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To serve and protect: a new heart patrolling and recycling role for macrophages

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Commentary on 'A network of macrophages supports mitochondrial homeostasis in the heart' by J.A. Nicolás-Ávila *et al.* 2020, *Cell* 183, 94–109.e23.

Genetic and acquired mitochondria dysfunction has a profound impact on cardiac performance and contributes to cardiovascular ageing, cardiovascular diseases, and ischaemia/reperfusion injuries evoked by coronary procedures. The pump function of the human adult heart requires enormous amount of ATP daily. To ensure this supply, cardiac myocytes are packed with mitochondria engaged with oxidation of fuels, such as fatty acid. Mitochondria are also the primary source of reactive oxygen species and regulate cell death and survival. Mitochondria homeostasis is ensured by the balance between the processes of mitochondrial fusion, fission (fragmentation), biogenesis, and mitophagy. New evidences by Nicolás-Ávila *et al.* suggest that the uptake of cardiomyocyte-released dysfunctional mitochondria by macrophages (Macs) could ensure an additional layer of control against the cardiac consequence of mitochondrial dysfunction.

In a stimulating study entitled 'A network of macrophages supports mitochondrial homeostasis in the heart' and recently published in *Cell*, the authors expand the list of skills and roles played by Macs in the heart. The paper reveals a potentially novel mechanism by which Macs support cardiac homeostasis by ensuring the clearance of dysfunctional mitochondria expelled from neighbouring cardiac myocytes into '*cardiac exopheres*', a potentially new type of extracellular vesicles (EVs).[1] According to the authors, '*cardiac exopheres*' are 'specifically' taken up by cardiac Macs (cMacs) using an active phagocytosis process mediated by Mertk (Tyrosine-protein kinase Mer). Experiments using an inducible genetic model of temporary Mac depletion ($CD169^{DTR}$ mice) have further characterized the importance of Macs to maintain cardiac function by ensuring cardiac mitochondrial fitness, proteostasis and ATP production, and avoiding the activation of the inflammasome system. An impressive combination of mouse models, including genetic models, bone marrow transplantation, parabiosis and the use of isoproterenol to induce cardiac stress, have been displayed to provide information on the cellular sources of mitochondria released by *exopheres* and the importance of the lysosomal degradation pathway (autophagy) and Mertk in respectively, triggering the newly proposed mitochondria shedding mechanism and allowing exosphere incorporation by Macs under homeostatic conditions and cardiac stress. Mitophagy enables the clearance of compromised mitochondria via their relocation into autophagosomes; this mechanism is essential in cardiomyocytes to ensure cellular energy production and optimal cardiac function. Several pathological stressors, including ischaemic, oxidative, and cardiotoxic injuries, can impair cardiomyocyte mitophagy, leading to the onset of cardiovascular disease, as extensively reviewed in ref.[2]

The study by Nicolás-Ávila *et al.* is important to confirm and reinforce the importance of multicellular communication within the cardiac tissue. Indeed, this work described an additional role for Macs in interacting with cardiovascular cells to further contribute to relevant homeostatic and reparative mechanisms (*Figure 1*). This new set of discoveries adds to other new and surprising Mac roles that have recently emerged, fuelling the interest for the crosstalk between Macs and their cardiac cell neighbours. On top of their established role in immunity, Macs have been recently shown to contribute to cardiac electrical conduction[3] and produce collagen during scar formation in zebrafish and mouse heart injury models.[4] A groundbreaking paper published this year by the Molkentin group has additionally shown that the temporal and regional induction of $CCR2^{+}$ and $CX3CR1^{+}$ macrophages mediates and can even replace adult stem cells-induced cardiac rejuvenation.[5] Changes in cMac activity might also help explain the reduced endogenous reparative potential of the mammalian heart after the first days of post-natal life, when the neonatal rodent heart responds to experimentally induced myocardial infarct with a substantial activation of cardiomyocyte proliferation and angiogenesis.[6]

Beyond the heart, understanding the intimate signalling relationship between Mac and tissue-resident cells could also be important to develop new approaches against peripheral vascular disorders. As an example, alteration in the secretory activity of Mac within the perivascular adipose tissue has been recently linked to development of endothelial lipids accumulation and obesity-associated hypertension,[7] substantiating the crucial influence of tissue-specific Macs as paracrine signalling hub towards neighbouring cells.

Nicolás-Ávila *et al.* have reported exciting findings. It is therefore understandable that some of them call for additional investigation in future studies. It would be particularly important to characterize the Macs contribution to the '*cardiac exosphere*' elimination and how Mac subsets contribute to the different cardioprotective activities showed in the paper.

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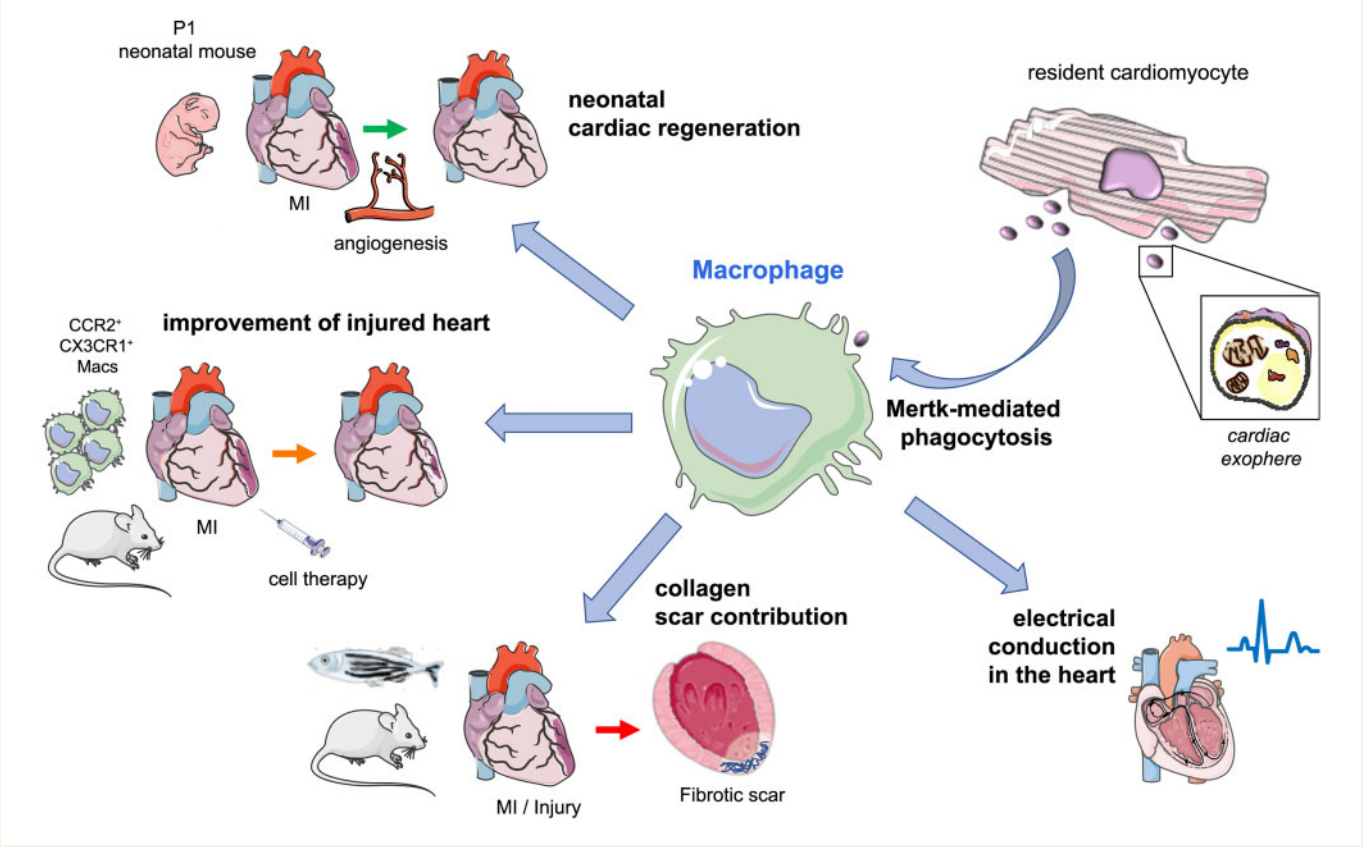

Figure 1 Schematic of different roles of macrophages within the cardiac tissue, in homeostatic vs. injured conditions, and in adulthood reparative models vs. neonatal regenerative ones. Macs, macrophages; MI, myocardial infarction/injury; P1, post-natal day 1. Schematic has been produced using Smart—Servier Medical Art (https://smart.servier.com).

The identification and targeting of the Macs present in the heart is still cumbersome. Authors have used a CX3CR1-GFP mouse model to image Macs in the heart and to study the contacts between cardiomyocytes and Macs. Then, they have globally depleted CD169-positive cells to study the functional roles of Mac in the heart. However, neither CX3CR1 nor CD169 is specific to cMacs. Indeed, CD169-positive cells are enriched in secondary lymph organs, such as lymph nodes and spleen, and reportedly contribute to adaptive immunity via interacting with B cells, T cells, and dendritic cells (DCs). This is stimulating because both Macs and DCs exert Mertk-mediated phagocytotic actions and use the autophagy–lysosomal pathway to degrade proteins and produce short peptides that are loaded and presented on major histocompatibility complex class I of cells, thus regulating T-cell activity. It would be therefore interesting to investigate the systemic immunological consequences of CD169 depletion and Mertk inhibition used by Nicolás-Ávila *et al.* This potential line of exploration is amplifed by a recent publication, where Forte *et al* reported that DCs respond to myocardial injury fuelling autoimmunity within the heart.[8] The Mertk-mediated phagocytosis used by Macs to incorporate exosphere may enlighten the correlation of genetic deficiency of with the increased accumulation of apoptotic cardiac cells and cardiac dysfunction, following cardiac injury. Likewise, Mertk activity has been associated with regulation of inflammatory response and efferocytosis with infarct size reduction.[9] Such evidence prompts additional mechanistic investigations on the putative cardioprotective function of Macs.

Nicolás-Ávila *et al.* have defined *cardiac exopheres* as sub-cellular particles released by cardiomyocytes. It would be important to attempt classifying *cardiac exopheres* complying with the guidelines defined by the International Society for Extracellular Vesicle (EV).[10] In particular, a more comprehensive analysis on *cardiac exopheres* structural organization and molecular signature under health and disease would help to fully appreciate their putative role in intercellular communication. Noteworthy, EVs have already been shown to transport functional mitochondria between cells; the EV-mediated transfer of mitochondria from progenitor cells to cardiomyocytes has been associated with therapeutic effects in the ischaemic myocardium, where it restored bioenergetic levels.[11] Mitochondria and mitochondria components are also transferred between cardiomyocytes using different EVs type and molecular nanotubes, dynamically contributing to continuous intercellular networking.[12] *Cardiac exopheres* resemble apoptotic bodies in dimensions. Of note, the apoptotic metabolite secretome has been reported to exert relevant beneficial effects by modulating inflammatory process and Mac functional phenotype in an inflammatory disease mouse model.[13] It is therefore important to integrate the *cardiac exosphere* uptake by Macs as part of a wider cell-to-cell communication system.

Overall, the study by Nicolás-Ávila *et al.* have provided new insights on the peculiar role of immune cells in pairing up with cardiomyocytes to preserve organ function under homeostatic conditions. The new finding candidates Macs as putative therapeutic targets to correct cardiomyocyte mitochondrial dysfunction. Future work should include

comprehensive investigations on the Mac behaviour using models of cardiac chronic disease, ischaemia/reperfusion myocardial injury, and cardiac rejuvenation.

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References

1. Nicolás-Ávila JA, Lechuga-Vieco AV, Esteban-Martínez L, Sánchez-Díaz M, Díaz-García E, Santiago DJ, Rubio-Ponce A, Li JL, Balachander A, Quintana JA, Martínez-de-Mena R, Castejón-Vega B, Pun-García A, Través PG, Bonzón-Kulichenko E, García-Marqués F, Cussó L, A-González N, González-Guerra A, Roche-Molina M, Martin-Salamanca S, Crainiciuc G, Guzmán G, Larrazabal J, Herrero-Galán E, Alegre-Cebollada J, Lemke G, Rothlin CV, Jimenez-Borreguero LJ, Reyes G, Castrillo A, Desco M, Muñoz-Cánoves P, Ibáñez B, Torres M, Ng LG, Priori SG, Bueno H, Vázquez J, Cordero MD, Bernal JA, Enríquez JA, Hidalgo A. A network of macrophages supports mitochondrial homeostasis in the heart. *Cell* 2020;**183**:94–109.e23.
2. Kaludercic N, Maiuri MC, Kaushik S, Fernández ÁF, Bruijn J, De Castoldi F, Chen Y, Ito J, Mukai R, Murakawa T, Nah J, Pietrocola F, Saito T, Sebti S, Semenzato M, Tsansizi L, Sciarretta S, Madrigal-Matute J. Comprehensive autophagy evaluation in cardiac disease models. *Cardiovasc Res* 2020;**116**:483–504.
3. Hulsmans M, Clauss S, Xiao L, Aguirre AD, King KR, Hanley A, Hucker WJ, Wülfers EM, Seemann G, Courties G, Iwamoto Y, Sun Y, Savol AJ, Sager HB, Lavine KJ, Fishbein GA, Capen DE, Da Silva N, Miquerol L, Wakimoto H, Seidman CE, Seidman JG, Sadreyev RI, Naxerova K, Mitchell RN, Brown D, Libby P, Weissleder R, Swirski FK, Kohl P, Vinegoni C, Milan DJ, Ellinor PT, Nahrendorf M. Macrophages facilitate electrical conduction in the heart. *Cell* 2017;**169**:510–522.e20.
4. Simões FC, Cahill TJ, Kenyon A, Gavriouchkina D, Vieira JM, Sun X, Pezzolla D, Ravaud C, Masmanian E, Weinberger M, Mayes S, Lemieux ME, Barnette DN, Gunadasa-Rohling M, Williams RM, Greaves DR, Trinh LA, Fraser SE, Dallas SL, Choudhury RP, Sauka-Spengler T, Riley PR. Macrophages directly contribute collagen to scar formation during zebrafish heart regeneration and mouse heart repair. *Nat Commun* 2020;**11**:600.
5. Vagnozzi RJ, Maillet M, Sargent MA, Khalil H, Johansen AKZ, Schwanekamp JA, York AJ, Huang V, Nahrendorf M, Sadayappan S, Molkentin JD. An acute immune response underlies the benefit of cardiac stem cell therapy. *Nature* 2020;**577**:405–409.
6. Aurora AB, Porrello ER, Tan W, Mahmoud AI, Hill JA, Bassel-Duby R, Sadek HA, Olson EN. Macrophages are required for neonatal heart regeneration. *J Clin Invest* 2014;**124**:1382–1392.
7. Zhu X, Wang Y, Zhu L, Zhu Y, Zhang K, Wang L, Bai H, Yang Q, Ben J, Zhang H, Li X, Xu Y, Chen Q. Class A1 scavenger receptor prevents obesity-associated blood pressure elevation through suppressing overproduction of vascular endothelial growth factor B in macrophages. *Cardiovasc Res* 2021;**117**:547–560.
8. Forte E, Perkins B, Sintou A, Kalkat HS, Papanikolaou A, Jenkins C, Alsubaie M, Chowdhury RA, Duffy TM, Skelly DA, Branca J, Bellahcene M, Schneider MD, Harding SE, Furtado MB, Ng FS, Hasham MG, Rosenthal N, Sattler S. Cross-priming dendritic cells exacerbate immunopathology after ischemic tissue damage in the heart. *Circulation* 2020.
9. McShane L, Tabas I, Lemke G, Kurowska-Stolarska M, Maffia P. TAM receptors in cardiovascular disease. *Cardiovasc Res* 2019; **115**:1286–1295.
10. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, Antoniou A, Arab T, Archer F, Atkin-Smith GK, Ayre DC, Bach J-M, Bachurski D, Baharvand H, Balaj L, Baldacchino S, Bauer NN, Baxter AA, Bebawy M, Beckham C, Bedina Zavec A, Benmoussa A, Berardi AC, Bergese P, Bielska E, Blenkiron C, Bobis-Wozowicz S, Boilard E, Boireau W, Bongiovanni A, Borràs FE, Bosch S, Boulanger CM, Breakefield X, Breglio AM, Brennan MÁ, Brigstock DR, Brisson A, Broekman ML, Bromberg JF, Bryl-Górecka P, Buch S, Buck AH, Burger D, Busatto S, Buschmann D, Bussolati B, Buzás EI, Byrd JB, Camussi G, Carter DR, Caruso S, Chamley LW, Chang Y-T, Chen C, Chen S, Cheng L, Chin AR, Clayton A, Clerici SP, Cocks A, Cocucci E, Coffey RJ, Cordeiro-da-Silva A, Couch Y, Coumans FA, Coyle B, Crescitelli R, Criado MF, D'Souza-Schorey C, Das S, Datta Chaudhuri A, de Candia P, De Santana EF, De Wever O, del Portillo HA, Demaret T, Deville S, Devitt A, Dhondt B, Di Vizio D, Dieterich LC, Dolo V, Dominguez Rubio AP, Dominici M, Dourado MR, Driedonks TA, Duarte FV, Duncan HM, Eichenberger RM, Ekström K, EL Andaloussi S, Elie-Caille C, Erdbrügger U, Falcón-Pérez JM, Fatima F, Fish JE, Flores-Bellver M, Försönits A, Frelet-Barrand A, Fricke F, Fuhrmann G, Gabrielsson S, Gámez-Valero A, Gardiner C, Gärtner K, Gaudin R, Gho YS, Giebel B, Gilbert C, Gimona M, Giusti I, Goberdhan DC, Görgens A, Gorski SM, Greening DW, Gross JC, Gualerzi A, Gupta GN, Gustafson D, Handberg A, Haraszti RA, Harrison P, Hegyesi H, Hendrix A, Hill AF, Hochberg FH, Hoffmann KF, Holder B, Holthofer H, Hosseinkhani B, Hu G, Huang Y, Huber V, Hunt S, Ibrahim AG-E, Ikezu T, Inal JM, Isin M, Ivanova A, Jackson HK, Jacobsen S, Jay SM, Jayachandran M, Jenster G, Jiang L, Johnson SM, Jones JC, Jong A, Jovanovic-Talisman T, Jung S, Kalluri R, Kano S-I, Kaur S, Kawamura Y, Keller ET, Khamari D, Khomyakova E, Khvorova A, Kierulf P, Kim KP, Kislinger T, Klingeborn M, Klinke DJ, Kornek M, Kosanović MM, Kovács ÁF, Krämer-Albers E-M, Krasemann S, Krause M, Kurochkin IV, Kusuma GD, Kuypers S, Laitinen S, Langevin SM, Languino LR, Lannigan J, Lässer C, Laurent LC, Lavieu G, Lázaro-Ibáñez E, Le Lay S, Lee M-S, Lee YXF, Lemos DS, Lenassi M, Leszczynska A, Li IT, Liao K, Libregts SF, Ligeti E, Lim R, Lim SK, Linē A, Linnemannstöns K, Llorente A, Lombard CA, Lorenowicz MJ, Lörincz ÁM, Lötvall J, Lovett J, Lowry MC, Loyer X, Lu Q, Lukomska B, Lunavat TR, Maas SL, Malhi H, Marcilla A, Mariani J, Mariscal J, Martens-Uzunova ES, Martin-Jaular L, Martinez MC, Martins VR, Mathieu M, Mathivanan S, Maugeri M, McGinnis LK, McVey MJ, Meckes DG, Meehan KL, Mertens I, Minciacchi VR, Möller A, Møller Jørgensen M, Morales-Kastresana A, Morhayim J, Mullier F, Muraca M, Musante L, Mussack V, Muth DC, Myburgh KH, Najrana T, Nawaz M, Nazarenko I, Nejsum P, Neri C, Neri T, Nieuwland R, Nimrichter L, Nolan JP, Nolte-'t Hoen EN, Noren Hooten N, O'Driscoll L, O'Grady T, O'Loghlen A, Ochiya T, Olivier M, Ortiz A, Ortiz LA, Osteikoetxea X, Østergaard O, Ostrowski M, Park J, Pegtel DM, Peinado H, Perut F, Pfaffl MW, Phinney DG, Pieters BC, Pink RC, Pisetsky DS, Pogge von Strandmann E, Polakovicova I, Poon IK, Powell BH, Prada I, Pulliam L, Quesenberry P, Radeghieri A, Raffai RL, Raimondo S, Rak J, Ramirez MI, Raposo G, Rayyan MS, Regev-Rudzki N, Ricklefs FL, Robbins PD, Roberts DD, Rodrigues SC, Rohde E, Rome S, Rouschop KM, Rughetti A, Russell AE, Saá P, Sahoo S, Salas-Huenuleo E, Sánchez C, Saugstad JA, Saul MJ, Schiffelers RM, Schneider R, Schøyen TH, Scott A, Shahaj E, Sharma S, Shatnyeva O, Shekari F, Shelke GV, Shetty AK, Shiba K, Siljander PR-M, Silva AM, Skowronek A, Snyder OL, Soares RP, Sódar BW, Soekmadji C, Sotillo J, Stahl PD, Stoorvogel W, Stott SL, Strasser EF, Swift S, Tahara H, Tewari M, Timms K, Tiwari S, Tixeira R, Tkach M, Toh WS, Tomasini R, Torrecilhas AC, Tosar JP, Toxavidis V, Urbanelli L, Vader P, van Balkom BW, van der Grein SG, Van Deun J, van Herwijnen MJ, Van Keuren-Jensen K, van Niel G, van Royen ME, van Wijnen AJ, Vasconcelos MH, Vechetti IJ, Veit TD, Vella LJ, Velot É, Verweij FJ, Vestad B, Viñas JL, Visnovitz T, Vukman KV, Wahlgren J, Watson DC, Wauben MHM, Weaver A, Webber JP, Weber V, Wehman AM, Weiss DJ, Welsh JA, Wendt S, Wheelock AM, Wiener Z, Witte L, Wolfram J, Xagorari A, Xander P, Xu J, Yan X, Yáñez-Mó M, Yin H, Yuana Y, Zappulli V, Zarubova J, Žėkas V, Zhang J-y, Zhao Z, Zheng L, Zheutlin AR, Zickler AM, Zimmermann P, Zivkovic AM, Zocco D, Zuba-Surma EK. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018;**7**:1535750.
11. Ikeda G, Santoso M, Tada Y, Vaskova E, O'Brien CG, Jung JH, Yang PC. Mitochondria containing extracellular vesicles from autologous induced pluripotent stem cell derived cardiomyocytes restore bioenergetics in ischemic myocardium. *J Am Coll Cardiol* 2020;**75**:3659.
12. Shen J, Zhang JH, Xiao H, Wu JM, He KM, Lv ZZ, Li ZJ, Xu M, Zhang YY. Mitochondria are transported along microtubules in membrane nanotubes to rescue distressed cardiomyocytes from apoptosis article. *Cell Death Dis* 2018;**9**:81.
13. Medina CB, Mehrotra P, Arandjelovic S, Perry JSA, Guo Y, Morioka S, Barron B, Walk SF, Ghesquière B, Krupnick AS, Lorenz U, Ravichandran KS. Metabolites released from apoptotic cells act as tissue messengers. *Nature* 2020;**580**:130–135.

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Biography: Dr Sveva Bollini graduated in Medical Biotechnology and got her PhD from the University of Padova in Italy, where she studied the cardiomyogenic differentiation potential of human amniotic fluid stem cells with Prof. Paolo De Coppi. During her post-doctoral work, she worked on the lineage characterization of reactivated epicardium-derived progenitor cells for cardiac repair under the supervision of Prof. Paul Riley, at UCL-University College London, London and at the University of Oxford, Oxford in UK. In 2014, she was presented with the '*Rita Levi Montalcini*' Young Investigator Award from the Italian Ministry of Research and Education (MIUR) and invited back to Italy to study the paracrine potential of the human amniotic fluid stem cell *secretome* (i.e. the whole of cell-secreted soluble factors and extracellular vesicles-EVs) to enhance cardiac repair.

Currently, she is Group Leader and Associate Professor in Experimental Biology in the Department of Experimental Medicine, University of Genova in Genova, Italy. Her research mainly focuses on the functional characterization of human foetal and perinatal stem cell secretome to unlock endogenous mechanisms of cardiac regeneration following injury and to rejuvenate myocardial renewal.

Dr Bollini is a member of the ESC Working Group on Cellular Biology of the Heart (2016) and on Cardiovascular Regenerative and Reparative Medicine (2017). In 2019, she became a member of the Cardiovascular Committee of the International Society for Cell & Gene Therapy and a member of the ESC Scientists of Tomorrow Nucleus.

Biography: Prof. Costanza Emanueli graduated in Biological Sciences from the University of Florence (Italy). She developed PhD studies in pharmacology (focusing on the kallikrein-kinin system) working with Prof Pierangelo Geppetti as part of an experimental multi-centric programme in Neurovegetative Medicine across Italian Universities, and when visiting the laboratory of Prof Nigel W. Bunnett at the University of California San Francisco. As an early career scientist, she focused on vascular gene therapy and therapeutic angiogenesis, working at the IDI Research Hospital in Rome (with Professor Maurizio Capogrossi), the Saint Elisabeth Medical Centre—Tufts University, Boston (Professor Jeffrey Isner), and the National Institute of Biostructures and Biosystems (INBB, an Italian intra-Universities consortium) and in Sassari-Sardinia (with Dr Paolo Madeddu). Prof Emanueli (from INBB) was part of the European Vascular Genomics Network (EVGN), a Network of Excellence funded by the European Union within its Framework Programme Six, implemented in 2004. The EVGN membership contributed to Prof Emanueli's decision to remain in (pre-Brexit) Europe, rather than pursuing a career in the USA. She subsequently obtained a British Heart Foundation (BHF) Lectureship award (contributing her salary and research programme budget—renewed in 2010 as a BHF Senior Research Fellowship), which allowed her relocation to the University of Bristol as Senior Lecturer in 2005. In 2010, she was promoted to Professor in Vascular Pathology. From 2015 to 2018, she served as British Heart Foundation Professor and Chair in Cardiovascular Science at the Bristol Heart Institute. She currently occupies the same role at the Imperial College London—National Heart and Lung Institute (NHLI).