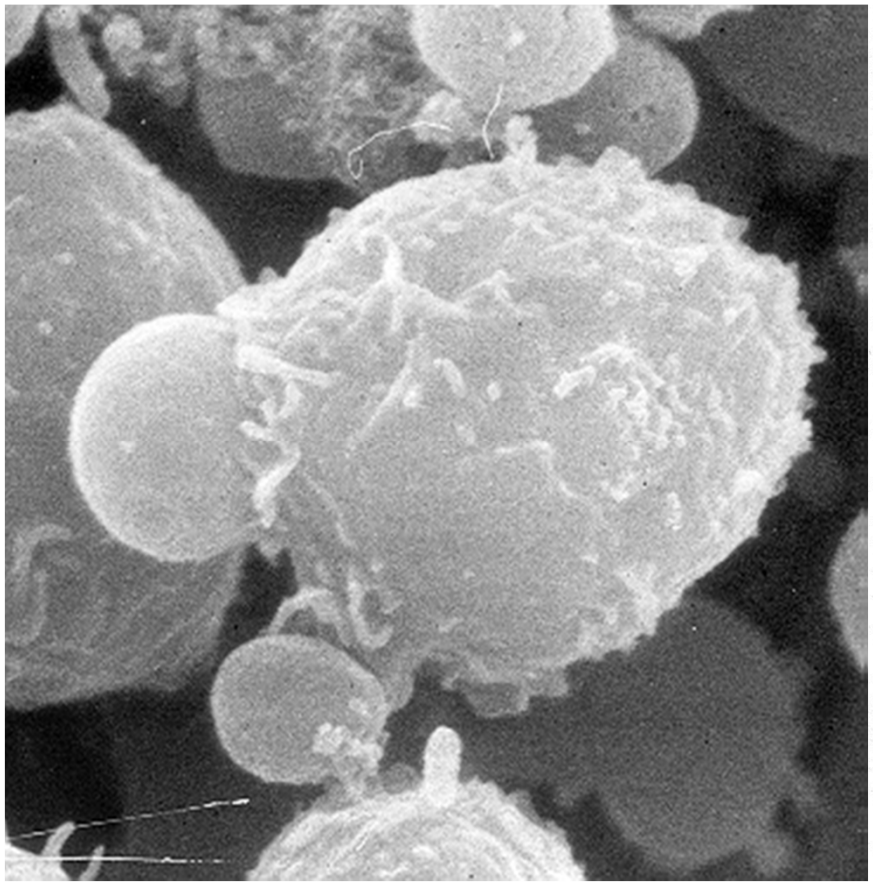
Granulocyte Transfusion (GTX):

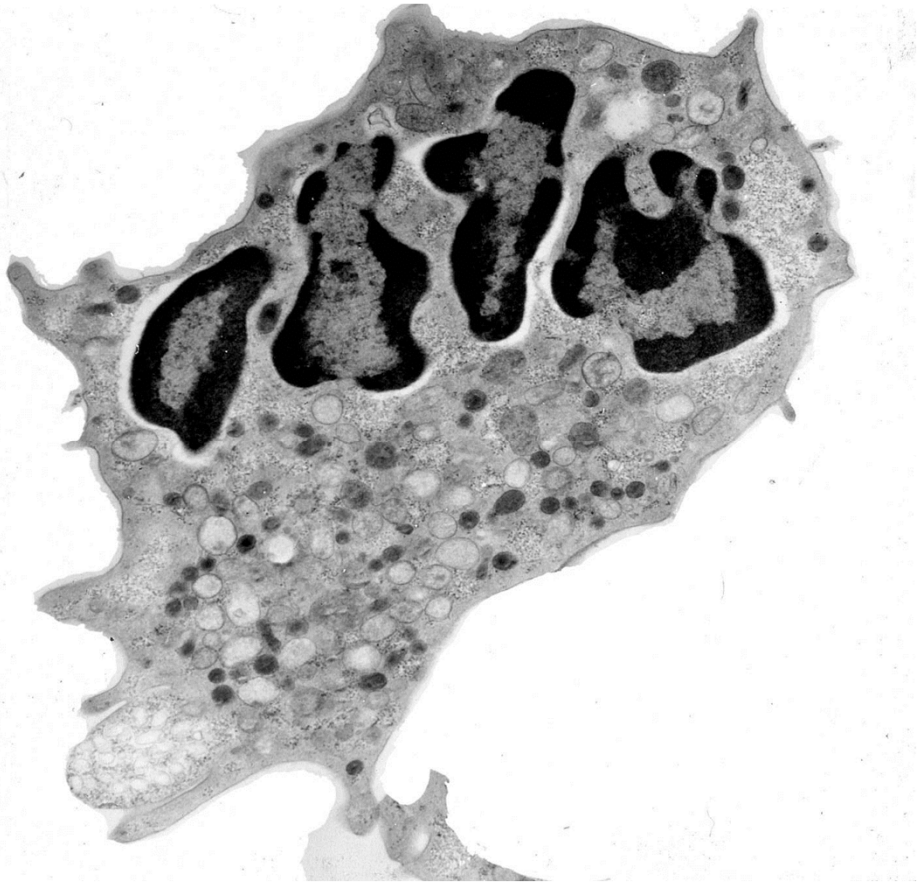
a bigger picture

Introduction to granulocyte transfusions

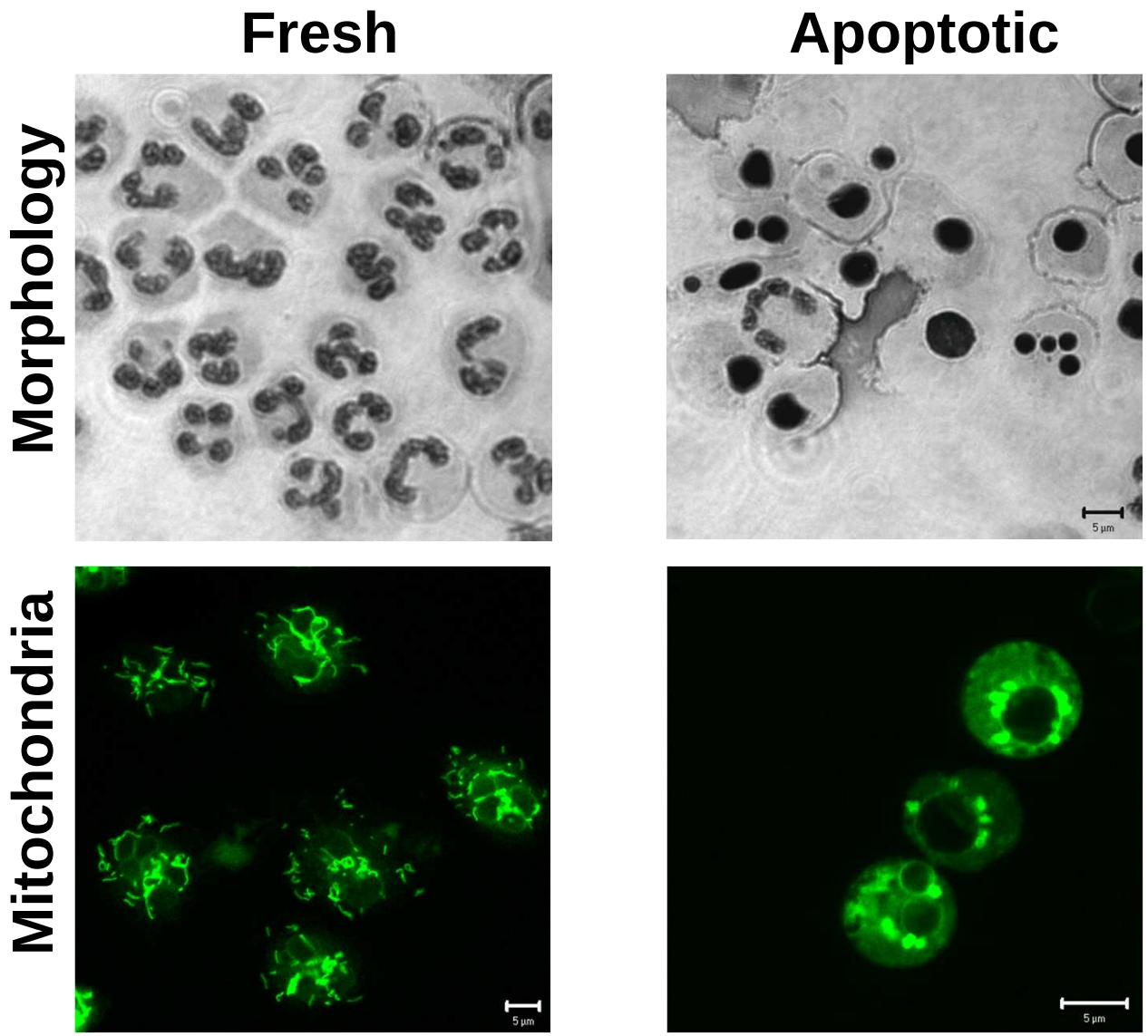
- **Neutrophils are the most frequent leukocyte cell type in the peripheral blood compartment**
- **Neutrophils are part of the innate immunity and play an important role in the host defense against bacterial and opportunistic fungal pathogens**
- **Production in healthy adults is about 10^{11} neutrophils per day**
- **The half-life of a neutrophil is about 8 hrs in the circulation. Once extravasated, neutrophils are assumed to dwell for about 24 hrs in the tissues or, at most, for about 48 hrs when activated by survival factors**

Neutrophil Function





Apoptotic Features in Neutrophils



Granulocyte Transfusions

Type of infection	# treated patients	# evaluable patients	# successfully treated (%)
Bacterial septicemia	298	206	127 (62)
Sepsis, organism unspecified	132	39	18 (46)
Pneumonia, organism unspecified	120	11	7 (64)
Localized infections, other	143	47	39 (83)
Invasive fungus - yeast infections	67	63	18 (29)
Nonspecific fever	184	85	64 (75)

Strauss, J Clin Apheresis 1995, 10:114-8.

Granulocyte Transfusions

Renewed interest in GTx for the following reasons:

- **Increased morbidity and mortality due to infections as a result of intensified chemotherapy and immunosuppressive treatment modalities**

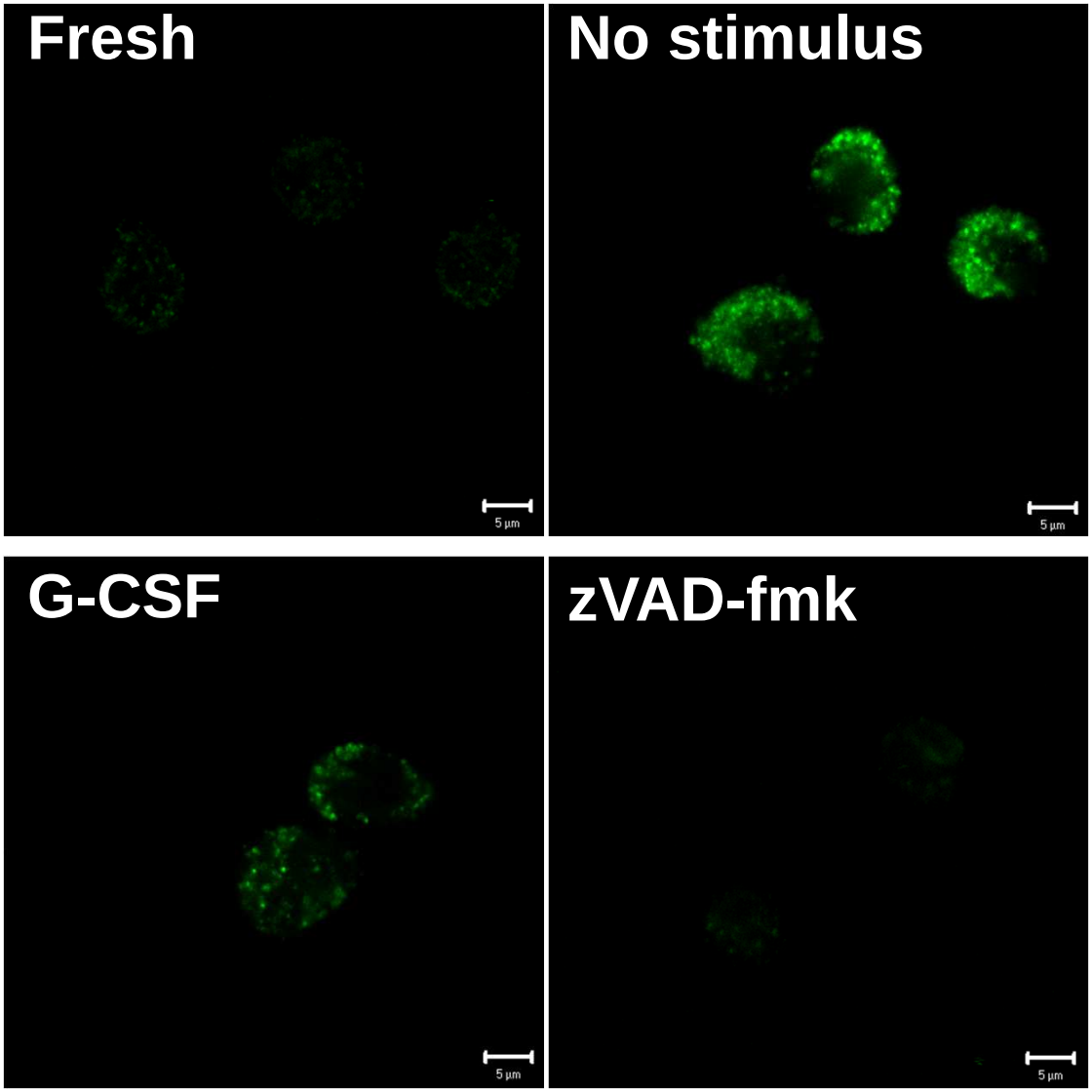
- **Novel antibacterial or antifungal drugs are not sufficient to completely prevent the increased morbidity and mortality**

- **Improvement of donor pretreatment (G-CSF & dexamethasone) and techniques for granulocyte collection result in better yields**

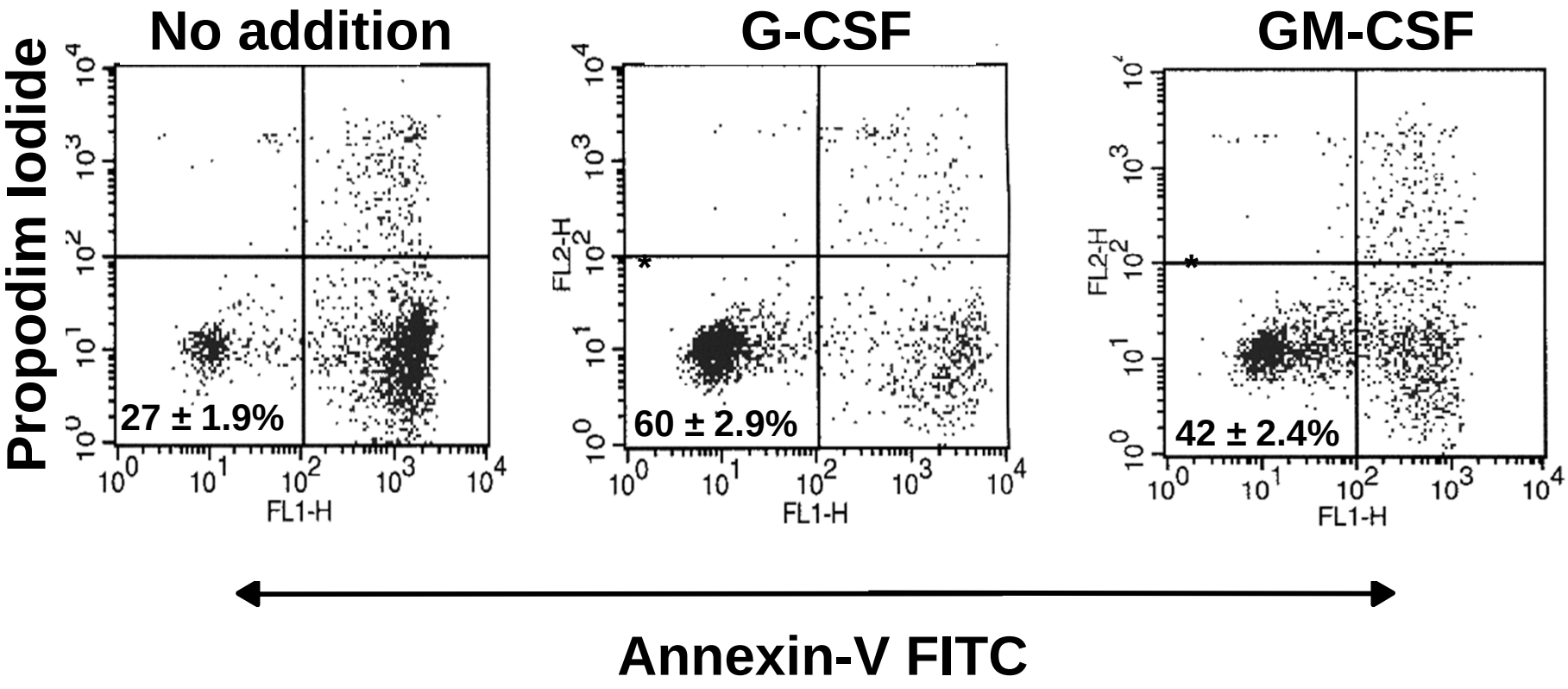
  untreated donors: 0.2-2.0 x 10^{10}; treated donors: 4.0-10 x 10^{10} per GTx

G-CSF effect on granulocyte functions?

Caspase-3 Activity in Neutrophils



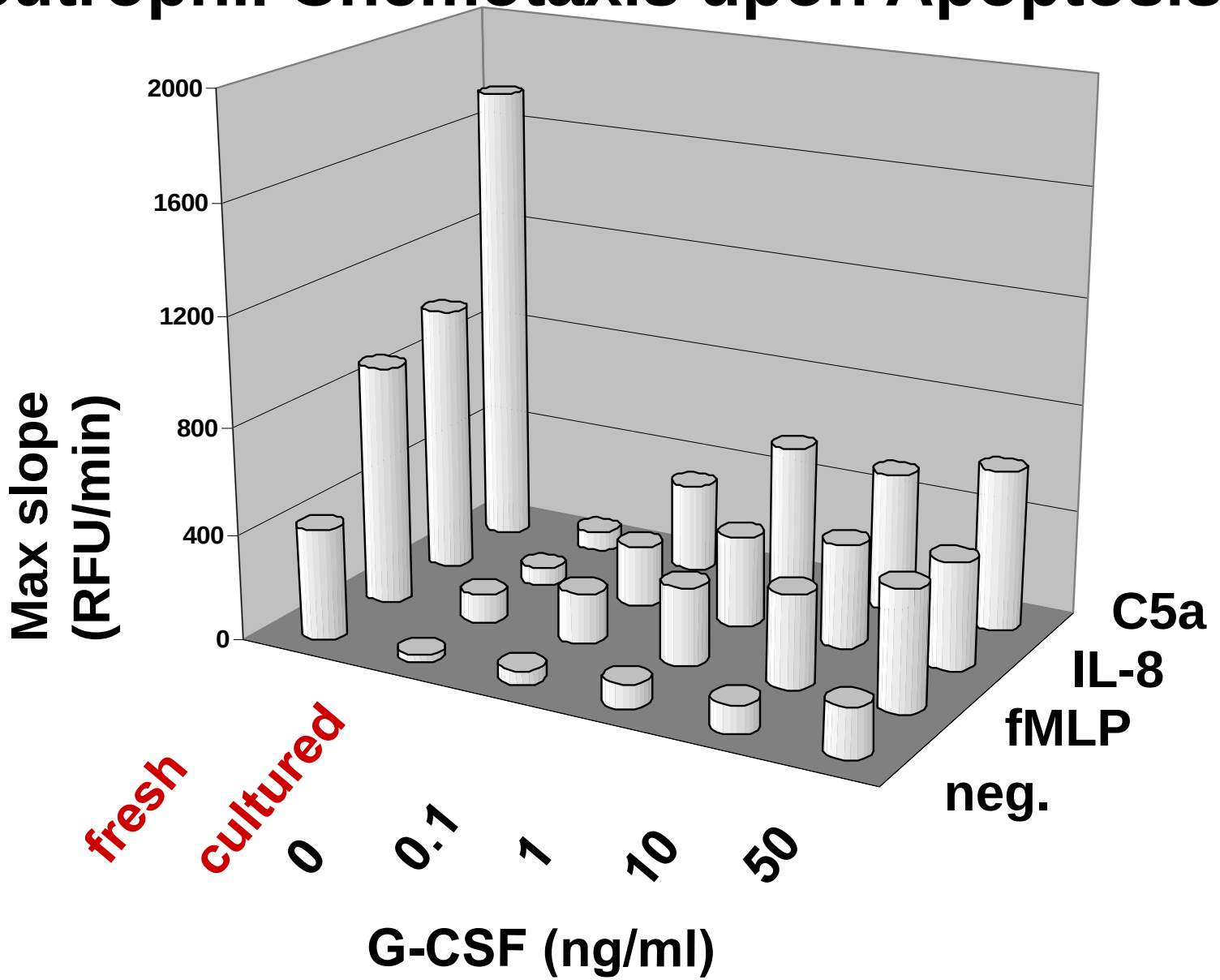
Neutrophil Apoptosis



NADPH oxidase in cultured Annexin^{neg} PMN

Stimulus	H_2O_2 release (% fresh cell activity)		
	Control	G-CSF	GM-CSF
PMA	43.9	61.4	58.0
STZ	67.5	79.9	74.2
fMLP	57.6	168.3	212.0

Neutrophil Chemotaxis upon Apoptosis



Functional Neutrophil Decay

-adhesion & chemotaxis almost absent

-degranulation strongly reduced

-phagocytosis impaired

- NADPH oxidase activity best preserved

in vivo use of G-CSF and dexamethasone: effects on granulocyte numbers & function

BLOOD BANK DONORS for GTX

Granulocyte Transfusions

Granulocyte Concentrates and Logistics

- **Relatives**
- **Unrelated community blood bank transfusion programs**
  Price *et al*. Blood 2000; 95:3302-9
- **Advantages and disadvantages**
  Hubel *et al*. Transfusion 2002; 42:1414-21:
  related donors: > 5 days before effective GTx was organized
  # donors and motivation
  higher increments
  minor HLA incompatibility in future HSCT / BMT

Granulocyte Transfusions

Adverse Events in the Patient:

- **Mild reactions in ~10%: fever and chills**
- **Severe side-effects ~1%: hypotension and respiratory distress (amphotericin B co-medication?)**

Granulocyte Transfusions

Adverse Events in the Patient:

- **TRALI in <0.1%: starting within 6 hrs after GTx**
- **Rapid alloimmunization: more prevalent in patients with neutrophil disorders compared with severely immunosuppressed patients**
- **Lack of neutrophil increments upon GTx**
- **Late leukocyte incompatibility: delayed or reduced myeloid engraftment after SCT**

(Adkins *et al.* Blood 2000; 95:3605-12; Zubair *et al*. Transfusion 2003; 43:614-21)

Granulocyte Transfusions

Adverse Events in the Donor:

- **Headache, bone pain, restlessness**
- **Hydroxyethyl starch (HES)-related severe itching**
- **Repeated G-CSF can result in strongly increased ANC; splenic rupture by repeated G-CSF has been reported**
- **Dexamethasone addition may induce early cataract (posterior capsule)**

(Strauss Br J Haematol 2012; 158:299-306)

Granulocyte Transfusions

Established or Recommended Policy:

- **ABO Rh match with the recipient is obligatory**
- **Prior irradiation with 15-30 Gy avoids problems of GVHD**
- **CMV infection: negative donors in negative recipients**
- **Screening recipients for HLA class I and II antibodies prior to GTx and afterwards, e.g. by using lymphocytotoxicity testing**

Granulocyte Transfusions

Indications: Prophylaxis or Therapy?

Granulocyte Transfusions

Indications: Therapeutic Use in Neutropenia?

- **Adequate dose of >0.5 x 10^9/kg is the only determinant of efficacy in neonates**
- **Adult patients benefit from GTx when:**
  - **survival rate of untreated "control" patients is below 40% (RR = 8.9)**
  - **the dosage is adequate (RR = 4.2)**
  - **neutropenia exists for > 2 weeks (RR = 12.3)**
- **Cross-matched compatible leukocytes were used (RR = 8.0)**

(Vamvakas & Pineda. J Clin Apheresis; 1996; 11: 1-9)

Granulocyte Transfusions

Indications: Therapeutic Use in Neutropenia?

- **Therapeutic transfusion of adequate doses of compatible leukocytes reduced the relative risk of infection, death and death from infection**
  (RR = 0.075, RR = 0.224, and RR = 0.168, resp.)

(Vamvakas & Pineda. J Clin Apheresis; 1997; 12: 74-81)

Primary intervention

Authors	Design	Patients #	Bacterial	Fungal	Infection control %
Dignani *et al.* 1997	uncontrolled	15	0	15	74
Lee *et al*. 2001	uncontrolled	25	13	11	40
Illerhaus *et al.* 2002	uncontrolled	18	8	10	66
Hubel *et al*. 2002	matched pairs	74 vs 74	17 vs 17	57 vs 57	44 vs 59
Rutella *et al*. 2003	uncontrolled	20	11	7	50
Mousset *et al*. 2005	uncontrolled	44	13	31	82

Secondary prophylaxis

Authors	Design	Patients #	Bacterial	Fungal	Reactivation %
Illerhaus *et al.* 2002	uncontrolled	8 (HR 1)	5	2	0
Hubel *et al*. 2002	matched pairs	9 vs 9 (HR 2)	0	7 vs 7	0
Mousset *et al*. 2005	uncontrolled	23 (HR 1)	2	20	0

Granulocyte Transfusions

Indications: Prophylaxis in HSCT or BMT setting?

- **Prophylactic transfusions did not result in significant differences with regard to infectious parameters**
- **Median number of Plt Tx during the course of neutropenia was reduced ($p<0.02$)**

(Illerhaus *et al*. Ann Hematol. 2002; 81: 273-81)

- **Transfusions did not result in differences in mortality between interventional or prophylactic treatment at day 30 (64 vs 65%)**
- **Shift from predominant use for bacterial containment toward prevention or treatment of fungal disease**

(Mousset *et al*. Ann Hematol; 2005; 84: 734-41)

Alternative for GTx ?

In case of prophylaxis in HSCT or BMT settings

- **Myelosuppressive instead of myeloablative HSCT regimens**
- **Secondary antifungal prophylaxis with voriconazole in leukemic patients and HSCT recipients**

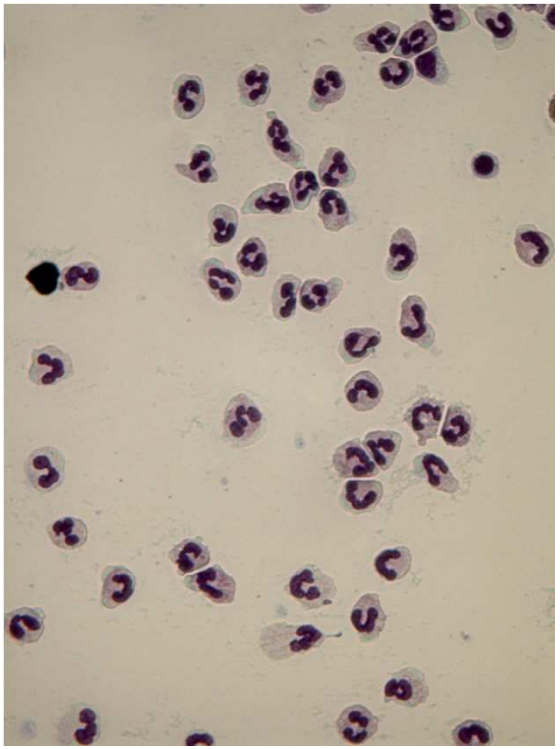
  (Cordonnier *et al*. Bone Marrow Transplant 2004; 33: 943-8)

- **Development of novel antimicrobial drugs**

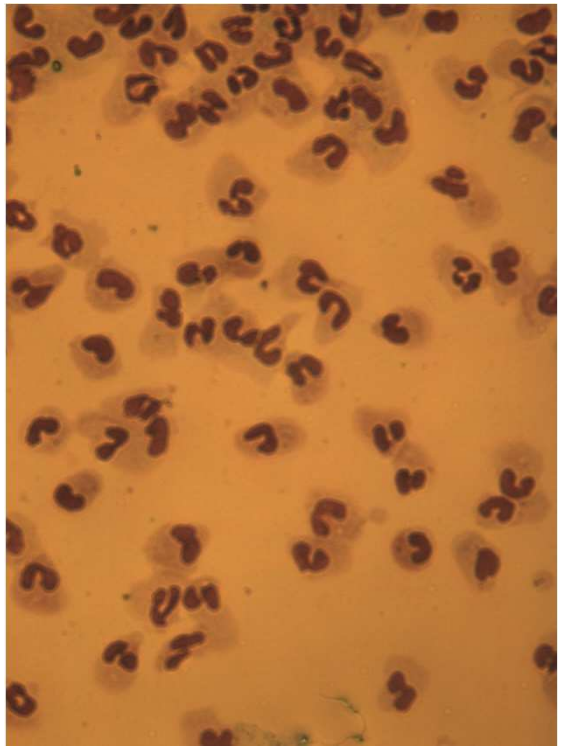
in vivo use of G-CSF and dexamethasone: effects on granulocyte numbers & function

After 24h storage…

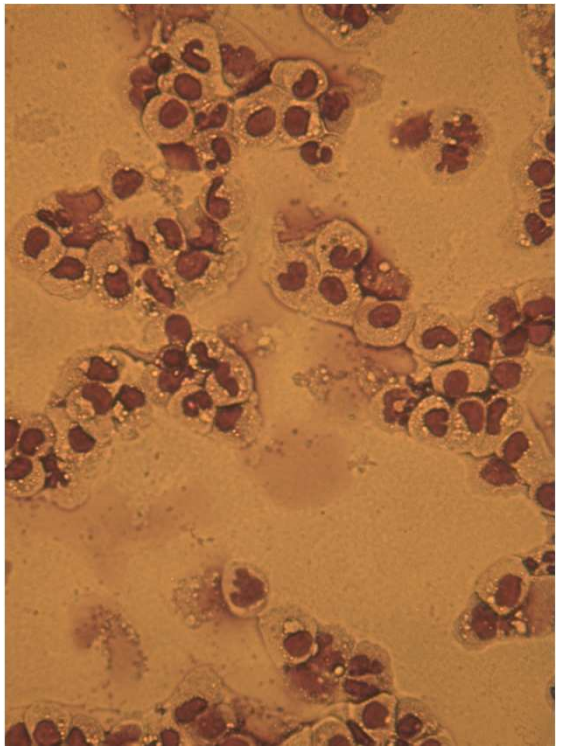
0 hrs



24 hrs



48 hrs

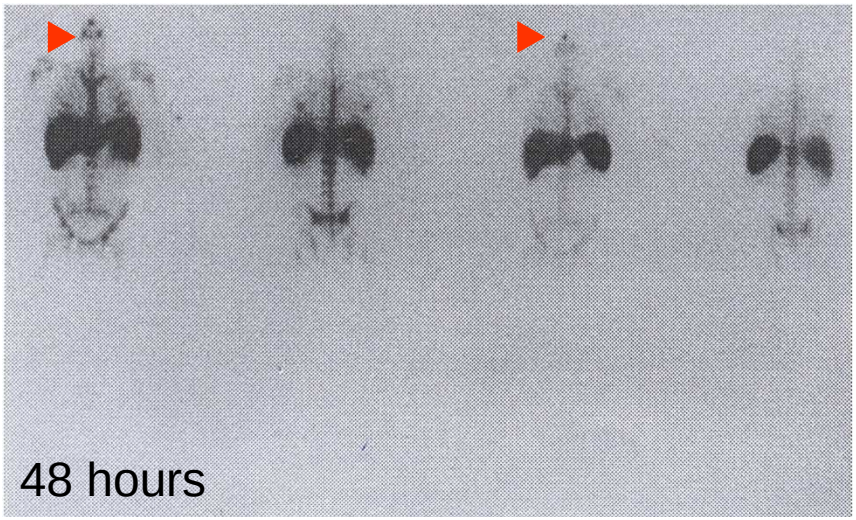


Neutrophil Chemotaxis *in vivo*

^{111}Indium-labeled WBC scans to sites of tissue damage

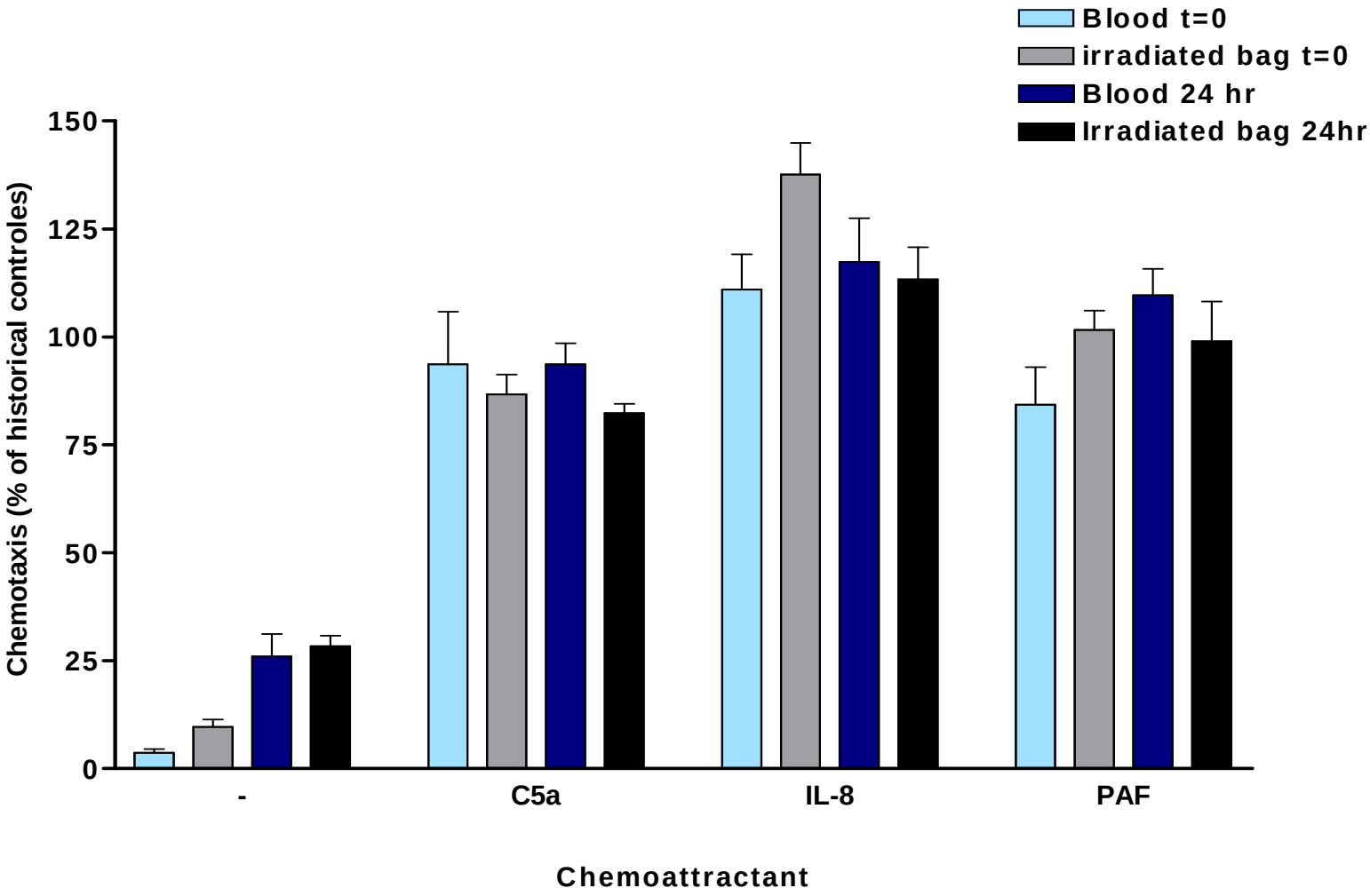
Patient 1

severe mucositis



Adkins *et al*. Bone Marrow Transpl. 1997;19:809-12

Granulocyte Concentrates:
neutrophil chemotaxis after 24 hours of storage



Unstimulated motility is slightly enhanced after 24h / directed chemotaxis is unaltered

Changes in gene expression of granulocytes during in vivo granulocyte colony-stimulating factor/dexamethasone mobilization for transfusion purposes

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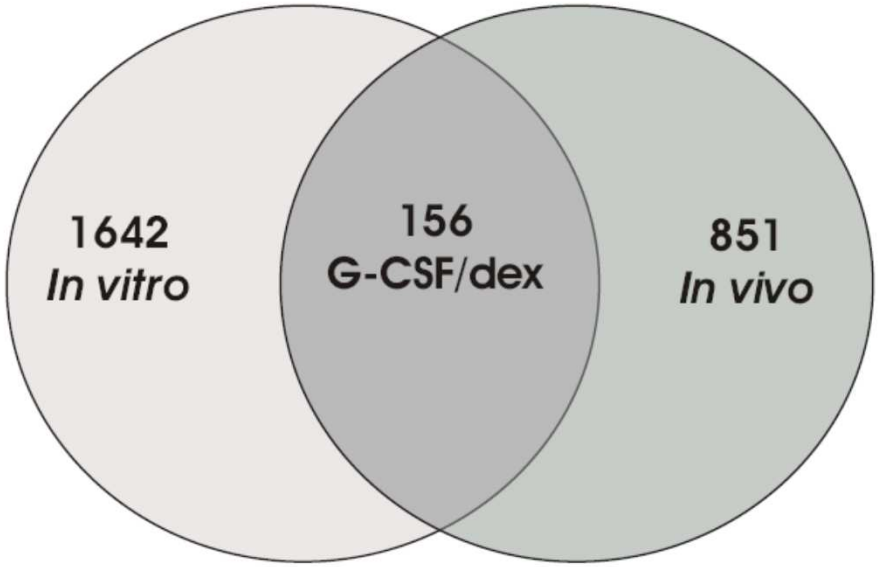
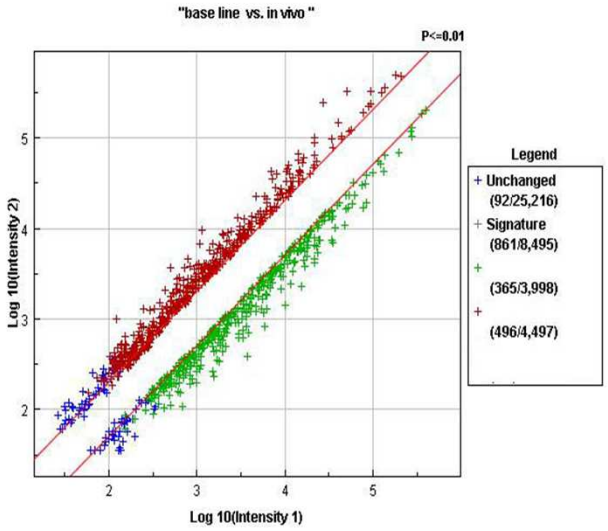
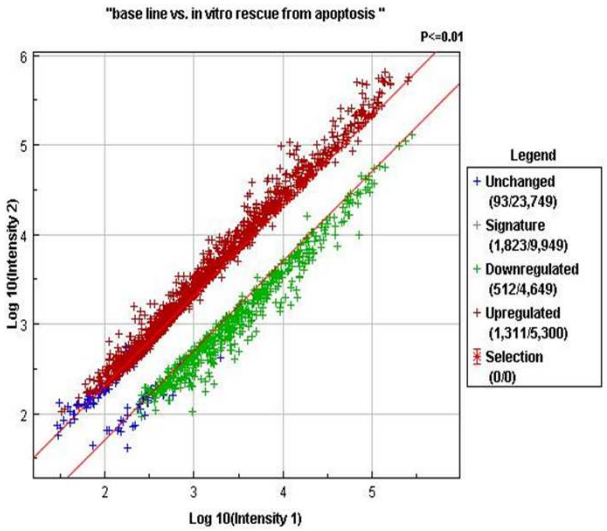
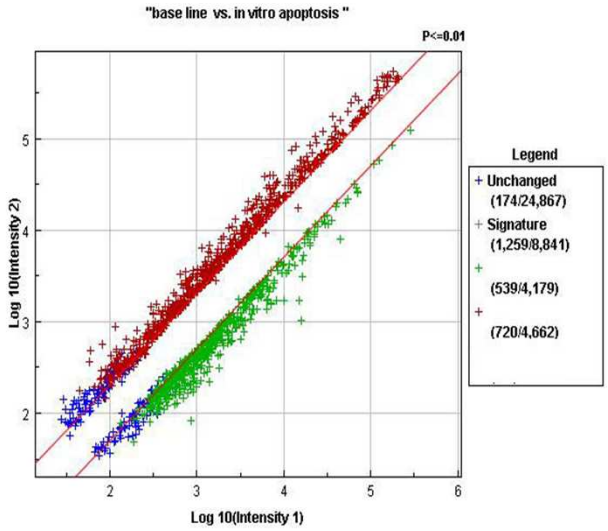
[1]Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, Amsterdam; [2]Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam; and [3]Department of Neurogenetics, Academic Medical Center, Amsterdam, The Netherlands



Drewniak *et al*. Blood 2009; 113: 5979-5998





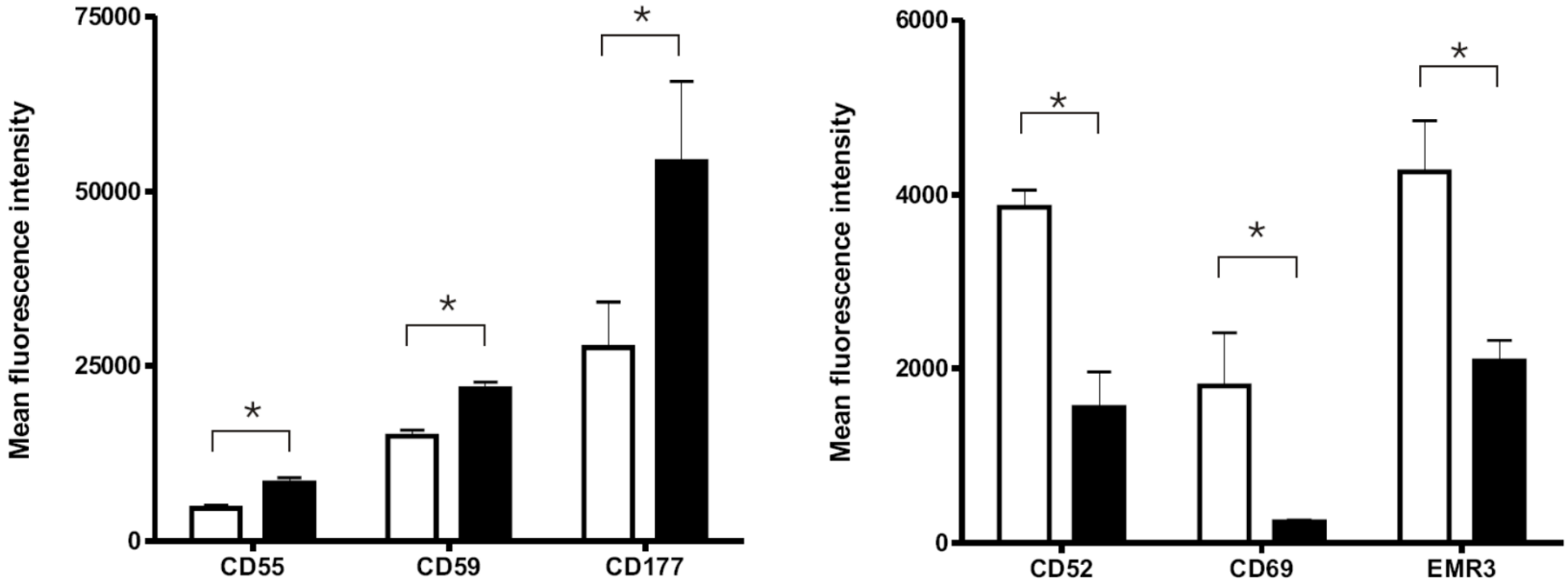


Treatment	Changed	Upregulated	Down-regulated
G-CSF/dexamethasone (*in vivo*)	861	365	496
G-CSF/dexamethasone (*in vitro*)	1823	512	1311
No treatment (*in vitro)*	1259	539	720

Fold change ≥ 3

P value ≤ 0.01

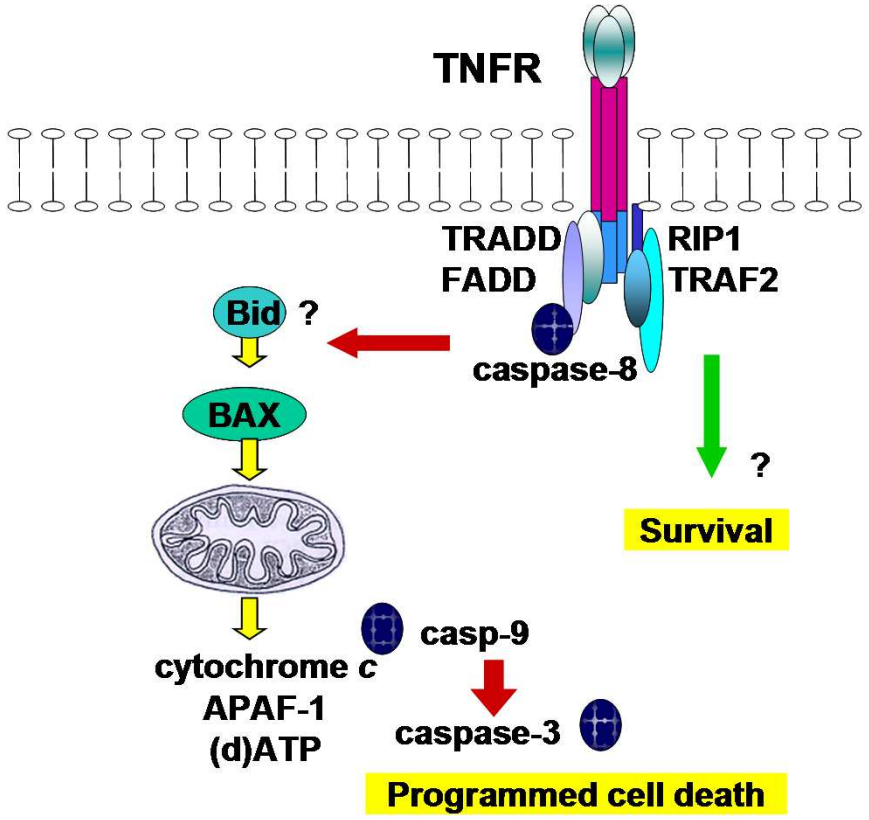
Expression of surface proteins: verification of up- and downregulation



Apoptosis

Up-regulated

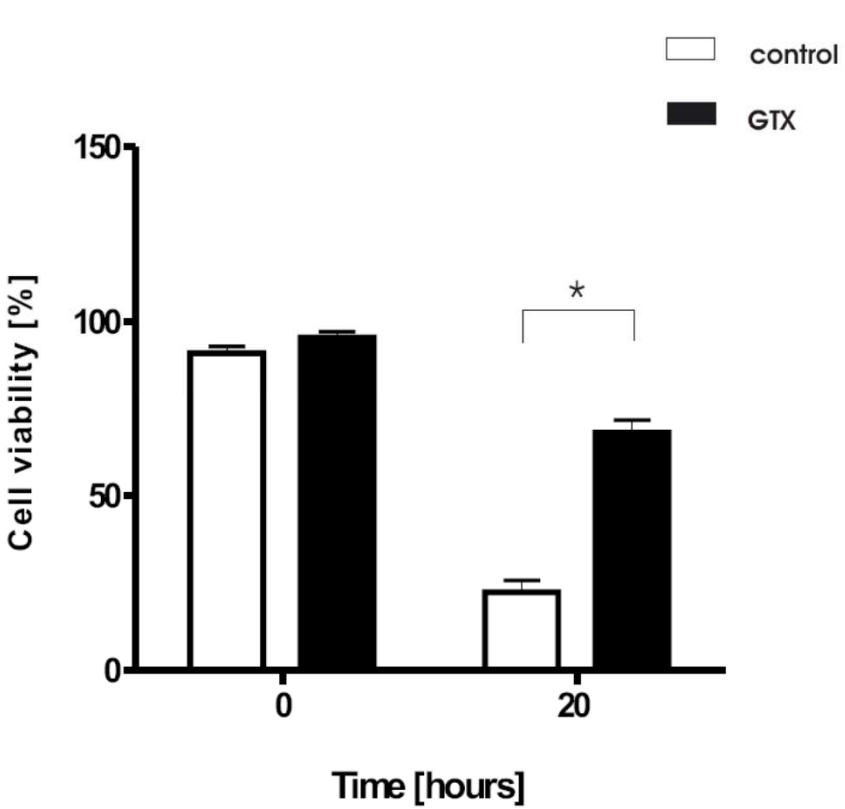
NAIP
NALP3
GADD45A
HIP1
HIPK2
ANXA1
IL10
CARD12
ELMO2
SPP1
STK3
CALPASTATIN
CARD6
TNFRSF10A
IHPK2
GALECTIN1
DDAH2
SH3GLB1
PERC

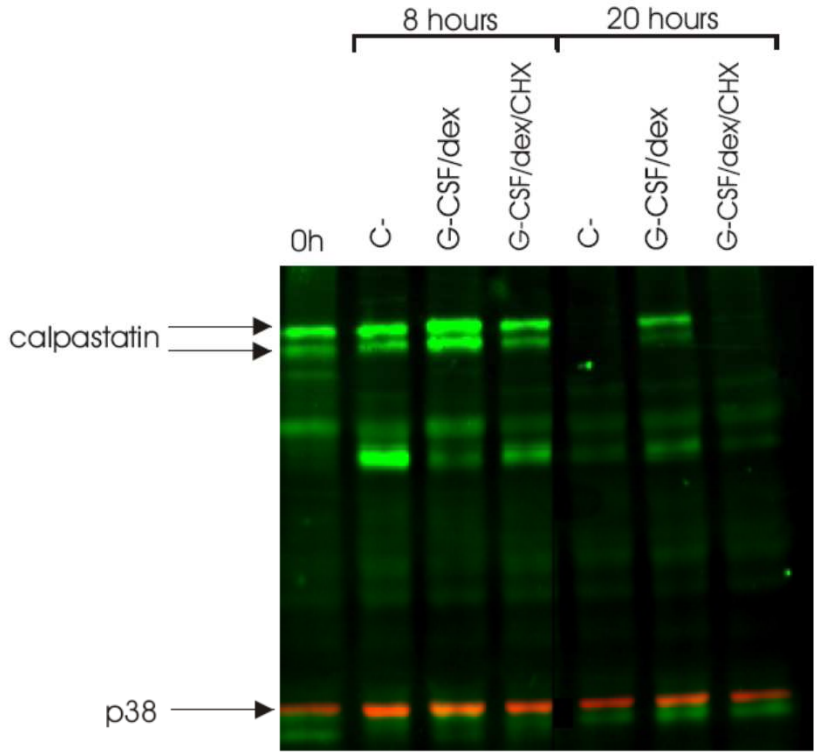


Down-regulated

CARD4
STEX
GSTP1
GZMB
AZU1
PCB4
CARD9
STAT1
PRDX2
GALECTIN 12
TNFRSF25
TNFSF12
SQSTM1
FAIM3

Expression of survival and function: verification of changes





Toll-like receptor–induced reactivity and strongly potentiated IL-8 production in granulocytes mobilized for transfusion purposes

Agata Drewniak,[1,2] Anton T. J. Tool,[1] Judy Geissler,[1] Robin van Bruggen,[1] Timo K. van den Berg,[1] and Taco W. Kuijpers[1,2]

[1]Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory, and [2]Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Drewniak *et al*. Blood 2010; 115: 4588-4596