Appendix 1 (as supplied by the authors): Methodologic details for updated meta-analysis, blood counts, stem cell mobilization and left ventricular diastolic function

Inclusion Criteria

Studies included in the analysis had to be prospective randomized placebo controlled trials of G-CSF for patients sustaining AMI with blinded assessment of LVEF. The primary follow-up endpoint was LVEF, as assessed by any modality, and secondarily we looked for major adverse cardiac events (MACE – defined as death, reinfarction, severe CHF, revascularization of any vessel, or cerebrovascular accident). No required minimum time to follow-up was outlined and in the event of serial publications based on a single trial the most recent analysis was used for analysis. Only data from published trials were included in this analysis and the language was restricted to English.

Trials that were retrospective, non-randomized, quasi-randomized, lacked placebo control or that the necessary follow up data were not available were excluded.

Literature Search

For accumulating relevant information, a search was performed of PubMed, MEDLINE (1966 until present), the Cochrane Library Databases, and SCOPUS. For all databases, permutations of the following keywords were used: G-CSF, clinical trial, myocardial and Filgrastim. For MEDLINE, human trials and publications in English were selected. Secondarily, additional references were identified using the related article feature in Pubmed, and all references of identified studies as well as citing articles were

Appendix to: Hibbert B, Hayley B, Beanlands RS, et al. Granulocyte colony-stimulating factor therapy for stem cell mobilization following anterior wall myocardial infarction: the CAPITAL STEM MI randomized trial. *CMAJ* 2014. DOI 10.1503/cmaj.140133.

identified utilizing the SCOPUS database. Trials that were included in this study were those that were available as of March 30th, 2014.

Data extraction

Two independent investigators conducted the literature search, confirmed eligibility of the trials, and extracted the data. Results of these processes were then compared and disagreements resolved by consensus (**Supplementary Figure 1**). A third investigator reviewed the identified manuscripts and confirmed eligibility and data extraction.

Blood Counts and Stem Cell Mobilization:

Baseline and follow-up complete blood counts and leukocyte differentials were performed. CD34+ cells were enumerated using the ISHAGE methodology following staining with anti-CD34 and anti-CD45 monoclonal antibodies on a LSR flow cytometer (Becton-Dickson) as previously described.(1) Baseline blood counts were obtained and blood work analyzed on day 0 (prior to treatment), day 4, day 7, at 6 weeks and at 26 weeks in the Ottawa Hospitals clinical laboratory.

Left Ventricular Diastolic Function:

Diastolic function was assessed at baseline and at 6 month follow-up by transthoracic echocardiography. Detailed exams where performed using modern echocardiographic systems capable of 2-D, conventional Doppler and tissue Doppler

measurements. Standard echocardiographic views were obtained by multifrequency transducers to assess: (i) biplane left atrial volume,(2) (ii) pulse Doppler trans-mitral inflow velocities, and (iii) tissue Doppler mitral annular velocities.(3) Trans-mitral inflow measurements included the peak early filling velocity (E), peak late filling velocity (A), deceleration time of the peak early filling velocity (DT) and the ratio of peak early and late filling velocities (E/A ratio). Tissue Doppler peak early diastolic mitral annular velocity (e') and late diastolic mitral annular velocity (a') were measured at the septal and lateral mitral annulus and an average measurement obtained from the two sites. The E/e' ratio, a measure of left atrial pressure, was computed from the average of the septal and lateral e' as this approach has been shown to provide the optimal accuracy in patients with regional wall motion abnormalities.(4). Diastolic dysfunction was also graded as normal, grade 1 (impaired LV relaxation), grade 2 (pseudonormal filling) and grade 3 (restrictive filling) as per established guidelines from the American Society of Echocardiography.(3) All echocardiographic analyses were performed off-line on Xcelera workstations (Philips, Andover, Massachusetts).

Complete baseline and follow-up diastolic parameters were available in 34 of the G-CSF and 31 of the placebo treated patients. No baseline differences between diastolic parameters were present between the placebo and G-CSF groups. At 6 month follow-up, differences between the placebo and G-CSF groups were present for the lateral E' (8.3 ± 2.3 vs 9.7 ± 2.4, $p=0.02$), the E/e' ratio (9.2 ± 3.1 vs 7.7 ± 3.5, $p=0.01$), and the left atrial volumes (44.3 ± 14.0 vs. 57.4 ± 23.5, $p=0.04$). However, there was no difference in the E/A ratio, the deceleration time, or percent of patients with grade 2/3 diastolic

dysfunction at 6 month follow-up. Importantly, there were no significant differences in the change in diastolic function parameters from baseline to 6 month follow-up between the placebo and G-CSF arms and no difference in the percent of patients with a $\geq$1 grade improvement in diastolic dysfunction.

References

1. Hibbert B, Ma X, Pourdjabbar A, et al. Pre-procedural atorvastatin mobilizes endothelial progenitor cells: clues to the salutary effects of statins on healing of stented human arteries. PLoS One 2011; 6:e16413.

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3. Nagueh SF, Appleton CP, Gillebert TC, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Eur J Echocardiogr 2009; 10:165-93.

4. Rivas-Gotz C, Manolios M, Thohan V, Nagueh SF. Impact of left ventricular ejection fraction on estimation of left ventricular filling pressures using tissue Doppler and flow propagation velocity. Am J Cardiol 2003; 91:780-4.