Flow-cytometric analysis - raw data and gating strategy

Gating strategy

In all flow cytometric gating strategies we used defined range (RN1) for debris elimination.





The CytoID autophagy detection kit is based on the determination of cells positive for the presence of acidic organelles co-localized with LC-3b protein. For negative control we used bafilomycin A1 treated cells. Bafilomycin A1 prevents organelles acidification by inhibiting vacuolar H+ ATPase. CytoID positive cells were divided into two populations, based on the signal strength (red cluster depicts CYTO--ID++ population, blue cluster Cyto-ID+ population). Weak Cyto-ID-positivity is presented even after Bafilomycin A1 treatment due to residual activity of acidic organelles (blue cluster).



For the determination of "normal" ("healthy"), "early apoptotic", "late apoptotic/necrotic" cells and "cellular fragments", classical four quadrant gating strategy was performed.



For the determination of large, SYTO16 highly positive cells, two gating strategies were applied. In first strategy (SSC/-FL1), cells were divided into three population according their positivity for SYTO 16 staining (negative/slightly positive/highly positive), and granularity. After that, second gating strategy (FSC/FL1) was performed for determining of SYTO16 positive-large sized population of cells.



1000 Gate: RN1 400 100 Gate: RN 320 80 RN 100 **g**240 60 SSC 160 -10 80 20 0 10 100 1000 100 1000 1003 1000 10 10 FSC SSC FSC 1000 Gate: R 200 Gate: RN1 200 Gate: RN1 160-160 100 s120 · **≨**¹²⁰ coun SSC 80 80 10 40 40 0 100 10 FL3 0.1 100 1000 0.1 10 FL1 CytoID 100 1000 0.1 1000 10 FL1 CytoID R3: 1.27% R1: 90.80% R2: 7.96% CV-y% 401.06 Gate %Gated Region Count Count/ml Mean-x CV-x% Mean-v 86.06 12.07 R1 RN1 11350 0.17 88.70 12.77 R2 RN1 3.37 RN1 1592 135.71 83.47 144.03

timepoint 40 min plumbagin treatment



timepoint 90 min plumbagin treatment

49.93

17.80

197.39

152.02

78.89

173.28

65.94

1.91

<None>

RN1

R3

13188

252

timepoint 0 h plumbagin treatment

File: X0h_cytolD_at_030.FCS Date: 11-11-2014 Time: 11:57:36 Particles: 20000 Acq.-Time: 249 s



partec PAS

timepoint 4 h plumbagin treatment

File: X4h_cytolD_50f_2.FCS Date: 03-11-2014 Time: 13:29:57 Particles: 20000 Acq.-Time: 20 s Concentration: 53650 / ml partec PAS



timepoint 6 h plumbagin treatment File: X6h_Cyto_1.FCS Date: 03-11-2014 Time: 15:34:38 Particles: 20000 Acq.-Time: 7 s partec PAS 1000 Gate: RN1 200 100 Gate: RN 160 RN 80 100 **월**^{120 -} 60 SSC 80 10 40 20 0 10 100 1000 100 1000 10 1003 1000 10 FSC SSC FSC 400 Gate: RN1 400 Gate: RN1 1000 Gate: RN1 320-320 100 \$240 160 strong 160 SSC 80 80 0 100 100 10 FL3 1000 10 FL1 100 1000 10 FL1 1000 0.1 0.1 0.1 R3: 6.03% R1: 65.80% R2: 28.57% CV-y% 183.31 Gate Count CV-x% Mean-y Region Count/m %Gated Mean-x Regi R1 R2 RN1 RN1 8997 57.20 0.31 73.35 5.49 RN1 5708 36.29 4.43 101.74 115.34 96.91 <None> 15729 78.64 78.51 156.21

147.29

61.26

336.19

timepoint 10 h plumbagin treatment

6.90

18.58

R3

RN1

1085



timepoint 8 h plumbagin treatment



timepoint 16 h plumbagin treatment







timepoint 20 h plumbagin treatment



timepoint 36 h plumbagin treatment





timepoint 0 h plumbagin treatment



timepoint 40 min plumbagin treatment



timepoint 20 min plumbagin treatment



timepoint 90 min plumbagin treatment



timepoint 0 h plumbagin treatment



timepoint 20 min plumbagin treatment



timepoint 40 min plumbagin treatment



timepoint 90 min plumbagin treatment



partec PAS

40 -

0

320-

on 240

160

80

Region Q1

Q2 Q3 Q4

RN1

0.1

Q4: 14.99%

Gate

RN1

RN1

RN1

RN1

<None>

400 TGate: RN1

10

FSC -

10 FL1 ·

Count

349

1618

10515

2822

15304

timepoint 6 h plumbagin treatment

File: 6hod annex.FCS Date: 20-05-2014 Time: 14:58:54 Particles: 20000 Acq.-Time: 43 s



timepoint 9 h plumbagin treatment





timepoint 7 h plumbagin treatment



timepoint 11 h plumbagin treatment



partec PAS

timepoint 13 h plumbagin treatment





timepoint 48 h plumbagin treatment



timepoint 28 h plumbagin treatment



timepoint 90 min plumbagin treatment





10

1000 Gate: RN1

100

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Q1: 4.51% 2530 / ml SSC

ŝŝ

1000

100

Q2: 15.26% 8550.7ml

5575*1* ml

80 40 0.1 10 FL1 10 FL1 100 100 1000 0.1 1000 R3: 17.79% R1: 62.63% R2: 19.56% Region Q1 Q2 Q3 Q4 R1 R2 R3 RN1 Gate RN1 Count 646 Count/ml 2530 %Gated 4.51 Mean-x 6.89 CV-x% 81.57 64.18 Mean-y 105.75 145.09 CV-y% 44.42 53.28 RN1 2188 8550 15.26 208.17 RN1 10171 40390 70.94 1.20 249.37 10.92 105.92 RN1 1333 5575 27585 9.30 93.83 105.05 29.01 55.96 7124 3660 49.69 0.19 3.97 63.06 119.74 6.34 23.62 729.19 191.88 RN1 25.53 RN1 15205 3548 14225 24.75 163.80 81.60 RN1 96.71 95.27 14338 57045 71 69 37.35 160.98

100

1000

10

Gate: RN1

FSC

160

80 -

200 -

160

ရွ 120 ·

no

timepoint 4 h plumbagin treatment



File: NNN4h_Syto50fsuper.FCS Date: 03-11-2014 Time: 13:22:50 Particles: 20000 Acq.-Time: 15 s Concentration: 61405 / ml partec PAS

partec PAS

1000

FSCR3 100

10 FL1 100 1000

1000

1000

10

R2

1000 Gate: RN1

100

0.1

ŝŝ

partec PAS

1000

1000



timepoint 8 h plumbagin treatment File: NN8h syto 2.FCS Date: 03-11-2014 Time: 17:09:31 Particles: 2000 Acq.-Time: 38 s



timepoint 16 h h plumbagin treatment



timepoint 10 h plumbagin treatment



partec PAS

1000

1000

partec PAS

RN1

RN1

RN1

<None:

2815

13204

<Non

RN1

4713

3935

15047

timepoint 20 h plumbagin treatment



timepoint 24 h plumbagin treatment File: NNN24h_syto_1.FCS Date: 04-11-2014 Time: 09:21:32 Particles: 20000 Acq.-Time: 10 s 1000-Gate: RN1 320 RN1 80 100 stunoo 160 **£** 60 SSC 40 80 20



71.15

67.41

112.47

86.61

185.46

11.26

123.69

190.22

106.99

4.34

179.83

67.71

26.15 75.23 timepoint 48 h plumbagin treatment

31.32



217.16

68.27

66.02

63.20

169.77

138.71

84.82

timepoint 36 h plumbagin treatment

24.04

72.59

3491

14510

10416

407

8006

3014

3009

14032

RN1

RN1

RN1

<None

R2

R3

RN1

R3

RN1

RN1

File: NNN36h svto 002.FCS Date: 04-11-2014 Time: 21:33:44 Particles: 20000 Acg.-Time: 22 s

170.53

78.26

86.80

186.97

125.83

102.72



270.56

35.87

22.00

108.05

7.42

56.50

66.37

88.44

116.66

193.60

49.56

0.17

4.20

183.44

73.82

57.06

21.48

21.44

70.16

106.96

41.74

741.00

385.57

108.48

1000