SUPPLEMENTAL MATERIAL

Supplemental Methods

Retrospective Clinical Data

All STEMI patients undergoing PPCI admitted to a large United Kingdom (UK) tertiary center between April 2008 and February 2010 were identified retrospectively from the local PPCI database. The diagnosis for STEMI was based on the criteria of chest pain of onset within 12 hours associated with persistent ST segment elevation of 0.1mV in at least two contiguous leads or new left bundle branch block (LBBB). A total of 1531 consecutive patients were identified, and clinical data obtained from the local database. The long-term outcome following hospital discharge was the main outcome of interest from this component of the study, and, as such, 63 patients were excluded due to in-hospital mortality. A further 91 cases were excluded due to previous inclusion from a prior admission (n=29), known malignancy (30), acute inflammatory of infectious disease (n=4), organ transplantation (n=5) or unavailability of data (n=23), leaving a total of 1377 patients included in the final analysis.

The blood results (full blood counts) of each patient were identified on the local pathology database, and the time of PPCI procedure used as a reference point to determine and record the results of tests. Those recorded were the full blood count closest to time of PPCI, within and nearest to 24 hours post PPCI, and within and nearest to 48 hours post PPCI. The minimum lymphocyte count during the admission for PPCI was also identified. Mortality data was then obtained using data provided by the Office of National Statistics, who record all mortality in the UK. This information was already linked to the hospital database using the unique identifier National Health Service (NHS) number for each patient. Mortality was assessed up to July 2011 giving follow up of 40 months (mean 25.2 months).

Statistical analysis was performed using SPSS (version 21) and graphs generated using GraphPad Prism (version 6). Survival distributions for the time to event were estimated using the Kaplan-Meier method and compared using the log-rank test. The effects on long-term mortality of known prognostic variables, as well as all other baseline variables that significantly differed between the minimum lymphocyte tertile groups, were examined using stepwise Cox proportional hazards regression analysis. The variables of gender, age, minimum lymphocyte tertile, hypertension, previous stroke/transient ischemic attack (TIA), smoking status, previous angina, previous MI, previous PCI, cardiogenic shock, anterior vs. other infarct locations, multivessel PCI, body mass index (BMI), serum creatinine, serum cholesterol, hemoglobin, and use of the drugs aspirin, clopidogrel, statins, beta-blockers, ACE-inhibitors/angiotensin receptor blockers (ARBs) and glycoprotein IIb/IIIa inhibitors were all entered as covariates in this analysis.

Prospective Lymphocyte Subset Characterization Data

Patient Populations and Blood Sampling

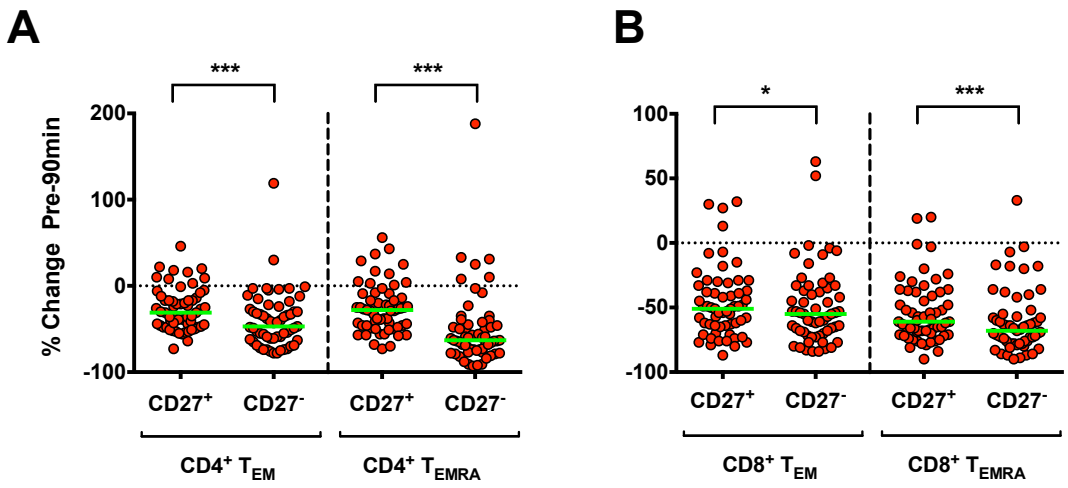
A cohort of 59 STEMI patients admitted to a single large tertiary center were prospectively identified and enrolled in the study following informed consent at the time of admission. Inclusion criteria were chest pain of onset within 6 hours with new ST segment elevation of 0.1mV in at least two contiguous leads. Patients with any of the following were excluded from the study: cardiogenic shock, previous myocardial infarction or coronary artery bypass grafting (CABG), known active malignant process, active infection, chronic inflammatory conditions requiring treatment with immunosuppressive agents, any pre-existing contraindication to cardiac MRI scanning (e.g. pacemaker, severe claustrophobia, breathlessness or frailty likely to limit tolerability of scan), patent arterial flow (Thrombolysis in Myocardial Infarction (TIMI) grade 2 or 3 flow) in infarct related artery on initial angiography, presence of visible

collateral circulation supplying infarct region, or inability or unwillingness to give informed consent.

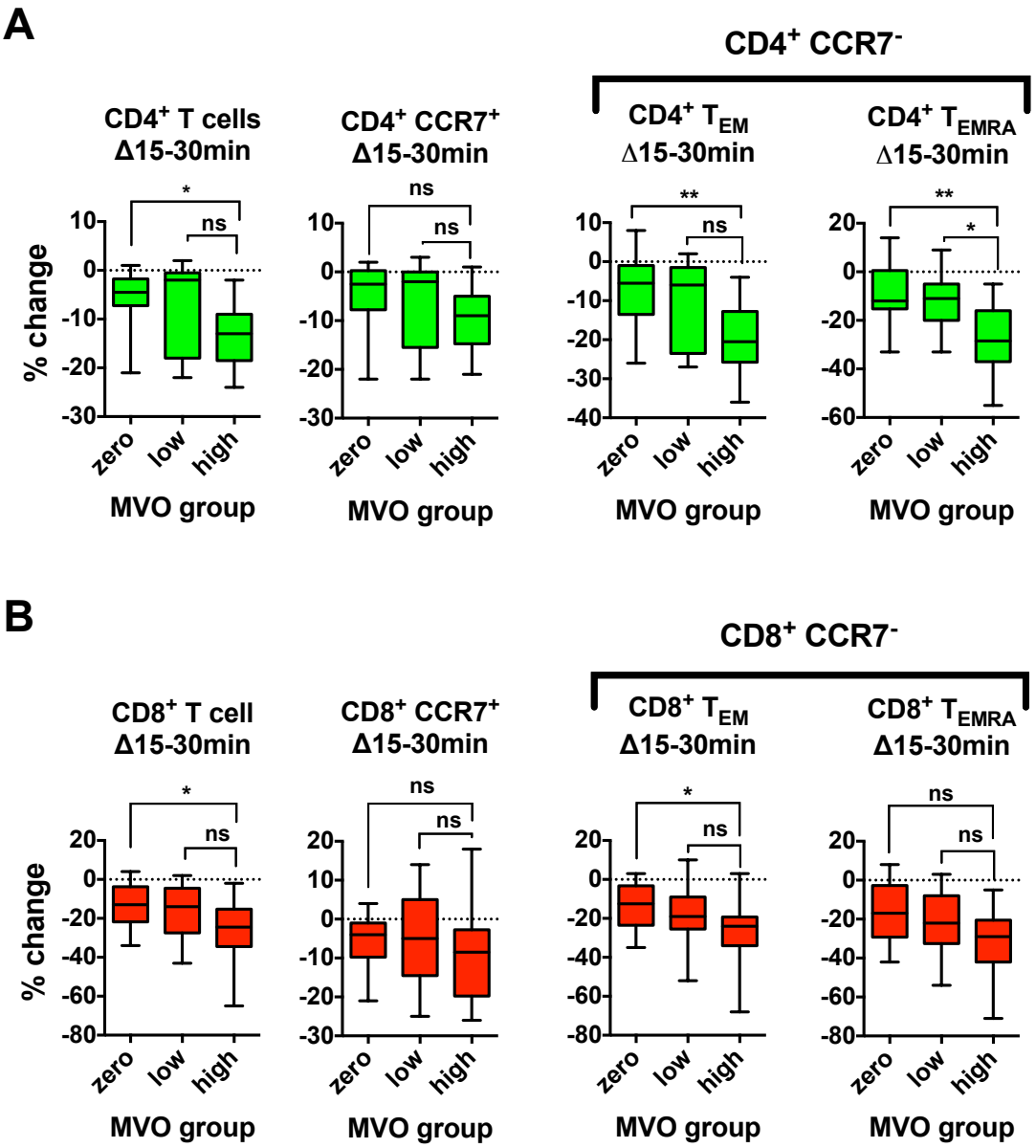
Coronary angiography and PPCI were performed as per standard clinical care, with arterial access via the radial or femoral artery. Arterial blood was acquired for the study via the arterial sheath or catheter at the start of the procedure, then at 15, 30 and 90 minutes following reperfusion, as determined by restoration of TIMI 2 or 3 flow. A further blood sample was obtained by venipuncture in all STEMI patients at 24 hours, and at 3-6 months in a subset of 23 patients.

A control group of 15 patients admitted with non-ST elevation myocardial infarction (NSTEMI) undergoing non-emergency angiography ± PCI were also enrolled in the study. Exclusion criteria for this group were the same as for the STEMI group, with the exception of MRI contraindications, as no scan was obtained in this group, and TIMI flow grade, previous MI/CABG and total ischemic time. Arterial blood samples were acquired at the start of the procedure, and at 15, 30 and 90 minutes after culprit vessel instrumentation (i.e. angioplasty wire insertion), or initial sampling in cases of no intervention. A further 5 NSTEMI patients undergoing PCI were subsequently recruited for analysis of lymphocyte chemokine receptor expression, with arterial blood taken at the start of the procedure.

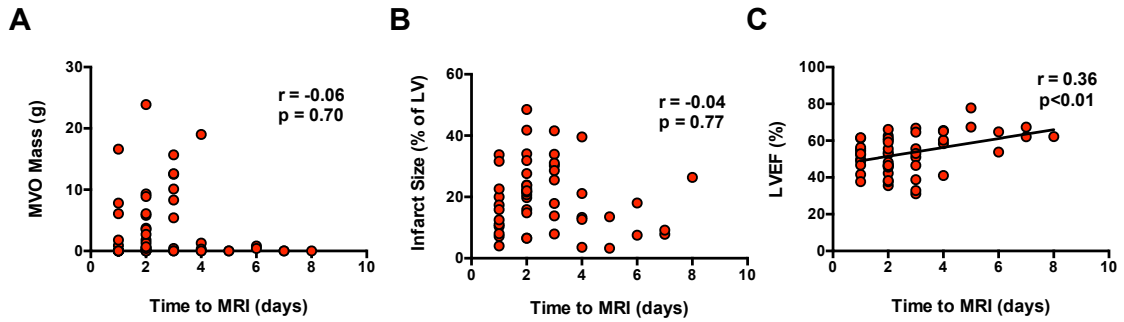
Supplemental Figures



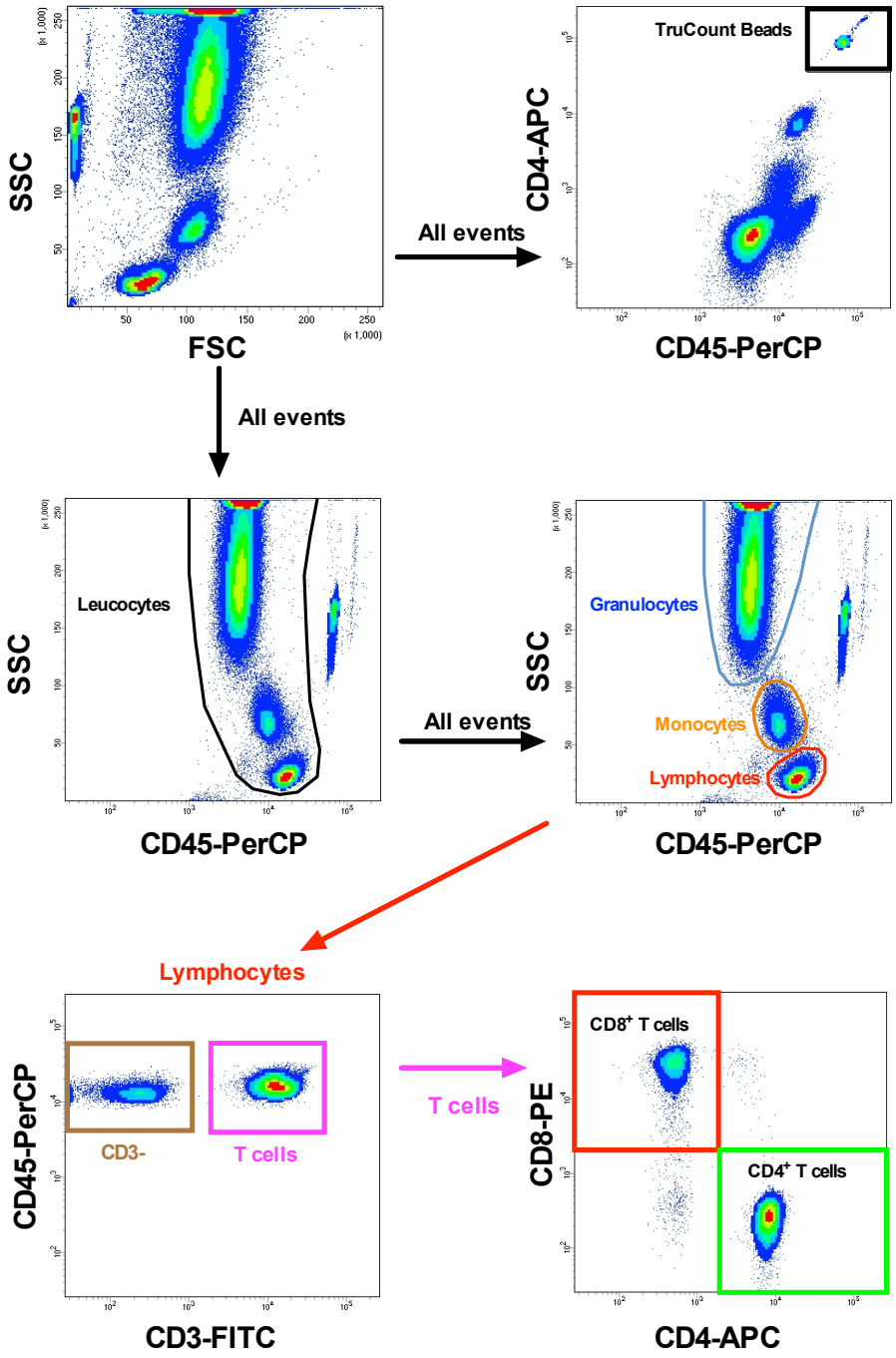
Supplemental Figure 1. Percentage change in effector T cell subsets according to CD27 expression. Changes in circulating counts between pre-reperfusion and 90 minutes in STEMI patients in **A**: CD4+ T cell subsets and **B**: CD8+ T cell subsets, with effector (T_{EM} and T_{EMRA}) subsets further divided based on expression of the co-stimulatory molecule CD27 (n=59). Statistics refer to Wilcoxon signed rank test for indicated populations, *** p<0.001.



Supplemental Figure 2. Relationship between early depletion of T cell subsets post reperfusion and development of MVO in STEMI patients with TIMI grade 0 arterial flow prior to PPCI. Patients were divided into groups based on extent of MVO (zero: n=14, low: n=13, high: n=16) **A:** Change in cell counts between 15 and 30 minutes post reperfusion, for total CD4+ T cells, CD4+ CCR7+ (T_N and T_{CM} combined) cells, and CCR7- effector (T_{EM} and T_{EMRA}) subsets. **B:** As above for total CD8+ T cells and subsets. Box plots display median (central line), 25th and 75th (limits of box), and 5th and 95th percentiles (error bars). Statistics refer to differences between MVO groups as indicated (Kruskal-Wallis test with Dunn's multiple comparisons test). (n=43) * p<0.05, ** p<0.01, ***p<0.001, ns = not significant.



Supplemental Figure 3. Relationship between MRI findings and timing of MRI scan. **A**: MVO. No significant relationship was seen between the timing of MRI scanning and the presence and extent of MVO. **B:** Infarct size expressed as % of left ventricle. No significant relationship was seen between the timing of MRI scanning and infarct size. **C:** Left ventricular ejection fraction (LVEF). The LVEF determined by CMR increased significantly with an increase in time from reperfusion to scanning. Statistics refer to Spearman correlation coefficient (n=50).



Supplemental Figure 4. Example of gating strategy for TruCount assay: Enumeration of major leukocyte populations. All leukocytes, as well as the major populations of granulocytes, monocytes and lymphocytes were gated on CD45 expression and scatter characteristics. Lymphocytes were then divided into T cells and $CD3^-$ lymphocytes, and T cells were classified into $CD4^+$ and $CD8^+$ populations. TruCount beads were gated based on their very high fluorescence in APC and PerCP channels, and absolute counts of each population calculated using the formula:

$$\text{cell count (cells/µl)} = \frac{\#\text{events in cell population}}{\#\text{TruCount bead events}} \times \frac{\#\text{beads per tube}}{\text{blood volume per test(µl)}}$$

Supplemental Tables

Supplemental Table 1: Cox regression analysis in retrospective cohort of patients discharged alive following PPCI for STEMI. Variables included in full stepwise analysis were gender, age, minimum lymphocyte tertile, hypertension, previous stroke/transient ischemic attack (TIA), smoking status, previous angina, previous MI, previous PCI, cardiogenic shock, anterior vs. other infarct locations, multivessel PCI, body mass index (BMI), serum creatinine, serum cholesterol, hemoglobin, as well as use of aspirin, clopidogrel, ACE-inhibitor/angiotensin receptor blocker, beta blocker, statin and glycoprotein IIb/IIIa inhibitor (n=1076 after exclusion of 301 cases with missing values). All covariates not shown in the table were non-significant and, therefore, excluded from the final step.

Covariate	p value	Hazard Ratio	95% CI for Hazard Ratio	
			Lower	Upper
Age (per 10 years)	<0.001	1.50	1.22	1.84
Previous angina	<0.001	2.30	1.49	3.54
Serum creatinine (per 100 μmol/l)	<0.001	1.52	1.23	1.88
Hemoglobin (per 10g/l)	0.007	0.83	0.72	0.95
Glycoprotein IIb/IIIa inhibitor	0.007	0.52	0.33	0.84
Lymphocyte tertile	0.020			
Lymphocyte tertile (low vs. high)	0.009	2.42	1.25	4.71
Lymphocyte tertile (med. vs. high)	0.054	1.97	0.99	3.92

Supplemental Table 2: Baseline (pre-PPCI) leucocyte counts in STEMI patients with and without preadmission statin therapy.

	Cell Count (cells/μl ± SEM)		
Leucocyte Subset	**No Statin Pre-admission (n=49)**	**Statin Pre-admission (n=10)**	**P value**
Granulocytes	9243 ± 510	9507 ± 1790	0.840
Monocytes	638 ± 41	621 ± 62	0.832
Lymphocytes	2187 ± 140	2822 ± 285	**0.045**
B cells	287 ± 28	283 ± 39	0.671
NK cells	484 ± 36	585 ± 84	0.308
T cells	1417 ± 102	1954 ± 203	**0.019**
$CD4^+$ T cells	878 ± 69	1215 ± 157	**0.025**
$CD4^+ CCR7^+$ T cells	586 ± 48	821 ± 112	0.055
$CD4^+ CCR7^-$ T cells	292 ± 34	395 ± 62	**0.041**
$CD8^+$ T cells	462 ± 55	648 ± 83	**0.005**
$CD8^+ CCR7^+$ T cells	74 ± 9	79 ± 25	0.960
$CD8^+ CCR7^-$ T cells	388 ± 50	570 ± 84	**0.005**

Data expressed as mean ± SEM. Groups compared using Mann-Whitney U test.

Supplemental Table 3: Change in leucocyte counts between pre-reperfusion and 90 minutes (ΔPre-90min) in STEMI patients with and without preadmission statin therapy

	Change in Cell Count Pre-reperfusion to 90 minutes (% ± SEM)		
Leucocyte Subset	**No Statin Pre-admission (n=49)**	**Statin Pre-admission (n=10)**	**p value**
Granulocytes	+21.5 ± 7.8	+47.4 ± 23	0.317
Monocytes	-15.2 ± 4.4	-10.4 ± 8.5	0.353
Lymphocytes	-39.8 ± 2.9	-36.9 ± 6.4	0.686
B cells	-23.3 ± 2.5	12.6 ± 6.6	0.203
NK cells	-57.0 ± 3.4	-47.1 ± 10.6	0.473
T cells	-35.8 ± 3.3	-36.5 ± 6.8	0.864
$CD4^+$ T cells	-26.5 ± 3.2	-26.0 ± 7.3	0.887
$CD4^+$ $CCR7^+$ T cells	-22.3 ± 2.7	-20.7 ± 7.3	0.944
$CD4^+$ $CCR7^-$ T cells	-31.8 ± 4.4	-37.2 ± 7.2	0.762
$CD8^+$ T cells	-48.3 ± 3.5	-50.4 ± 8.0	0.716
$CD8^+$ $CCR7^+$ T cells	-20.4 ± 3.0	-19.6 ± 8.9	0.887
$CD8^+$ $CCR7^-$ T cells	54.4 ± 3.6	-54.4 ± 7.9	0.936

Data expressed as mean ± SEM. Groups compared using Mann-Whitney U test.

Supplemental Table 4: Mean coefficient of variation (CV) for TruCount assay for each major cell population quantified, during testing of duplicates for 23 separate blood samples.

Cell Population	n (of duplicates)	Mean C.V. (%)
Leukocytes	23	1.4
Lymphocytes	23	1.5
T cells	23	1.5
$CD8^+$ T cells	23	2.0
$CD4^+$ T cells	23	1.6
$CD3^-$ Lymphocytes	23	1.7
Granulocytes	23	1.5
Monocytes	23	2.3

Supplemental Table 5: Baseline data for prospective cohort patients undergoing cardiac MRI, according to MVO groups.

	MVO group			
	None (0g)	**Low (0.1-2.7g)**	**High (>2.7g)**	**p value**
n	19	14	17	n/a
Age	57.5 ± 7.3	61.0 ± 12.1	57.5 ± 11.3	0.698
Sex (male:female)	15:4	12:2	14:3	0.882
BMI	28.0 ± 5.0	25.0 ± 4.0	27.2 ± 4.4	0.087
Diabetes mellitus	0 (0)	1 (7.1)	3 (17.6)	0.148
Family history of CAD	9 (47.4)	3 (21.4)	8 (47.1)	0.455
Active smoker	12 (63.2)	6 (42.9)	8 (47.1)	0.453
Hypertension	8 (42.1)	1 (7.1)	7 (41.2)	0.063
Anterior MI	6 (31.6)	9 (64.3)	11 (64.7)	0.077
Door-to-balloon time (minutes)	29.8 ± 18.2	26.1 ± 13.1	25.1 ± 12.1	0.738
Onset-to-reperfusion time (minutes)	173.2 ± 85.6.	158.5 ± 66.8	147.6 ± 83.1	0.455
Time to MRI (days)	3.11 ± 2.3	2.79 ± 1.6	2.29 ± 0.85	0.896
LVEF (%)	59.6 ± 8.1	51.8 ± 9.6	47.4 ± 10.2	0.002
Infarct size (% of LV)	11.5 ± 6.6	18.7 ± 7.1	30.9 ± 8.5	<0.001

Continuous variables expressed as mean ± SD, categorical variables as n (%). Continuous variables compared using Kruskal-Wallis test, categorical variables using chi-square test (χ^2). Note: 15 minute blood samples were not successfully obtained in 3 patients due to concurrent clinical requirements, hence higher n than in figure 6 owing to necessary exclusion of those patients from that figure.

Supplemental Table 6: Medication preadmission and treatment during PPCI for prospective cohort patients undergoing cardiac MRI, according to MVO groups.

	MVO group			
	None (0g)	**Low (0.1-2.7g)**	**High (>2.7g)**	**p value**
n	19	14	17	n/a
Preadmission Regular Medication				
Statin	4 (21.1)	1 (7.1)	3 (17.6)	0.545
B-blocker	1 (5.3)	0 (0)	0 (0)	0.435
Aspirin	1 (5.3)	0 (0)	3 (17.6)	0.169
ACE-inhibitor/ARB	2 (10.5)	0 (0)	3 (17.6)	0.264
Treatment During PPCI				
Aspirin	19 (100)	14 (100)	17 (100)	n/a
Additional antiplatelet (prasugrel/clopidogrel/ticagrelor)	17/2/0	12/1/1	15/0/2	0.430
Heparin	18 (94.7)	14 (100)	17 (100)	0.435
Abciximab	9 (47.4)	3 (21.4)	10 (58.8)	0.105
Tirofiban	5 (26.3)	5 (35.7)	4 (23.5)	0.738
Bivalirudin	2 (10.5)	5 (35.7)	3 (17.6)	0.193
Aspiration catheter	16 (84.2)	11 (78.6)	14 (82.4)	0.916

Categorical variables as n (%) unless otherwise stated. All variables compared using chi-square test (χ^2).

Supplemental Table 7: Early change in cell counts between 15 and 30 minutes post reperfusion, in all populations, according to MVO group.

	MVO Group			
Population	**None (0g) (n=17)**	**Low (0.1-2.7g) (n=13)**	**High (>2.7g) (n=17)**	**p value**
Granulocytes	+6.7 ± 4.2%	+3.2 ± 2.4%	3.4 ± 2.7%	0.647
Monocytes	+1.7 ± 2.5%	-1.9 ± 3.0%	-8.0 ± 3.6%	**0.049**
B cells	+3.8 ± 4.2%	-0.2 ± 6.1%	-5.5 ± 7.7%	0.714
NK cells	-22.1 ± 3.5%	-19.6 ± 7.4%	-32.3 ± 5.0%	0.197
T cells	-8.3 ± 1.5%	-10.9 ± 2.6%	-20.4 ± 3.0%	**0.003**
$CD4^+$ T cells	-6.0 ± 1.4%	-7.5 ± 2.5%	-14.3 ± 1.8%	**0.006**
$CD4^+$ T_{Naive}	-4.1 ± 1.5%	-6.2 ±2.6%	-9.1 ± 1.8%	0.162
$CD4^+$ T_{CM}	-5.5 ±1.7%	-6.5 ± 2.5%	-11.2 ±2.0%	0.068
$CD4^+$ T_{EM}	-8.6 ± 2.1%	-11.6 ± 3.2%	-21.1 ± 2.3%	**0.004**
$CD4^+$ T_{EMRA}	-7.8 ± 2.9%	-11.3 ± 3.1%	-27.8 ± 2.3%	**<0.001**
$CD4^+$ $CCR7^+$	-4.9 ± 1.5%	-6.3 ± 2.4%	-10.2 ± 1.8%	0.108
$CD4^+$ $CCR7^-$	-8.8 ± 2.0%	-11.3 ± 3.1%	-22.3 ± 2.4%	**0.002**
$CD8^+$ T cells	-13.9 ±2.5%	-17.0 ± 3.8%	-27.1 ± 3.6%	**0.019**
$CD8^+$ T_{Naive}	-7.1 ± 2.0%	-3.5 ± 3.3%	-9.8 ± 3.2%	0.313
$CD8^+$ T_{CM}	-2.5 ±3.7%	-9.2 ± 4.2%	-9.8 ± 4.1%	0.341
$CD8^+$ T_{EM}	-14.5 ± 2.6%	-19.0 ± 4.3%	-26.7 ± 3.8%	**0.031**
$CD8^+$ T_{EMRA}	-17.8 ± 3.5%	-21.5 ± 4.7%	-31.8 ± 3.9%	**0.045**
$CD8^+$ $CCR7^+$	-5.8 ± 1.6%	-4.9 ± 3.1%	-10.4 ± 3.1%	0.260
$CD8^+$ $CCR7^-$	-16.4 ±3.1%	-20.9 ± 4.3%	-30.2 ± 3.8%	**0.030**

All data expressed as mean % change in cell count between 15 and 30 minutes post reperfusion ± SEM. P value displayed is that for overall Kruskal-Wallis test for relevant population (n=47).

Supplemental Table 8: Influence of medication before admission and during PPCI on MRI findings.

	Pre-admission Statin Group		p value
	No Statin (n=42)	**No statin (n=8)**	
LVEF (%±SEM)	53.2 ± 1.5	53.8 ± 5.4	0.866
Infarct Size (%LV±SEM)	20.2 ± 1.7	19.4 ± 3.7	0.969
MVO (g)	3.9 ± 0.9	3.3 ± 1.4	0.825

	Additional Antiplatelet Group (all also received aspirin)			
	Prasugrel (n=44)	**Clopidogrel (n=3)**	**Ticagrelor (n=3)**	
LVEF (%±SEM)	52.9 ± 1.6	56 ± 10.2	56 ± 5.0	0.672
Infarct Size (%LV±SEM)	20.1 ± 1.8	18.8 ± 5.8	22.0 ± 2.1	0.817
MVO (g)	3.8 ± 0.9	0.03 ± 0.03	7.0 ± 2.1	0.079

	IV Antithrombotic Group				
	None (n=4)	**Abciximab (n=22)**	**Tirofiban (n=14)**	**Bivalirudin (n=10)**	
LVEF (%±SEM)	62.5 ± 7.4	50.3 ± 2.3	55.5 ± 2.6	53.1 ± 2.2	0.207
Infarct Size (%LV±SEM)	16.6 ± 4.6	20.7 ± 2.2	20.6 ± 3.6	19.5 ± 3.5	0.922
MVO (g)	0.5 ± 0.5	5.0 ± 1.3	3.0 ± 1.7	3.4 ± 1.6	0.404

All STEMI patients who underwent cardiac MRI were divided into groups based on treatment strategies received, in each of the three categories shown. MRI parameters for each treatment group were compared using Mann-Whitney-U test for comparison of two groups, or Kruskal-Wallis test for more than two groups. There were no significant differences in MRI parameters for any of the treatment groups.

Supplemental Table 9: Correlation between T cell subset expression of chemokine receptors (n=5) and observed drop in respective subsets in STEMI patients following reperfusion (n=59).

Chemokine Receptor	Correlation (r^2) Between T Cell Subset Expression and Change in Cell Count (ΔPre-90min)	p value
CCR2	0.264	0.487
CCR4	0.187	0.460
CCR5	0.709	0.158
CXCR1	0.948	**0.026**
CXCR2	0.954	**0.023**
CXCR3	0.209	0.543
CXCR4	0.074	0.729
CXCR6	0.928	**0.037**
CX3CR1	0.987	**0.006**

Correlation as determined by linear regression and Pearson's correlation coefficient.

Supplemental Table 10: Quantitative real-time PCR data for expression of mRNA of chemokine receptors and adhesion molecules in STEMI patients.

Molecule	n	Pre-reperfusion	90 min	24 hr	3 months	P value
CX3CR1	15	0.39 ± 0.12	0.37 ± 0.07	1.38 ± 0.24	1.00	**<0.0001**
CXCR1	15	1.21 ± 0.33	1.48 ± 0.73	4.12 ± 1.45	1.00	0.114
CXCR3	15	1.37 ± 0.34	1.37 ± 0.67	2.09 ± 0.65	1.00	**0.028**
CXCR4	15	2.11 ± 0.21	1.37 ± 0.21	1.23 ± 0.24	1.00	**0.003**
CD11a	9	0.81 ± 0.16	0.84 ± 0.11	1.14 ± 0.22	1.00	0.200
CD11b	9	1.23 ± 0.29	1.50 ± 0.17	1.47 ± 0.22	1.00	0.324
CD62L	9	0.56 ± 0.07	1.01 ± 0.15	1.02 ± 0.14	1.00	**0.008**
CCR4	9	0.77 ± 0.21	0.99 ± 0.63	1.38 ± 0.33	1.00	0.413
CCR5	9	0.68 ± 0.21	0.74 ± 0.26	1.38 ± 0.40	1.00	0.081
CCR7	9	0.68 ± 0.09	0.89 ± 0.12	1.15 ± 0.17	1.00	0.108

Expressed relative to 3 month level (post-morbid baseline) (mean ± SEM, p value from Friedman test.

Supplemental Table 11: Mean coefficient of variation (C.V.) for 8-color assay for each cell population quantified.

Cell Population	n (of duplicates)	Mean C.V. (%)
$CD16^-$ Monocytes	30	0.8
$CD16^+$ Monocytes	30	4.7
$CD8^+$ T_N cells	31	4.9
$CD8^+$ T_{CM} cells	31	10.2
$CD8^+$ T_{EM} cells	31	3.0
$CD8^+$ T_{EMRA} cells	31	1.7
$CD4^+$ T_N cells	31	2.0
$CD4^+$ T_{CM} cells	31	2.7
$CD4^+$ T_{EM} cells	31	2.4
$CD4^+$ T_{EMRA} cells	31	7.4
B cells	30	1.4
NK cells	30	2.0