation and showed sustained benefits. The next step, a larger study called ADVANCE, enrolling 375 patients will give greater statistical power. Eighteen month results for a similar first-inhuman trial of ARDCs for ischaemic heart failure, PRECISE, although not yet published, have been presented at the AHA Scientific Sessions 2010.[40] Here, 27 patients were randomised to receive transendocardial ADRCs or placebo. Results at 6 months showed a significant reduction in infarct size in the treatment group relative to the controls but with no difference in LVEF. Out to 18 months, cell therapy was found to be safe with no difference in adverse outcomes between the two groups and found to significantly improve both NYHA and Canadian cardiovascular society (CCS) class symptoms, metabolic equivalents and peak oxygen consumption, in the treatment group.

nim inovacijama, a pokazali su postojanu prednost. Sljedeći korak je veliko istraživanje nazvano ADVANCE, koje obuhvaća 375 bolesnika i koje će pridonijeti statističkoj snazi istraživanja. Rezultati dobiveni nakon 18 mjeseci u sličnoj studiji (PRECISE)koja je provedena po prvi put na ljudima u kojoj su primijenjene ARDC stanice kod ishemijskog zatajivanja srca, nisu još objavljeni, ali su predstavljeni 2010. na znanstvenim skupovima AHA.[40] U ovoj studiji 27 bolesnika je randomizirano za primanje transendokardno ADRC ili placeba. Rezultati nakon 6 mjeseci pokazali su znatno smanjenje veličine zone infarkta u liječenoj skupini u usporedbi s kontrolnom skupinom, dok nije bilo nikakve razlike u EF. Nakon 18 mjeseci pokazalo se da je liječenje stanicama sigurno i da između dvije skupine nema nikakve razlike u nepovoljnim ishodima te se pokazalo da su u liječenoj skupni ublaženi simptomi prema NYHA stupnjevanju i CCS klasifikaciji (CCS, prema eng. *Canadian Cardiovascular Society*) te da su poboljšani metabolički ekvivalenti i maksimalna potrošnja kisika.

Osim autolognih MSC i alogene MSC su također nedavno proučavane kao potencijalno nova strategija liječenja koja bi bila dostupna po potrebi. MSC imaju sposobnost izbjegavanja imunološkog prepoznavanja, što znači da kod ovih bolesnika nije potrebno suprimirati imunološku reakciju. U prvoj fazi randomizirane kontrolirane studije provedene po prvi put na ljudima uspoređivani su alogene MSC i placebo u kontekstu prvog infarkta miokarda i disfunkcije lijeve klijetke kod 53 bolesnika.[41] Ono što je važno je da je istraživanje pokazalo da nema nikakve razlike u nepovoljnim događajima, ponovnoj hospitalizaciji i aritmiji između skupina. Nakon 18 mjeseci liječena skupina zabilježila je znatno bolju EF u odnosu na kontrolnu skupinu. Preliminarni rezultati druge faze randomizirane kontrolirane studije alogenih MSC kod ishemijskog zatajivanja srca objavljeni su 2011. na znanstvenim skupovima AHA.[22] Istraživanje je obuhvatilo 60 bolesnika praćenih tijekom razdoblja od 12 mjeseci i potvrdilo je sigurnost tehnologije. Iako nije bilo nikakve razlike u EF lijeve klijetke između dvije skupine, u liječenoj skupini učestalost velikih neželjenih srčanih događaja, smrtnosti i simptoma bili su znatno manji, što podupire tezu da EF nije pogodan zamjenski parametar za praćenje ishoda.

Nakon što je otkriveno da odraslo srce ima svoju rezervu progenitornih stanica, mogućnost iskorištavanja matičnih stanica srca (CSC, prema eng. *cardiac stem cell*) koje mogu ponovno izrasti u zdravo tkivo srca činila se privlačnom. Opisane su dvije glavne populacije CSC, populacija c-kit+ i stanice porijeklom iz područja srca, koje predstavljaju prirodnu kombinaciju podpopulacija stanica porijeklom iz srca, u koje se ubrajaju c-kit+/CD90- i srčane MSC c-kit-/CD90. Iako nije sigurno hoće li se one pokazati korisnijima od drugih vrsta matičnih stanica, osobito ako budu djelovale na stanice u neposrednoj okolini, obje populacije su proučavane u kliničkoj primjeni.

Nedavno objavljena studija SCIPIO (prema eng. *cardiac stem cells in patients with ischaemic cardiomyopathy*, matične stanice srca kod bolesnika koji pate od ishemijske kardiomiopatije) je studija u prvoj fazi, koja se provodi po prvi put na ljudima, i koje ispituje korisnost c-kit+ CSC kod ishemijskog zatajivanja srca nakon CABG.[42] Autologne c-kit+ stanice aurikule atrija izolirane su tijekom CABG i umnožene, a ponovno se ugrađuju 3-4 mjeseca nakon operacije. Važno je reći da nije bilo nikakve razlike u učestalosti neželjenih događaja između liječene i kontrolne skupine. Nakon 8 mjeseci kod liječenih bolesnika znatno se smanjila veličina zone infarkta, a poboljšala EF. Studija CADUCEUS (prema eng. cardiosphere-derived autologous stem cells to reverse ventricular dysfunction, autologne matične stanice porijeklom iz područja srca u reverziji ventrikularne disfunkcije) prati utjecaj intrakoronarne aplikacije autolognih stanica sr-

Allogeneic as opposed to autologous MSCs have also recently been evaluated as a potential novel therapeutic strategy allowing for 'off-the-shelf' logistical ease. MSCs are able to evade immune detection meaning immunosuppression is not required for these patients. The first-in-human phase I randomised controlled study comparing allogeneic MSCs with placebo in the setting of first AMI and LV dysfunction enrolled 53 patients.[41] Importantly, the study demonstrated no difference in adverse events, rehospitalisation or arrhythmia between the groups. At 18 months, the treatment group conferred significant improvement in LVEF relative to controls. The preliminary results of a phase II randomised controlled trial assessing allogeneic MSCs in the setting of ischaemic heart failure were presented at the AHA Scientific Sessions 2011.[22] The study consisted of 60 patients with a 12 month follow-up period and confirmed safety of the technology. While there was no difference in LVEF between the two groups, there was a significantly lower incidence of major adverse cardiac events, mortality and symptoms in the treated group supporting the concept of LVEF not being a useful surrogate marker for outcome.

The attractive opportunity to exploit cardiac stem cells (CSC) which are capable of regrowing healthy heart tissue was realised with the discovery that the adult heart contains its own reservoir of progenitor cells. There are two main CSC populations that have been described, the c-kit+ population and cardio-sphere-derived cells, which are a natural mix of heart derived cell subpopulations including c-kit+ /CD90- and cardiac MSCs c-kit-/CD90. Although it is uncertain as to whether these will prove advantageous over other stem cell types, particularly if they act in a paracrine manner, both populations have been studied in the clinical setting.

The recently published SCIPIO trial (Cardiac stem cells in patients with ischaemic cardiomyopathy) is a first-in-human phase I trial assessing the value of c-kit+ CSCs in ischaemic heart failure post-CABG.[42] Here, autologous atrial appendage c-kit+ cells are isolated and expanded at the time of CABG and re-infused 3-4 months after surgery. Importantly, there was no difference in adverse event rate between treatment and control arms. At 8 months, there was a significant improvement in infarct size and LVEF in treated patients. The CADUCEUS trial (cardiosphere-derived autologous stem cells to reverse ventricular dysfunction) assessed the impact of intracoronary infusion of autologous cardiosphere-derived cells harvested from endomyocardial biopsies in patients 2-3 months post-AMI in a phase I clinical trial.[43] Here, LVEF was significantly improved at 12 months compared with controls and there was a major reduction in scar mass on Cardiac magnetic resonance imaging (CMR) in the treated but not the control group. There was no difference in adverse outcome between the groups. Importantly, this is one of the first trials of cell therapy to suggest that the benefits seen in relation to myocardial repair are explained by a regenerative process. The results of a phase II trial will be eagerly awaited.

Although the ultimate goal of cell therapy is to restore cardiac function and thereby improve quality of life and survival, the mechanism by which this is achieved using cell therapy continues to remain a topic of debate depending on the cell type used. This area of research has nonetheless led to a better understanding of how cells can in vitro be made to differentiate into a phenotype that may improve cardiac repair. The first results of this approach in humans have recently been published. In the C-Cure trial, the investigators have

ca koje su dobivene endomiokardnim biopsijama kod bolesnika 2-3 mjeseca nakon akutnog infarkta u prvoj fazi kliničke studije.[43] Nakon 12 mjeseci znatno se poboljšala EF u usporedbi s kontrolnom skupinom, a površina ožiljka je bila znatno manja na magnetskoj rezonanciji srca u liječenoj skupini, ali ne i u kontrolnoj skupini. Nije bilo nikakve razlike u neželjenom ishodu između skupina. Važno je reći da je ovo jedna od prvih studija koje ukazuju da je povoljan učinak liječenja stanicama u obnavljanju miokarda zapravo regenerativni proces. Rezultati druge faze studije nestrpljivo se iščekuju.

Iako je krajnji cilj liječenja stanicama oporavak srčane funkcije pa time i poboljšanje kvalitete života i preživljavanje, način na koji se to postiže liječenjem stanicama i dalje ostaje predmet rasprave ovisno o tome koja se vrsta stanica koristi. Ipak, ovo područje istraživanja dovelo je do boljeg razumijevanja kako se stanice mogu diferencirati in vitro u fenotip koji može poboljšati obnavljanje miokarda. Prvi rezultati primjene na ljudima objavljeni su nedavno. U studiji C-Cure znanstvenici su potaknuli diferencijaciju BMMNC u specifične progenitorne stanice srca koristeći kardiogene proteine prije presađivanja stanica transendokardijalnim putem[44] kod 45 bolesnika s ishemijskim zatajivanjem srca. Nakon 6 mjeseci praćenja EF lijeve klijetke se značajno poboljšala, a volumen lijeve klijetke se smanjio uz znatno ublažavanje simptoma prisutnih kod šestominutnog testa hodanja u liječenoj skupini u usporedbi s kontrolnom skupinom. Nije bilo nikakve značajne razlike u nepovoljnom ishodu. U tijeku je druga faza studije.

Zaključak

Istraživanje liječenja stanicama otvara mogućnosti za potpuno novi pristup liječenju u kardiologiji. U posljednje 2 godine prati se sustavni napredak kliničkih studija iz prve u drugu fazu ispitivanja koristeći provjerene vrsta stanica, uz pojavu i novijih vrsta stanica u prvoj fazi istraživanja, što sve je postalo izvedivo zahvaljujući brzoj translaciji istraživanja za primjenu kod ljudi. Što se tiče pragmatičnog pristupa liječenju matičnim stanicama porijeklom iz koštane srži, novija meta-analiza ponovno potvrđuje potencijal povoljnog učinka, čemu je sada usmjerena treća faza praćenja ishoda, koja će ujedno i standardizirati metode obrade i načina primjene stanica. Ostale vrste stanica će morati prijeći sličan put ispitivanja, a nesumnjivo je da će studije matičnih stanica porijeklom iz koštane srži postaviti standarde po kojima će se potom ocjenjivati druge vrste stanica kao i primjenjene tehnike.

driven the differentiation of BMMNCs into lineage-specific cardiac progenitor cells using cardiogenesis proteins before cell transfer via the transendocardial route[44] to 45 patients with ischaemic heart failure. At 6 month follow-up, there was significant improvement in LVEF and reduction of LV volumes as well as significant symptomatic improvement evidenced by the 6 min walk test in the treated group compared with the control group. There were no significant differences in adverse outcome. The second phase of this trial is ongoing.

Summary

Cell therapy research offers the prospect of a completely new therapeutic approach in cardiology. The last 2 years has seen a systematic move from phase I to phase II clinical trials using established cell types together with the emergence of new cell types in phase I studies that have only become feasible due to the research that has been driven by the early translation into humans. For the pragmatic approach of bone marrow derived cell therapy, recent meta-analysis again confirms the potential for benefit and this will now be addressed in a phase III outcome study that will also standardise the technique of cell processing and administration. Other cell types will need to follow a similar path of investigation and no doubt the trials of bone marrow derived cells will set the standards by which different cell types and techniques will be judged.

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