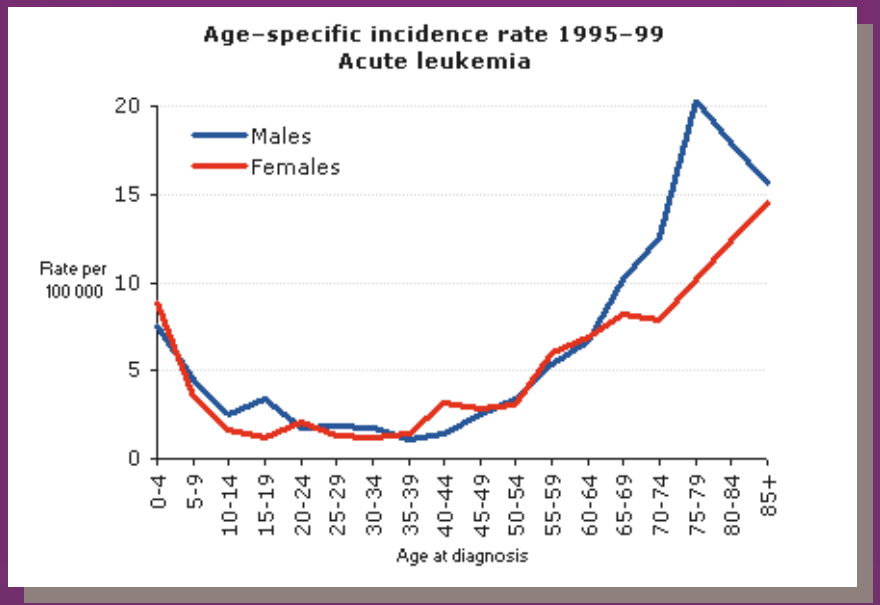
CHEMOKINES IN ACUTE MYELOGENOUS LEUKEMIA

Øystein Bruserud

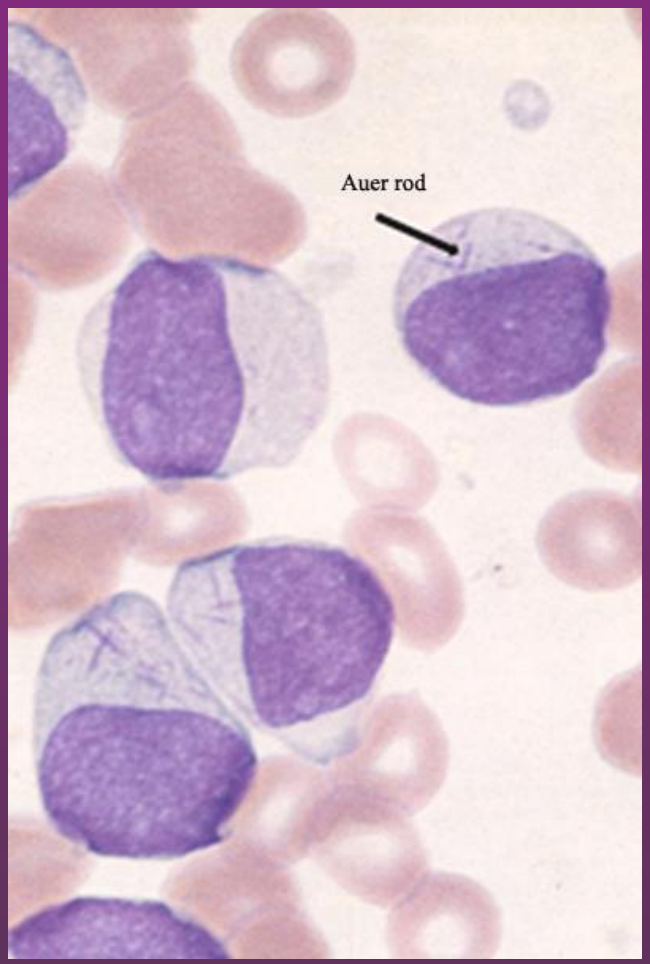
Acute leukemia in Norway



Kreftregisteret
institute of population-based cancer research

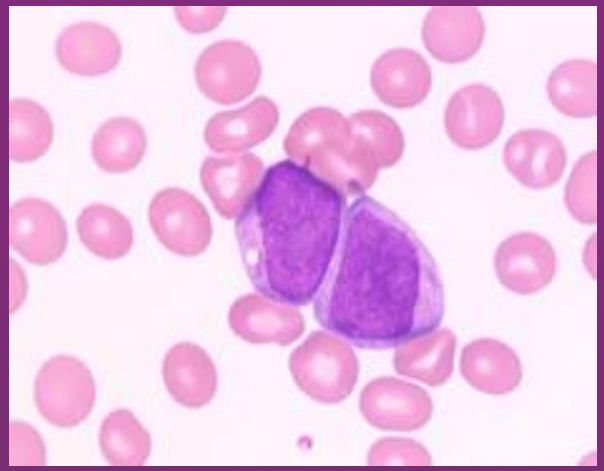
Diagnosis of AML

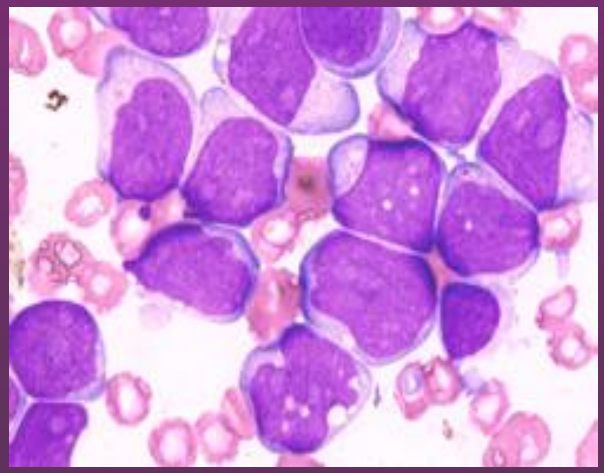
- At least 20% or 30% of leukemia blasts in the bone marrow
- Auer-rods
- Morphological signs of differentiation (FAB classification



AML – DIAGNOSIS

- Bone marrow infiltration
- Peripheral blood leukemization
- Bone marrow failure with anemia, neutropenia and thrombocytopenia

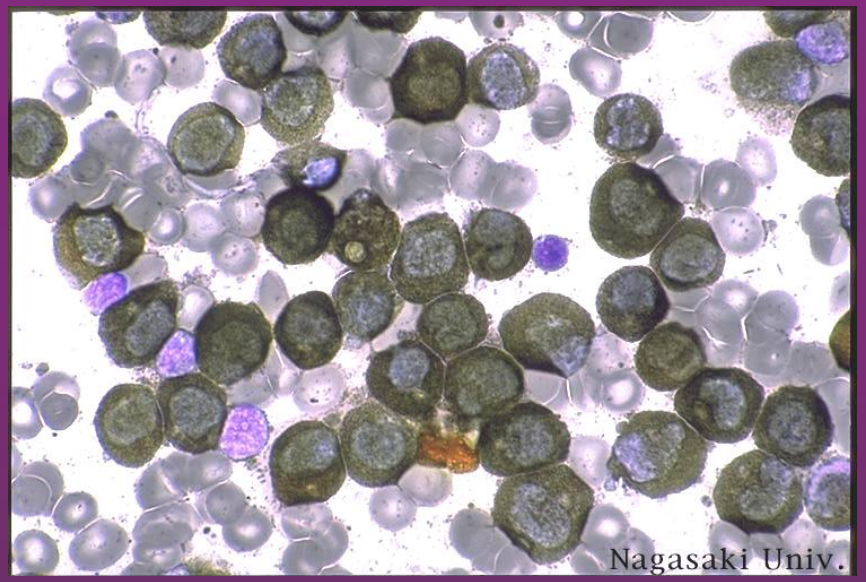




AML-DIFFERENTIATION

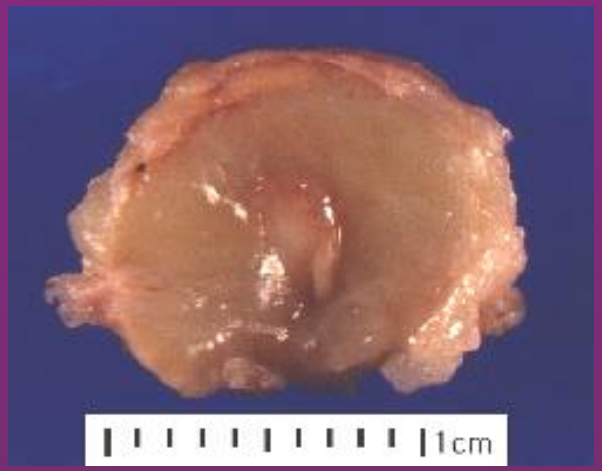
- Histochemistry for evaluation of differentiation

- Flow-cytometric analysis of membrane molecule expression

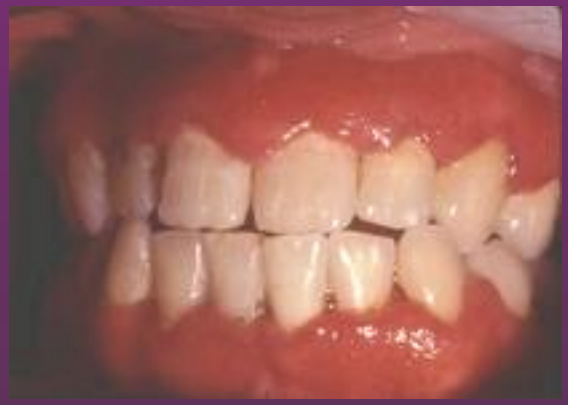


AML – organ manifestations

- Extramedullary disease is very uncommon



- Gingival infiltration or CNS affection in monocyte variants



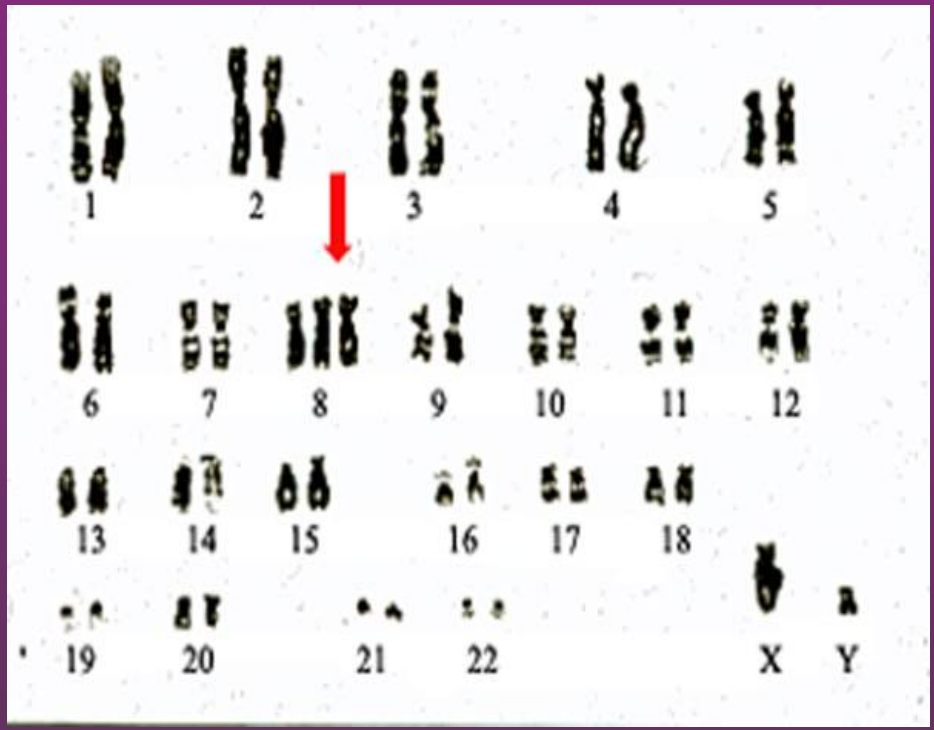
AML – CYTOGENETIC ANALYSIS

Cytogenetic analysis and AML-free survival

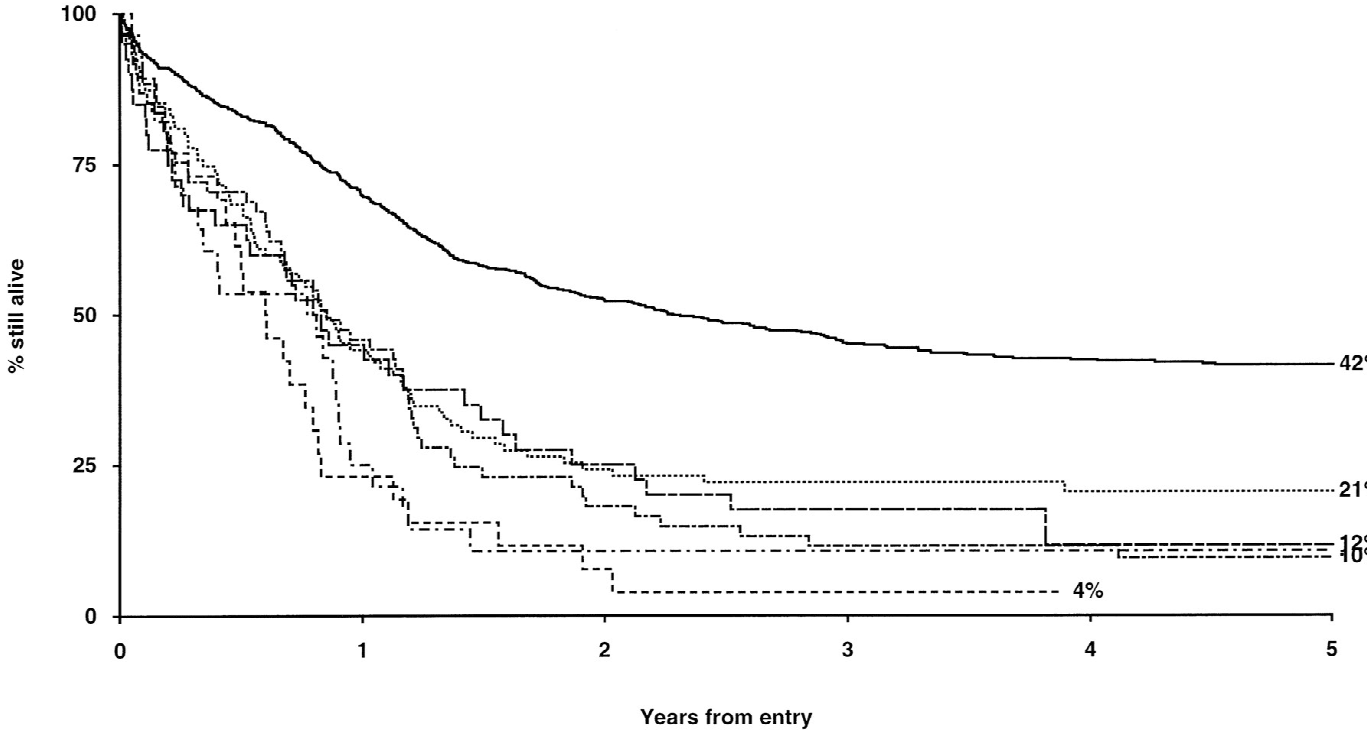
High risk 10-30%

Low risk 70-80%

Normal/intermediate risk: 40-50%



AML: survival with high risk cytogenetics



Normal (n=680)

Complex (n=95)
abn(3q) (n=40)
del(5q) (n=28)
-7 (n=61)
-5 (n=26)

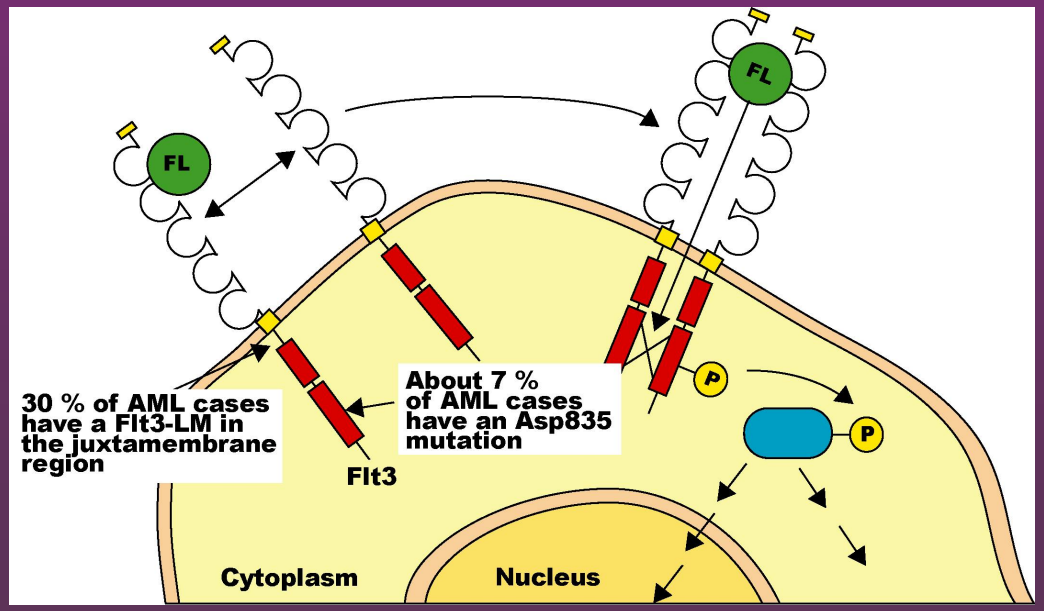
Total n=1612

Grimwade et al. 1998

Fetal liver tyrosine kinase/Fms-like tyrosine kinase (Flt3)

- Type III receptor tyrosine kinase
- Active Flt3 has an anti-apoptotic effect (Stat5, Bcl-2)
- Mutated (LM or point mutation) Flt3 in 30 – 40 % of AML and causes constitutive activation

- Negative prognostic factor if mutated (LM)



CHEMOKINES

- Chemotactic cytokines
- Small molecular weight (8-14 kDa)
- Structurally categorized into four major subfamilies (CCL, CXCL, CX3C, C)
- Approcimately 50 chemokines
- At least 18 chemokine receptors

Chemokines

- Two major families: CCL and CXCL
- Two receptor families: CCR and CXCR
- A chemokine often binds many receptors
- A receptor often binds many chemokines
- Involved in the regulation of leukocyte chemotaxis, angiogenesis, proliferation

CHEMOKINE RECEPTORS

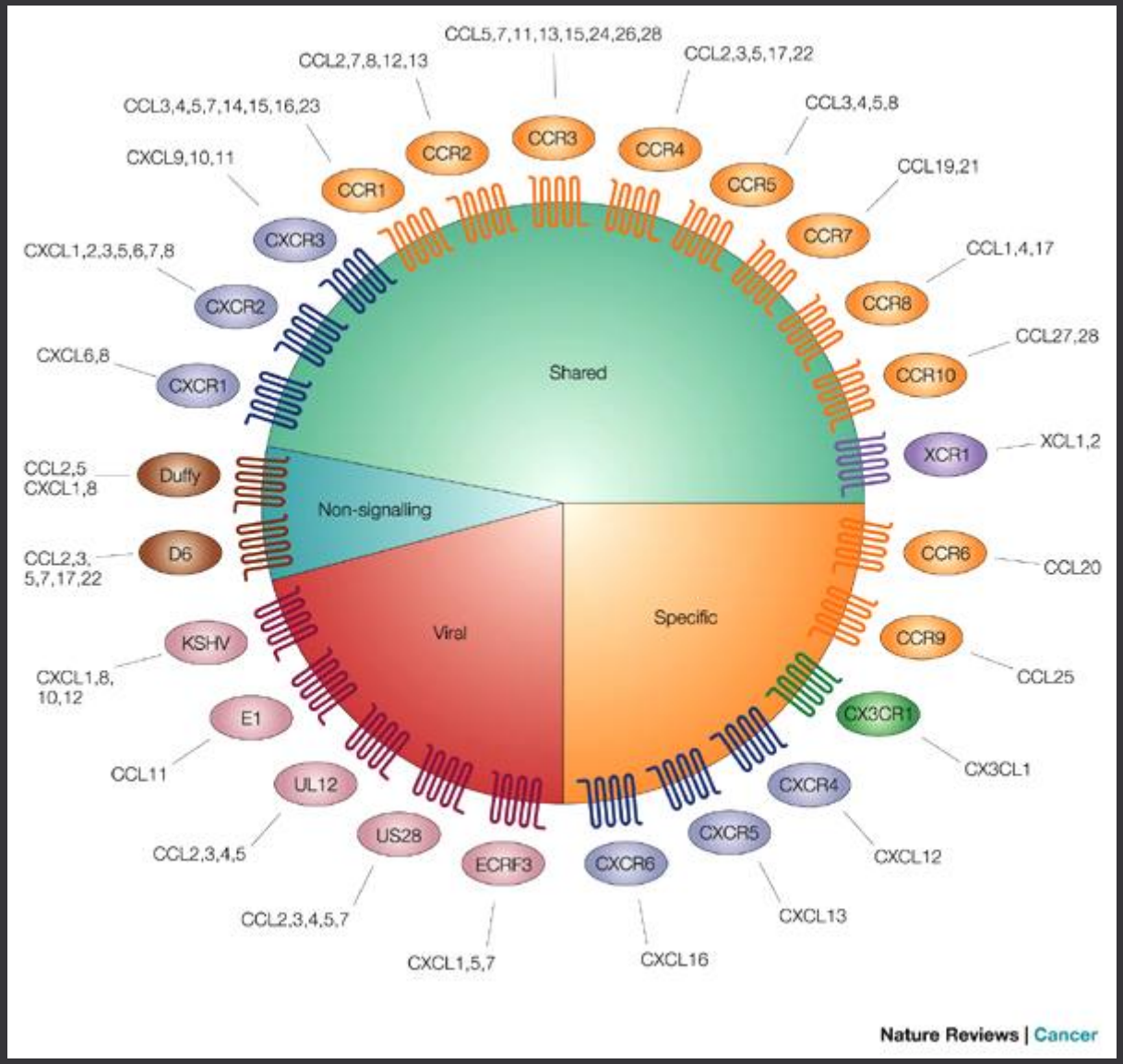
- At least 18 chemokine receptors
- Each receptor binds ligand(s) from only one chemokine family
- Four receptor subfamilies
- G-protein coupled receptors

CHEMOKINES
Inflammatory chemokines

- Not constitutively released
- Induced and upregulated by inflammation
- Recruit leukocytes
- Controlled by the local proinflammatory cytokine network
- Receptor promiscuity: Receptors bind several chemokines, chemokines bind several receptors

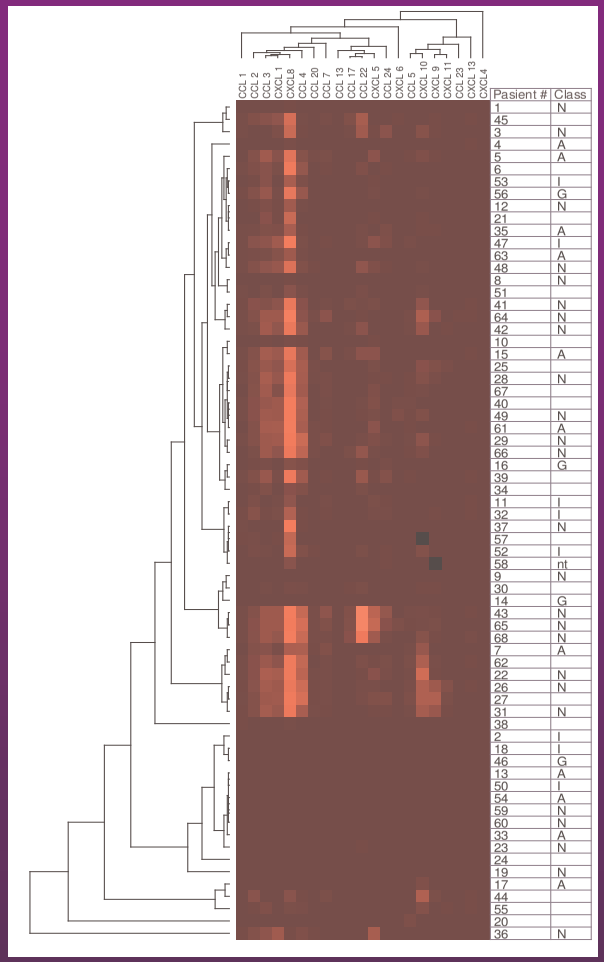
CHEMOKINES
Homeostatic chemokines

- Constitutively expressed
- Development and homeostasis of the hematopoietic and immune system
- Lymphoid chemokines, i.e. act preferentially on lymphoid and dendritic cells
- Bind to a single receptor
- The receptors bind single chemokines


CCL5,7,11,13,15,24,26,28
CCL2,7,8,12,13
CCL2,3,5,17,22
CCL3,4,5,7,14,15,16,23
CCL3,4,5,8
CXCL9,10,11
CCL19,21
CXCL1,2,3,5,6,7,8
CCL1,4,17
CXCL6,8
CCL27,28
CCL2,5
CXCL1,8
XCL1,2
CCL2,3,
5,7,17,22
CCL20
CXCL1,8,
10,12
CCL25
CCL11
CX3CL1
CCL2,3,4,5
CXCL12
CCL2,3,4,5,7
CXCL13
CXCL1,5,7
CXCL16
CCR1
CCR2
CCR3
CCR4
CCR5
CCR7
CCR8
CCR10
XCR1
CCR6
CCR9
CX3CR1
CXCR4
CXCR5
CXCR6
ECRF3
US28
UL12
E1
KSHV
D6
Duffy
CXCR3
CXCR2
CXCR1
Shared
Non-signalling
Viral
Specific
Nature Reviews | Cancer

Chemokines - AML cell release

- Enriched AML cells
- 68 patients
- Constitutive cytokine release
- Concentrations in supernatants
- Subsets defined based on chemokine release



Constitutive chemokine release by primary human AML cells

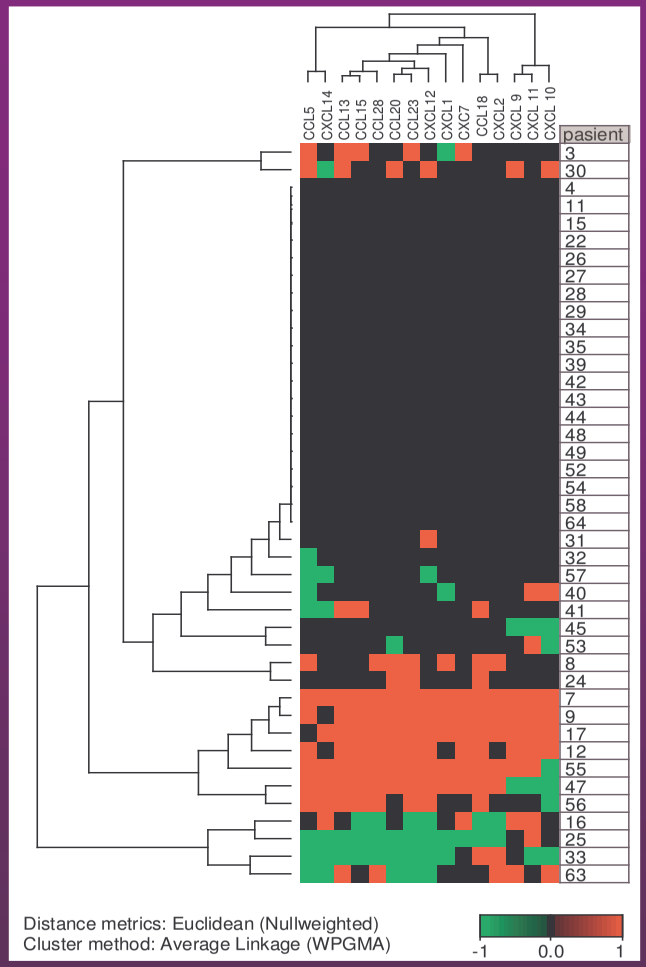
- Relatively few chemokines are released at high levels for most patients
- CXCL8 (IL8) is usually released at highest levels
- Three major chemokine release clusters:
  CCL2-4/CXCL1,8

  CCL5, CXCL9-11

  CCL13, 17, 20, 24 CXCL5

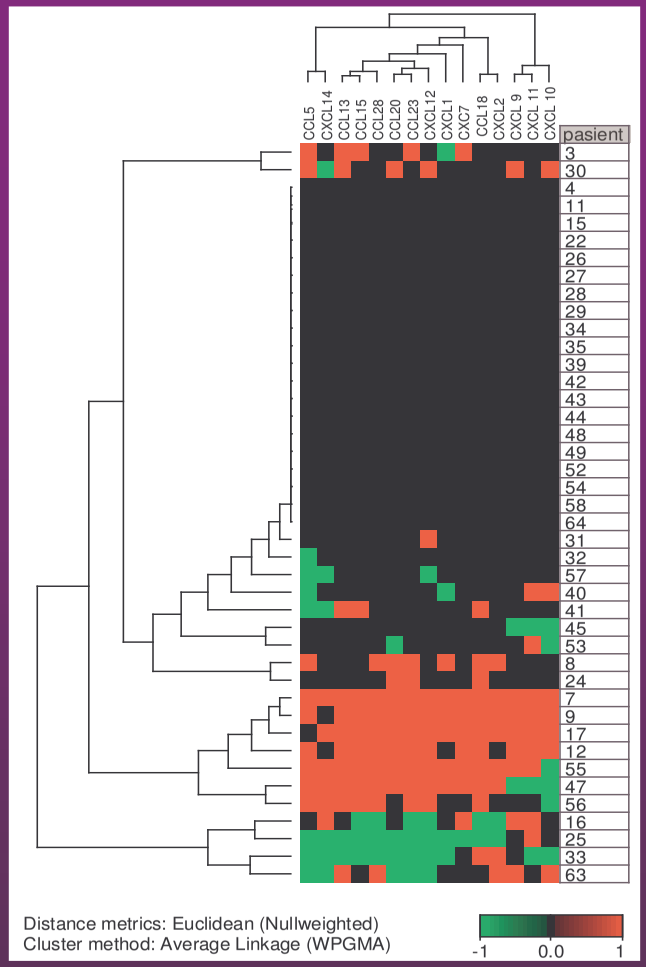
Chemokines - AML cell proliferation

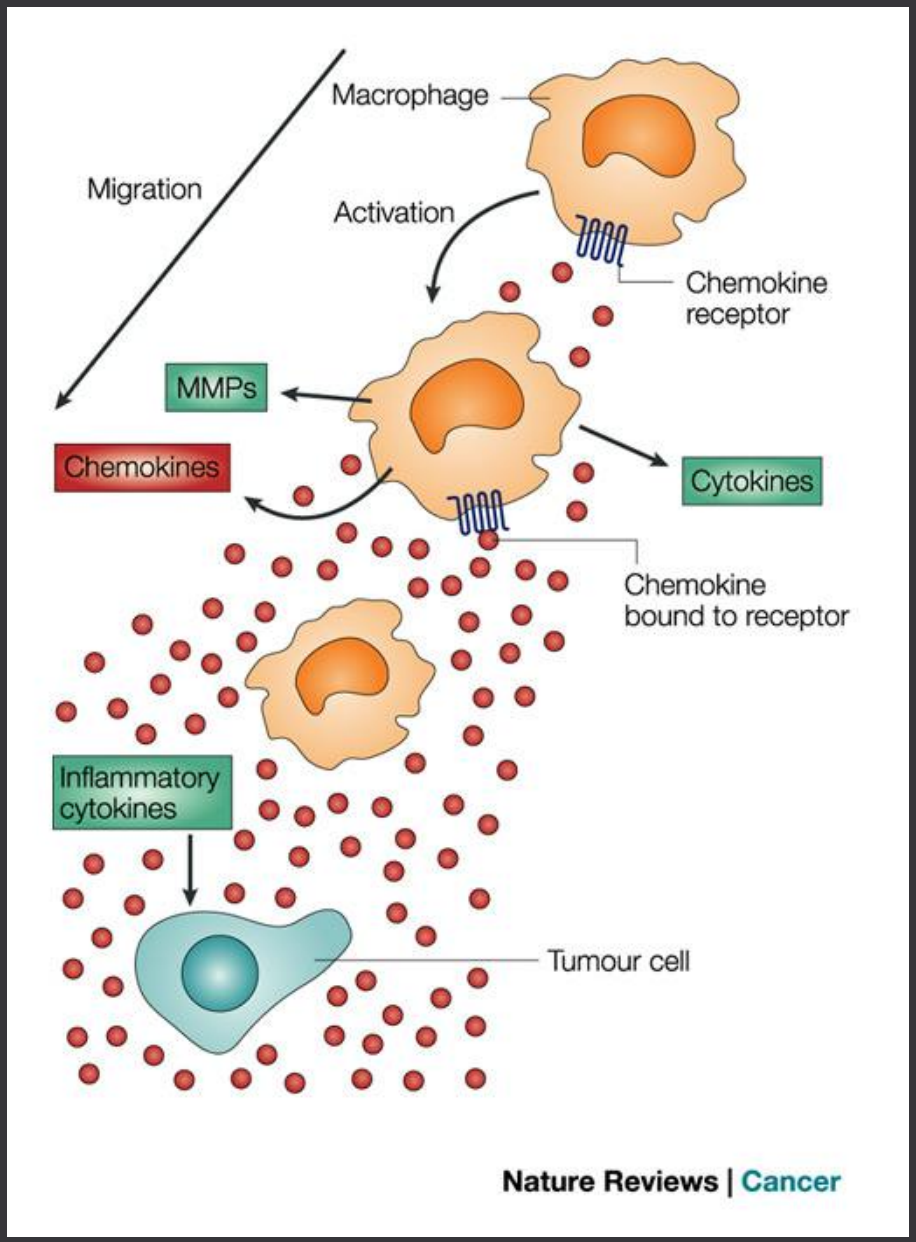
- Enriched AML cells
- 64 patients
- Cytokine-dependent in vitro proliferation
- Effect of exogenous chemokines
- Subsets defined based on chemokine responsiveness



Chemokines - AML cell proliferation

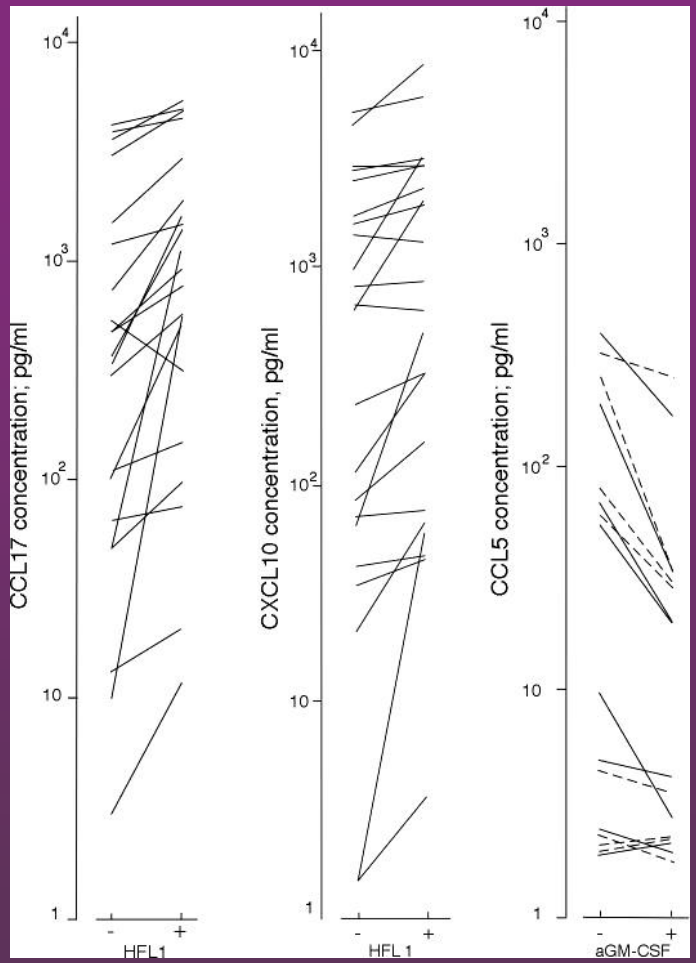
- Exogenous chemokines affect proliferation only for a small subset
- Mediated through several receptors
- CCR1-10
- CXCR1-6




Macrophage
Migration
Activation
Chemokine receptor
MMPs
Chemokines
Cytokines
Chemokine bound to receptor
Inflammatory cytokines
Tumour cell
Nature Reviews | Cancer

T cell chemotactic chemokine release Coculture with fibroblasts

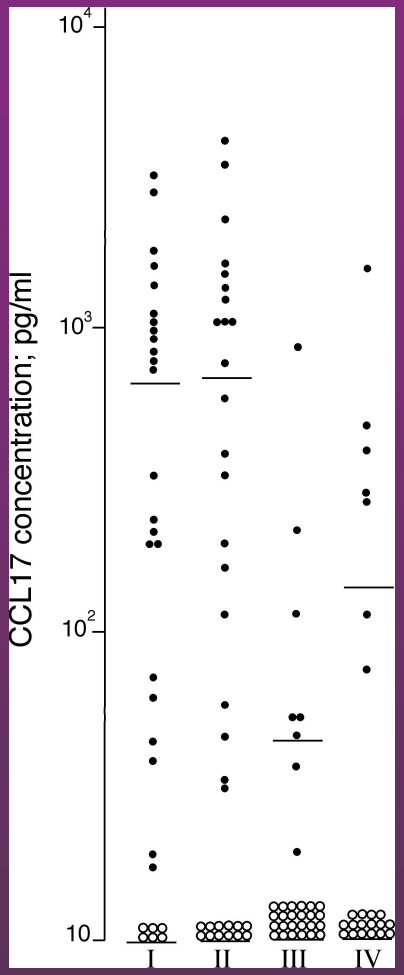
- Native AML blasts in upper chamber
- HFL1 fibroblasts in lower chamber
- Transwell cultures
- Chemokine levels determined after 7 days of coculture



T cell chemotactic chemokines CCL17 serum levels

CCL17 levels before and during therapy

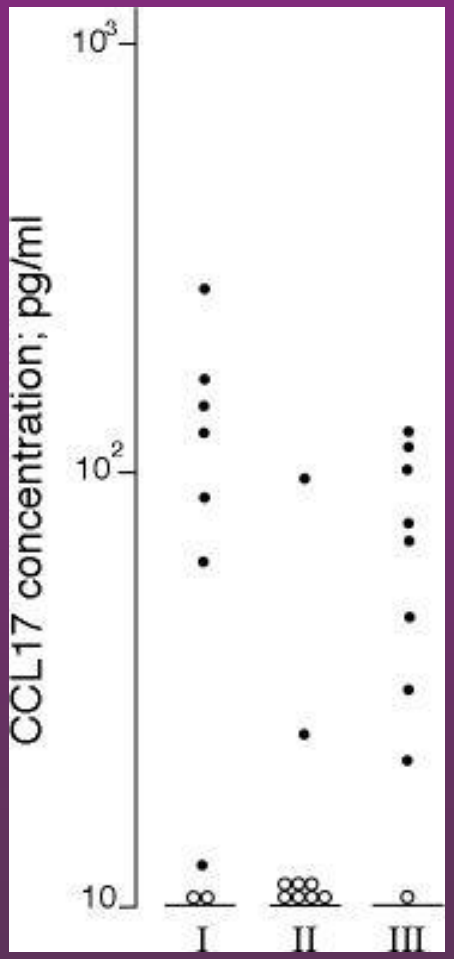
I Before therapy
II 1st day neutropenia
III Neutropenia day 8-10
IV Neutropenia day 14-16



T cell chemotactic chemokines CCL17 serum levels

CCL17 serum levels during neutropenia

I Before infection
II During infection
III After infection during clinical improvement



T cell chemotaxis in AML
Serum chemokine levels

- Altered levels in patients with untreated AML
- Wide variation between patients
- Age-dependent differences
- Increased before consolidation therapy
- Altered by chemotherapy
- Altered levels during cytopenia

Angiogenesis in AML

- Increased vessel density in AML bone marrow
- AML blast levels of VEGF have a prognostic impact in human AML
- Serum endostatin levels have a prognostic impact in AML
- Antiangiogenic therapy may have antileukemic effects

Angioregulatory mediators released by human AML blasts

Proangiogenic mediators:

- CXCL8/IL8
- Other CXCR2 chemokines
- VEGF
- HGF

Antiangiogenic mediators:

- IL12
- CXCL4
- CXCL9-11
- Endostatin (rarely)

Chemokines-angiogenesis-AML

- Proangiogenic chemokines
  CXCR2 ligands:
  CXCL1-3, CXCL5-8
  CXCL8 most important in AML

- Antiangiogenic chemokines
  CXCR3B ligands – CXCL9-11
  CXCR3B ligands – CXCL4

Constitutive AML cell release of CXCL8 varies between <10 and >10,000 pg/ml

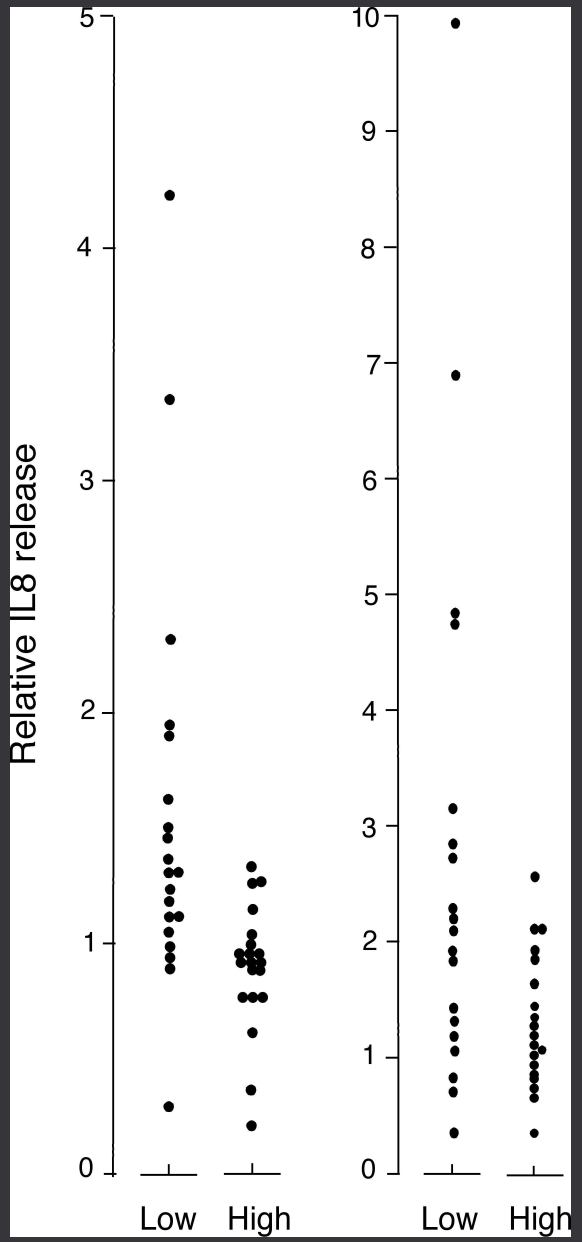
Coculture of primary AML cells with osteoblasts increase local levels of proangiogenic CXCL8/IL8

Low: Less than median

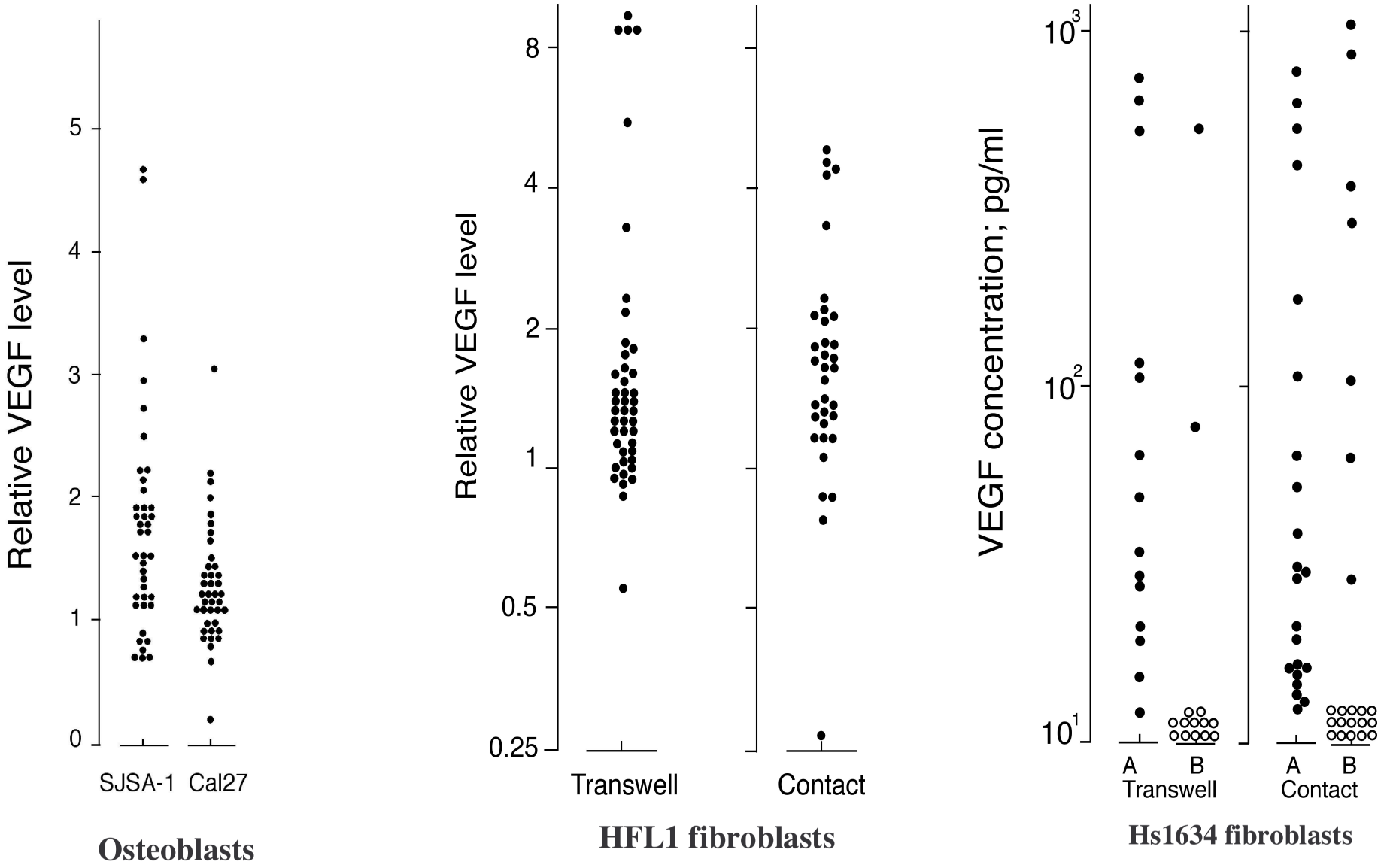
High: Exceeding median

Left: Cal72

Right: SJSA1



Coculture of AML and stromal cells increase VEGF levels



Angioregulators in AML

- Proangiogenic VEGF and CXCL8 are increased during coculture of AML cells and normal bone marrow stromal cells
- Interferon-gamme increases CXCL8 and decreases CXCL9-11
- Endothelial cells support AML cell survival and proliferation

AML-Chemokines

- Chemokines are probably involved in leukemogenesis
- Chemokines are probably important for antileukemic immune reactivity
- Chemokines are probably involved in angioregulation in AML