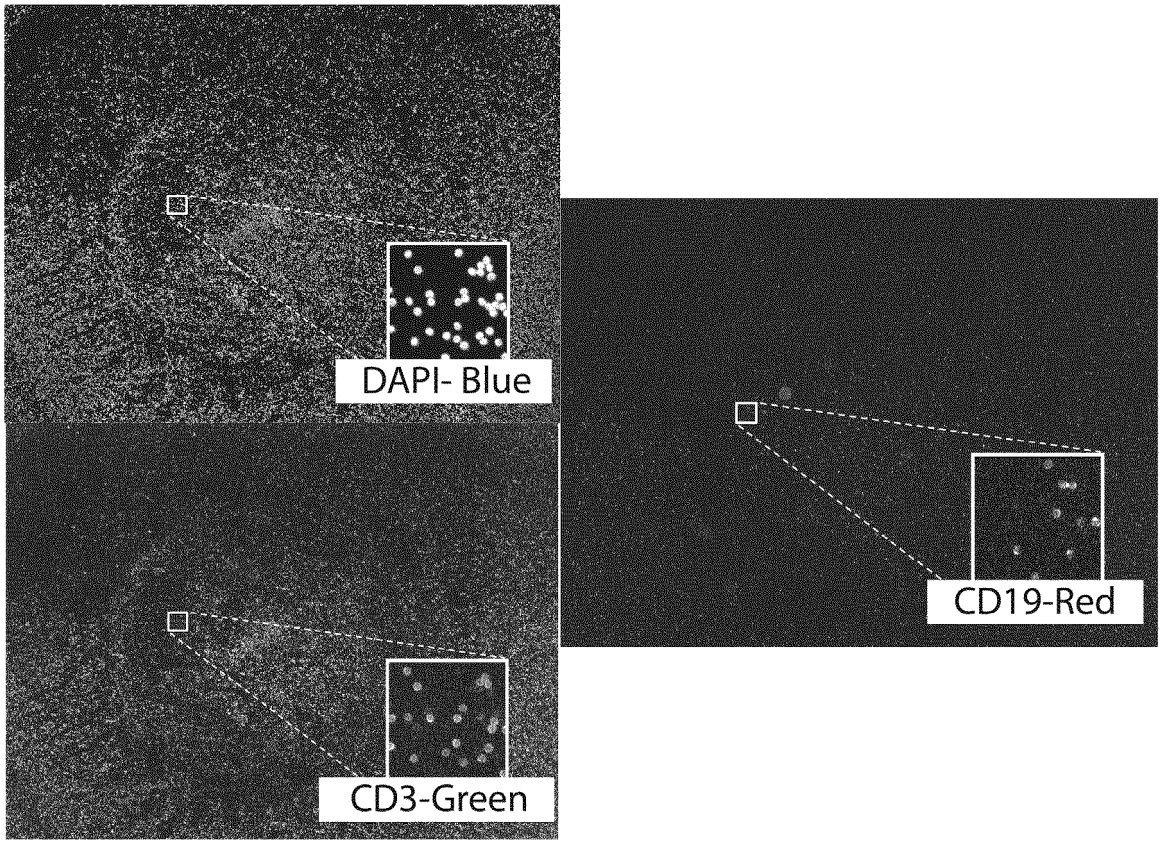


Figure 1

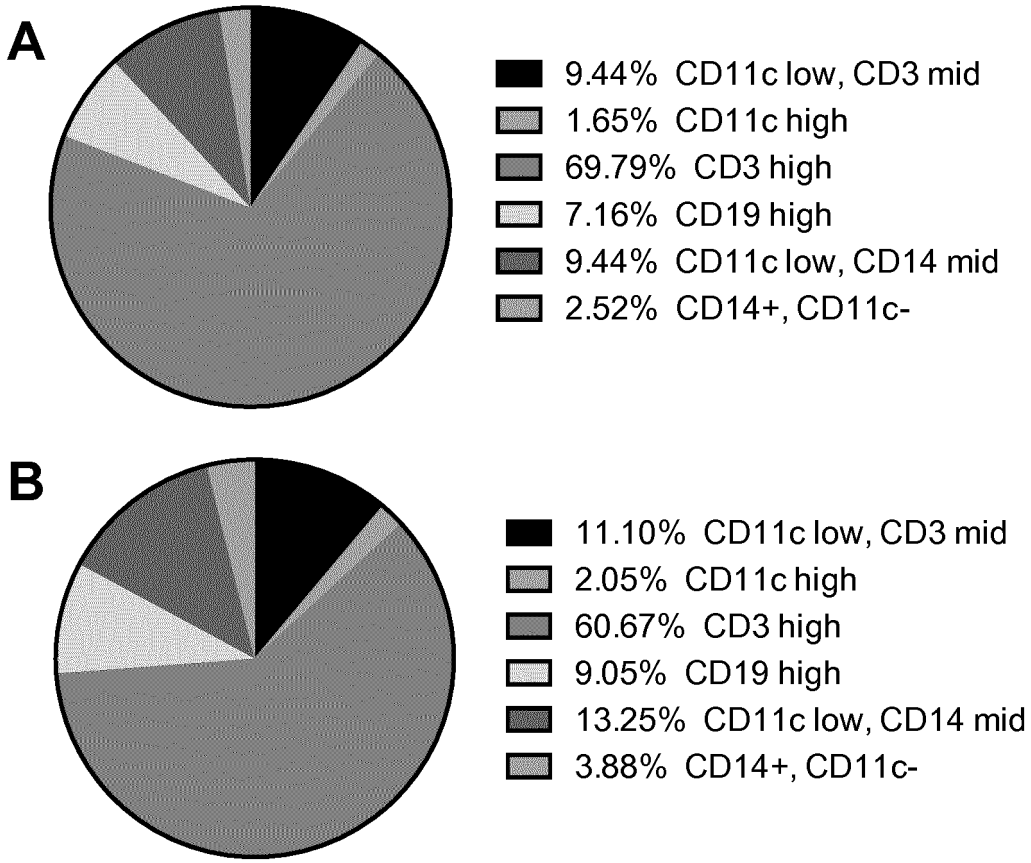


Figure 2

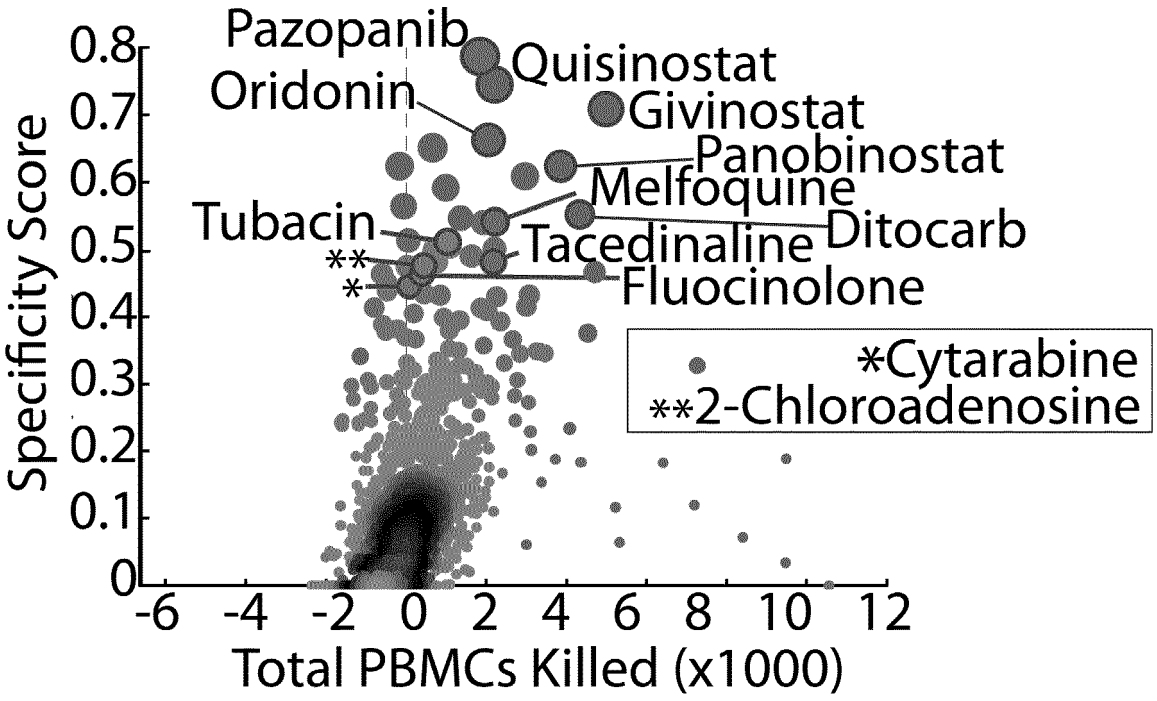


Figure 3

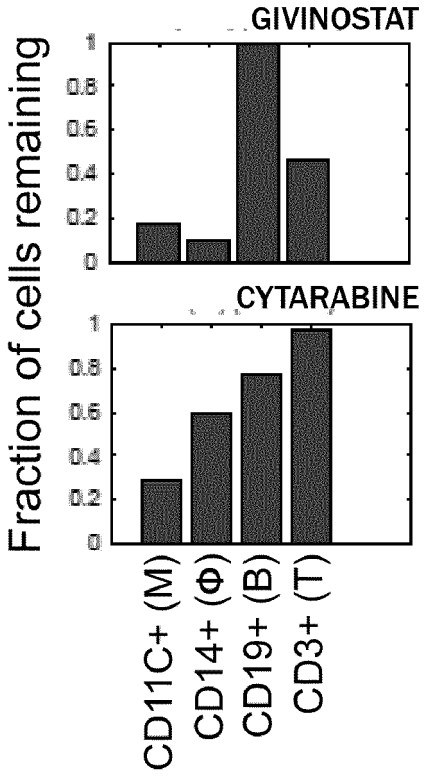


Figure 4

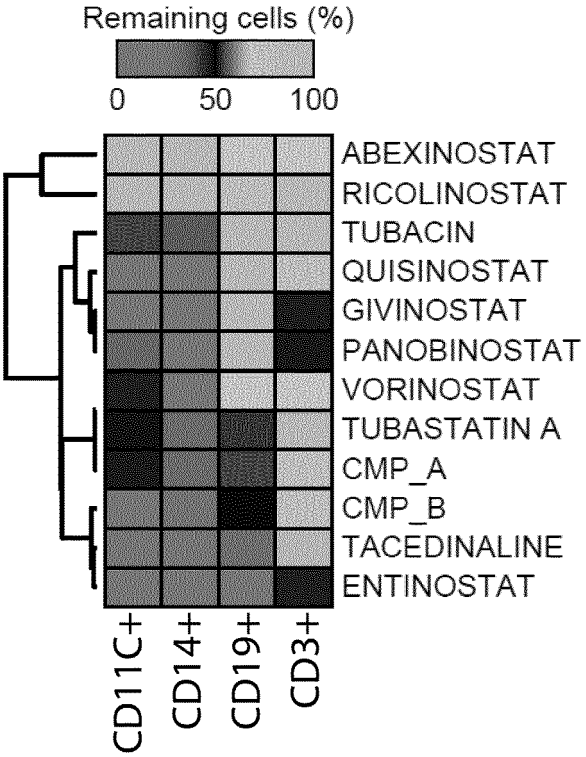


Figure 5

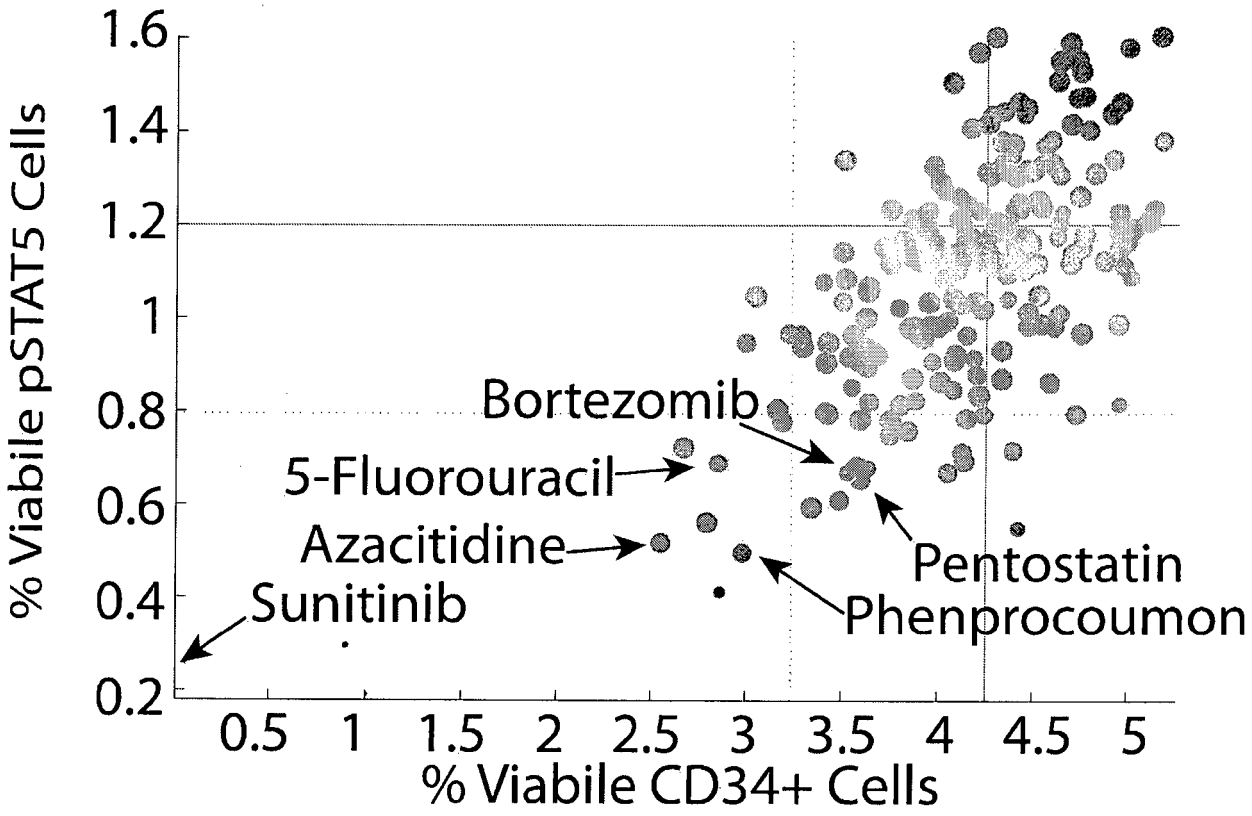


Figure 6

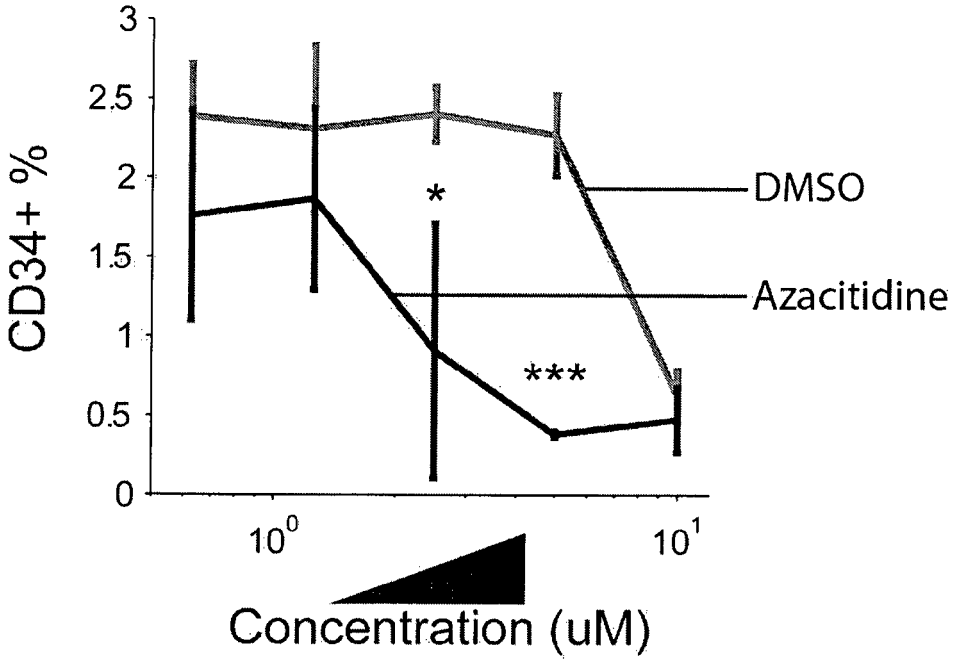


Figure 7

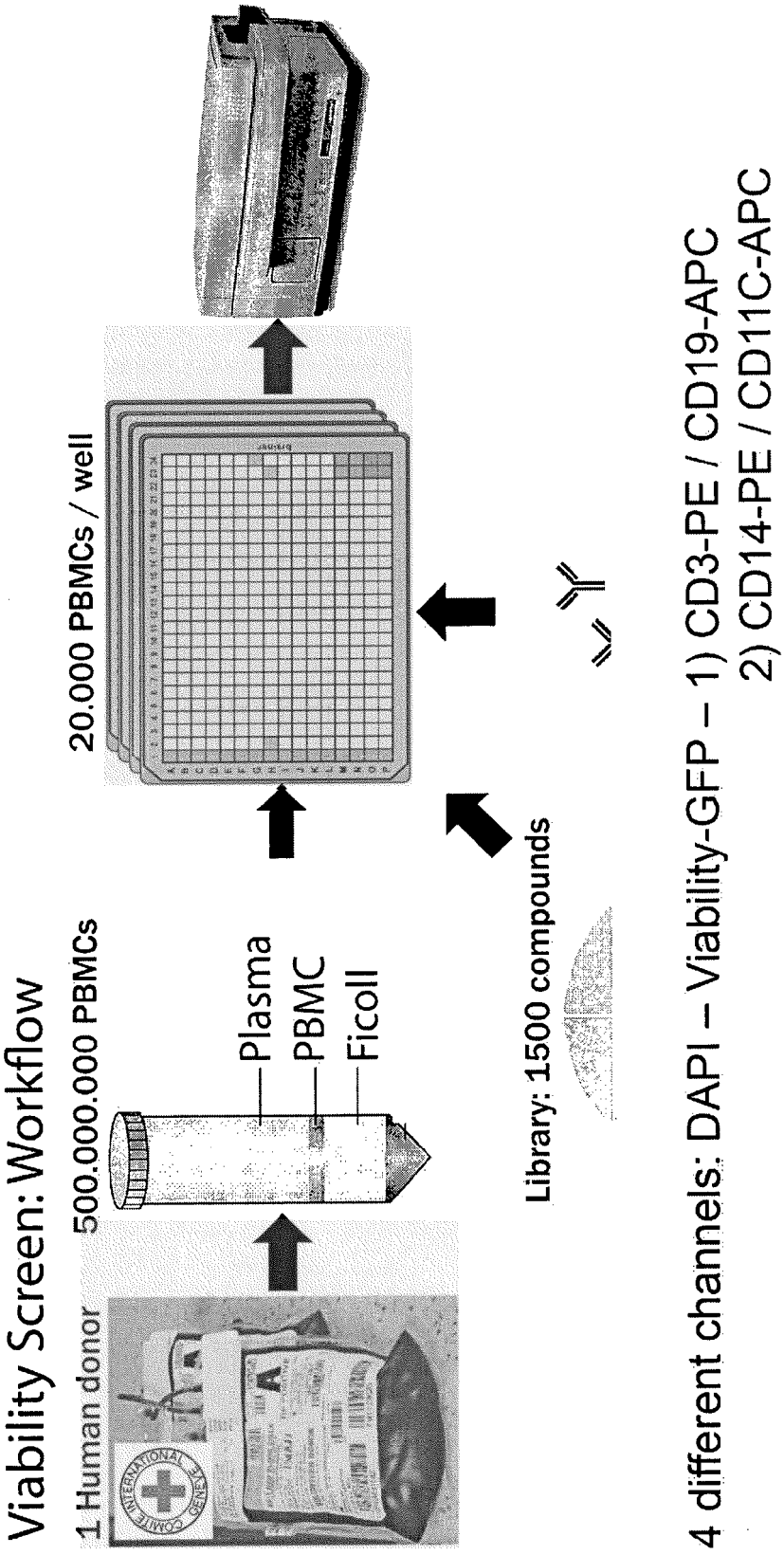

Viability Screen: Workflow
1 Human donor
500.000.000 PBMCs
Plasma
PBMC
Ficoll
20.000 PBMCs / well
Library: 1500 compounds
4 different channels: DAPI – Viability-GFP – 1) CD3-PE / CD19-APC
2) CD14-PE / CD11C-APC
Randomized Duplicates over plates for consistency
Clinical trials (185)
Others (806)

Figure 8a

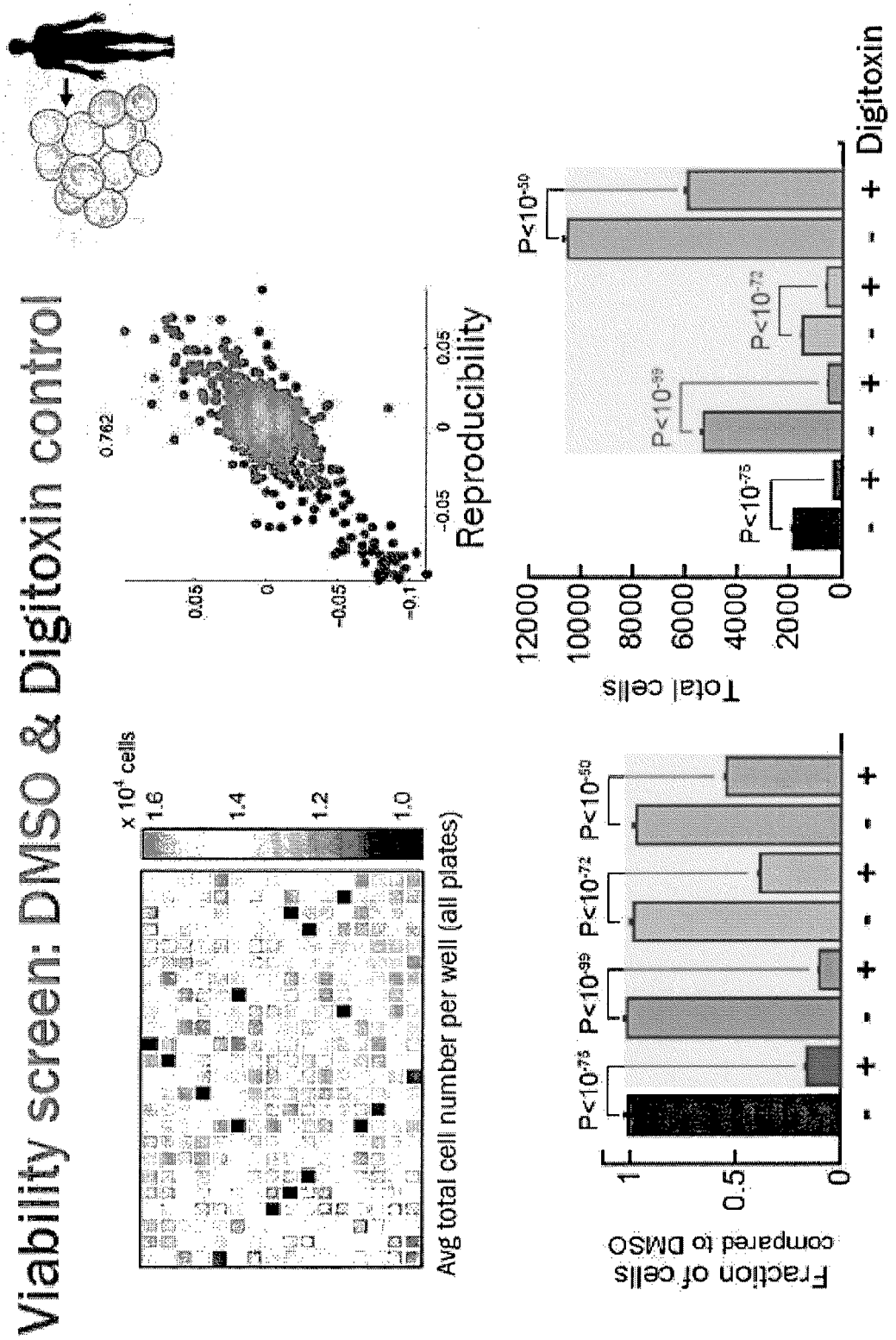

Viability screen: DMSO & Digitoxin control
Avg total cell number per well (all plates)
x 10⁴ cells
1.6
1.4
1.2
1.0
Reproducibility
0.762
0.05
0
-0.05
-0.1
Total cells
12000
10000
8000
6000
4000
2000
0
P<10⁻⁵⁰
P<10⁻⁷²
P<10⁻⁹⁹
P<10⁻⁷⁵
- + - - + - + + Digitoxin
Fraction of cells compared to DMSO
1
0.5
0
P<10⁻⁷⁵ P<10⁻⁹⁹ P<10⁻⁷² P<10⁻⁵⁰
- + - - + - + +

Figure 8b

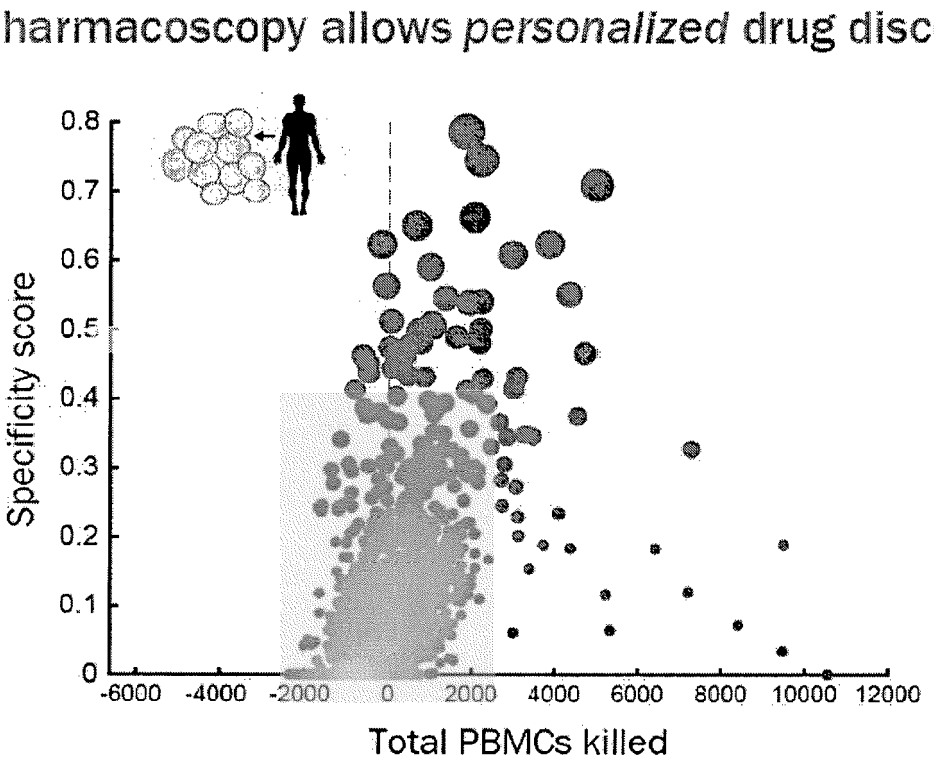


Figure 8c

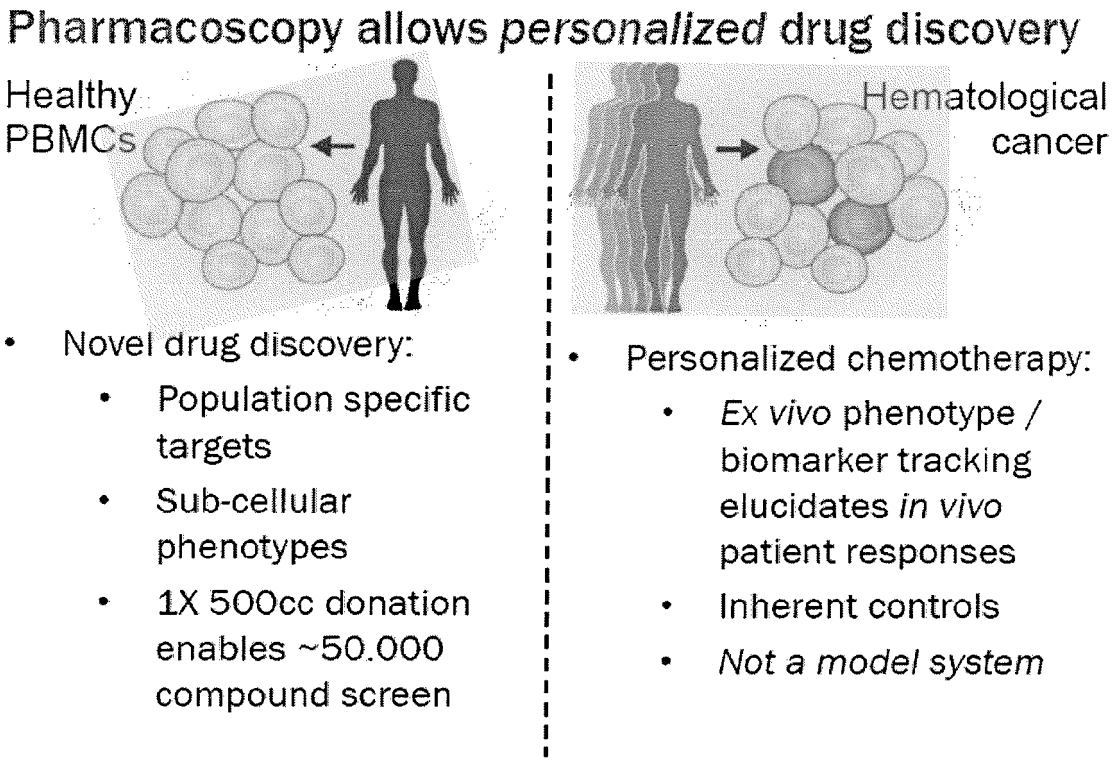


Figure 8d

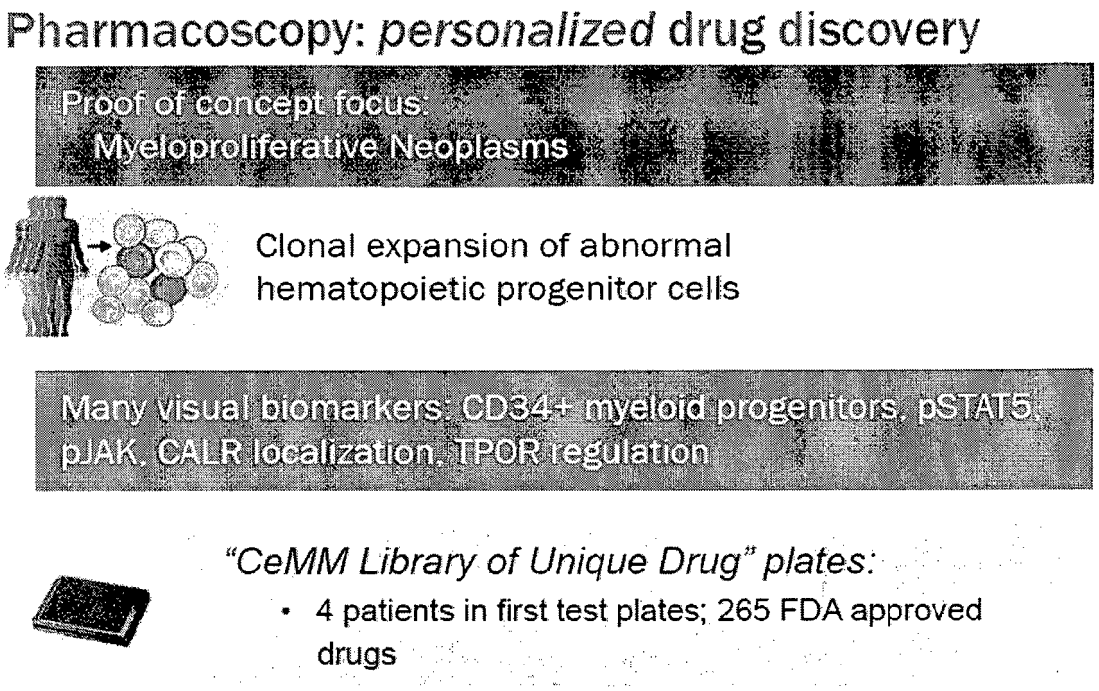

Pharmacoscopy: *personalized* drug discovery
Proof of concept focus:
Myeloproliferative Neoplasms
Clonal expansion of abnormal
hematopoietic progenitor cells
Many visual biomarkers: CD34+ myeloid progenitors, pSTAT5,
pJAK, CALR localization, TPOR regulation
"CeMM Library of Unique Drug" plates:
• 4 patients in first test plates; 265 FDA approved
drugs

Figure 8e

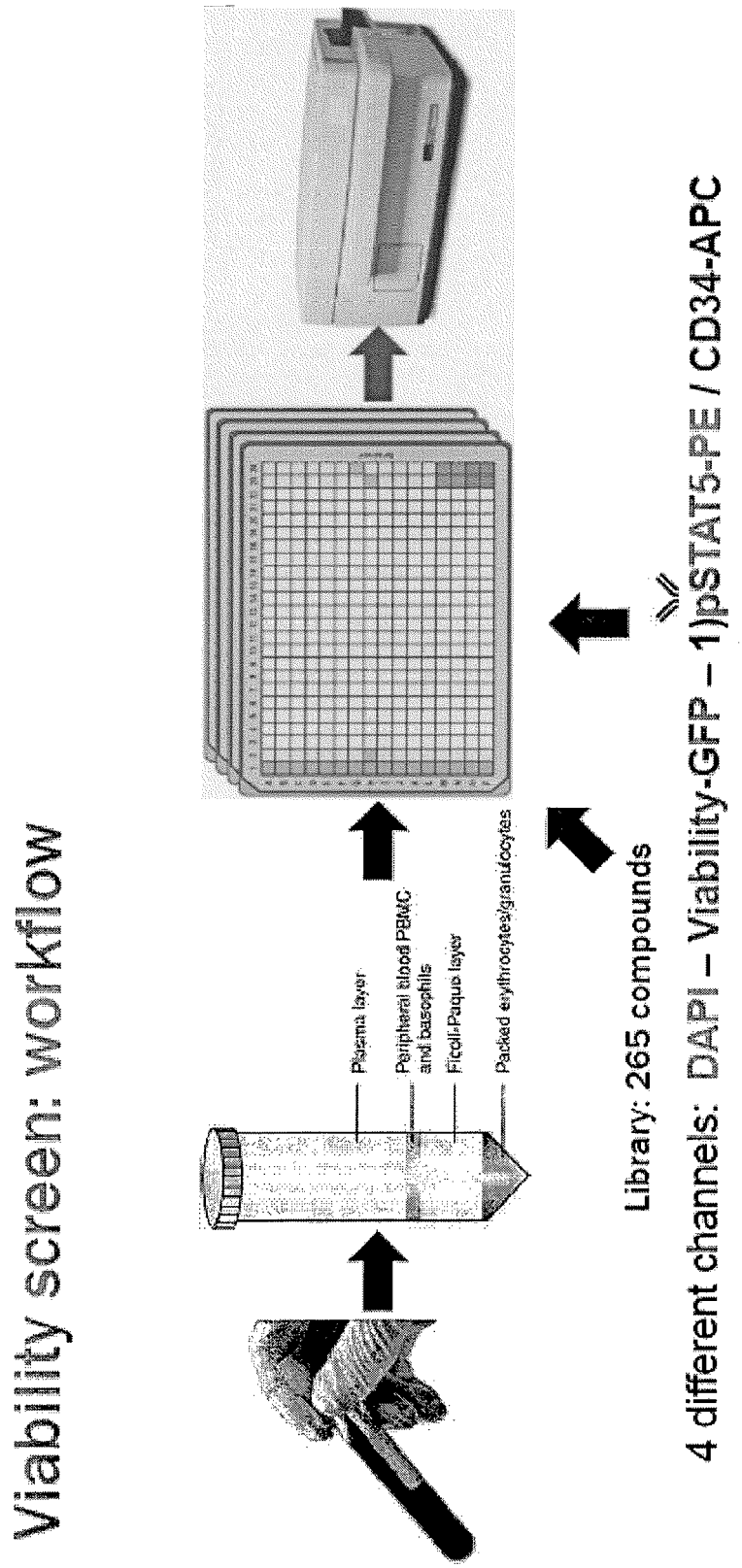

Viability screen: workflow
Plasma layer
Peripheral blood PBMC and basophils
Ficoll-Paque layer
Packed erythrocytes/granulocytes
Library: 265 compounds
4 different channels: DAPI – Viability-GFP – 1)pSTAT5-PE / CD34-APC

Figure 8f

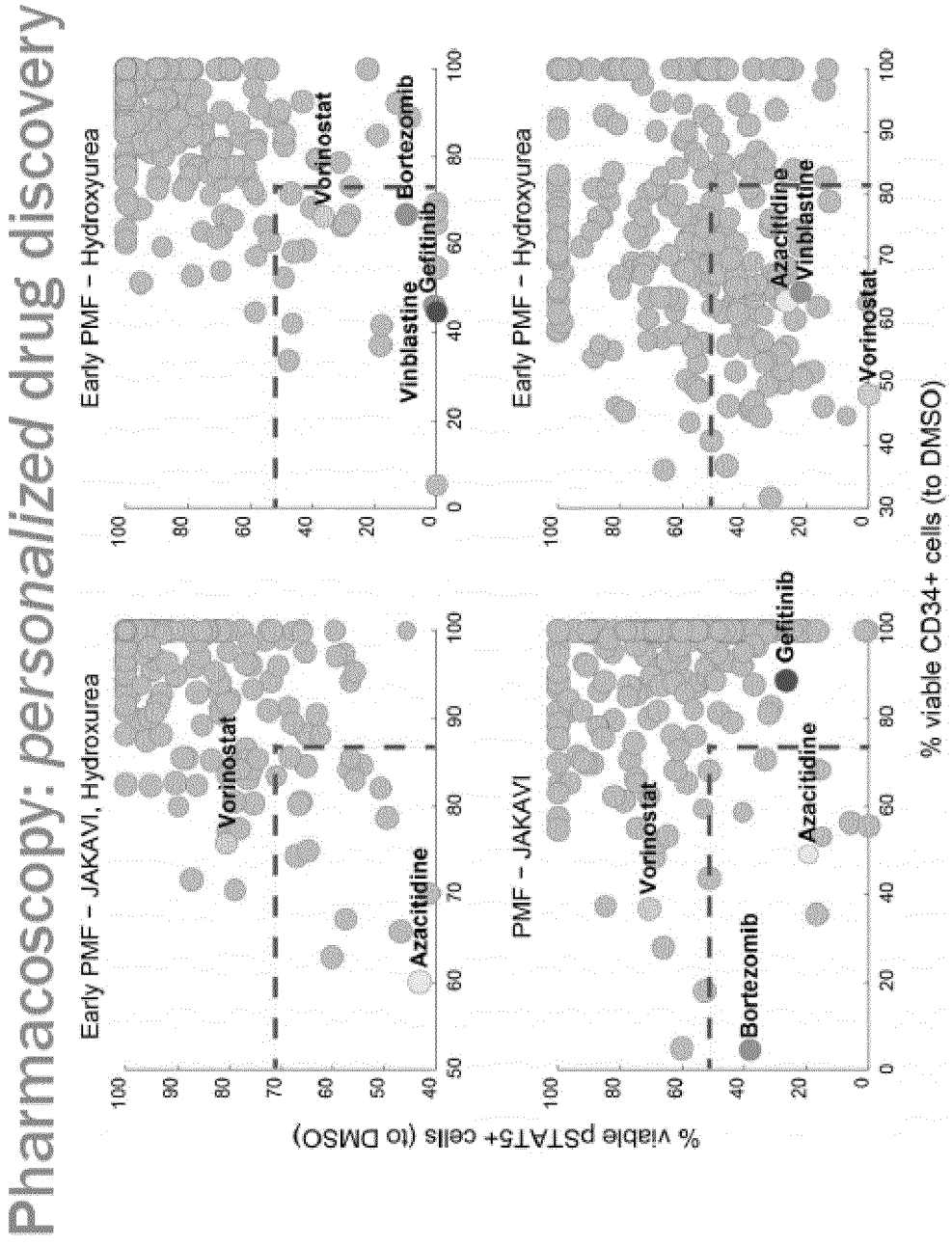

Pharmacoscopy: personalized drug discovery
Early PMF – JAKAVI, Hydroxurea
Early PMF – Hydroxyurea
PMF – JAKAVI
Early PMF – Hydroxyurea
Vorinostat
Azacitidine
Vinblastine
Gefitinib
Bortezomib
% viable pSTAT5+ cells (to DMSO)
% viable CD34+ cells (to DMSO)

Figure 9

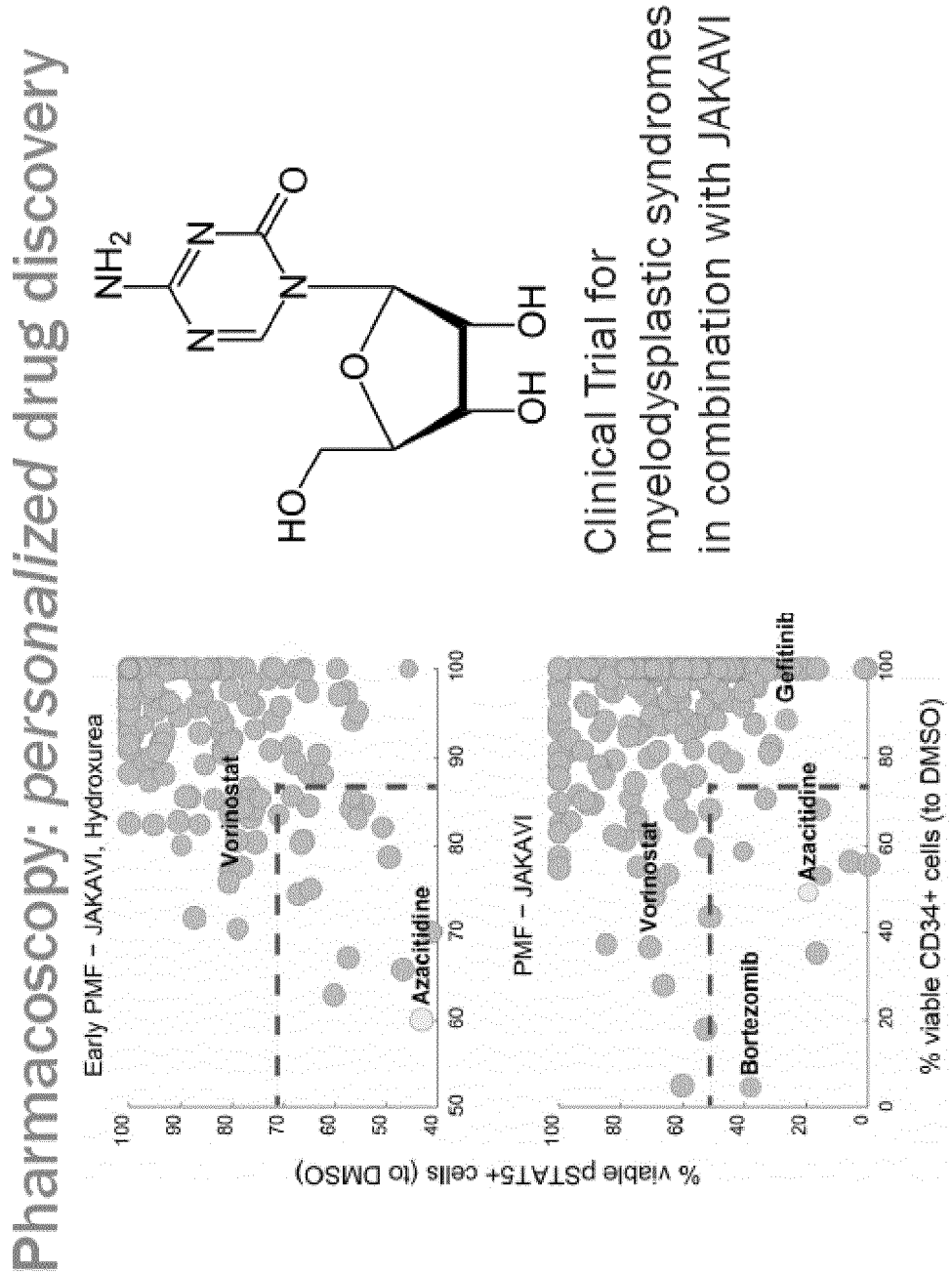

Pharmacoscopy: personalized drug discovery
Clinical Trial for
myelodysplastic syndromes
in combination with JAKAVI
NH2
HO
OH
OH
Early PMF – JAKAVI, Hydroxurea
Vorinostat
Azacitidine
PMF – JAKAVI
Vorinostat
Bortezomib
Azacitidine
Gefitinib
% viable pSTAT5+ cells (to DMSO)
% viable CD34+ cells (to DMSO)

Figure 10

Workflow

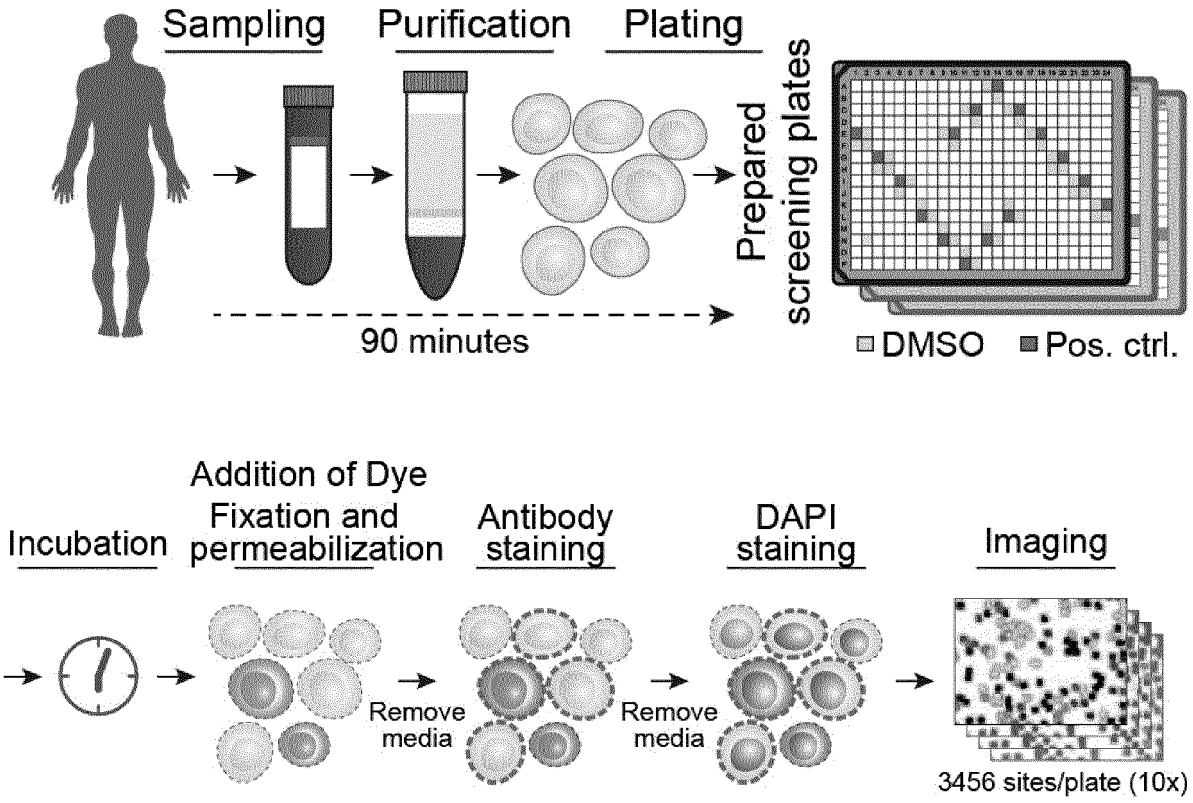


Figure 11

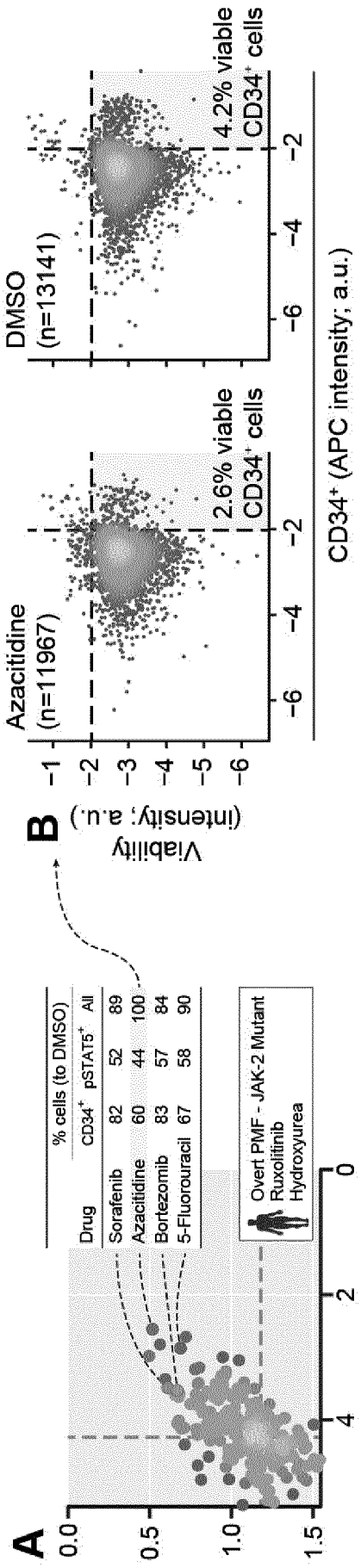


Figure 12 A and B

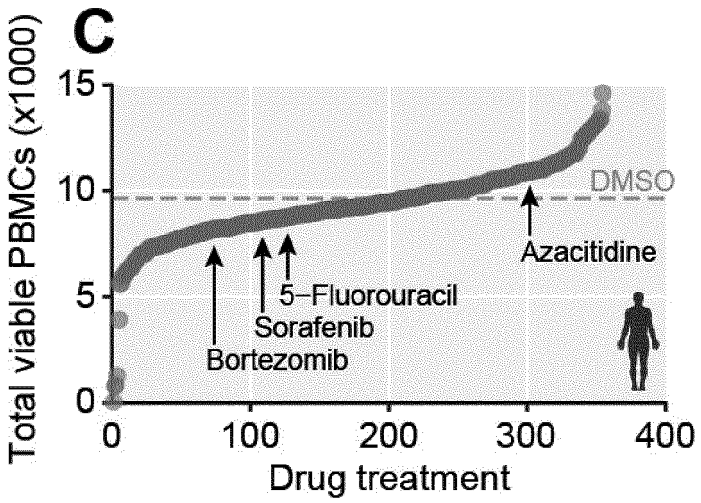


Figure 12 C

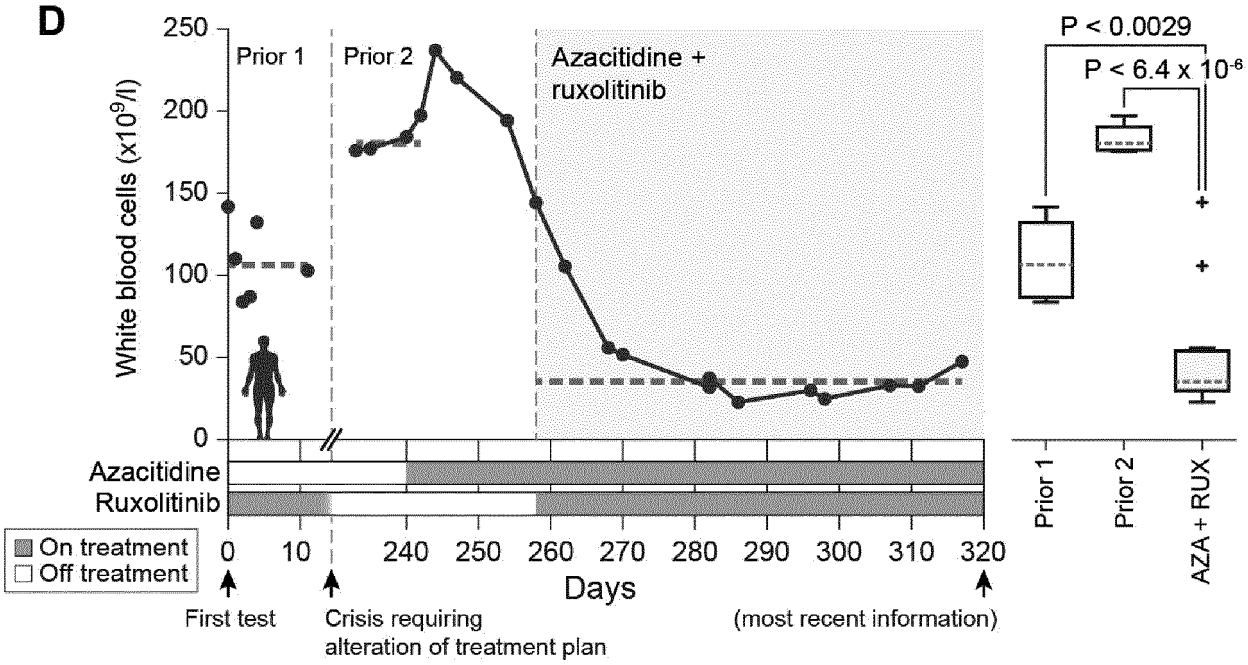


Figure 12 D

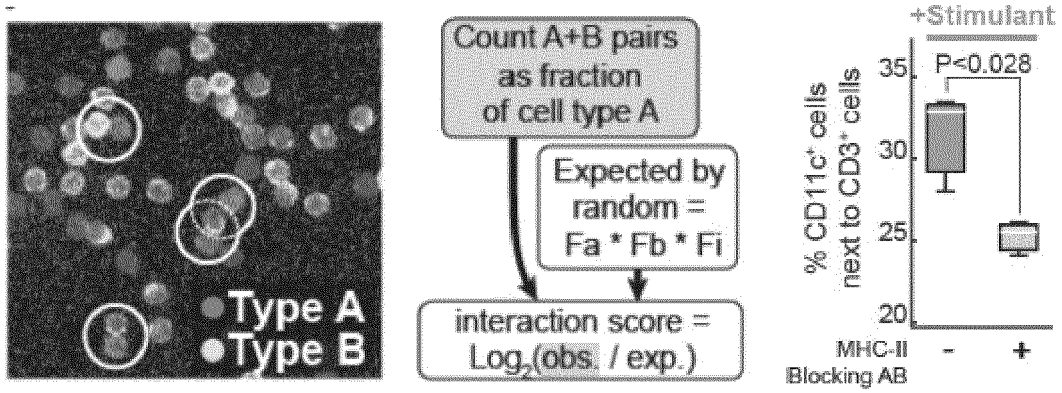
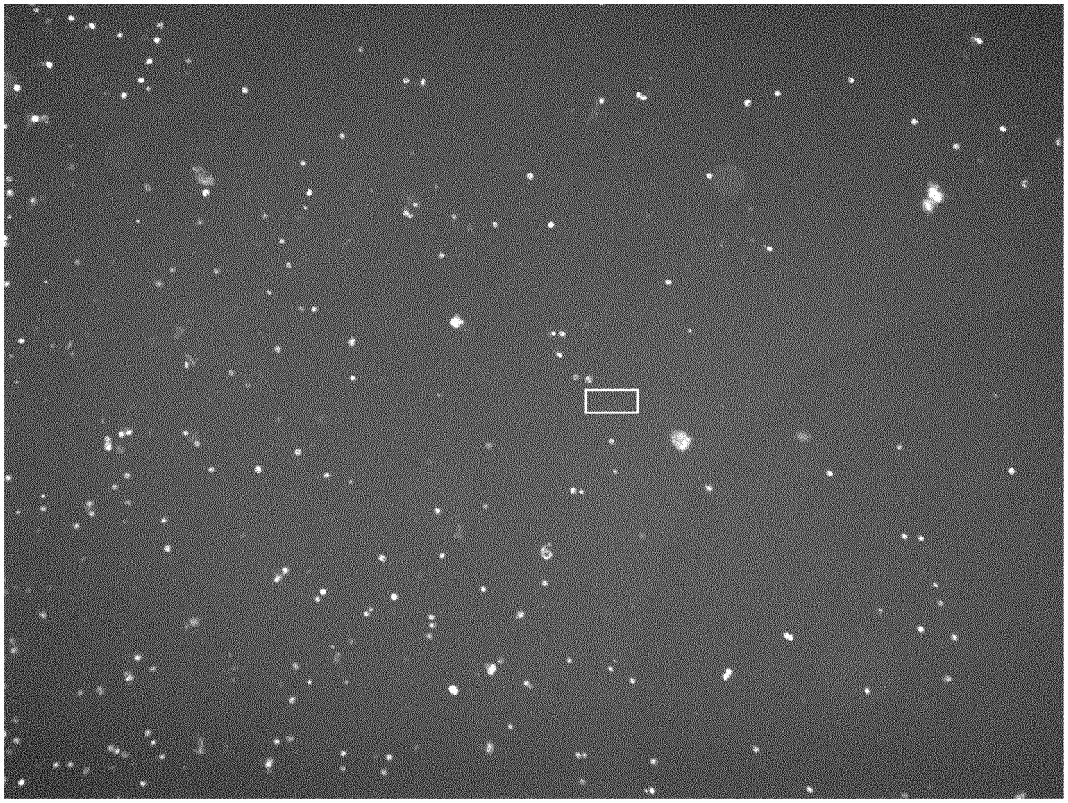
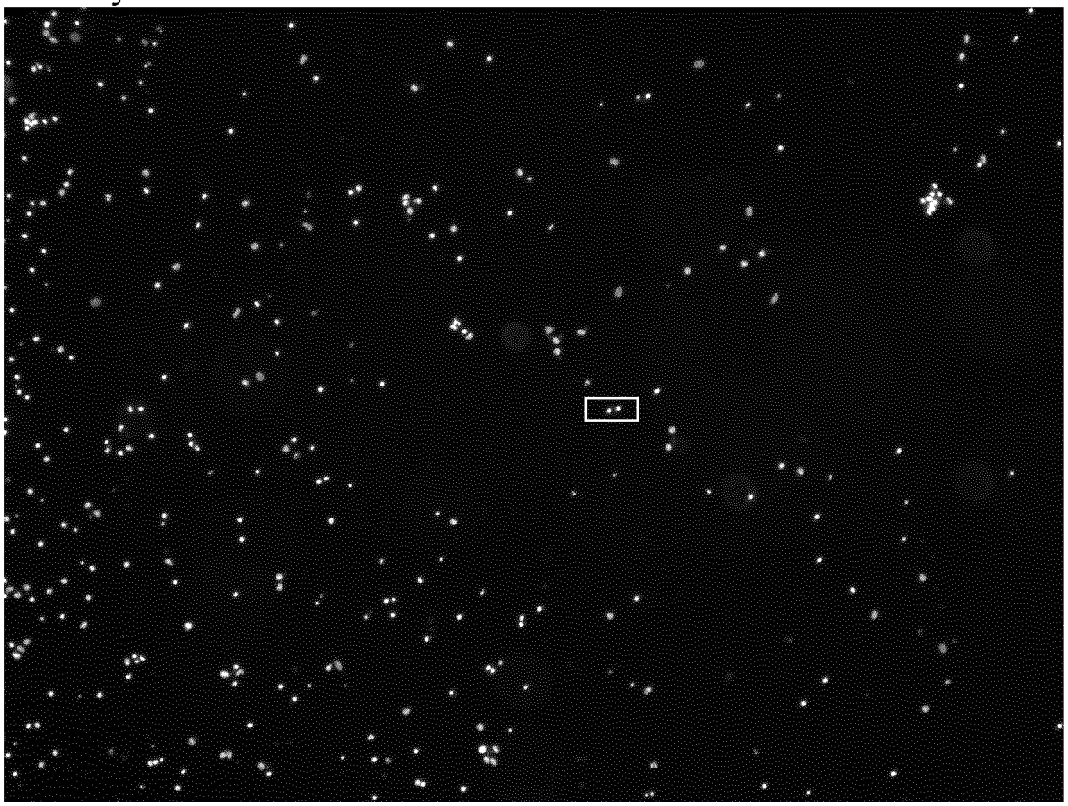

Type A
Type B
Count A+B pairs as fraction of cell type A
Expected by random = Fa * Fb * Fi
interaction score = Log2(obs. / exp.)
+Stimulant
P<0.028
% CD11c+ cells next to CD3+ cells
35
30
25
20
MHC-II Blocking AB
-
+

Figure 13

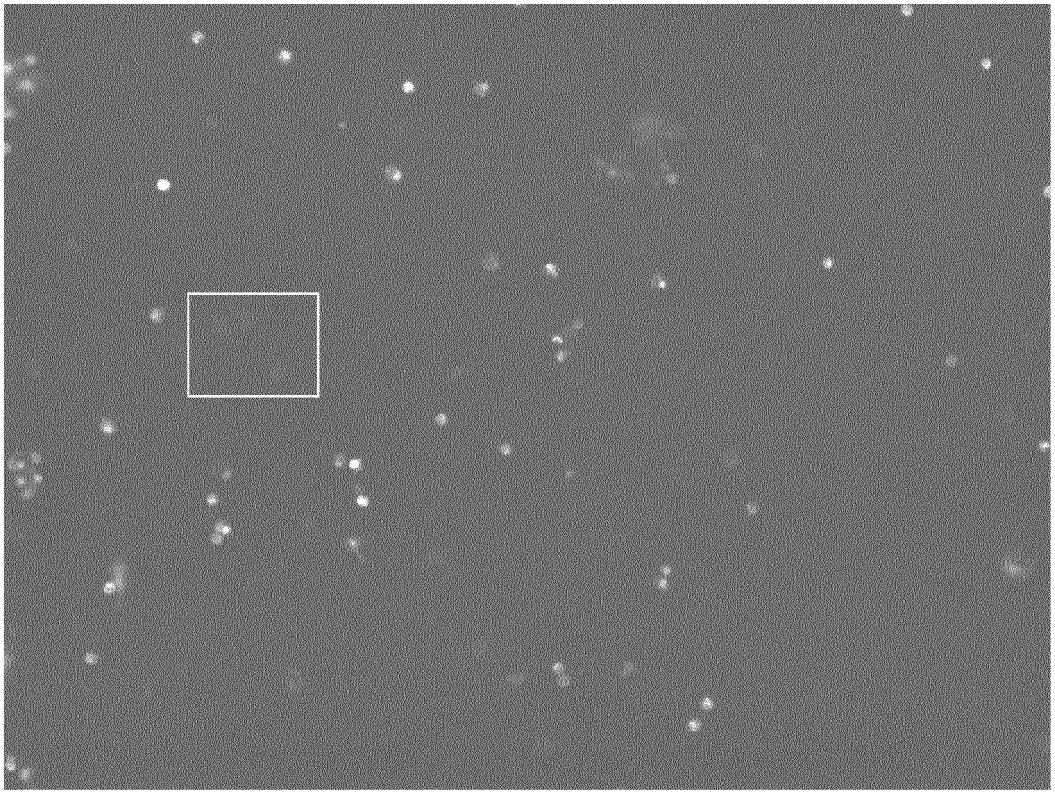


Viability Stain

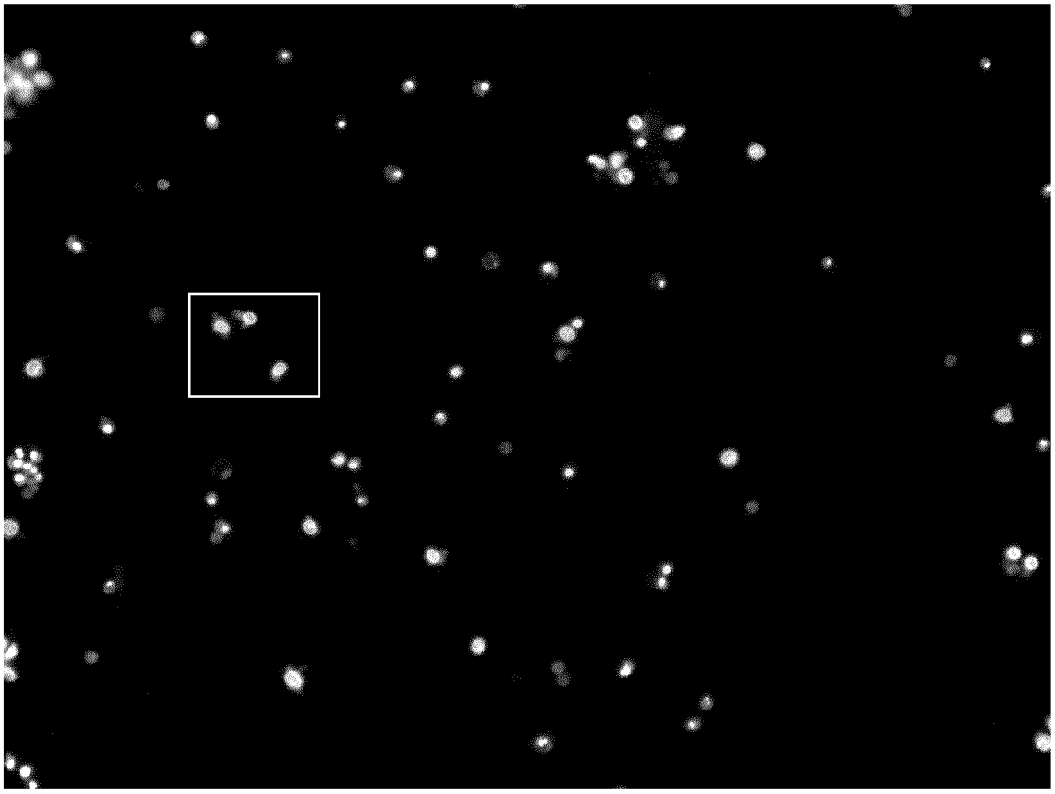


Nuclear Stain 10x

Figure 14A

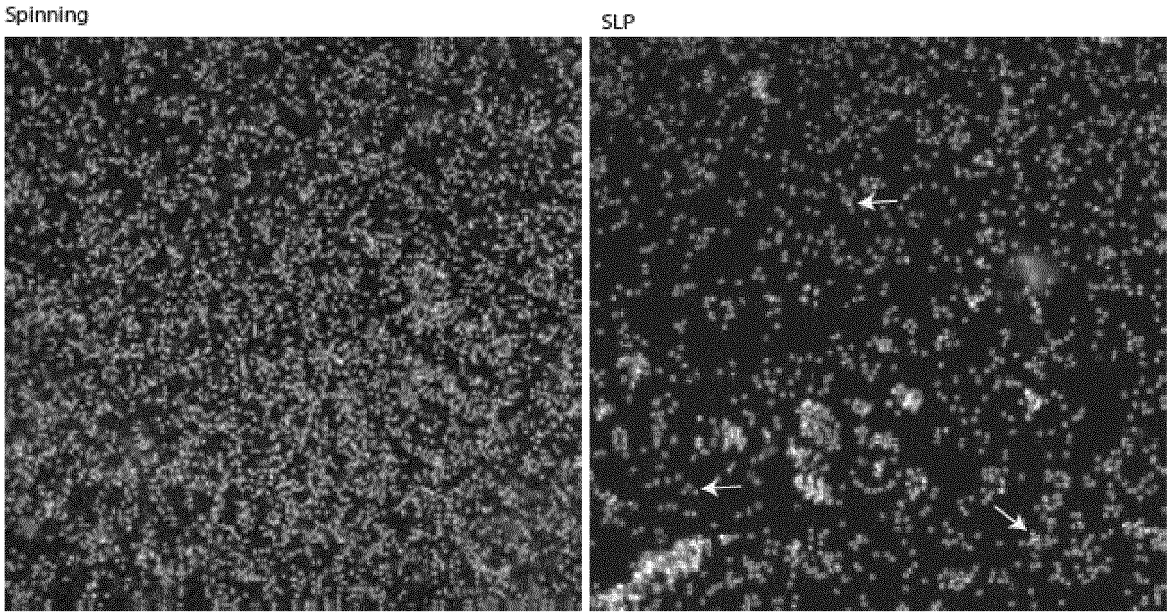


Viability Stain



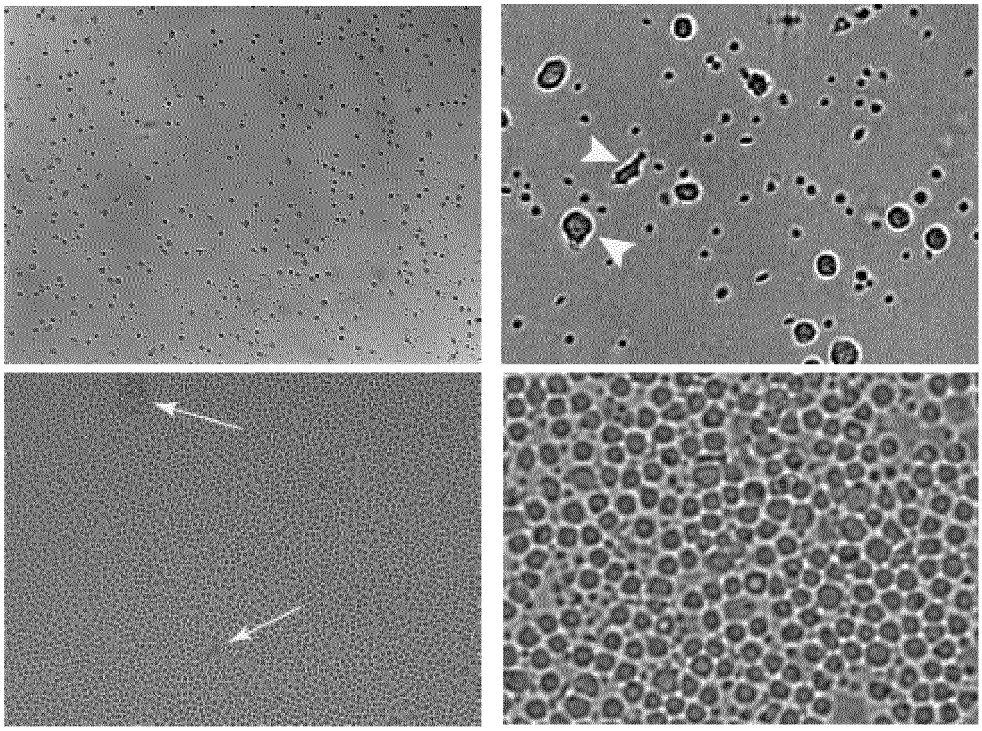
Nuclear Stain 20x

Figure 14B



Arrow - example natural interactions lost upon spinning of plate

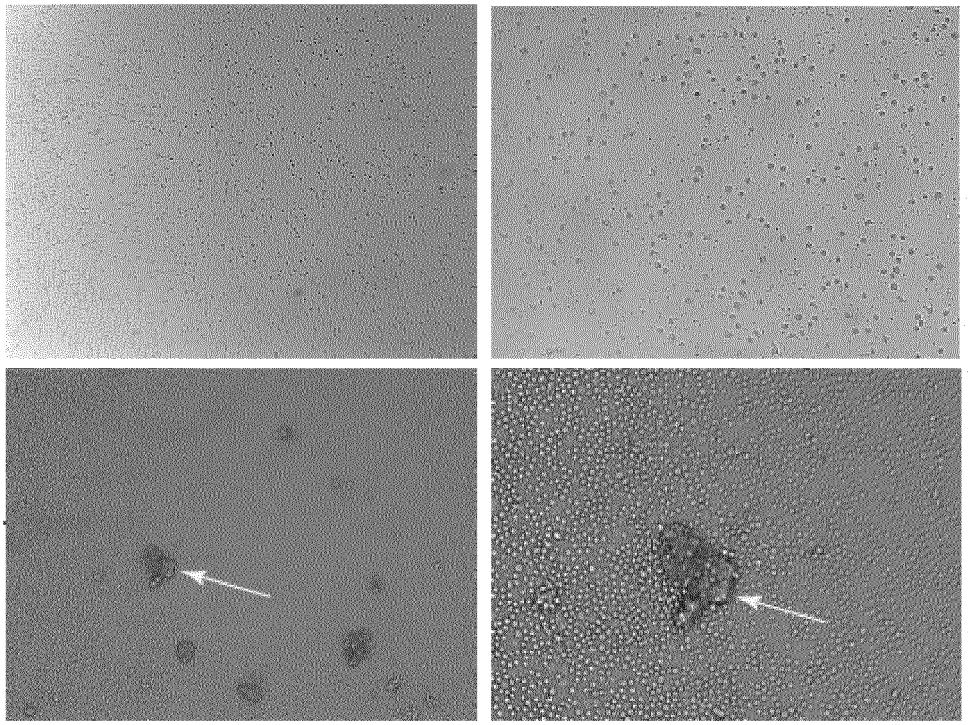
Figure 15A



10x 10x Zoom

Arrow - Clumping
Arrow head- morphology characteristics

Figure 15B



Arrow - Clumping

Figure 15C

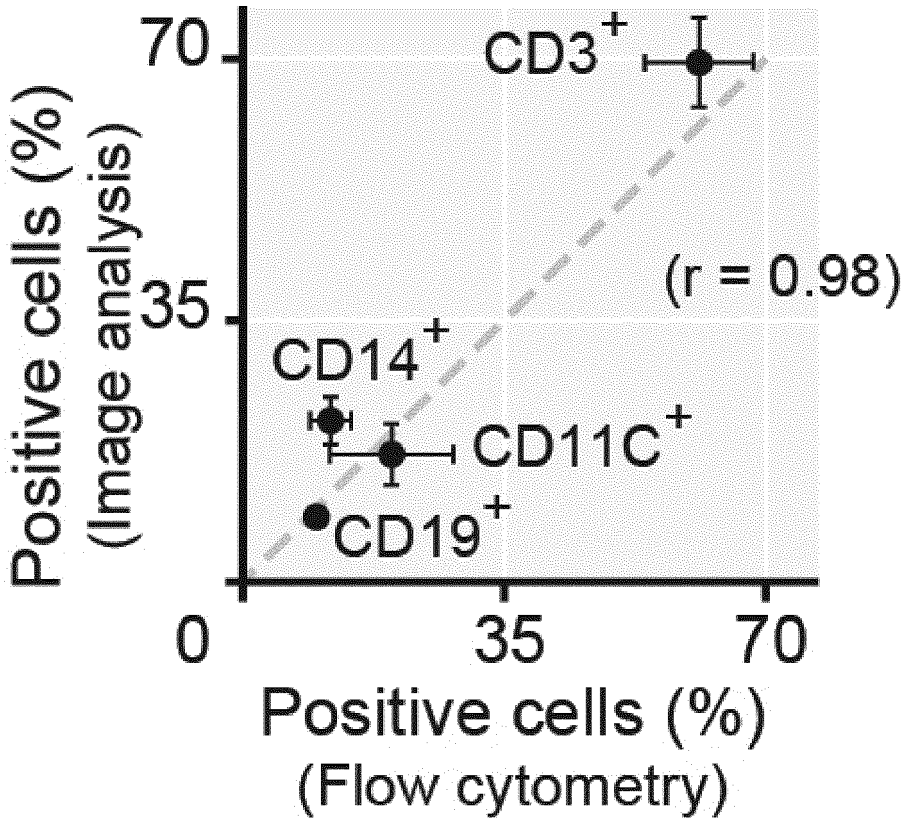

70
35
0
35
70
Positive cells (%)
(Image analysis)
Positive cells (%)
(Flow cytometry)
CD3+
(r = 0.98)
CD14+
CD11C+
CD19+

Figure 16

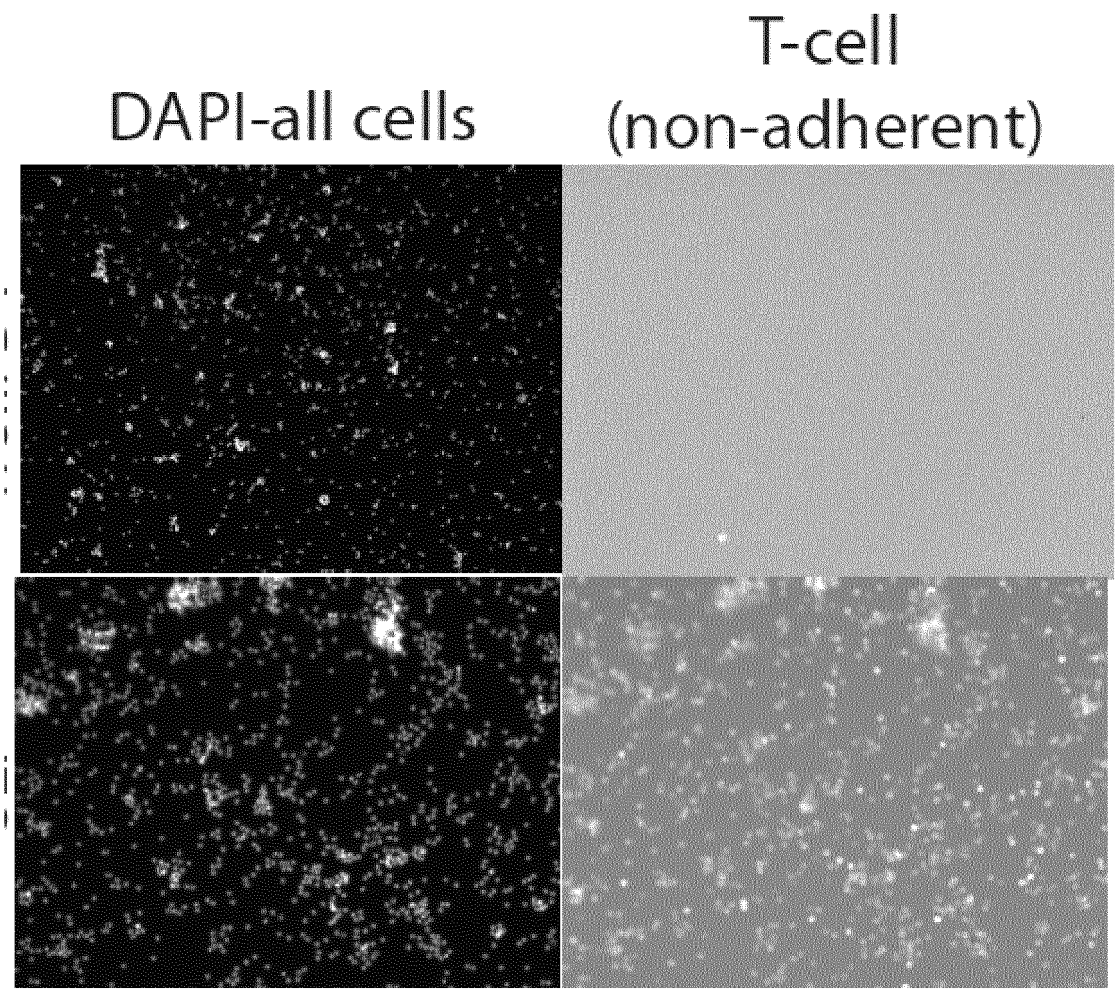


Figure 17