Granulocyte concentrates: who knows when to transfuse

Taco Kuijpers, MD PhD

Granulocyte transfusions

- Neutrophils are the most frequent leukocyte cell type in the peripheral blood compartment and are part of the innate host defense against bacterial and fungal pathogens
- Production in healthy adults is about 10^{11} neutrophils per day
- The half-life of a neutrophil in the circulation is about 8-12 hrs. Once extravasated, neutrophils are assumed to dwell for about 24 hrs in the tissues or 48-96 hrs when activated by survival factors
- Granulocyte transfusions (GTX) for neutropenic patients have been used for over 40 years, effectiveness and indications remain debated

Granulocyte transfusions

Type of infection	# treated patients	# evaluable patients	# successfully treated (%)
Bacterial septicemia	298	206	127 (62)
Sepsis, organism unspecified	132	39	18 (46)
Bacterial pneumonia	5	–	–
Pneumonia, organism unspecified	115	11	7 (64)
Invasive fungal infections	67	63	18 (29)
Localized infections	143	47	39 (83)
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
- Novel antibacterial or antifungal drugs are not sufficient to completely prevent the increased morbidity and mortality
- Improvement of donor pretreatment and techniques for granulocyte collection result in better yields

Granulocyte transfusions

	Donor stimulation	# Aphereses per donor	Mean PMN (10^9) per apheresis
Bensinger *et al.* 1993	G-CSF 5 μg/kg/day	4-12	42
Caspar *et al*. 1993	G-CSF 300 μg	1	44
Hester *et al.* 1995	G-CSF 5 μg/kg/day	4-5	32-66
Dale *et al*. 1998	G-CSF 600 μg Dexamethasone 8 mg	1	78
Jendiroba *et al*. 1998	G-CSF 5 μg/kg/day x 5	4-5	42-46
	or		
	Prednisone 60 mg/day x 5		29-32
Adkins *et al*. 2000	G-CSF 10 μg/kg on days 1, 4, 6, and 8	4	56-99
Hubel *et al*. 2000	G-CSF 600 μg	1	73

Granulocyte transfusions

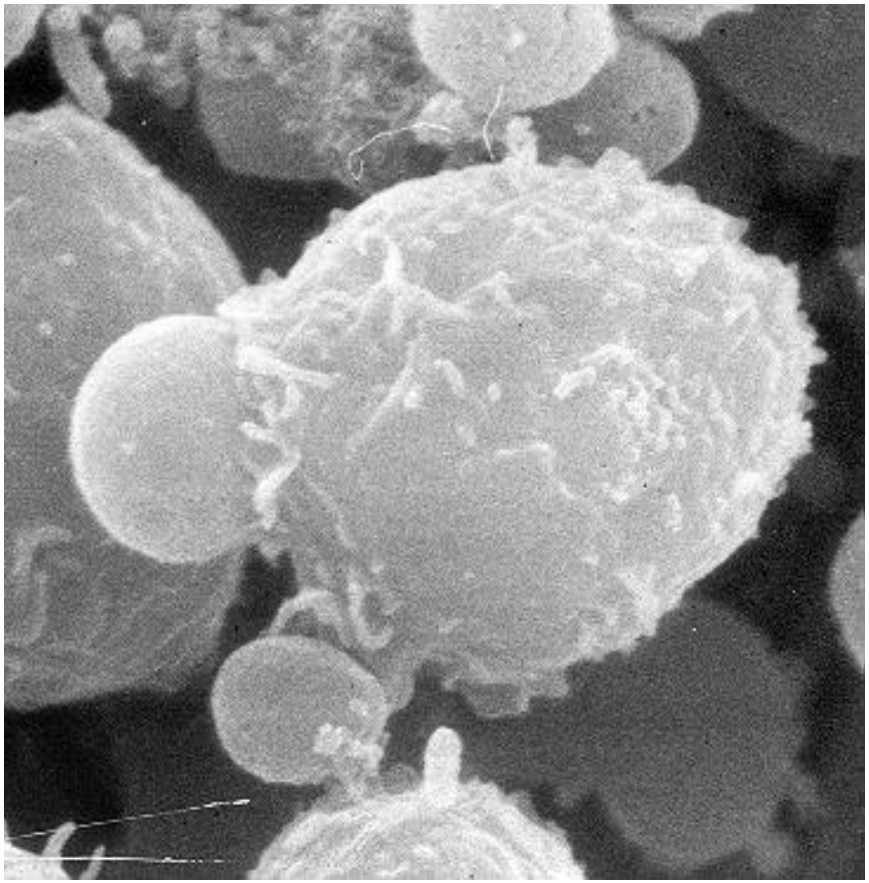
GTX & fungal infections after 1995?

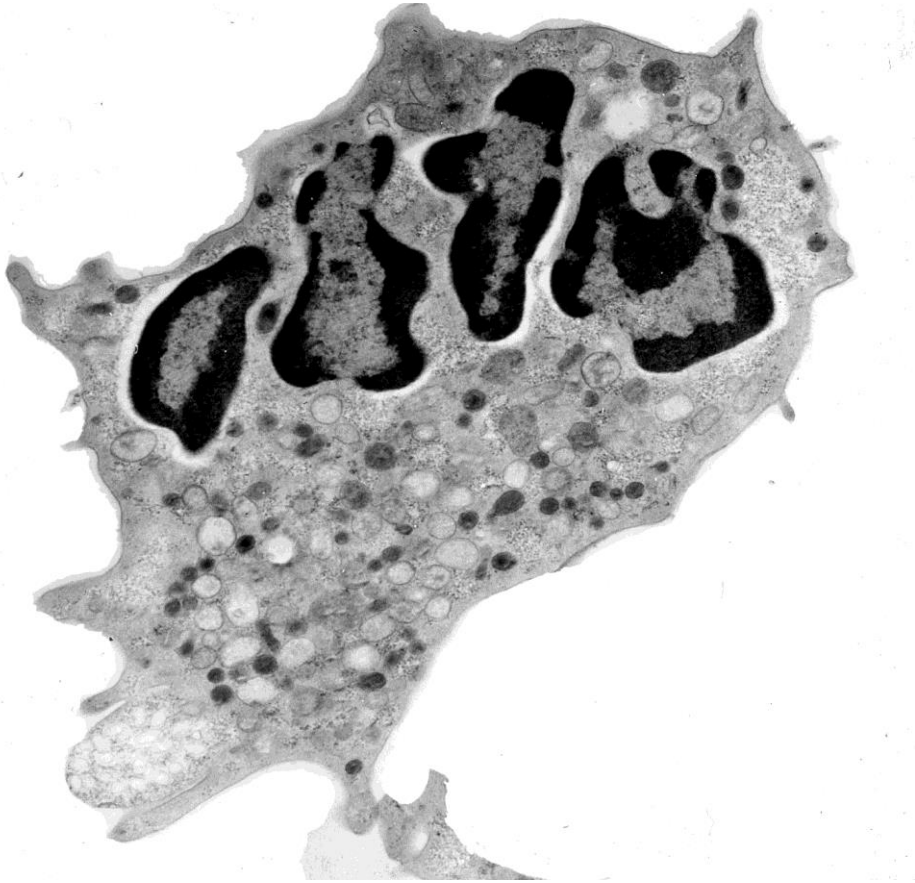
- Increased survival in patients unresponsive to standard therapy:
  9 of 15 patients (60%) showed objective improvement
  (Hester *et al*. J Clin Apheresis 1995;10:188-93)

- Impressive responses in 11 of 15 patients (80%) with invasive fungal diseases resistant to amphotericin B (Dignani *et al*. Leukemia 1997;11:1621-30)

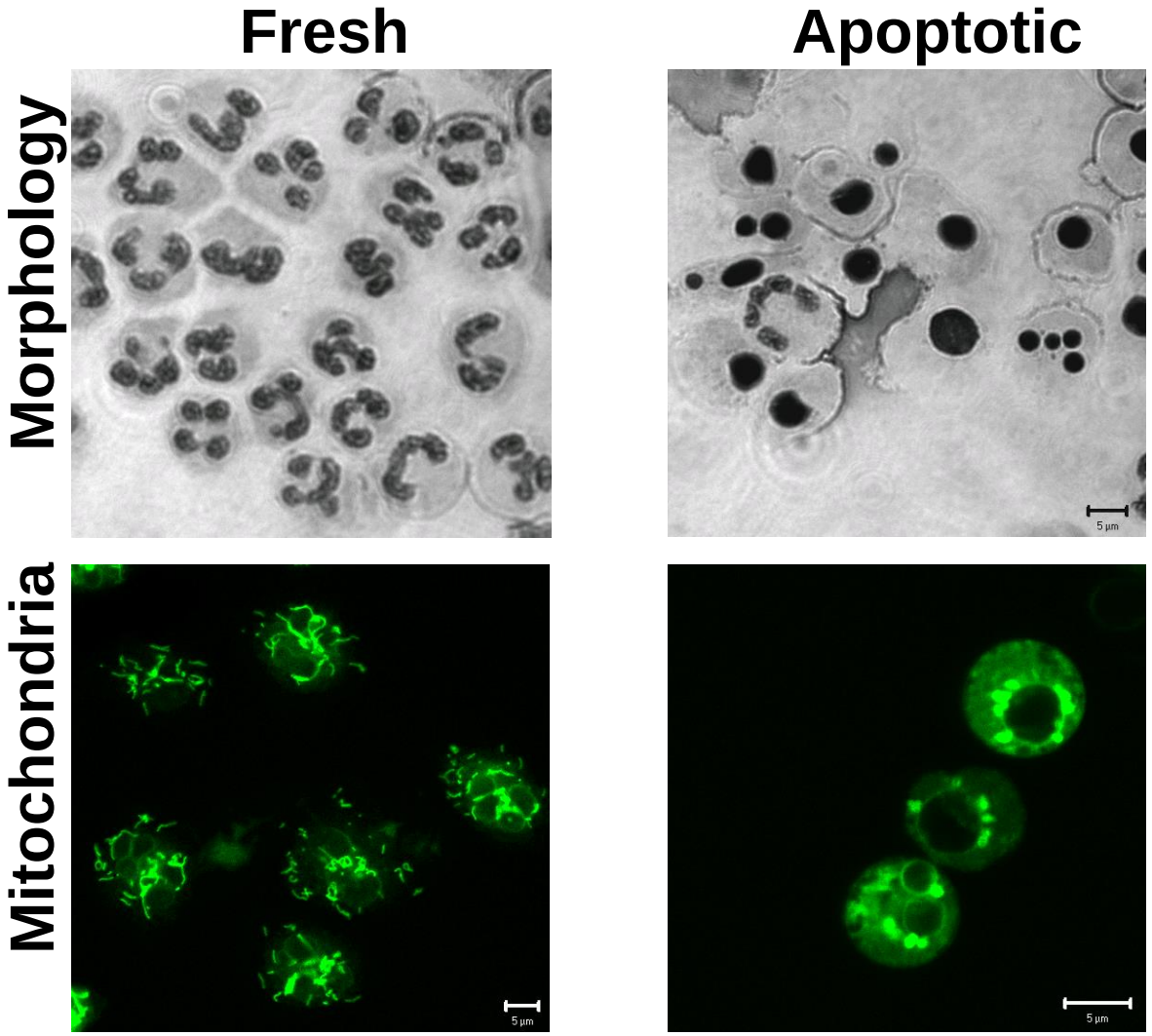
- Survival to day 100 of 14 of 23 patients (60%) with severe fungal infection (Peters *et al*. Br J Haematol 1999;106:689-96)

Neutrophil function



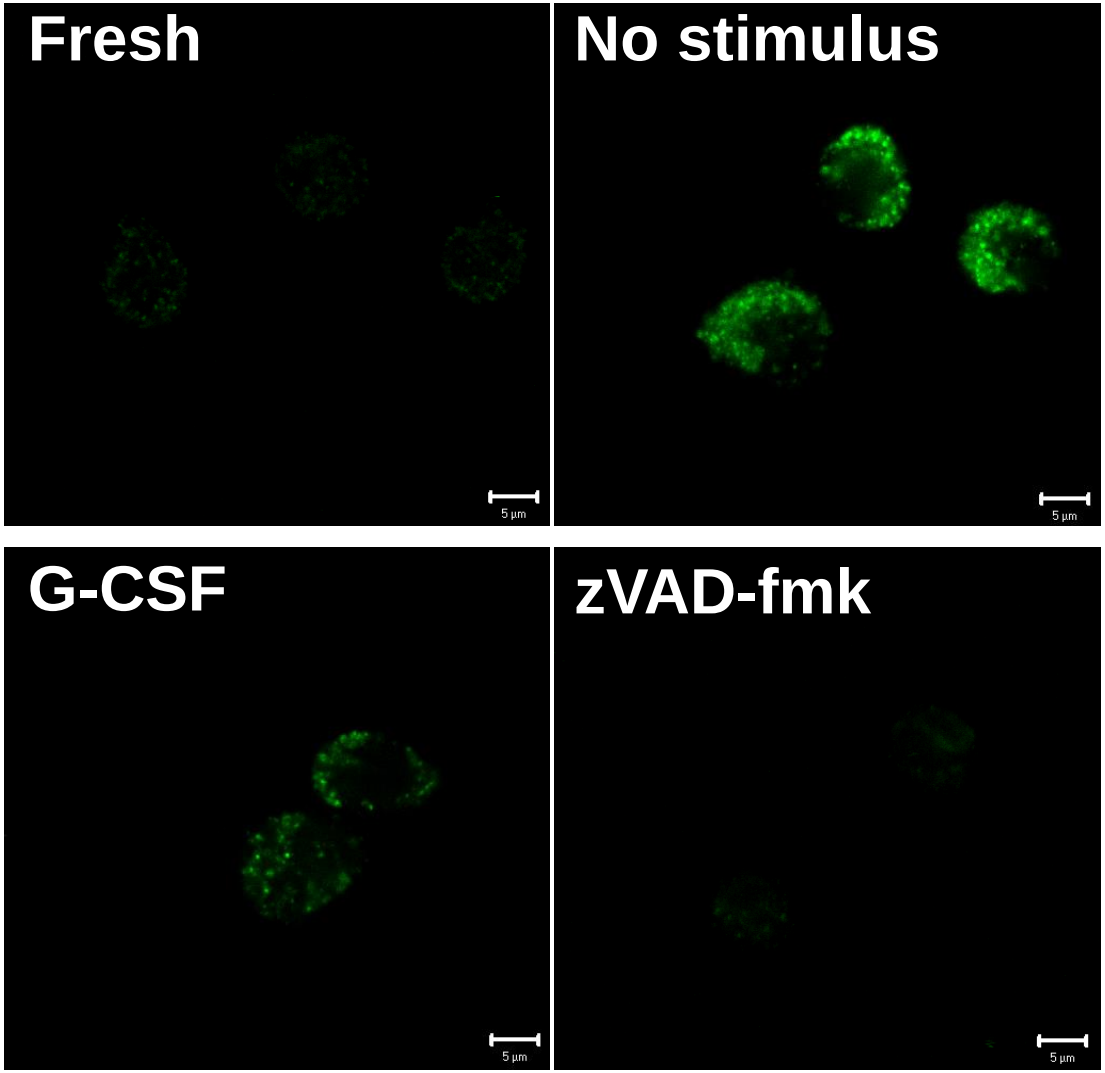


Apoptotic features in neutrophils



Maianski et al. Blood. 2002; 99:672-9.

Caspase-3 activity in neutrophils



Maianski et al. Blood. 2002; 99:672-9.

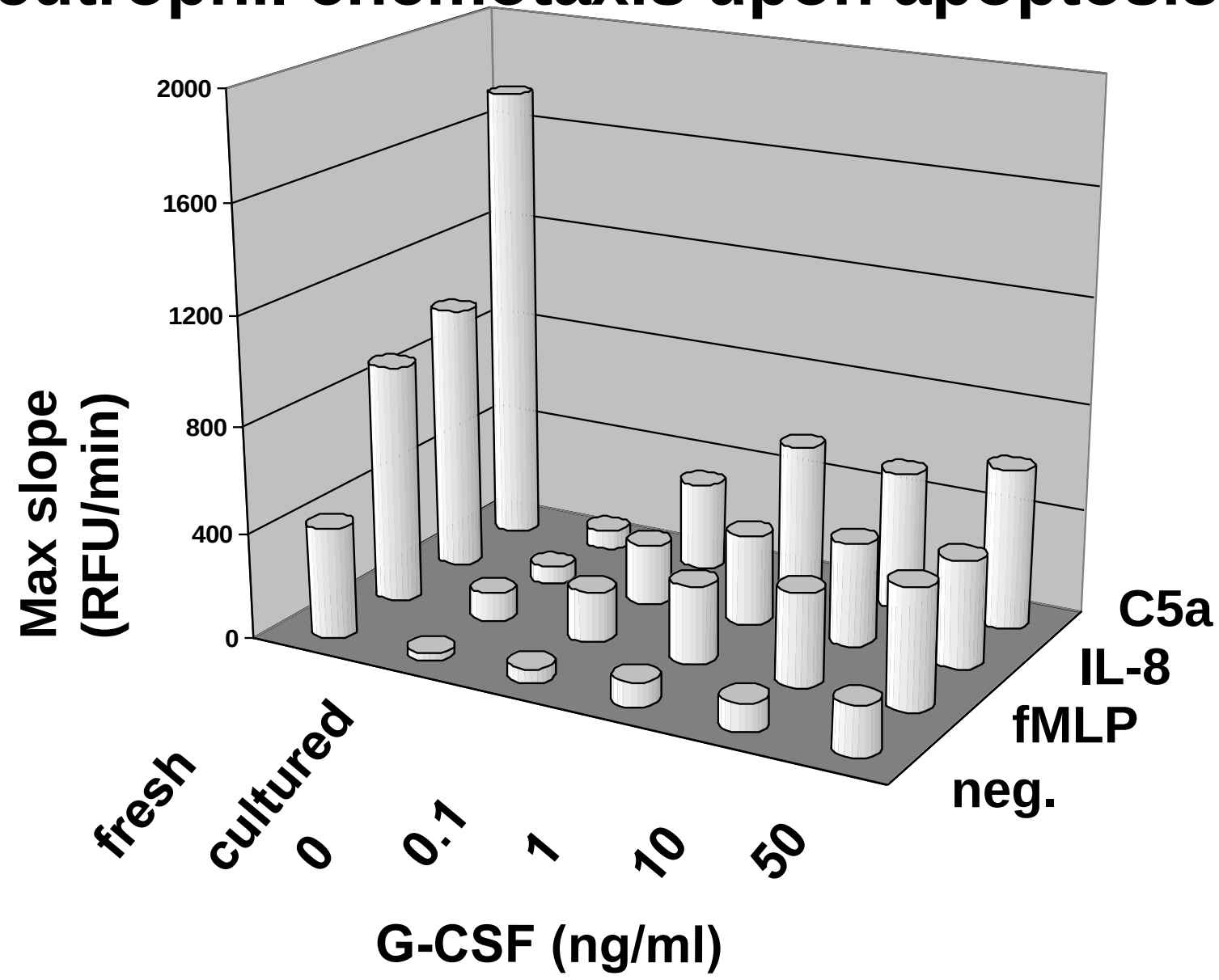
ROS activity by cultured neutrophils

non-apoptotic neutrophils*

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

* Negative for Annexin-V, Propidium Iodide and active caspase 3 staining

Neutrophil chemotaxis upon apoptosis



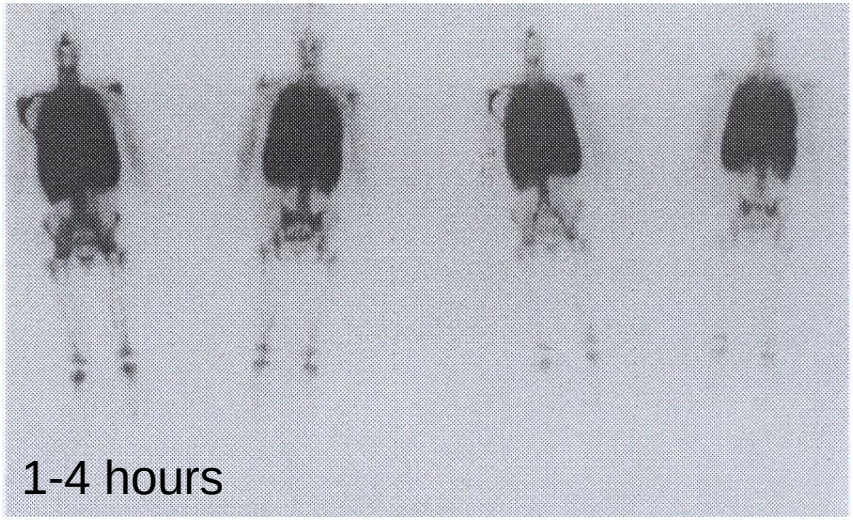
Functional neutrophil decay *in vitro*:

- **adhesion & chemotaxis <u>almost absent</u>**
- **degranulation strongly reduced**
- **phagocytosis impaired**
- **NADPH oxidase activity best preserved**

Wolach et al. Exp Hematol. 2007 Apr;35(4):541-50.

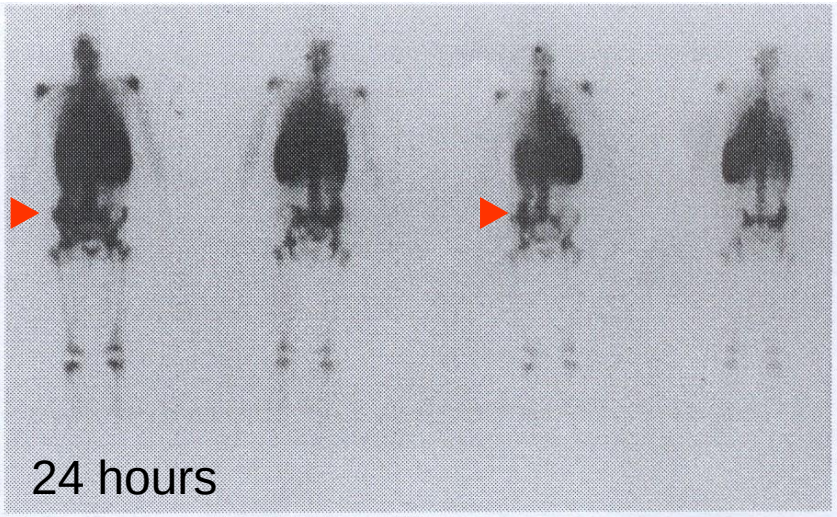
GTX neutrophil chemotaxis *in vivo*

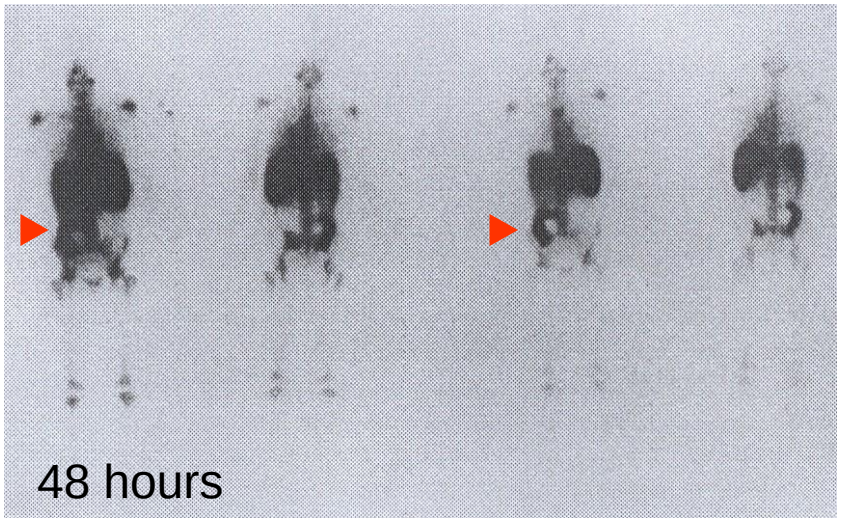
^{111}Indium-labeled WBC scans to sites of tissue damage



Patient

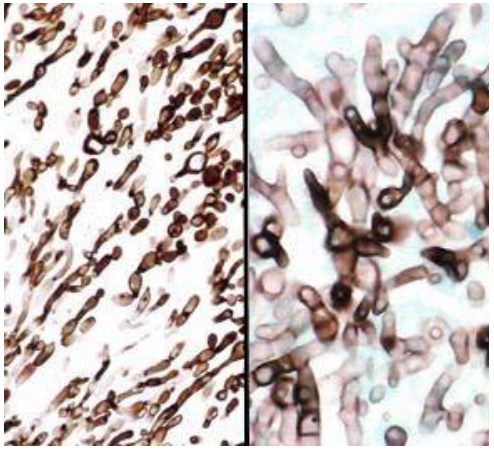
neutropenic colitis

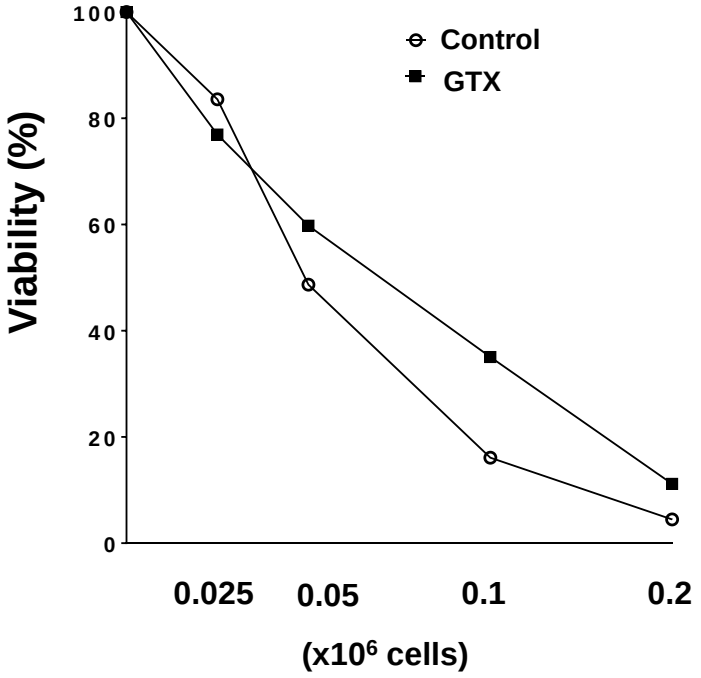




Adkins *et al*. Bone Marrow Transpl. 1997;19:809-12

G-CSF/dexa derived GTX neutrophils:
Killing of *Aspergillus fumigatus* hyphae





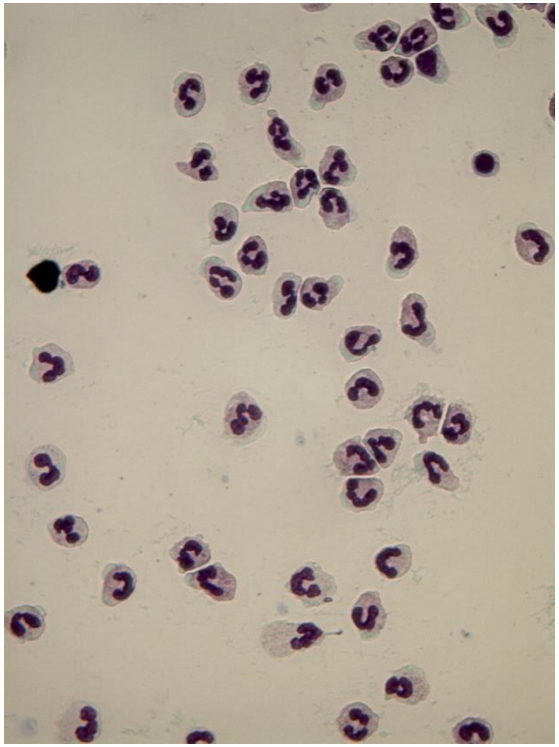
N=7

Van de Geer et al. Vox Sang. 2017 Feb;112(2):173-182

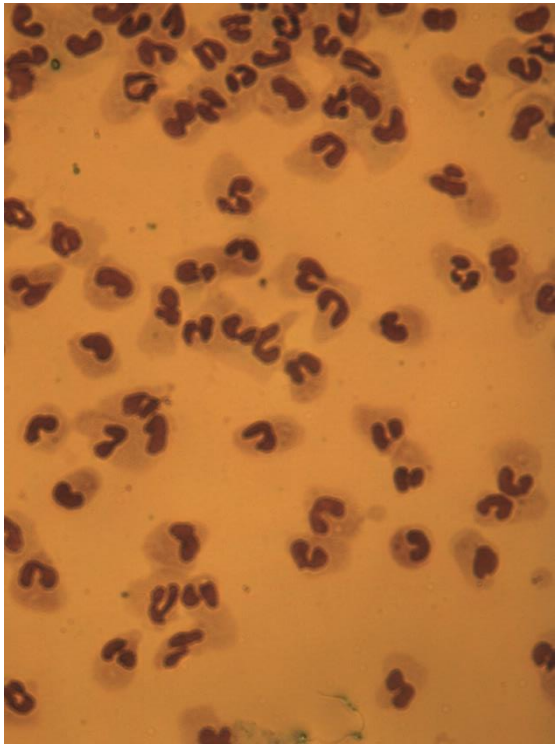
G-CSF/dexa derived GTX neutrophils:

After 24hr storage time…

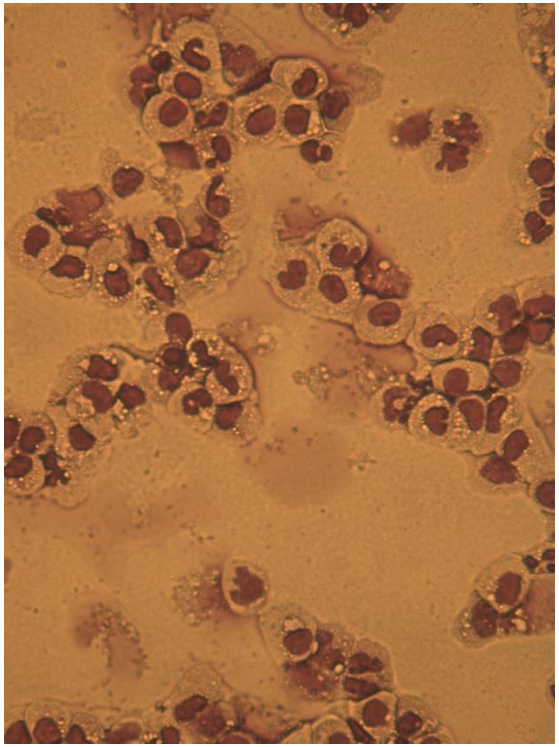
0 hrs



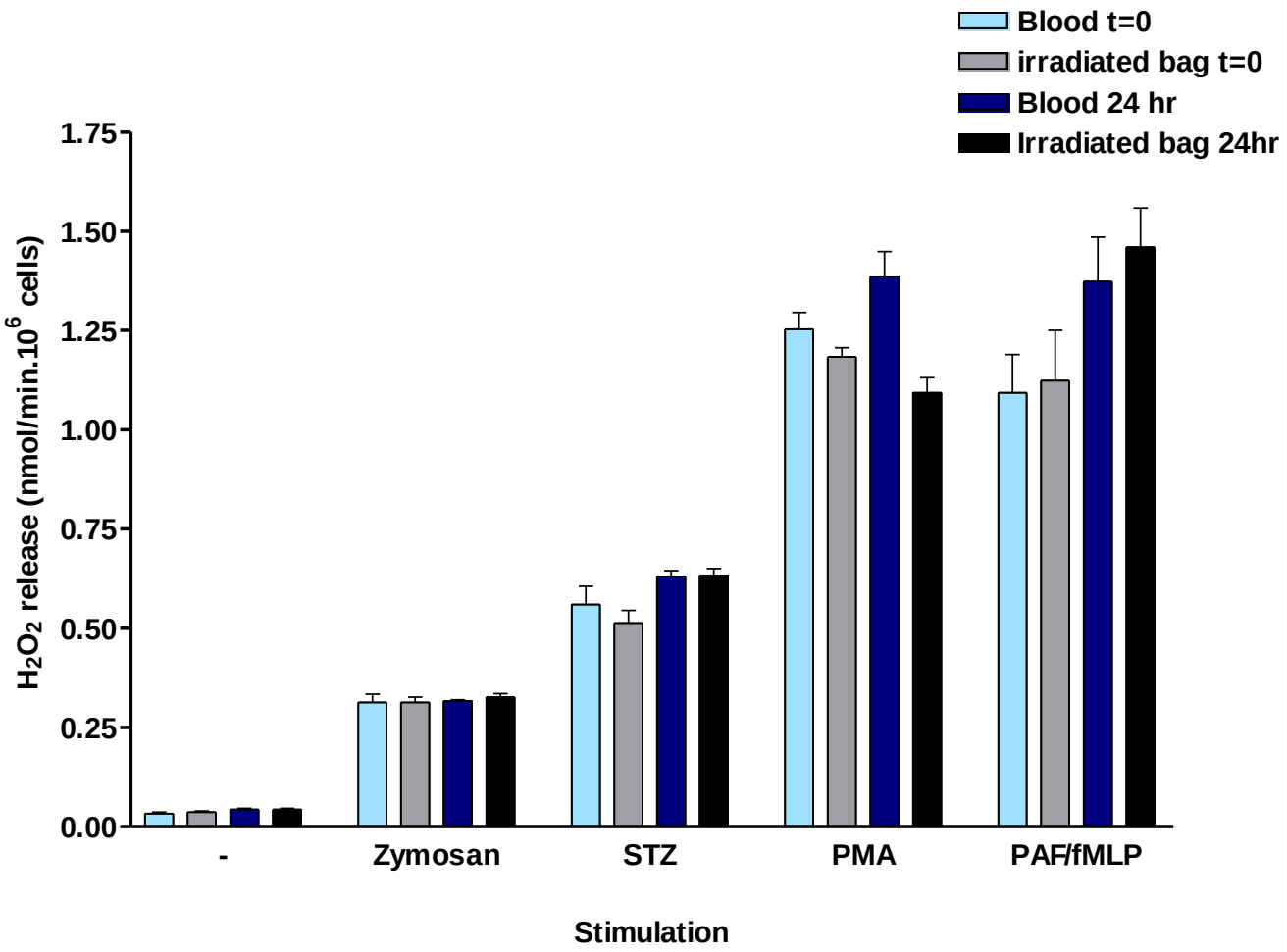
24 hrs



48 hrs

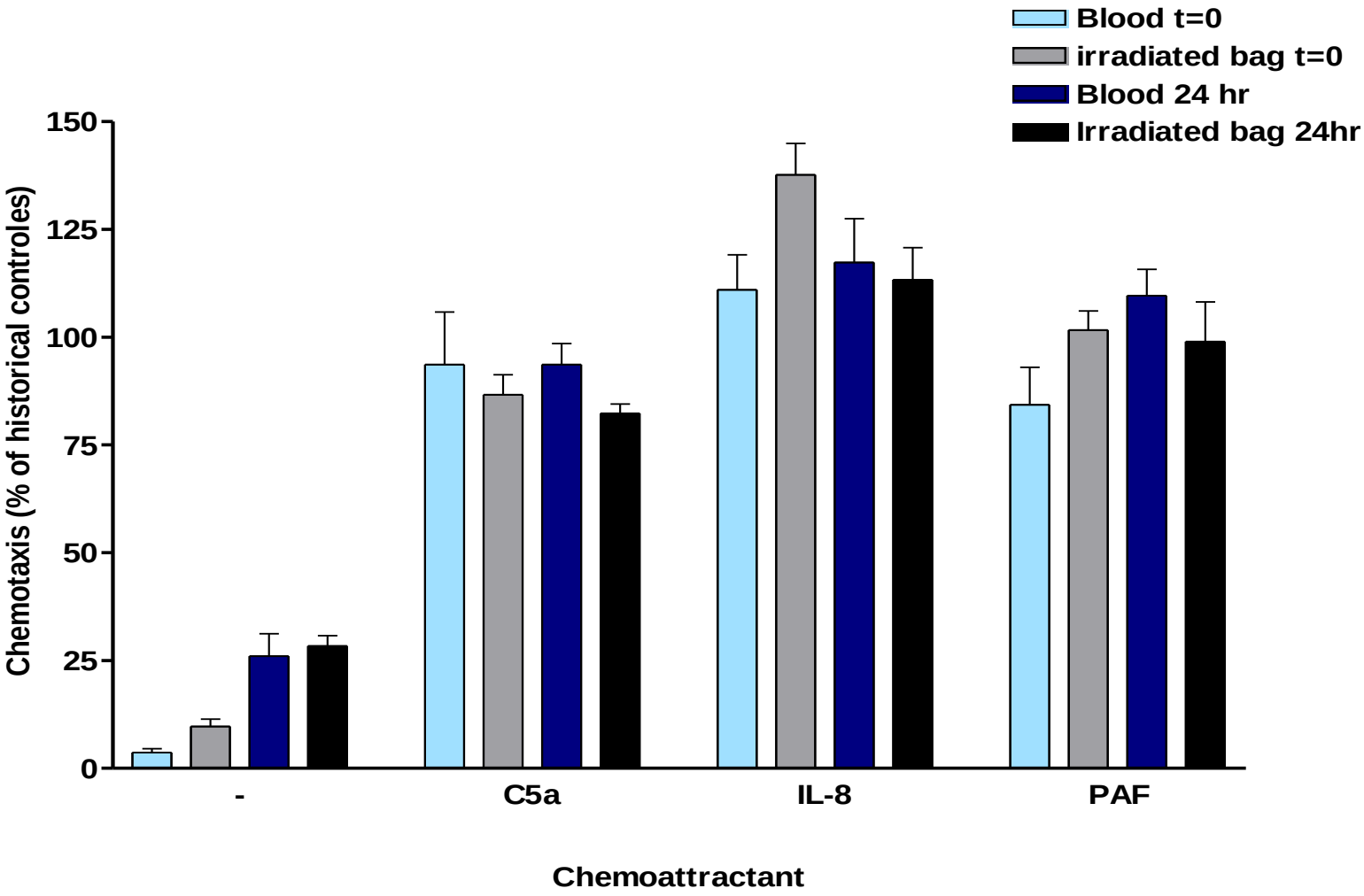


Granulocyte concentrates:
NADPH oxidase activity after 24 hrs storage



No significant changes in the respiratory burst

Granulocyte concentrates:
Neutrophil chemotaxis after 24 hrs storage



Unstimulated motility is slightly enhanced after 24hrs; directed chemotaxis is unaltered

Granulocyte concentrates

Functional behavior & storage conditions:

- Increased neutrophil survival due to prior G-CSF
- Neutrophil chemotaxis and NADPH oxidase activity are preserved after G-CSF mobilization of the donor – up to 24 hrs storage

  (Leavey *et al*. Transf. 2000; 40:414-9; Drewniak *et al*. Haematologica. 2008; 93:1058-67)
- Storage at 10°C better than at 22°C (Hübel *et al*. Blood 2000; 96:820a)
- Storage media differ in effect; culture media are best but not approved

  (Lightfoot *et al*. Vox Sanguis 2001; 80:106-11)

Granulocyte transfusions

Granulocyte concentrates & logistics

- Relatives
- Unrelated community blood bank transfusion programs
  Price *et al*. Blood 2000; 95:3302-9

- Advantages and disadvantages between donor choice

  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

Established or Recommended Policy:

- ABO Rh cross-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)

GTX: primary intervention

Authors	Design	Patients #	Bacterial	Fungal	Infection control %	
Dignani *et al.* 1997	**uncontrolled**	**15**	**0**	**15**	**74**	(d+21)
Lee *et al*. 2001	**uncontrolled**	**25**	**13**	**11**	**40**	(d+30)
Illerhaus *et al.* 2002	**uncontrolled**	**18**	**8**	**10**	**66**	(d+30)
Hubel *et al*. 2002	**matched pairs**	**74 vs 74**	**17 vs 17**	**57 vs 57**	**44 vs 59 (*p*=0.1)**	(d+30)
Rutella *et al*. 2003	**uncontrolled**	**20**	**11**	**7**	**50**	(d+30)
Mousset *et al*. 2005	**uncontrolled**	**44**	**13**	**31**	**82**	(d+30)
Sachs *et al*., 2006	**uncontrolled**	**27 ped**	**21**	**6**	**82**	(d+30)
Seidel *et al*., 2008	**RCT**	**40(t) / 39(c)**	**17**	**55**	**74 vs 72 (*p*=0.4)**	(d+100)
Atay *et al.* 2011	**uncontrolled**	**35 ped**	**17**	**18**	**82**	(d+30)
RING study, 2015	**RCT ***	**56(t) / 58(c)**	**21 vs 23**	**11 vs 15**	**43 vs 42 (*p*=1.0)**	(d+100)
Weingarten *et al.* 2016	**uncontrolled**	**21 ped**	**?**	**?**	**62**	(d+100)
Zhou *et al*. 2018	**uncontrolled**	**47 ped**	**44**	**32 (3)**	**66 / 57**	(d+30,+120)

* The success rate was 58% for patients given at least three GTX containing an average dose of 5 x 10^{10} neutrophils vs 11% success for patients given lower doses (p=0.01).

Granulocyte transfusions

General remarks on therapeutic use in neutropenia

- Earlier GTX after onset of neutropenia results in better outcome
- Higher GTX doses show better infection control rates
- Improved outcome in pediatric compared to adult patients

- No optimal strategy for the timing of when to start GTX

Granulocyte transfusions

Adverse events in the patient are mild:

- Mild reactions in ~10%: fever and chills
- Severe side-effects ~1%: hypotension and respiratory distress (amphotericin B co-medication?)
- TRALI in <0.1% (starting soon after GTX)
- Alloimmunization: more prevalent in patients with neutrophil disorders compared with severely immunosuppressed patients
- Late leukocyte incompatibility: delayed or reduced myeloid engraftment after HSCT

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

Granulocyte transfusions

Failure in RCT studies on therapeutic use of GTX

Main obstacles mentioned:

- Patients' and physicians' refusal to randomize in a life-threatening situation, especially if a potentially life-saving GTX was available
- Lack of available donors
- Availability of new and more effective antimicrobial drugs (including linezolid, carbopenems and antifungal agents such as caspofungin, micafungin and posaconazole)
- Lack of clear (predictive) indications to start GTX?

GTX: open questions about indications

General criteria for GTX:

- Neutrophil count < 0.5 x 10^9/L for more than 72 hr
- Life-threatening infection
- Infection not responding to systemic antimicrobial therapy ≥ 48 hr
- Fever (>38.0°C)
- Life expectancy of >3 months (in the absence of infection)
- Expecting to recover from the neutropenia

CME/SAM

The burden of invasive infections in neutropenic patients: incidence, outcomes, and use of granulocyte transfusions

Tanja Netelenbos,[1] Edwin Massey,[2] Liesbeth C. de Wreede,[3] Kay Harding,[2] Angela Hamblin,[4] Mallika Sekhar,[5] Anna Li,[5] Paula F. Ypma,[6] Lynn Ball,[7] Jaap Jan Zwaginga,[1,8] and Simon J. Stanworth[4]

Objective:

Describe the incidence of invasive infections and outcomes of mortality in inpatients with a hematologic malignancy

Demographics:

Cohort of 471 patients (70% male; median age 54 years), 569 neutropenic episodes

AML was the most common underlying hematologic diagnosis (30%)

HSCT in 305 patients (65%) at the time of the first neutropenic episode

Acute leukemia in 124 patients (30%) for induction or consolidation chemotherapy

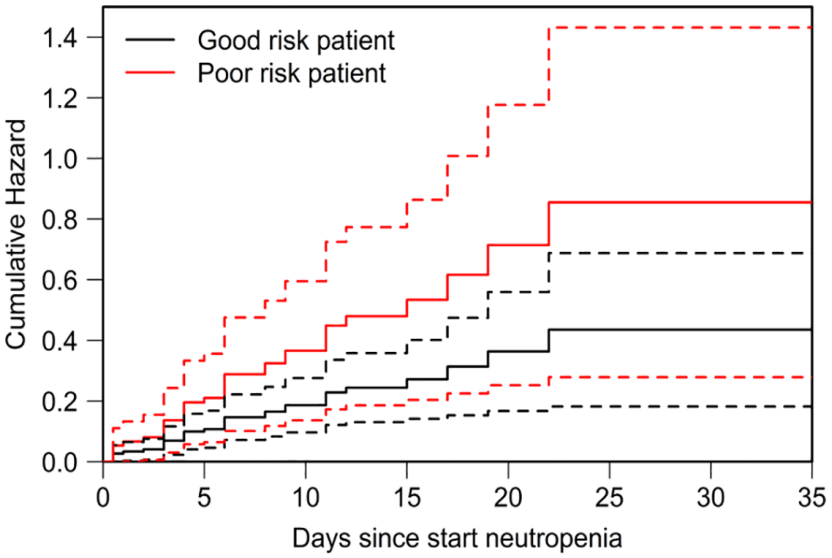
Most patients had a low comorbidity score at start (88.7% WHO 0 or 1 score)

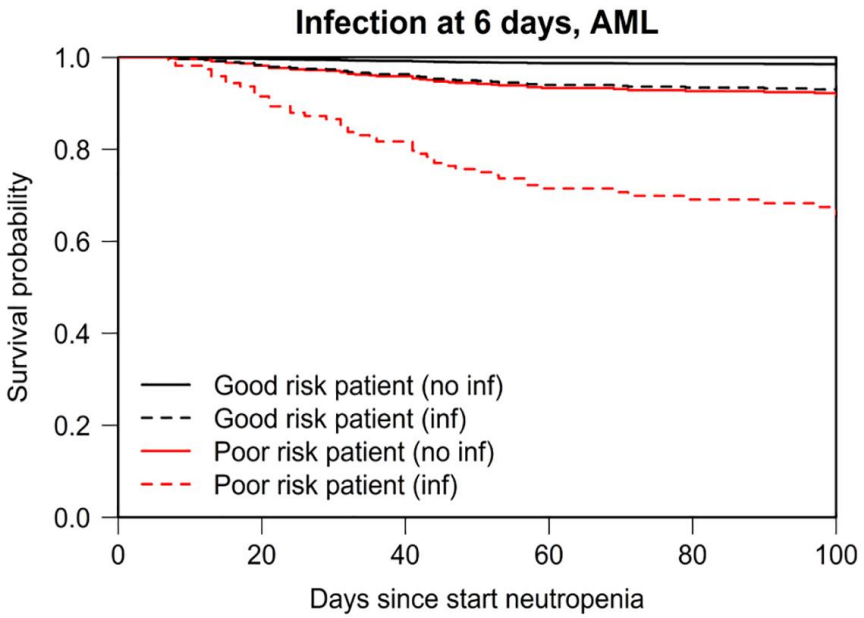
Real-world setting
across six hematology wards in two countries

	Invasive infections	NOT fulfilling GTX criteria	fullfilling GTX criteria	
All invasive infections	224 (166 pts)	173 (138 pts)	51 (34 pts)	
Bacterial infections	133 (59.4)	103 (59.5)	28 (54.9)	
Fungal infections	50 (22.3)	33 (19.1)	17 (33.3)	
Time of infection after start of neutropenia	6.0	6.0	4.5	0.118
Considered life threatening	47 (25.8)	11 (7.5)	36 (100)	<0.001
Estimated life expectancy >3 months	163 (89.6)	136 (93.2)	28 (77.8)	<0.001
Expectancy of bone marrow regeneration	164 (90.1)	137 (93.8)	28 (77.8)	<0.001
Infections unresponsive to antimicrobial therapy				
for 48 hr	71 (39.0)	35 (24.0)	36 (100)	<0.001
for 96 hr	53 (29.1)	22 (15.1)	31 (86.1)	<0.001
G-CSF use	73 (40.1)	53 (36.3)	20 (55.6)	0.059
Treatment with GTX	11 (6.0)	2 (1.4)	9 (25.0)	<0.001

In 2 patients GTX (5) were administered pre-emptively; 9 patients GTX (10) were treated therapeutically, and only 4 out of these 9 were alive after 100 days

Real-world setting
across six hematology wards in two countries





Left: cumulative hazard of infections with 95% CI depicted for reference patients with AML.
Right: model-based predicted survival is shown for reference patients with AML

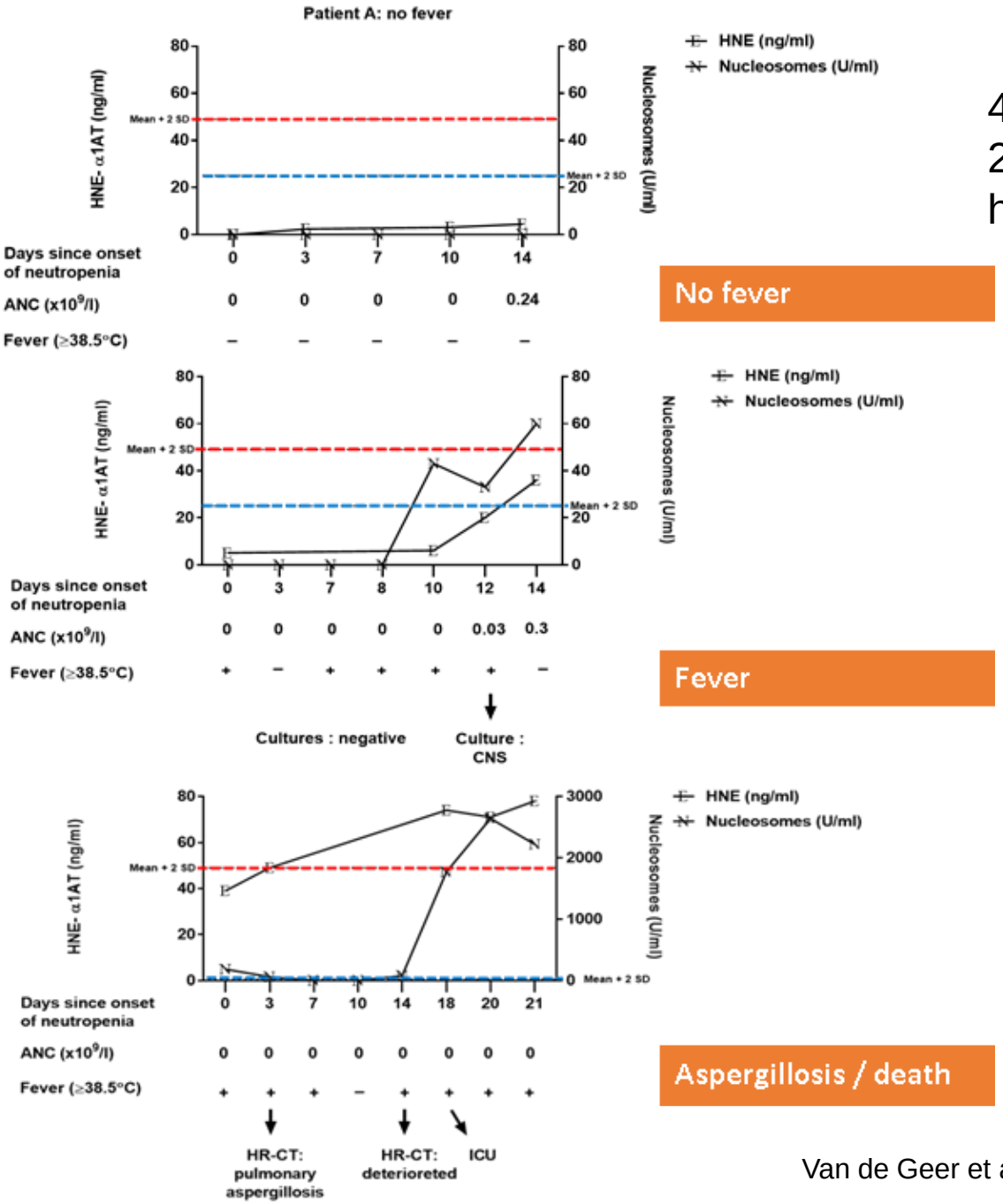
Good risk is defined as a patient with WHO comorbidity score 0 and HCT-CI score of 0.
Poor risk, WHO score ≥ 1, HCT-CI ≥ 2.

GTX: common practice

Conclusions from the prospective multicenter study:

- Approximately one-third of neutropenic patients following intensive chemotherapy developed invasive infections
- Infections were bacterial (59.4%) and fungal (22.3%)
- Despite the fact that 34 patients (6.3% of all episodes) appeared to have met criteria to receive GTX, only 9 patients (26%) were treated
- Invasive infections were associated with an increase in mortality up to 100 days after start of the neutropenia (HR 5.8 [2.5-13.0] in Cox proportional hazards models for infection and mortality).

GTX:



40 episodes in
26 patients with
high-risk AML

Van de Geer et al. 2019 in preparation

GTX: conclusions about concentrates

Functional behavior & storage conditions:

- Cells obtained from donors primed *in-vivo* with G-CSF and dexamethasone are superior to those primed *in-vitro*
- Buffy-coat derived GTX products are safe and ready to use but show limited survival compared to G-CSF/dexa derived GTX
- Survival and functional capacity of neutrophils from G-CSF/dexa derived GTX products seem remain well preserved for 24 hrs
- Can we improve GTX storage (buffy-coat or G-CSF/dexa derived) and use these stored granulocytes in patients?

GTX: one question less

Follow-up outcome of the RING study:

TRANSFUSION COMPLICATIONS

CME/SAM

WBC alloimmunization: effects on the laboratory and clinical endpoints of therapeutic granulocyte transfusions

Thomas H. Price,[1,2] Jeffrey McCullough,[3] Ronald G. Strauss,[4,5] Paul M. Ness,[6] Taye H. Hamza,[7] Ryan W. Harrison,[8] and Susan F. Assmann[7]

GTX: common practice to improve

BLOOD BANK DONORS for GTX

GTX: open questions about indications

Future perspective & policies:

- Is there still a need for GTX?
  - The largest patient category for GTX use consists of those being treated for hematologic malignancies and HSCT conditions
  - Severe infections can cause direct morbidity and mortality
  - Delays in receiving curative chemotherapeutic treatments in the right timing and doses cause additional risk of mortality
  - Increase in elderly patients and vulnerability to toxic regimens

- If yes, how to improve strategies to allocate GTX to the right patient?
  - Belief and advocacy
  - Biomarkers may be of help

Granulocyte transfusions – when?

Do not wait until it’ll be too late…

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