Pediatric leukemias

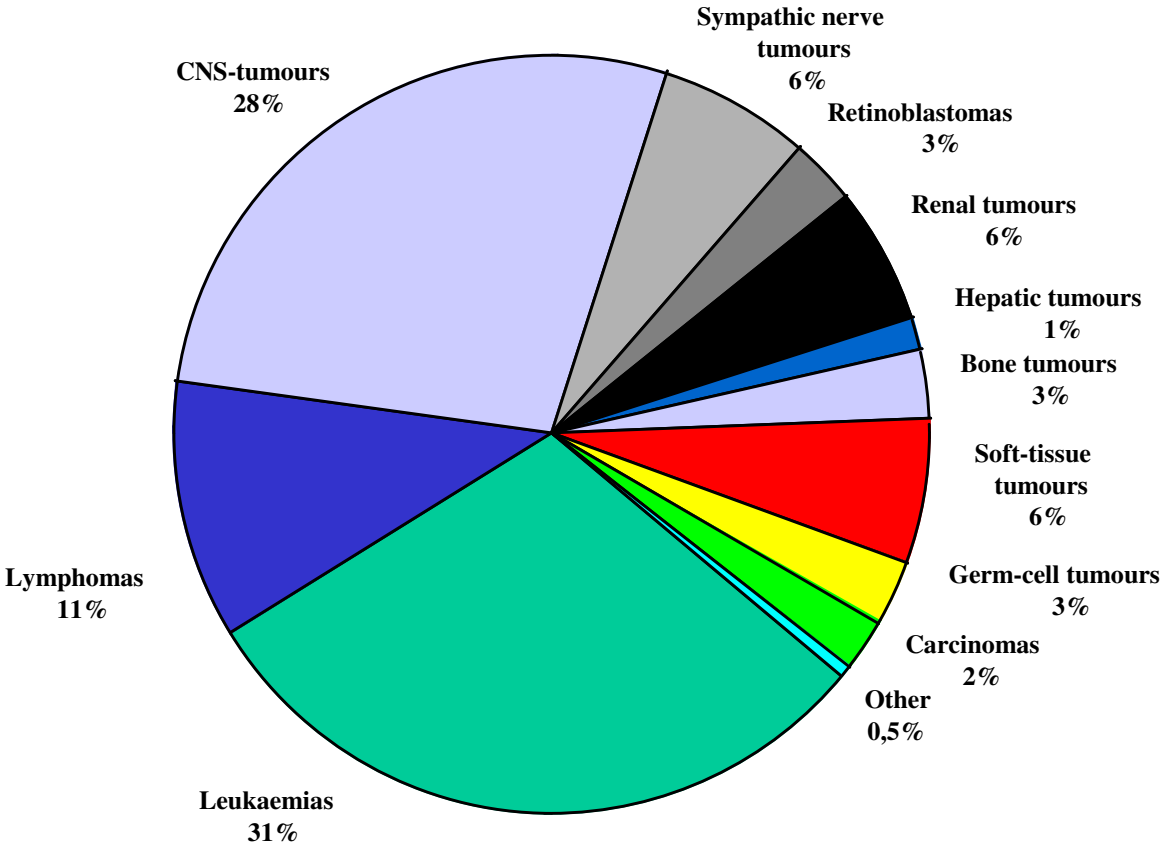
Overall survival
Prognostic factors
Treatments
Hematopoietic stem cell transplantation

Britt Gustafsson, professor of pediatrics/senior consultant pediatric hematology

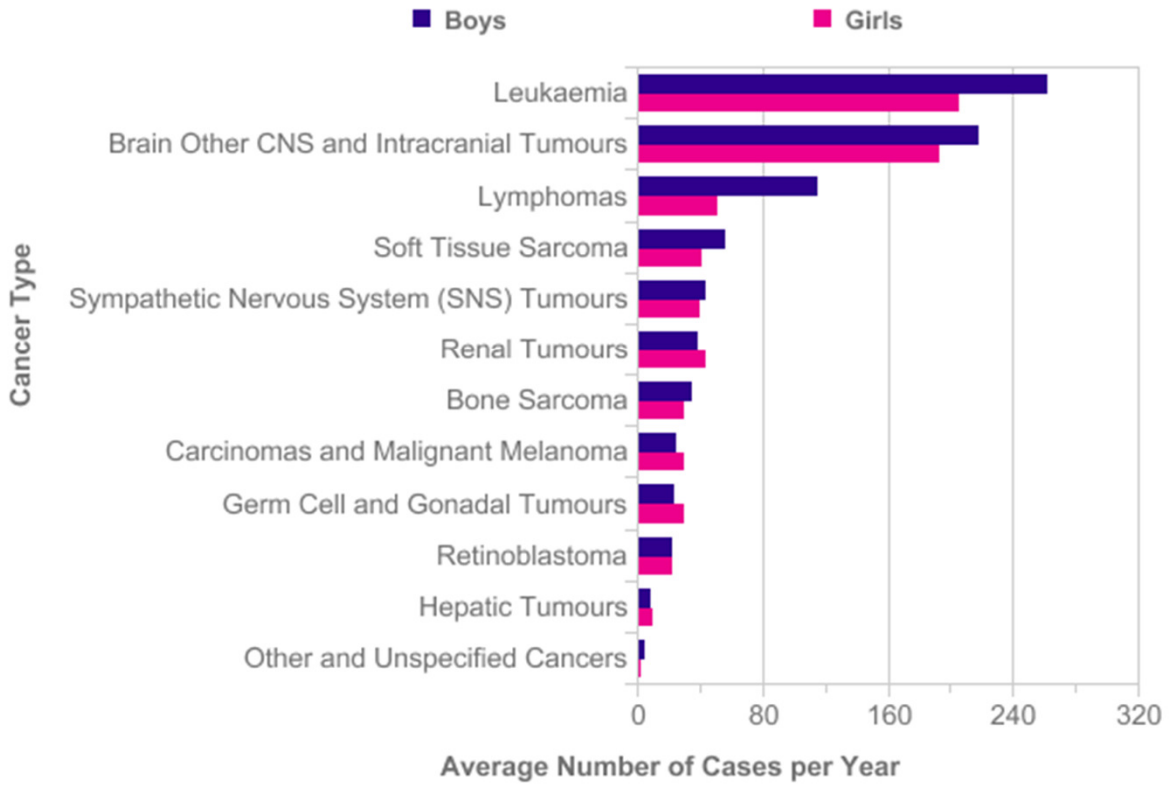
Centre for allogeneic stem cell transplantation

Karolinska University Hospital-Huddinge, Sweden

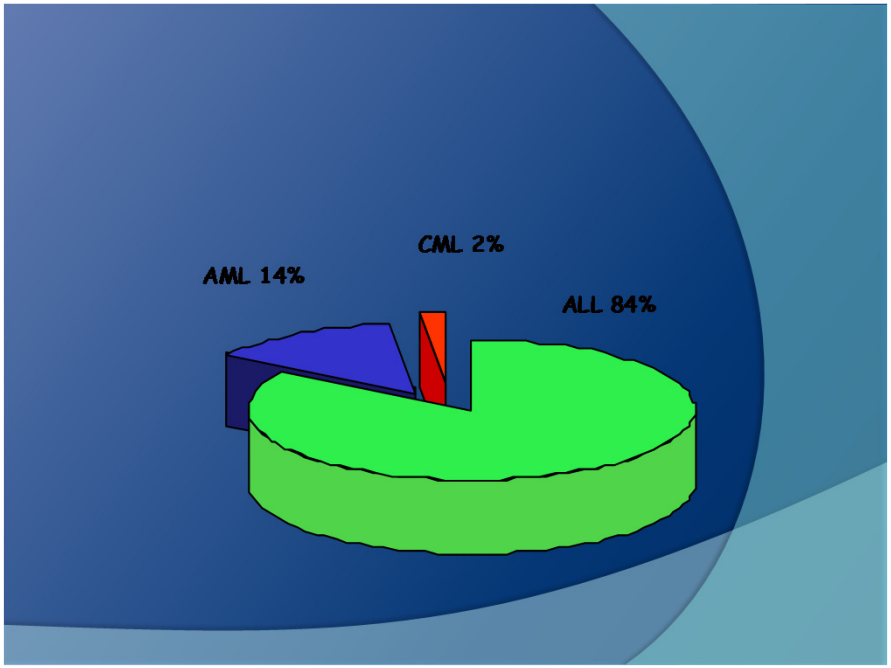
Incidence of different pediatric cancers

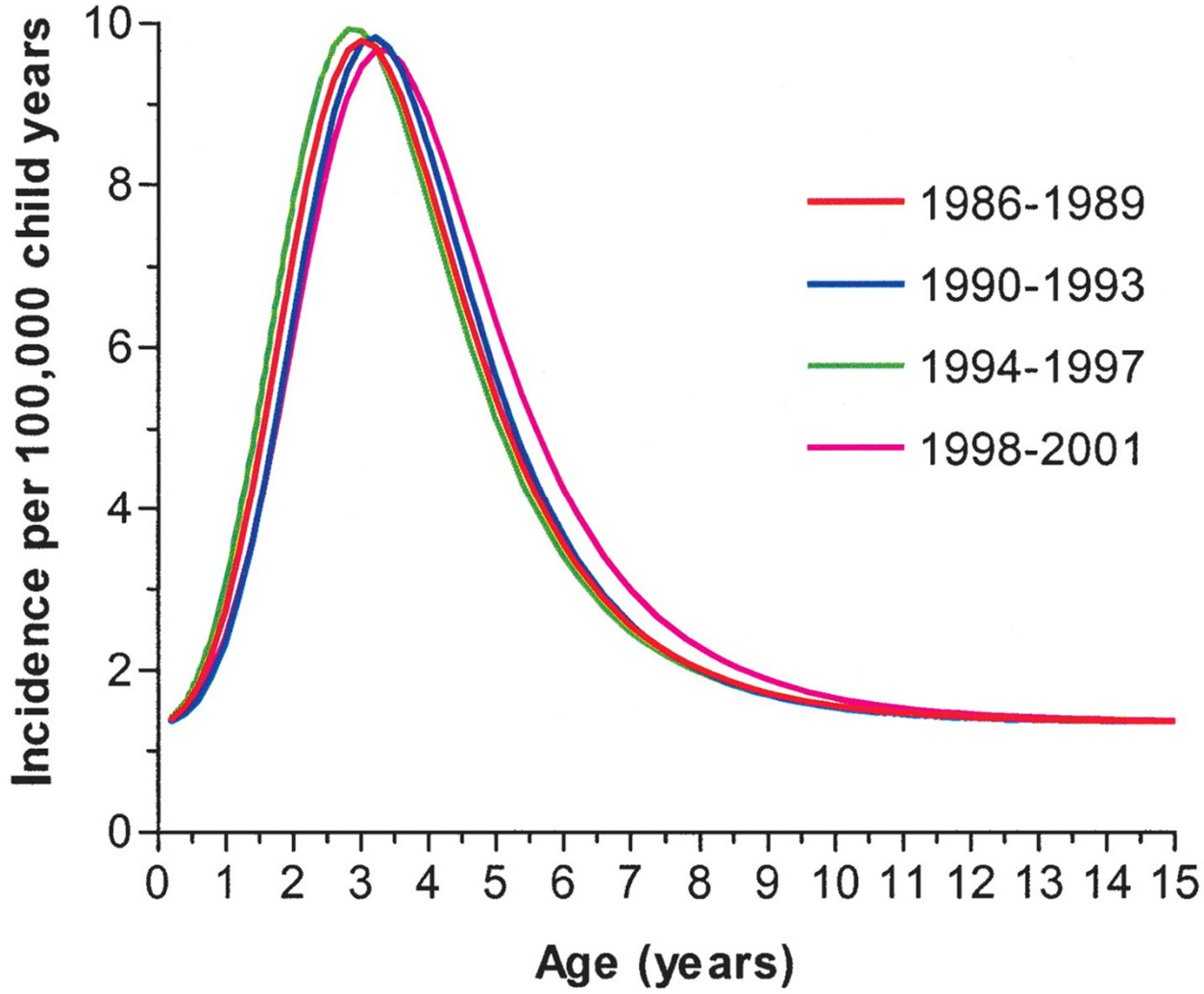


Karolinska Institutet



Childhood leukemias

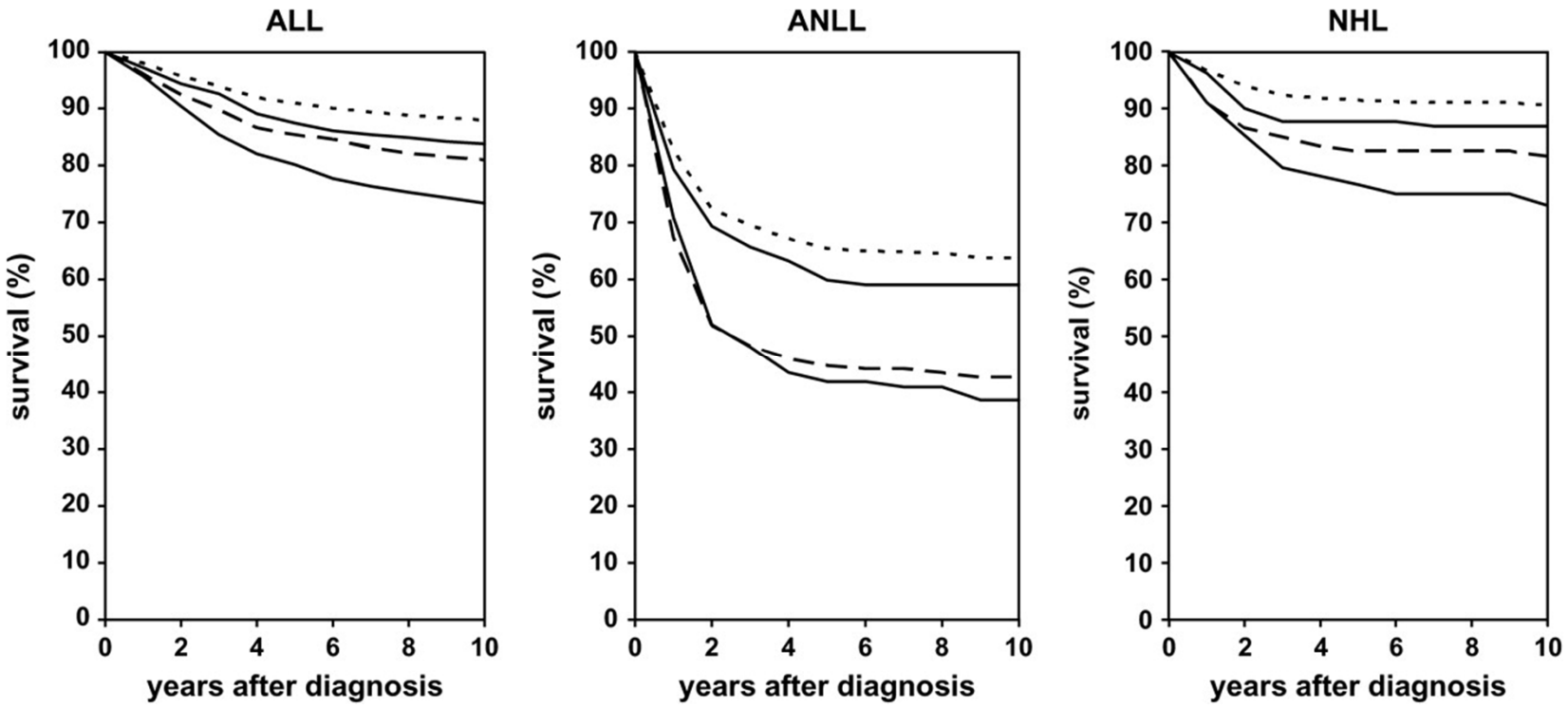




Hjalgrim, L. L. et al. J Natl Cancer Inst 2003;95:1539-1544

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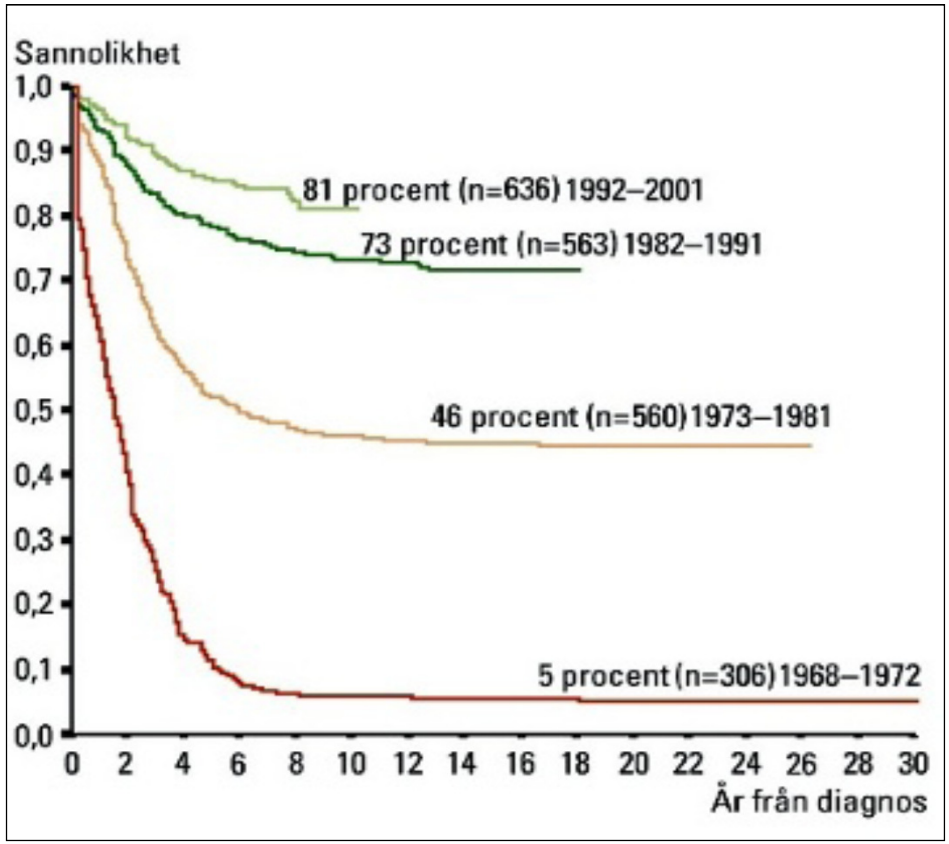
Ten-year survival curves for children with common hematologic malignancies.



Pulte D et al. JNCI J Natl Cancer Inst 2008;100:1301-1309

© The Author 2008. Published by Oxford University Press.

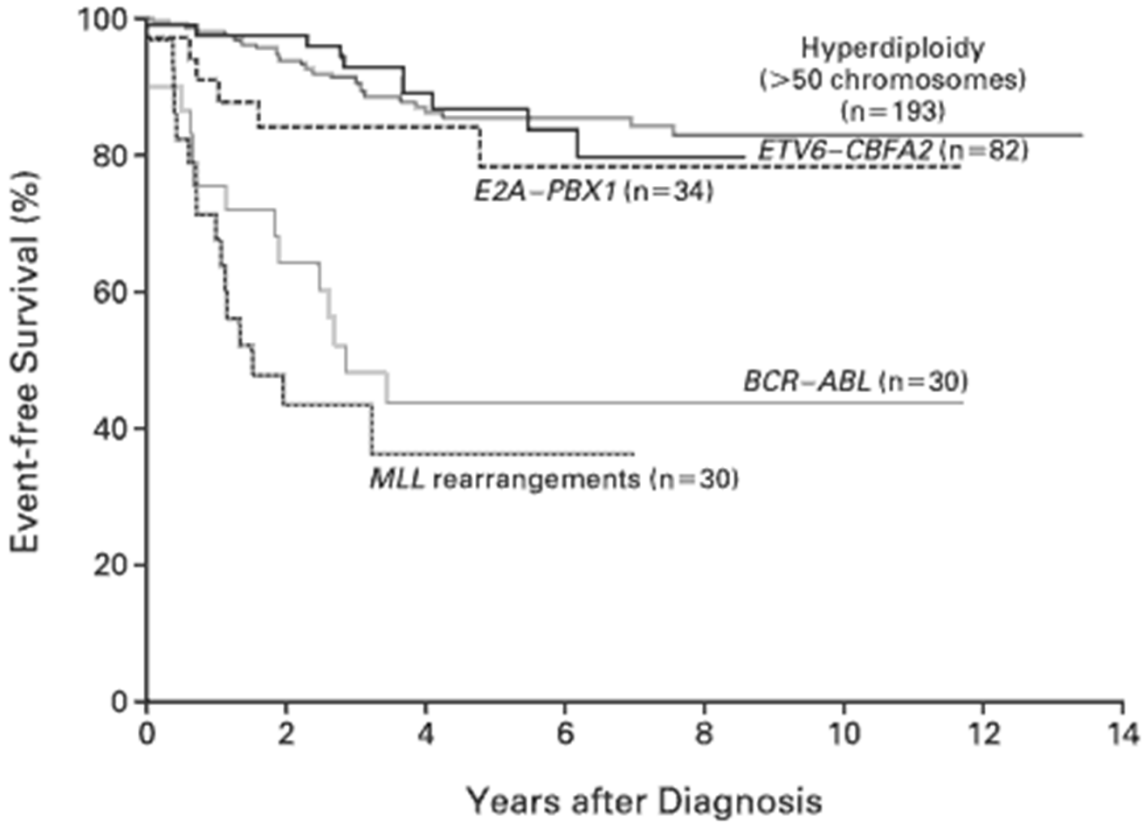
Factors affecting the 5-year overall survival in children



- Better diagnostic tools
- Intensified chemotherapy
- Intrathecal therapy
- Irradiation to CNS
- Supportive care
- Hematopoietic stem cell transplantation

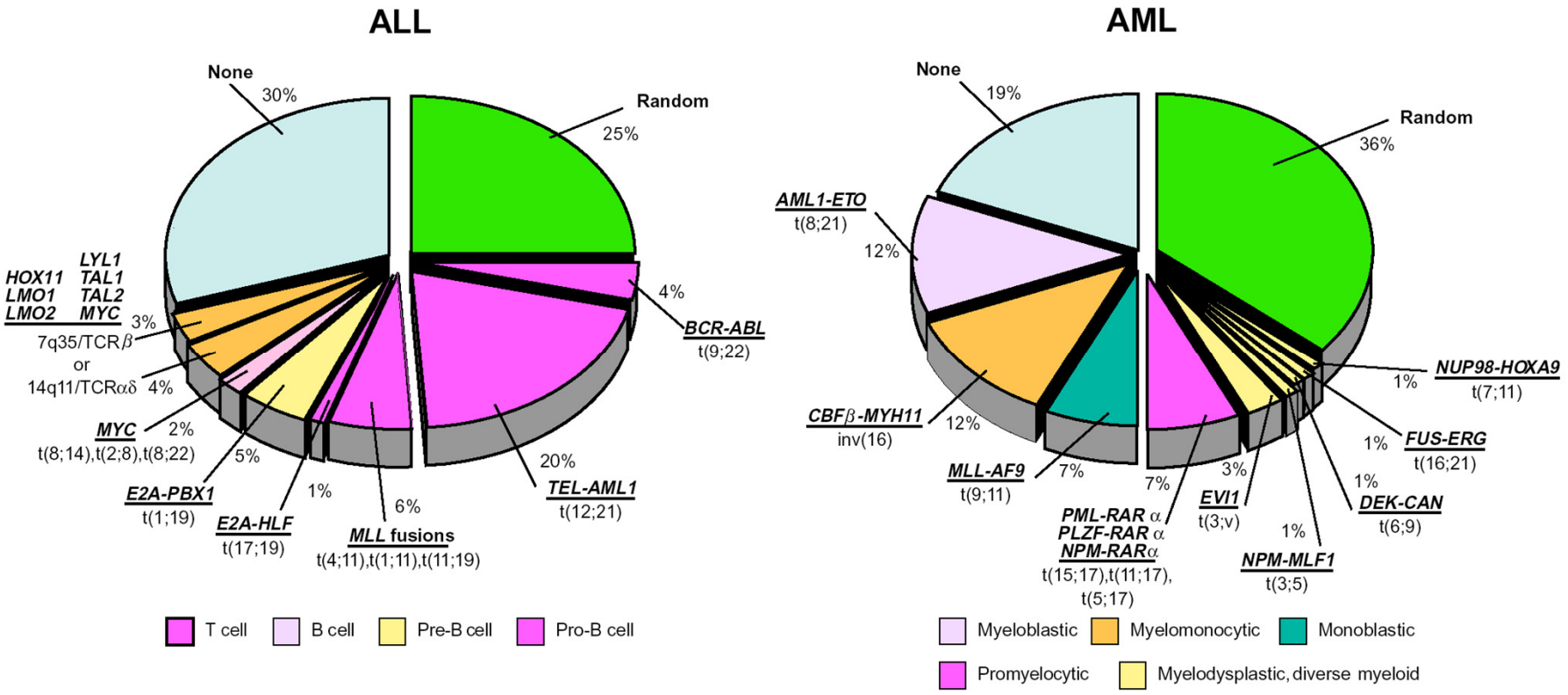
Karyotype leukemic cells and prognosis

Childhood ALL

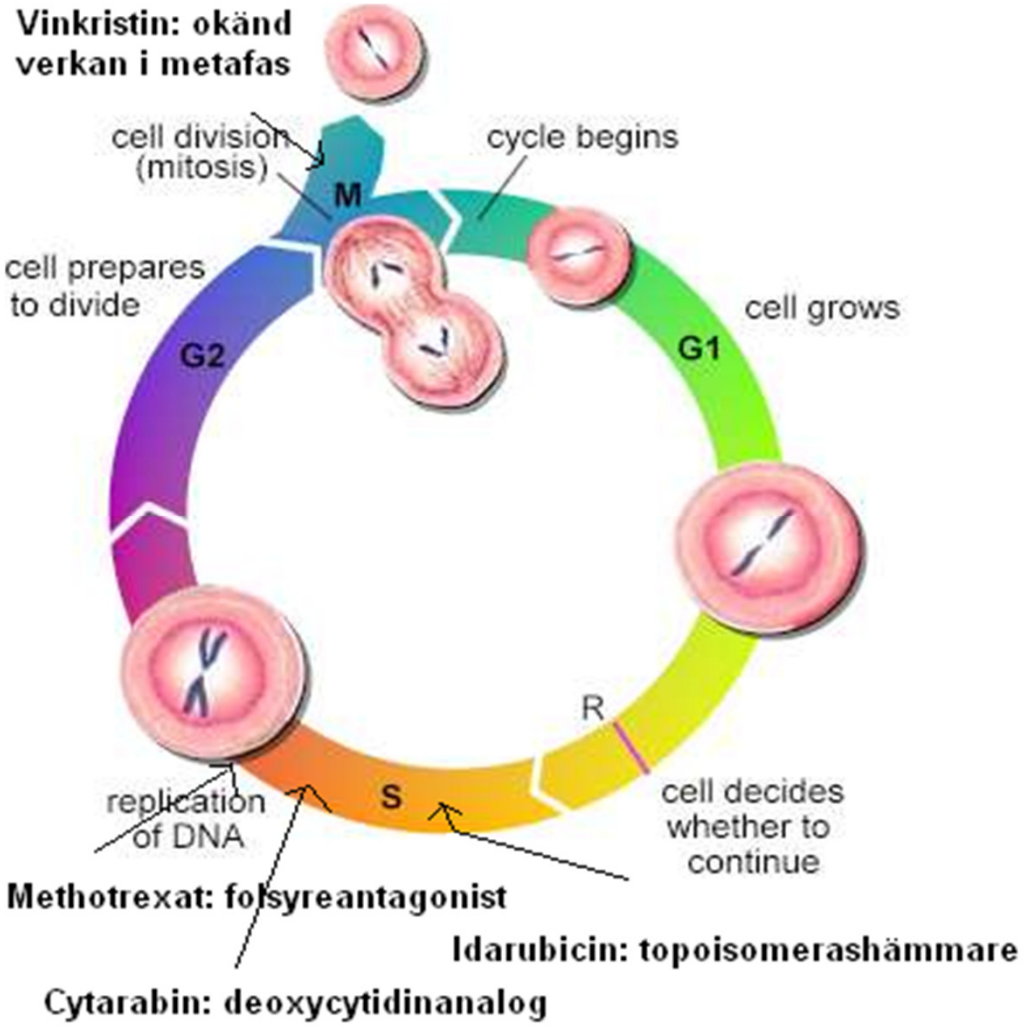


NEJM 1998 (339) : 605-615

Translocation-*generated* oncogenes are common among acute leukemias of children



Cytostatic drugs and the Cell cycle



CNS-prophylaxis?



Protect the CNS system

- **Intrathecal therapy**
  - → early intensification of intrathecal chemotherapy will reduce the risk of CNS relapse
  - → triple intrathecal chemotherapy (methotrexate, hydrocortisone, and cytarabine)
- CNS irradiation
  - → CNS irradiation can cause potentially serious neurotoxicity.



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Better supportive care

- **Infections**
  - →**Identify severe virus infections etc**
- **Dietary supplements**
  - →Percutaneous Endoscopic Gastrostomy (PEG)



- **Psychological care**
  - →Music therapy
  - →Parents involved
  - →Parents always present
  - →Clowns



Karolinska University Hospital-Huddinge

- 20-25 children/year

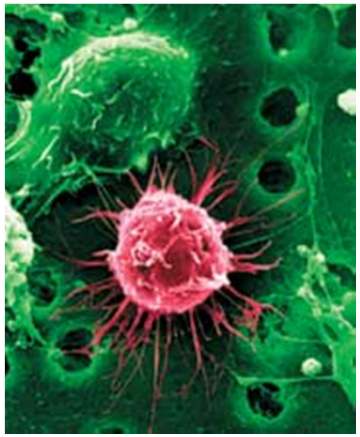
- Siblings
- MUD
- Haplo



Karolinska University Hospital-Huddinge

Stemcells source:

- Bone marrow
- Peripheral stem cells
- Cord blood



Indications for HSCT in malignant diseases, Acute Lymphoblastic Leukaemia (ALL):

- **ALL in 1st complete remission (CR1)** are based on response criteria only:

1. B-lineage ALL with white blood cell count (WBC) $<100x10^9/L$:
   a. A day 29 bone-marrow leukemic blast count of $\geq$5% (MRD)
   b. A day 79 MRD $\geq 10^{-3}$
2. T-ALL and/or B-cell ALL with WBC $\geq 100x10^9/L$:
   a. A day 15 bone-marrow leukemic blast count of $\geq$25%, and a bone-marrow leukemic blast count of $\geq$5% after block A.
   b. A day 29 bone-marrow leukemic blast count of $\geq$5%.
   c. A post-block B bone-marrow MRD $\geq 10^{-3}$.
   d. A precursor B-cell ALL or uncertain lineage with WBC $\geq 200x10^9/L$ and no MRD result available days 29 and79.

- **Relapse in ALL**

NOPHO ALL 2008

Indications for HSCT in myeloid diseases:

- Acute myeloid leukemia (AML)
  - → Poor responders
  - → Specific high risk markers
  - → Relapse

- Myelodysplastic syndrome (MDS)
- Juvenile myelomonocytic leukaemia (JMML)
- Chronic myeloid leukaemia (CML)

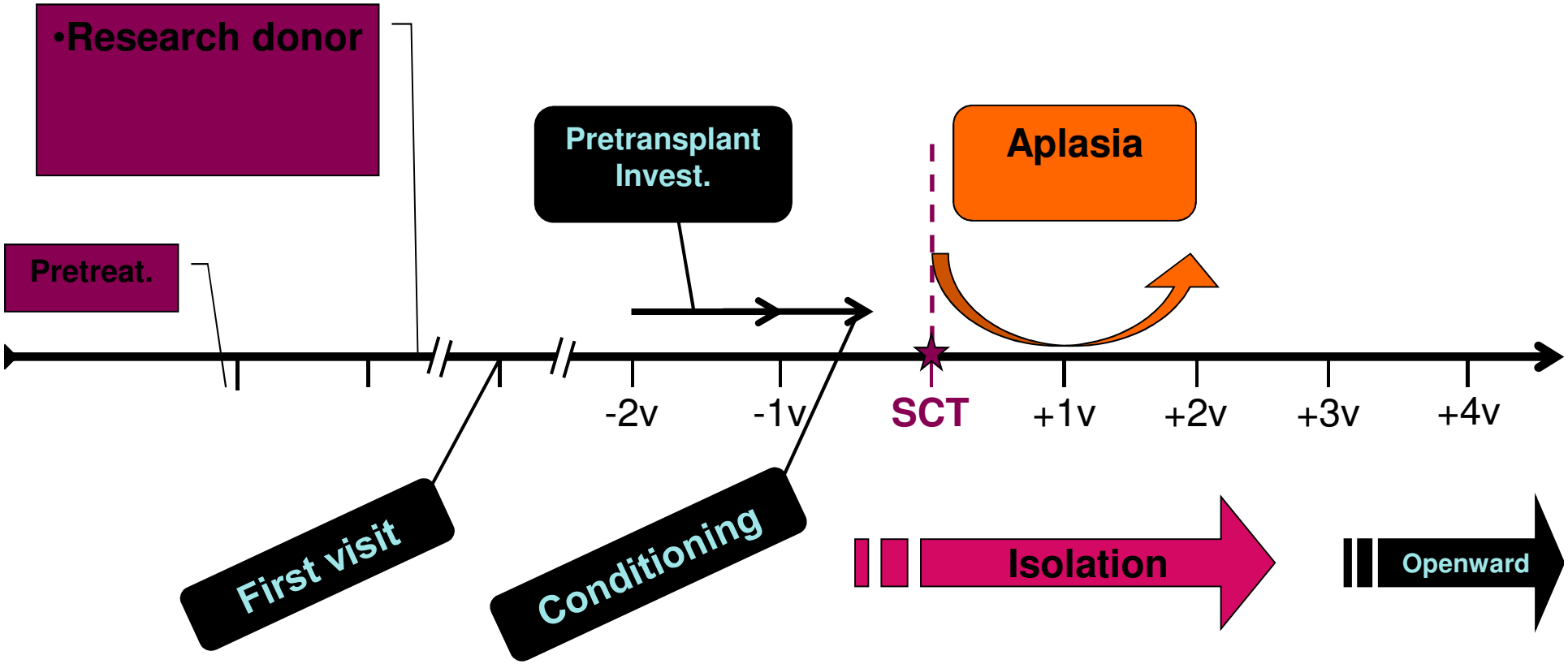
Haematopoietic Stem Cell Transplantation

- Conditioning (most common)
  →Total Body Irradiation (TBI) or Busulfan
  →Cyclofosfamide
  →Etoposide
- Infusion of cells - day 0
- Pre-engraftment period, susceptible to infections
- Engraftment day + 10-20.
- Out-patient care during the first year, from three months after HSCT. Continued immunosuppressive treatment and post-HSCT intrathecal chemotherapy.

Desired effects of Haematopoietic Stem Cell Transplantation

- Maximise irradiation and chemotherapy treatment
- Allogeneic anti-tumour effect (GVT), graft-versus-leukaemia effect (GVL)
- Defect cells are replaced by healthy cells
- Only now available “gene therapy”
- Cures > 20 lethal conditions

Stemcellstransplantation-procedure



Severe undesired side effects after Haematopoietic Stem Cell Transplantation

- Graft-versus-host-disease
- Severe infections
- **Relapse**



CMV Infections

- Adults
  - 70-80% seropositive
- Congenital
  - Birth prevalence 0.3-2.4%
  - 90% no symptoms
  - Prematurity, death, deafness, encefalitis



EBV

- Mononucleosis



- Adults >90% seropositive
- Onkogen potential
  - Burkitt lymfom
  - Tx patients



Relapse after Hematopoietic stem cell transplantation

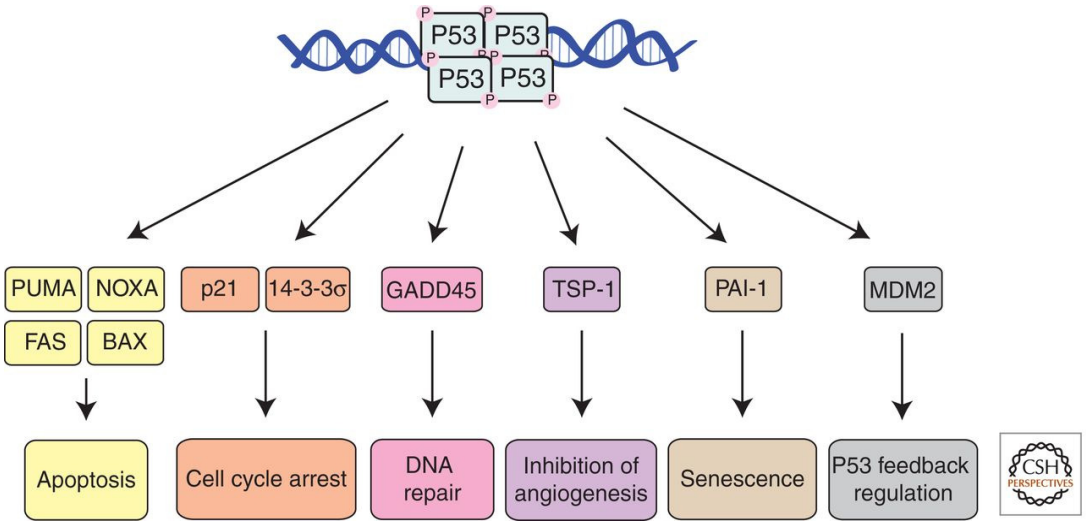
- Poor prognosis

  → Chimerism

  → Minimal Residual Disease

  → **Could over expression of p53 predict relapse?**

The tumor suppressor P53 acts as a transcriptional regulator.



Delbridge A R et al. Cold Spring Harb Perspect Biol 2012;4:a008789

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Part 1-
protein expression as prognostic marker after HSCT

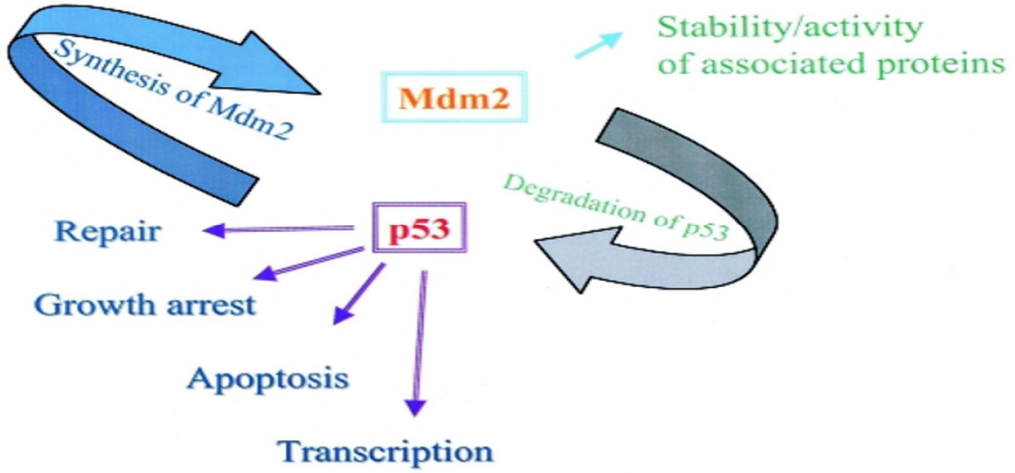
Does the protein expression of specific genes change in the bone marrow from diagnosis and before and after HSCT? Can these changes in expression predict prognosis?

TP53 (tumour suppressor gene 53)

- Guardian of the cell cycle

MDM2 (murine double minute protein-2)

- Key regulator of p53



Alarcon-Vargas D. and Ronai Z. p53-Mdm2--the affair that never ends. *Carcinogenesis* 2002; 23(4):541-7.

Background

- Few studies on p53 expression in hematological malignancies in children

  → Increased protein expression of p53 in child with MDS with poor outcome (Saito *et al*)

  → Increased protein expression of p53 and MDM2 in children with leukaemia compared to controls. Increased expression in ALL patients who relapsed compared to patients with > 5 years survival (Gustafsson *et al* 2001).

Studies of p53 protein overexpression as a predictor of relapse in a pediatric myeloid leukemic population before and after hematopoietic stem cell transplantation

Center for allogeneic stem cell transplantation, Karolinska University Hospital-Huddinge, Department of Clinical Science, Intervention and Technology, CLINTEC, Karolinska Institutet, Stockholm

MATERIAL

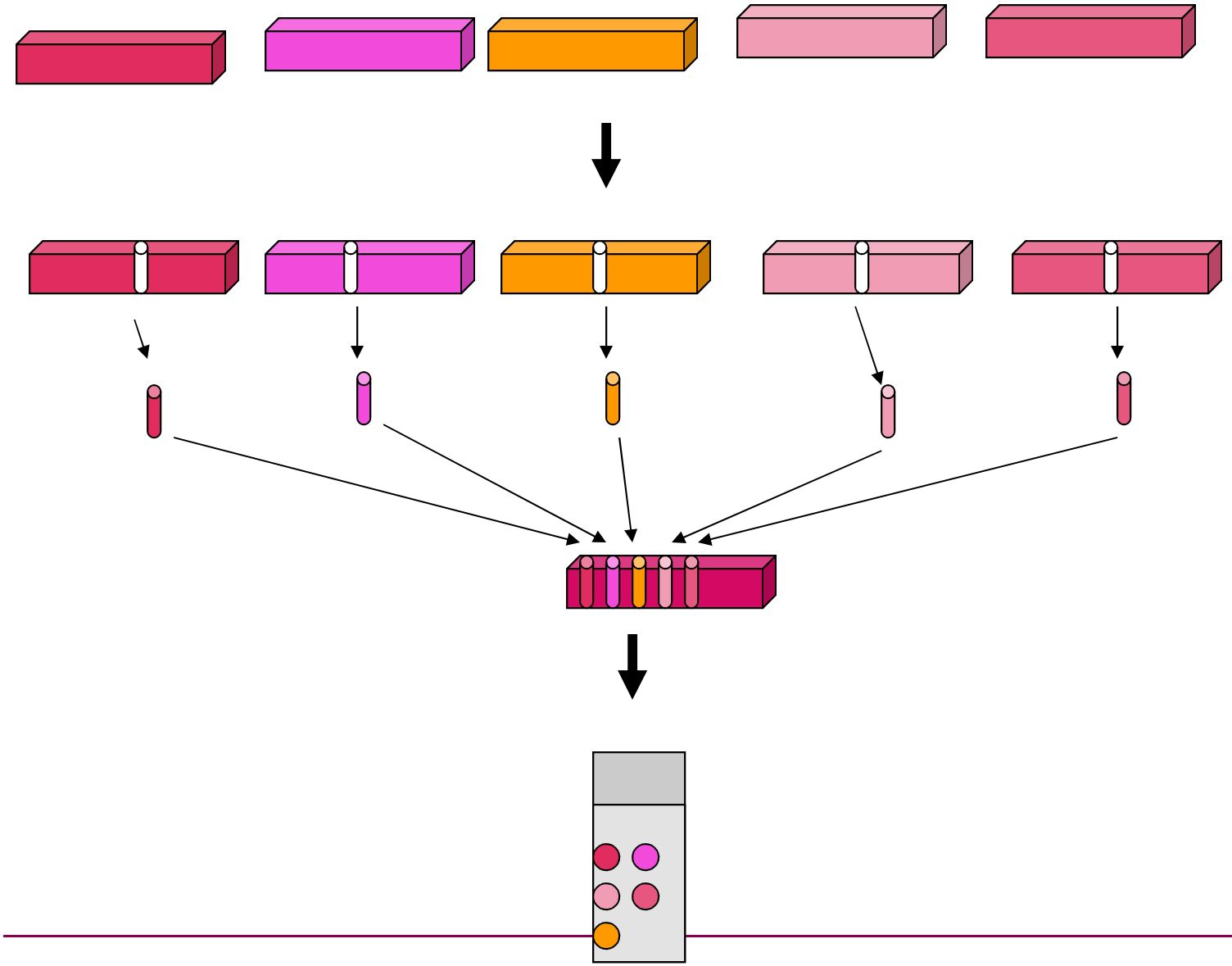
- Paraffin-embedded bone marrow tissue
- Taken at diagnosis, before and after HSCT=67 myeloid malignancies
- 55 non-malignant controls



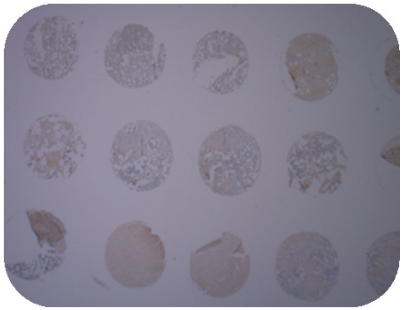
METHODS

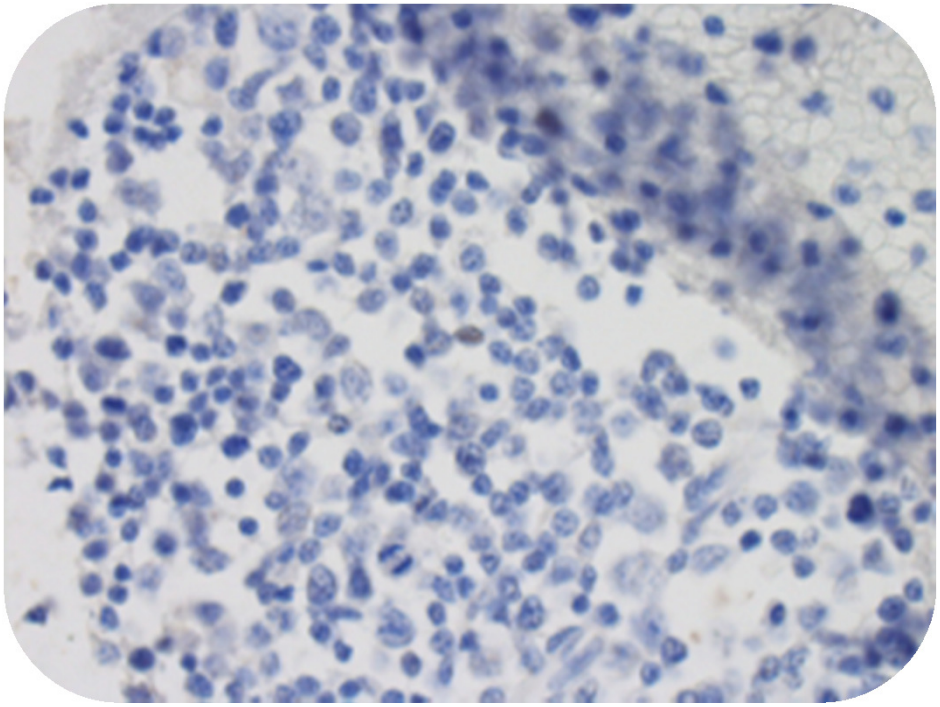
- Tissue micro array and full tissue sections
- Immunohistochemistry with monoclonal antibodies for p53, p16 and p21

Tissue micro arrays



Methods





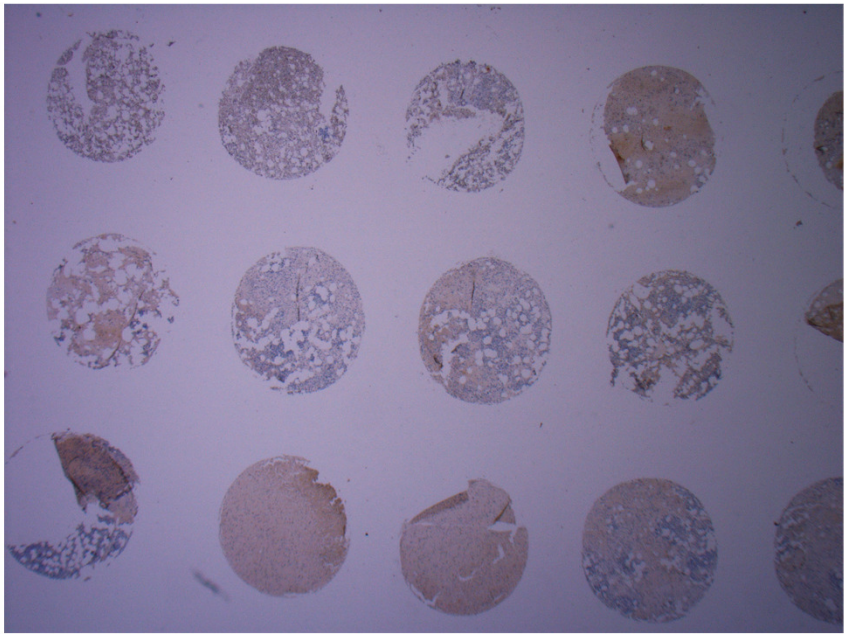
TMA

Benefits

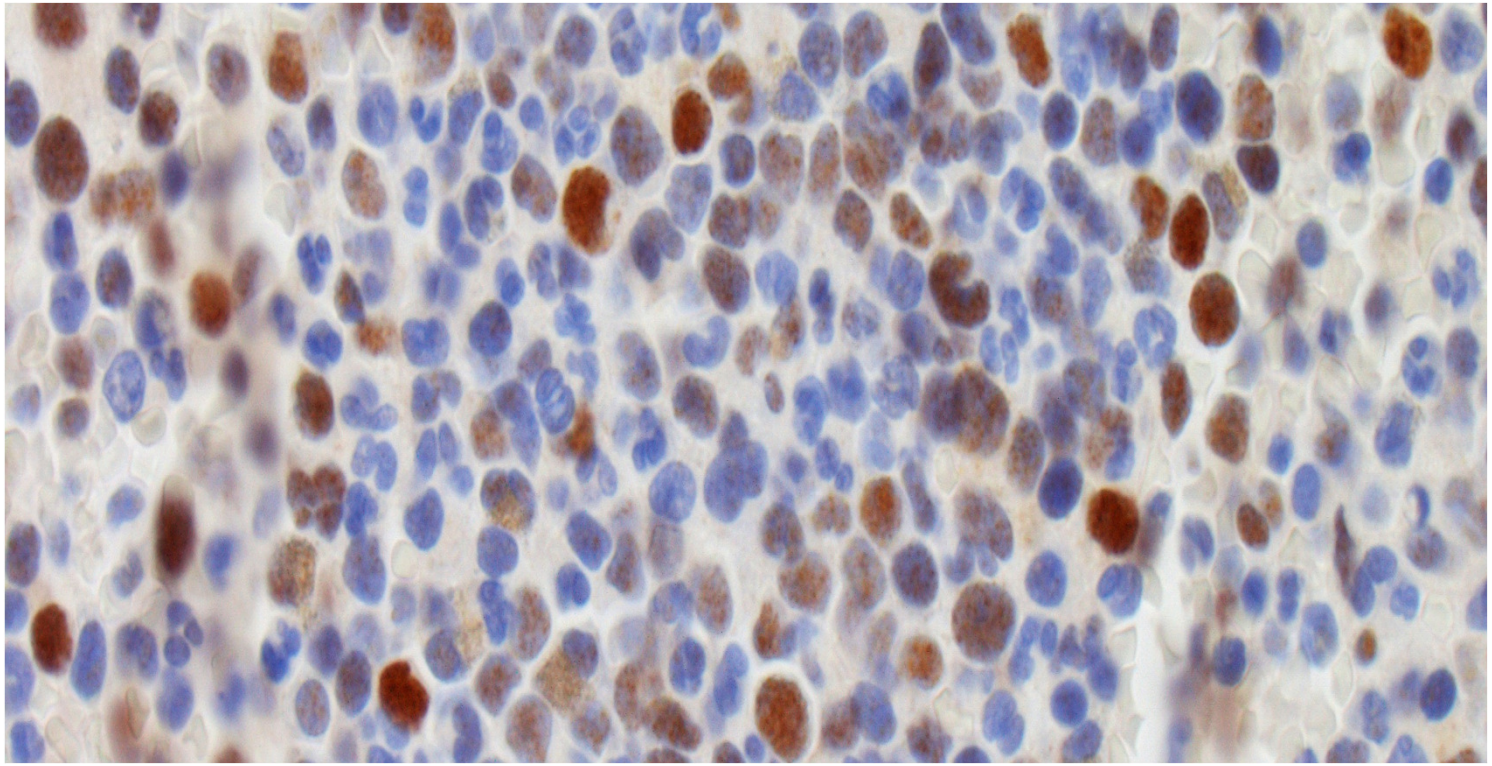
- Efficient and economic method for protein analysis
- Only small quantity of tissue used from each bone marrow sample
- All samples treated in the same way

Limitations

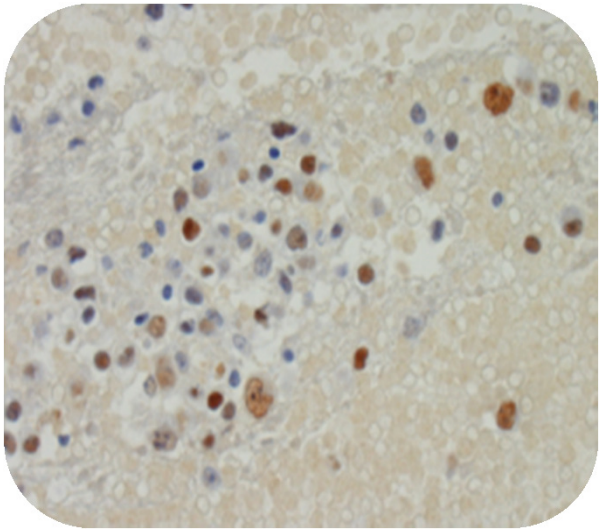
- Small quantity of tissue used makes it less sensitive

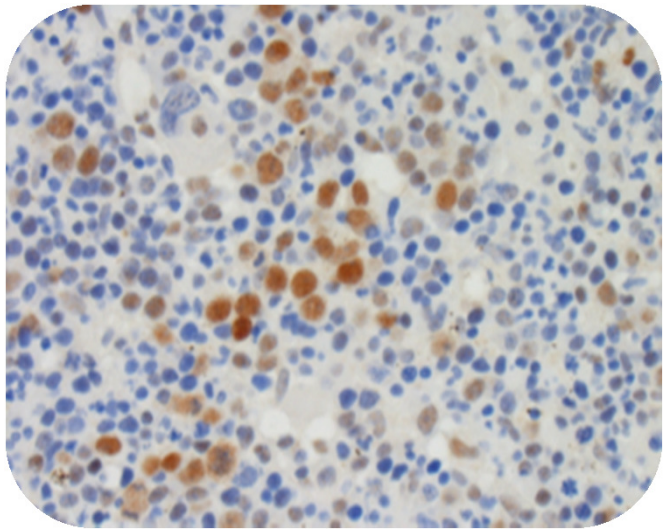


p53 efter immunhistokemi



Two children who relapsed after HSCT

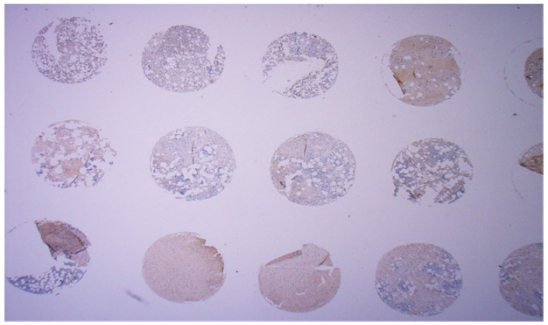
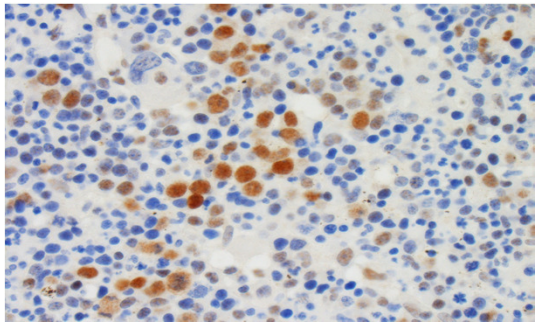
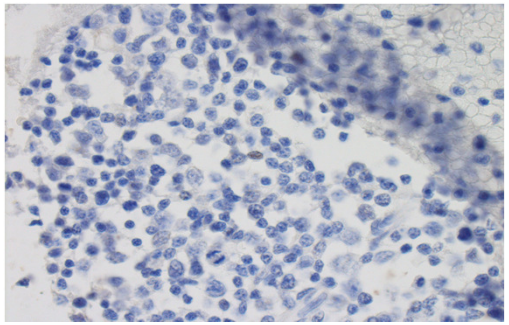




First study

- 33 patients with JMML, CML and MDS
- Paraffinembedded bone marrow samples from the patients transplanted at Karolinska University Hospital-Huddinge 1997-2010 were analyzed before and after HSCT by immunhistochemistry
- Elevated p53 protein expression could be a predictor of relapse in rare chronic myeloid malignancies in children?

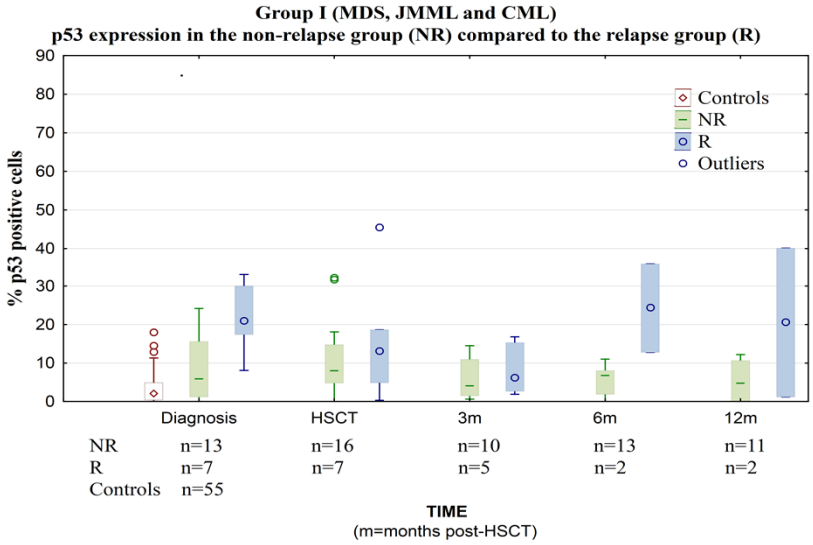
Second study

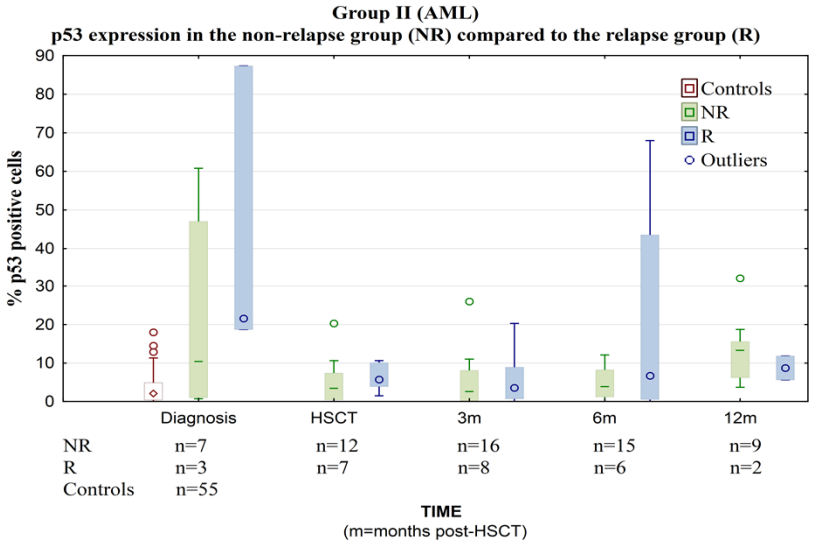




- 34 Children with AML
- Tissue micro array (TMA) och Immunohistochemistry
- P53
- Increased expression of p53 protein at 6 months post HSCT was associated with risk of relapse.

…results

- expressions of p53 protein in the relapse patients compared to the non-relapsed patients in both Group I and II.





Conclusion

Elevated p53 protein expression may be an indicator for children at risk of relapse and would therefore suggest that these children could benefit from more intense therapy.

In conclusion

- p53 has been analysed in two groups of myeloid malignancies
- Group I included MDS, JMML and CML (n=33)
- Group II included AML (n=34)

- Increased expression of p53 protein at diagnosis was associated with risk of relapse in Group I, (odds ratio 1.19, 95% CI: 1.02-1.40, p=0.028).
- Increased expression of p53 protein at 6 months post HSCT was associated with risk of relapse in Group 2 (with a significant difference for p53 at 6 months post HSCT, using T-test).

…….in conclusion

- Consider conditioning therapy, for more aggressive leukemias
- Earlier interventions :

  T-cell therapy

  Decrease the immunosuppression

Acknowledgments

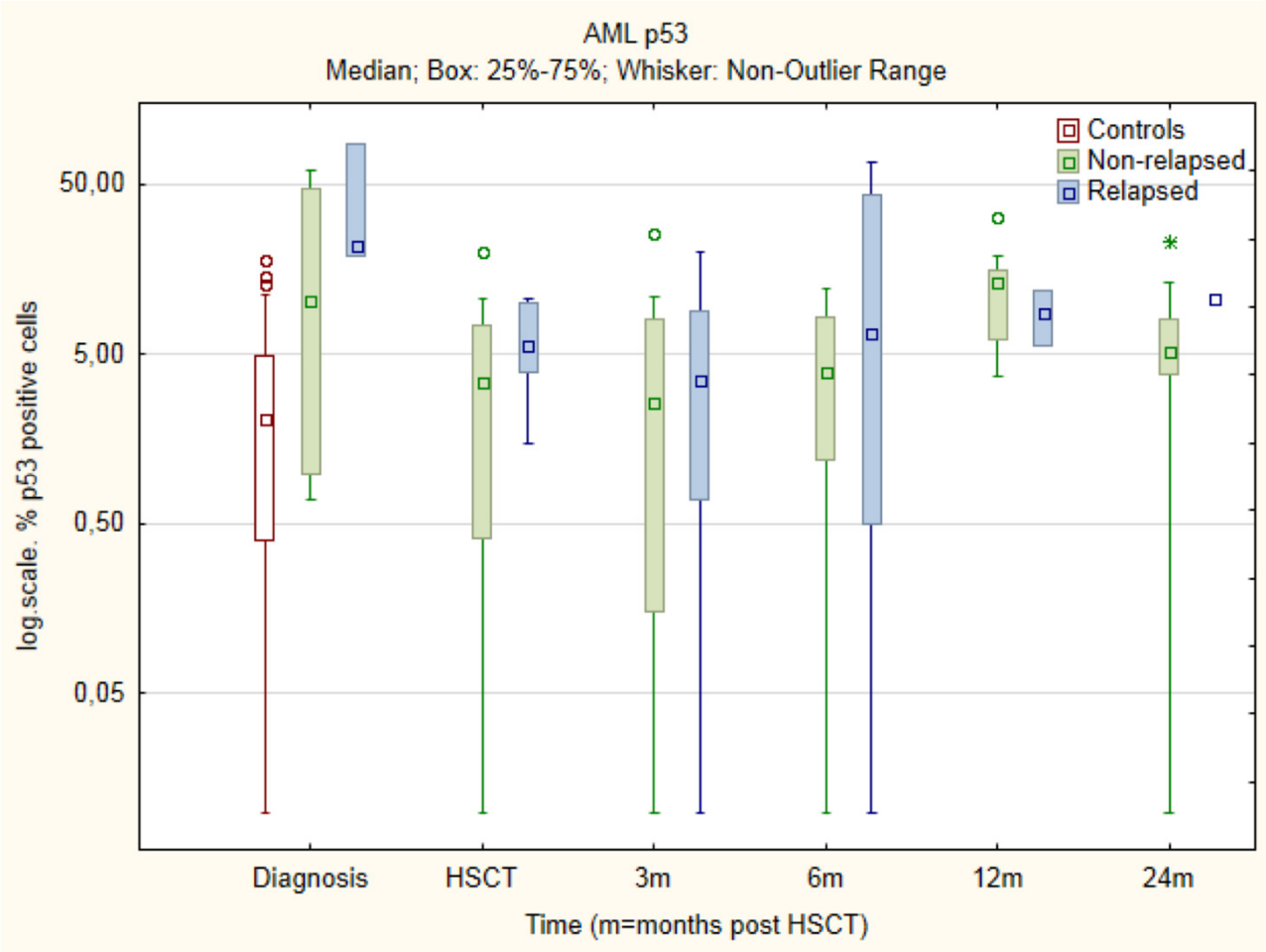
- Honkaniemi E
- Matttson K
- Barbany G
- Sander B

Swedish Childhood Cancer Society

Mary Béves foundation for childhood cancer reseach

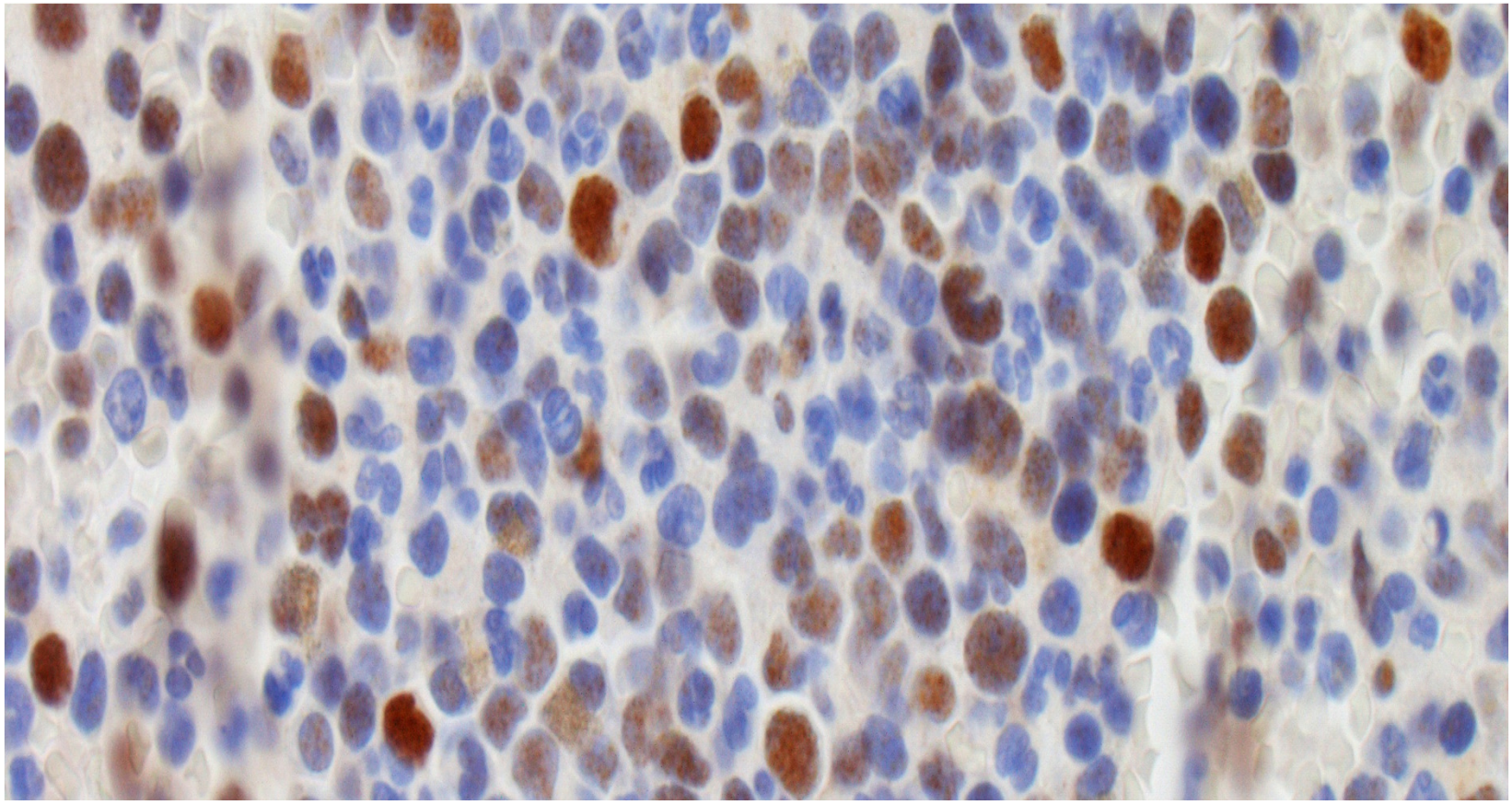
The Samariten foundation

Results: ”Elevated p53 protein expression could be a predictor of early relapse after hematopoietic stem cell transplantation in children with AML”



Could elevated p53 protein expression be a predictor of relapse in children with acute myelogenous leukemia after HSCT

- Paraffinembedded bone marrow samples from 34 patients diagnosed with AML were analyzed before and after HSCT
- 1997-2010
- Immunohistochemistry



RESULTS

- Protect the CNS system
- Cranial irradiation
- Intrathecal chemo therapy
- Intensified protocols
- Better diagnostic tools
- Supportive care
- Hematopoietic stem cell transplantation





Hematopoietic development

