ICCS e-Newsletter CSI Winter 2011

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e-CSI – Clinical history:

8 year old female presenting with fever and bruising on extremities

e-CSI – Complete blood count:

CBC parameter	Result	Units	Reference range
WBC	122.2	$x10^9/L$	(5.41 – 9.70)
RBC	3.01	$x10^{12}/L$	(3.88 – 4.72)
HGB	8.4	g/dl	(11.3 – 13.4)
HCT	25.3	%	(32.3 – 38.3)
MCV	84.0	fl	(79.5 – 85.2)
MCH	27.9	pg	(27.8 – 30.0)
MCHC	33.4	gm/dl	(34.3 – 35.8)
RDW	13.9	%	(12.8 – 13.9)
PLT	86	$x10^9/L$	(187 - 376)

WBC differential	%	Absolute	Reference range
Neutrophils	0	0	(2.58 – 5.95)
Lymphocytes	8		
Monocytes	1		
Eosinophils	0		
Others	91		

e-CSI – Work-up and evaluation:

Bone marrow aspirate and biopsy were procured

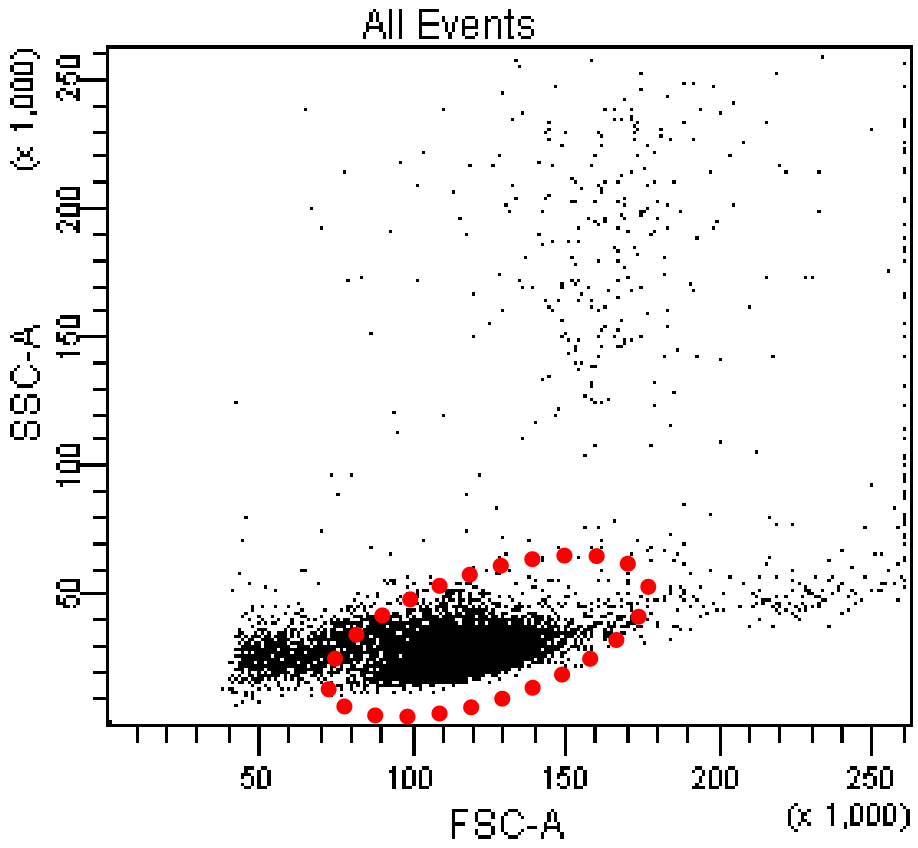
Flow cytometric analysis was performed on the marrow aspirate and results from selected 4-color tubes are provided for review

e-CSI – Flow cytometric approach:

Acquisition FACS CantoII, analysis DIVA
(FITC / PE / PerCP-Cy5.5 / APC)

Tube 1: HLA-DR / CD117 / CD45 / CD19
Tube 2: CD13+CD33 / CD7 / CD19 / CD2
Tube 3: CD20 / CD10 / CD45 / CD19
Tube 4: Lambda / Kappa / CD19 / CD22
Tube 5: CD15 / CD56 / CD19 / CD34

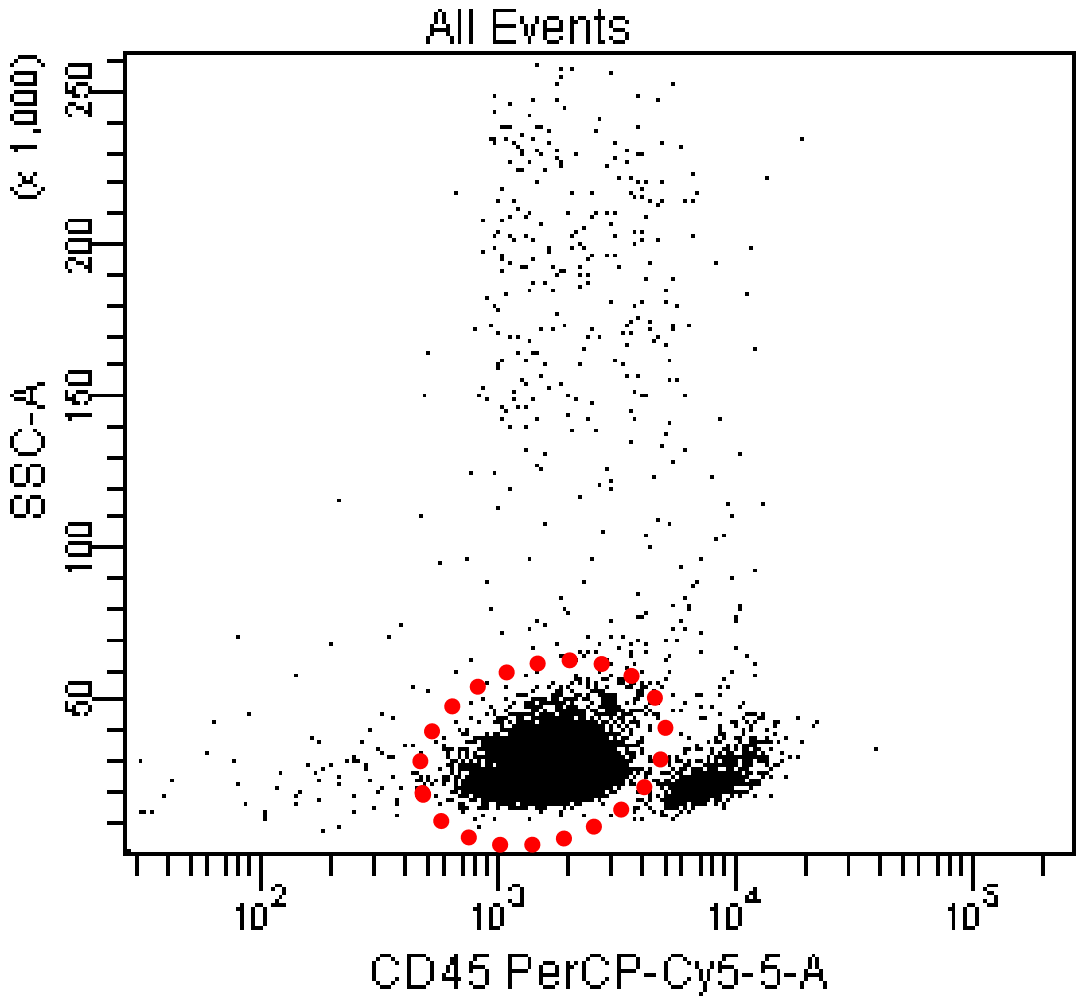
e-CSI – Flow cytometric analysis:



An expanded population of small non-complex cells (ie. low FSC and SSC) is detected.

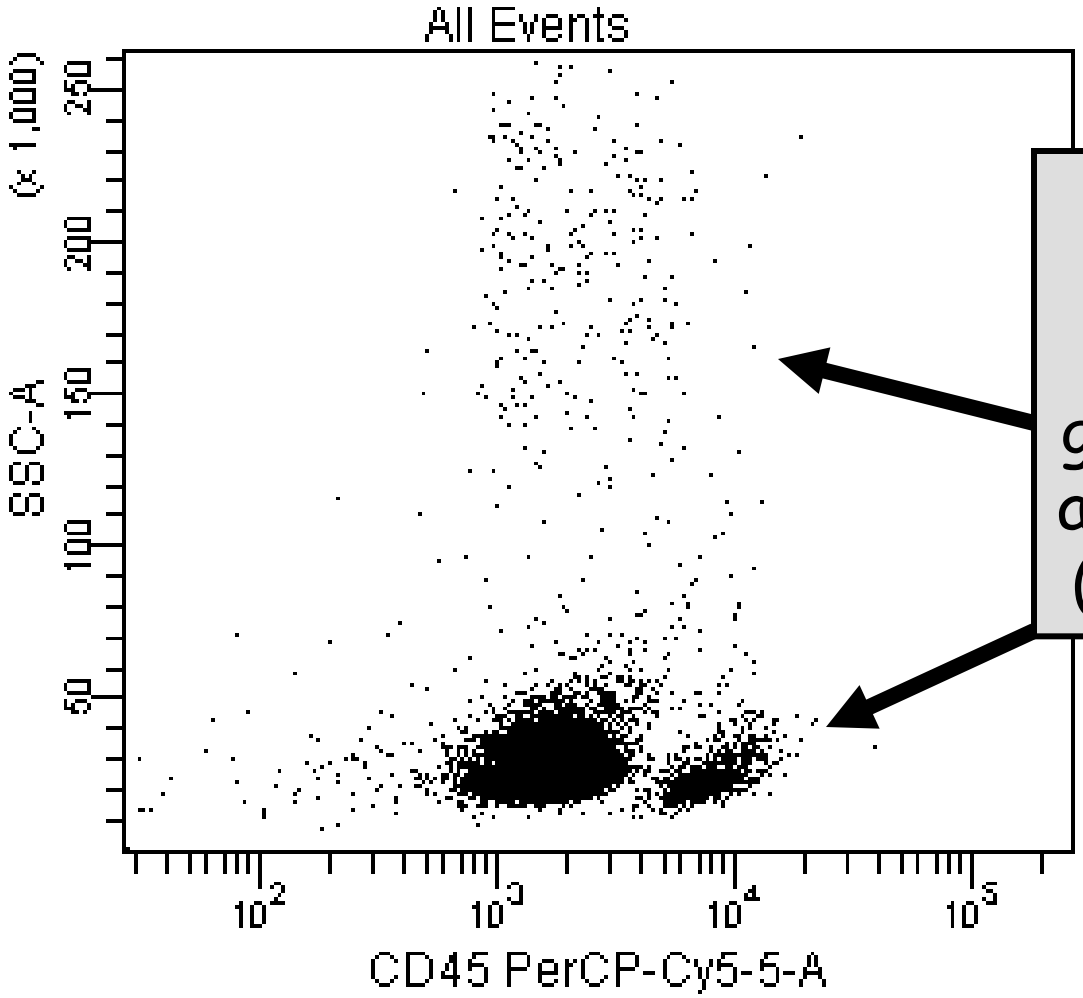
This profile is not typical of marrow where maturing granulocytic and monocytic elements should be present in abundance.

e-CSI – Analysis – tube 1:



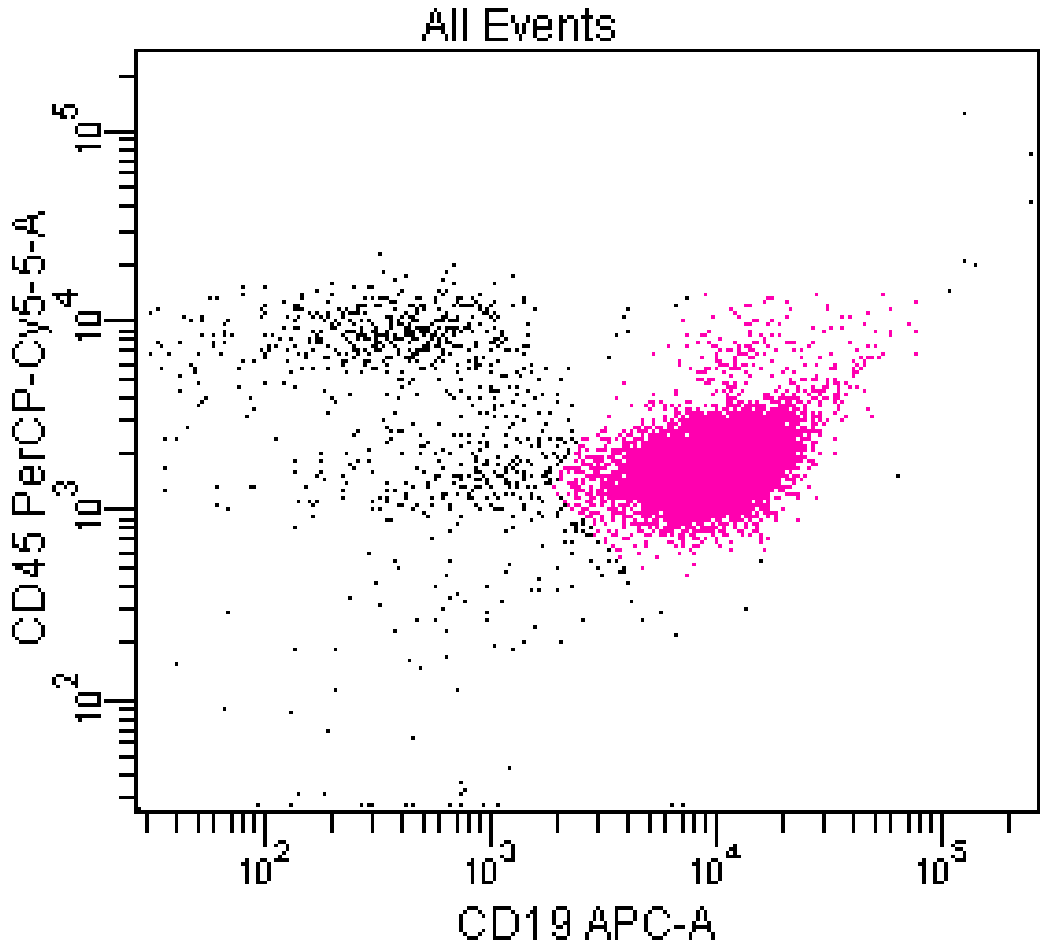
The majority of cells express dim CD45, indicating they are immature.

e-CSI – Analysis – tube 1:



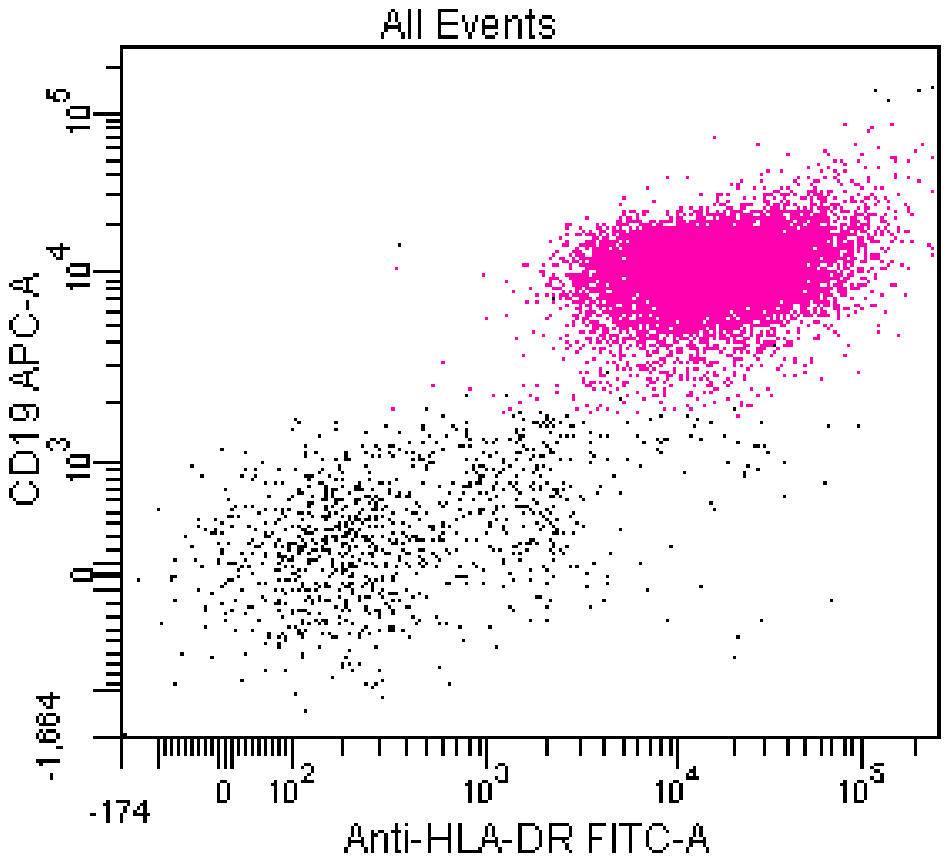
Note also small populations of presumptive granulocytes (high SSC) and mature lymphocytes (CD45 bright, low SSC)

e-CSI – Analysis – tube 1:



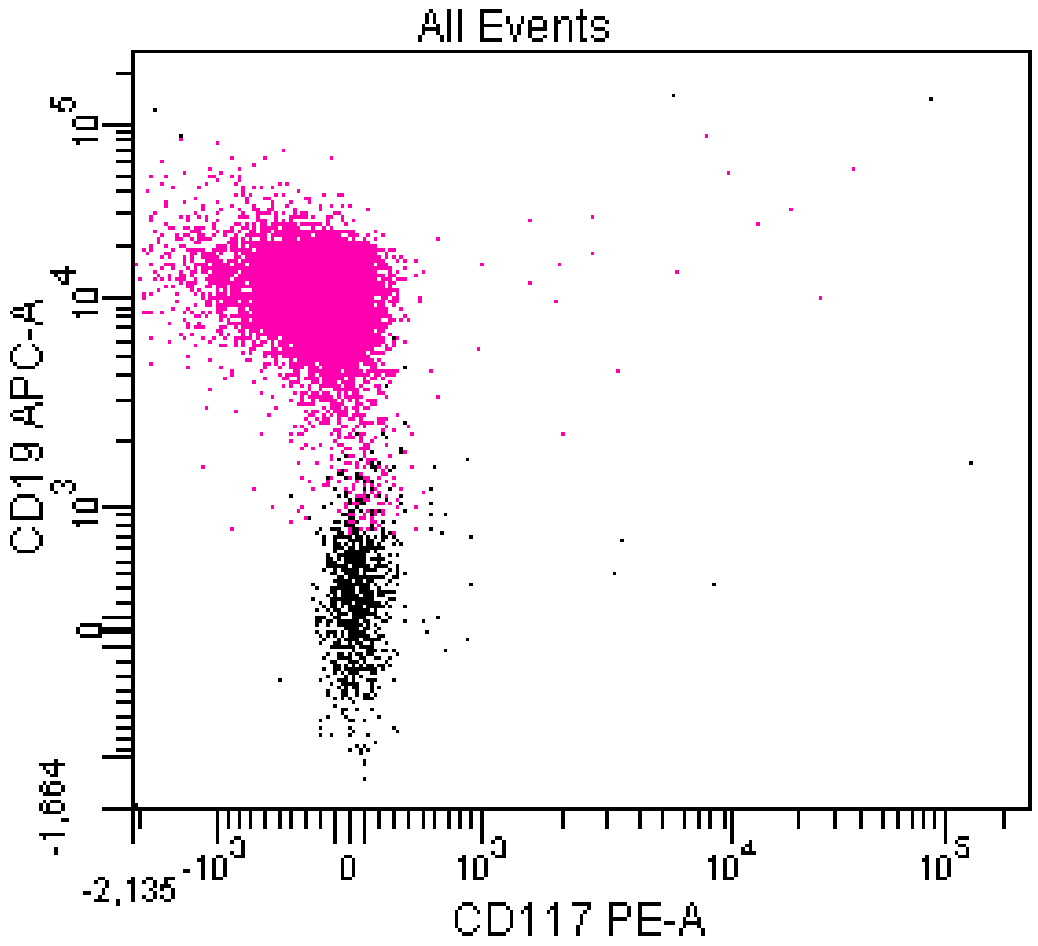
The CD45 dim population coexpresses CD19, an antigen that can be expressed normally on B-cells (in all stages of maturation and differentiation) as well as aberrantly on myeloblasts

e-CSI – Analysis – tube 1:



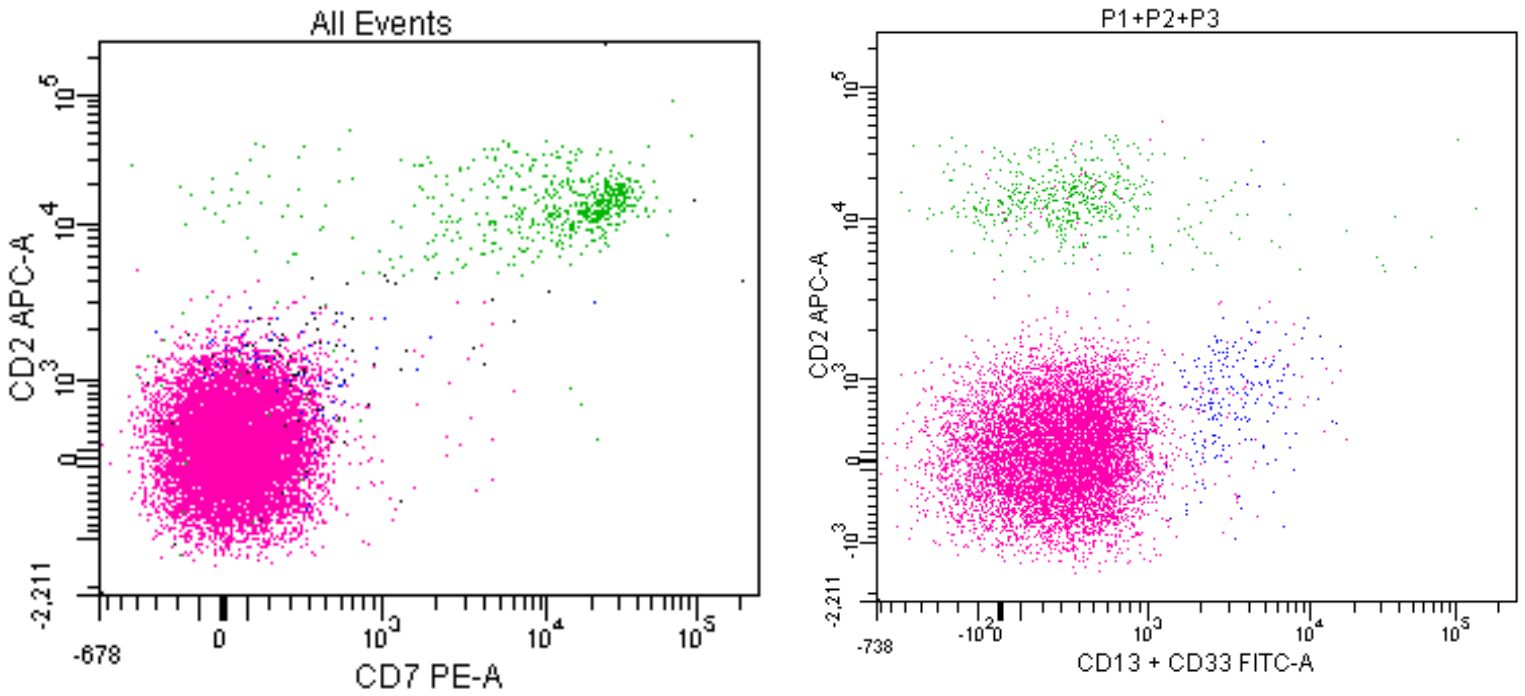
HLA-DR, which is seen in most acute myeloid leukemias as well as in B-lymphoblastic leukemias, is also coexpressed by these CD19 positive cells.

e-CSI – Analysis – tube 1:



This population is, however, negative for CD117, suggesting it is not of myeloid derivation.

e-CSI – Analysis – tube 2:



Absence of CD13/CD33 (myeloid antigens) offers further support against a myeloid process while lack of CD2 (T-cell) and CD7 (primarily a T-cell antigen, but can be seen in myeloblasts as well) indicates these cells are likely not of T-cell lineage. Note the presence of mature T-cells (green) and granulocytes (blue).

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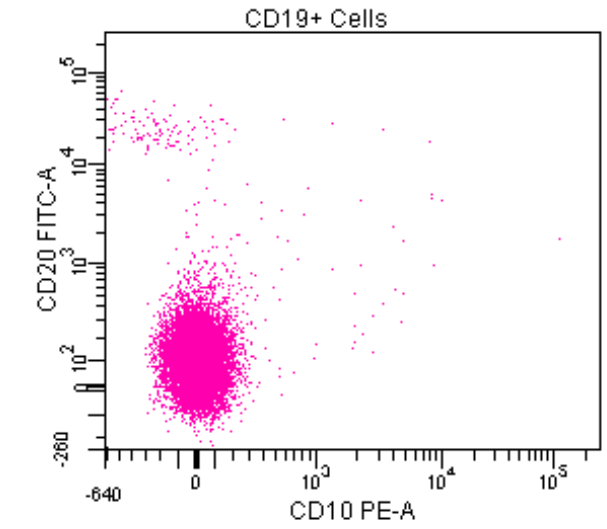
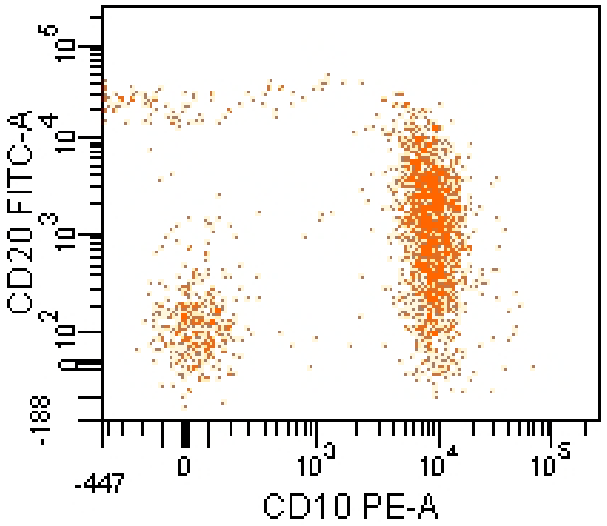
e-CSI – Analysis – tube 3:



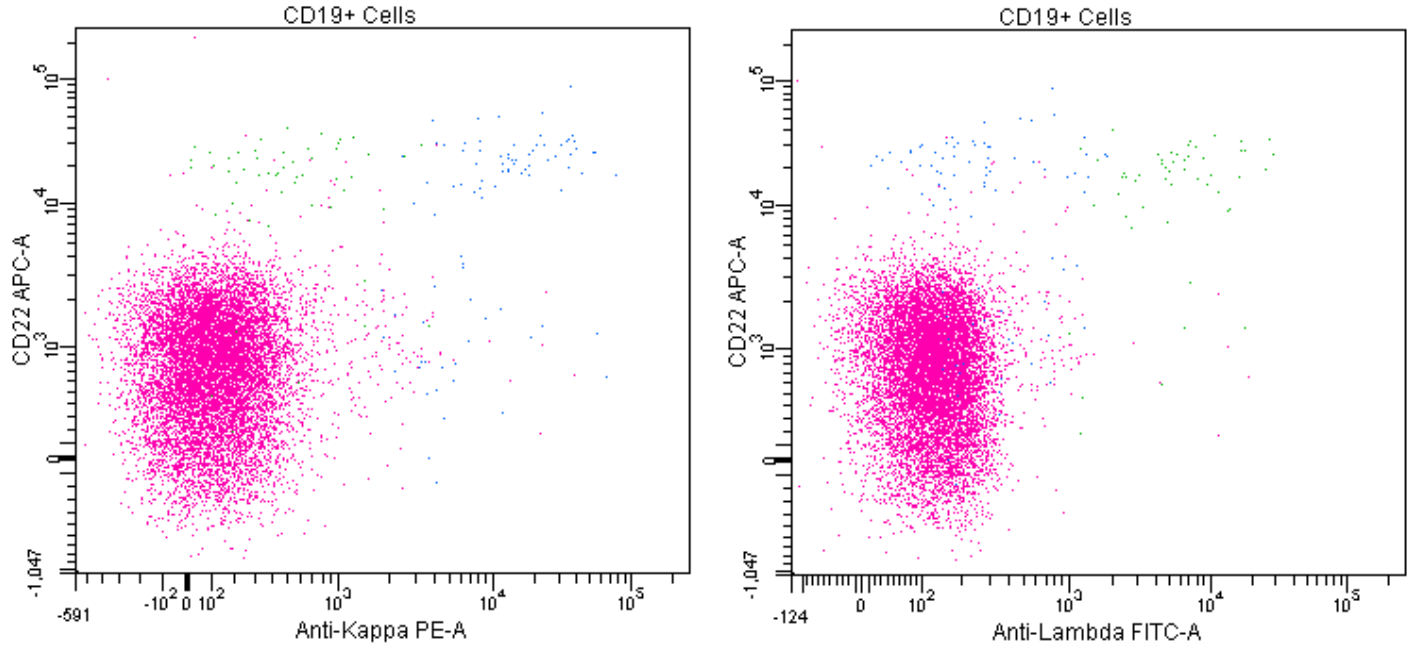


Although the cells are CD19 positive, other markers commonly seen in B-lymphoblastic leukemia (CD20 and CD10) are absent. Note that a minute population of mature B-cells (CD20 bright) is present.

Compare this with a profile of normal maturing B-cells (ie. hematogones) provided below.

e-CSI – Analysis – tube 4:

CD22 shows dim positivity, supporting a B-cell lineage.



Mature B-cells (CD22 bright) express polytypic surface immunoglobulin.

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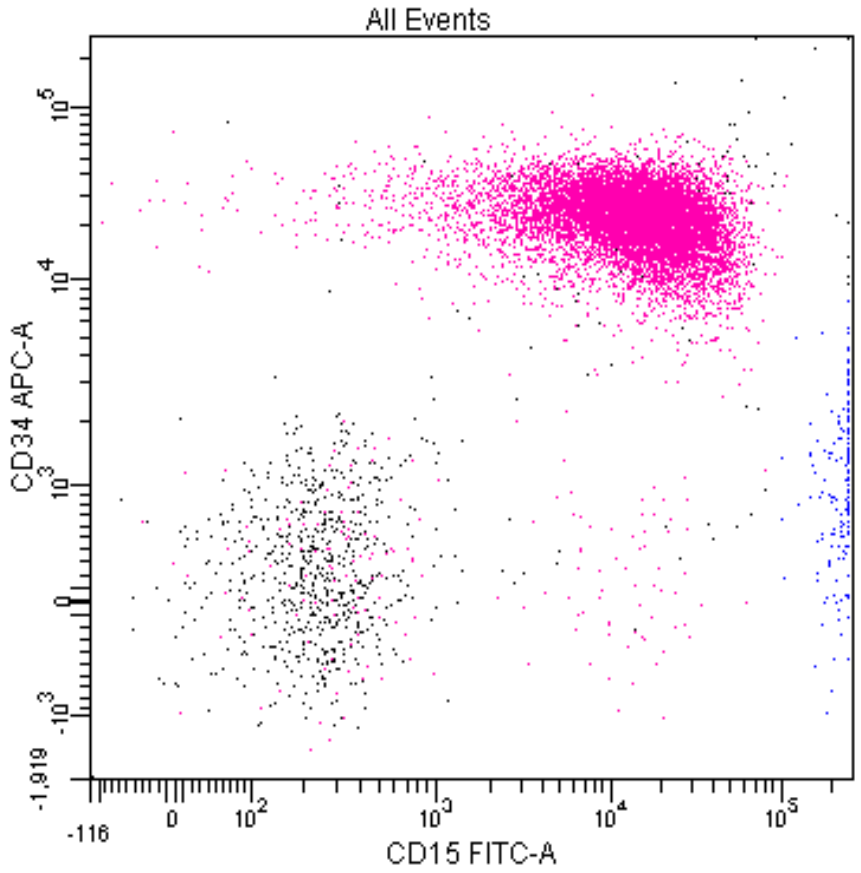
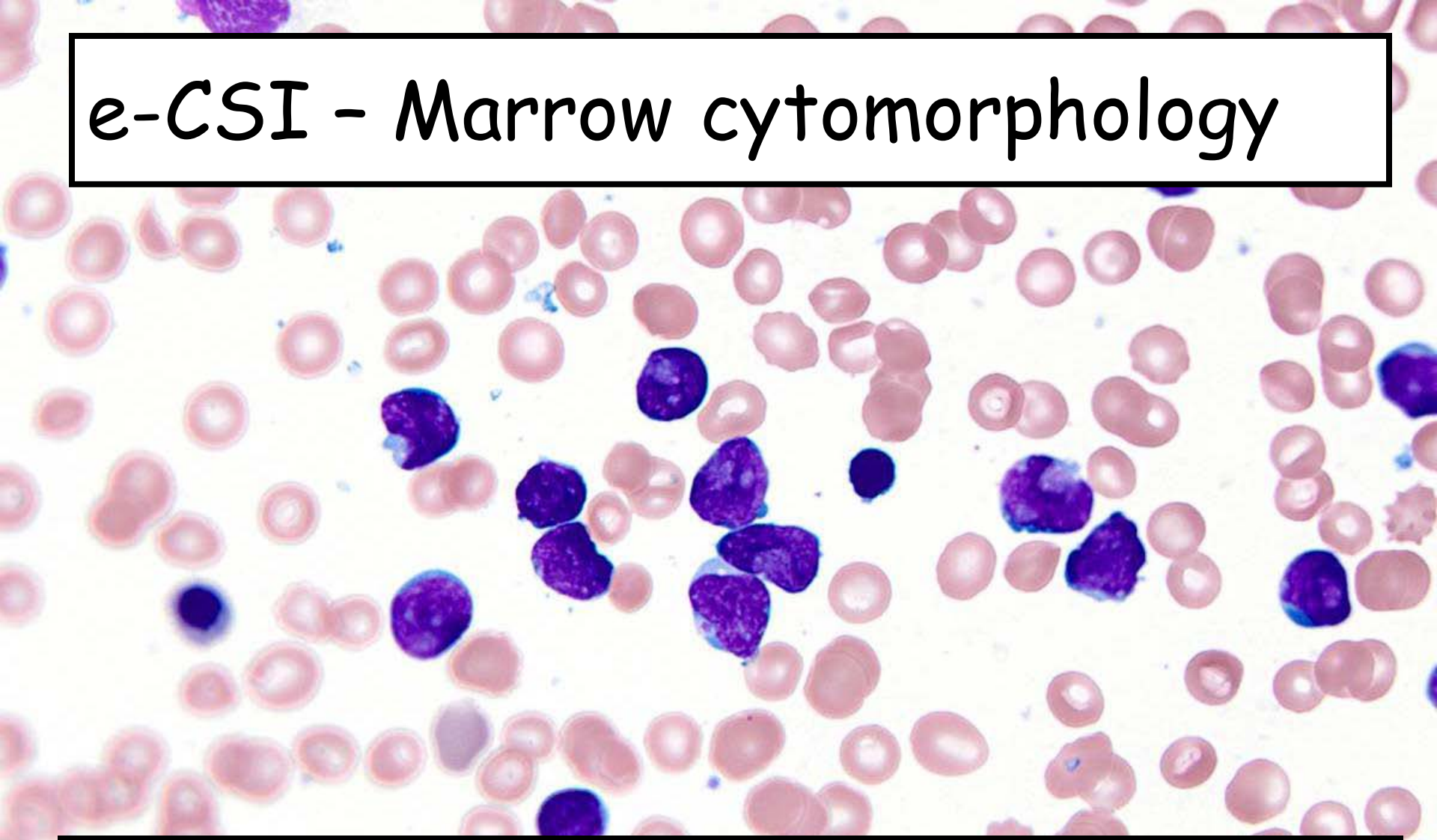
e-CSI – Analysis – tube 5:



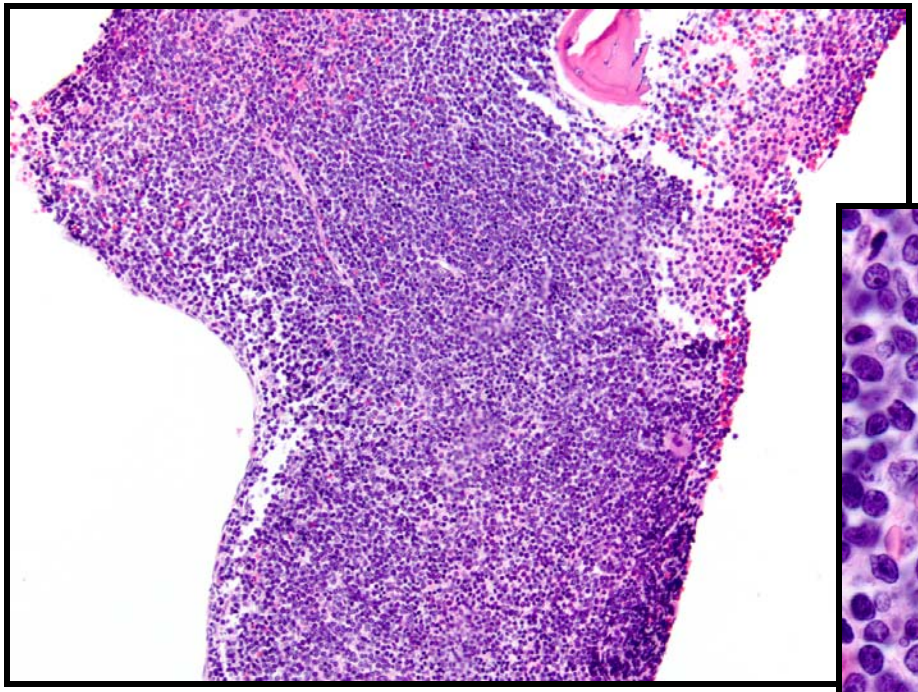
The CD19, CD22 positive cells also coexpress CD34, confirming that they are B-lymphoblasts and they exhibit aberrant expression of CD15, another myeloid antigen. Note the mature granulocytes (CD15 bright) in blue.

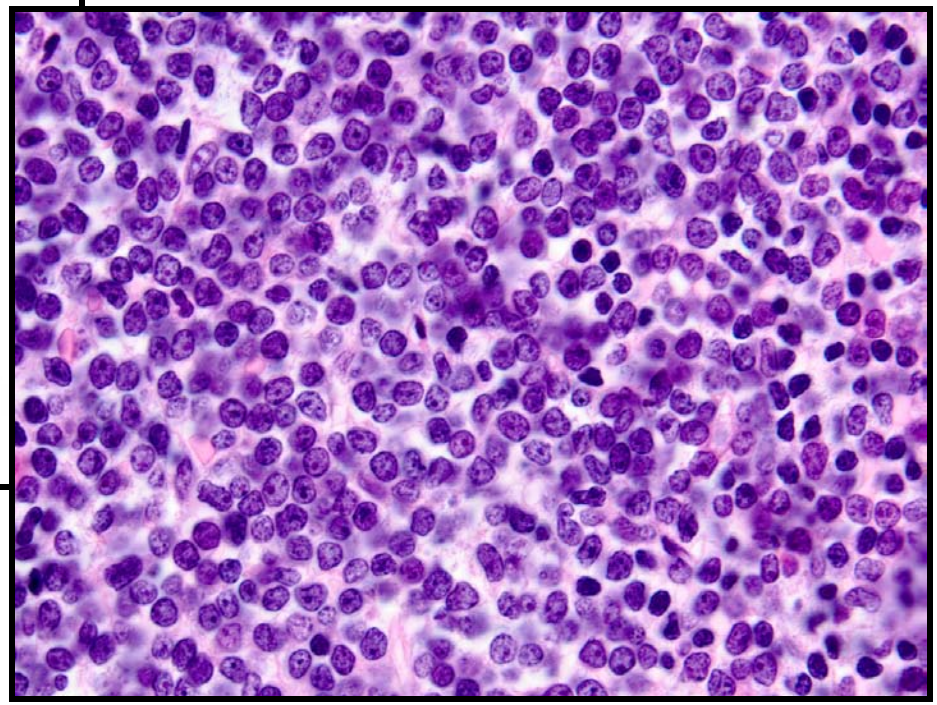
e-CSI – Marrow cytomorphology



e-CSI – Bone marrow aspirate smear demonstrates a predominant population of small to intermediate-sized mononuclear cells that have round to irregular/clefted nuclei, fine chromatin, inconspicuous to small nucleoli and scant cytoplasm, consistent with lymphoblasts.

e-CSI – Marrow cytomorphology





e-CSI – Bone marrow core biopsy demonstrates marrow replacement by sheets of lymphoblasts. Only rare erythroid, myeloid and megakaryocytic elements are present.

e-CSI – Broad diagnosis:

B-lymphoblastic leukemia

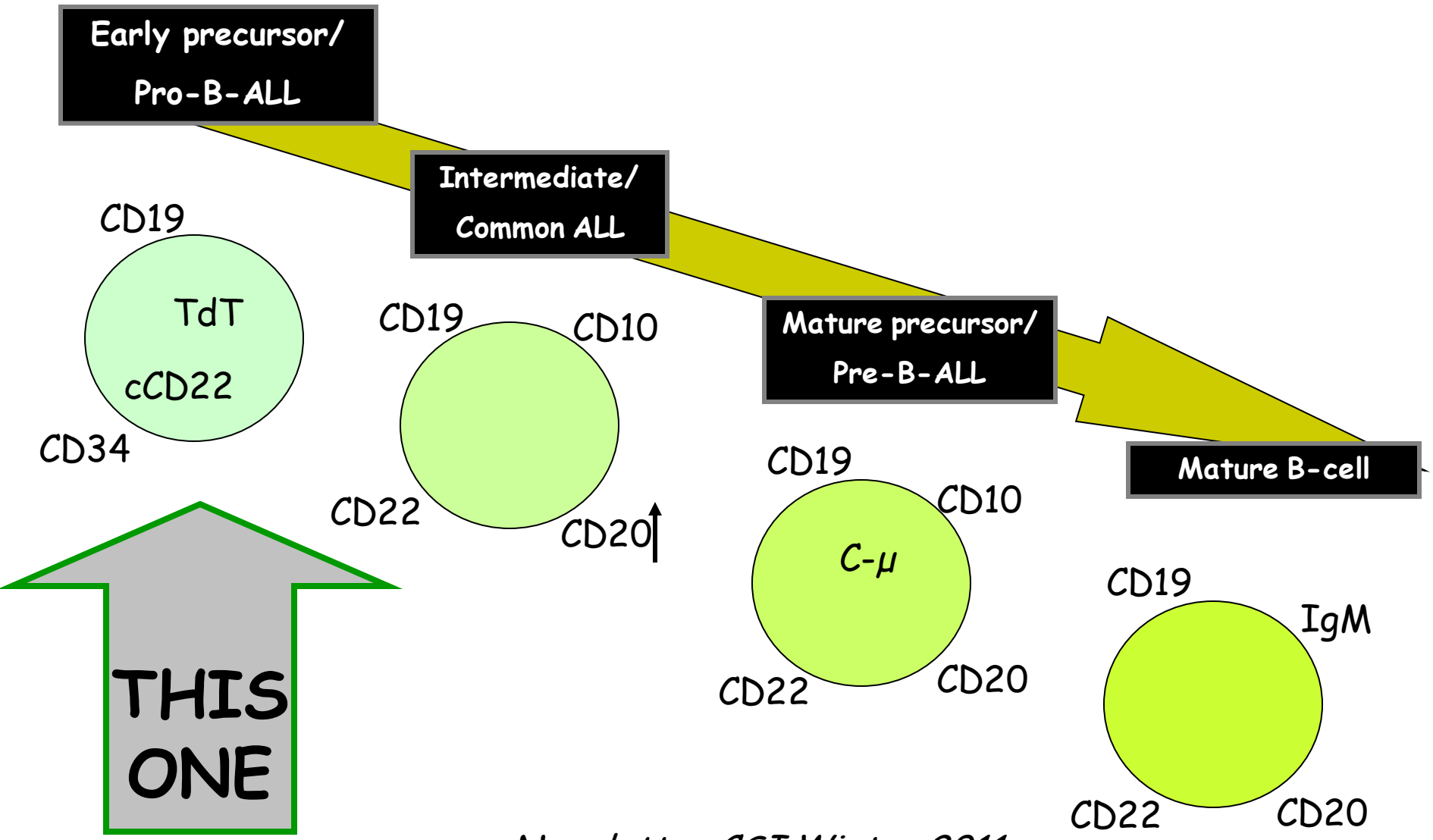
e-CSI – Question 1:

Given this immunophenotypic profile:

CD45 dim, HLA-DR, CD34, TdT (data not shown), CD19, CD22 dim and CD15 positive

What stage of B-cell maturation do these cells best represent?

e-CSI – B-cell development:



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e-CSI – Question 2:

Based upon this immunophenotype, can you predict the cytogenetic abnormality that was detected?

e-CSI – Cytogenetic results:

t(4;11)(q21;q23)

with an MLL [11q23] rearrangement

(this cytogenetic abnormality further subcategorizes B-lymphoblastic leukemia within the WHO 2008 classification schema)

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e-CSI – Question 3:

The clinical history was that of an 8 year old female – what is somewhat unusual about the age of presentation given the presence of a t(4;11) translocation?

e-CSI – Infantile ALL:

Acute lymphoblastic leukemia (ALL) with MLL rearrangement is the most common leukemia in infants less than 1 year of age, but is less common in older children.

So (playing the odds), one might have expected this patient to be an infant based upon the immunophenotypic profile.

e-CSI – Question 4:

The full karyotype is as follows:

45,XX,t(4;11)(q21;q23)[18]/
46,XY[2],donor

How do you reconcile this information?
What historical details were omitted?

e-CSI – Relapsed infantile ALL:

This child was diagnosed with MLL-associated ALL at 4 months of age.

She was in remission for 3 yrs, then relapsed, underwent re-induction, and was subsequently transplanted with unrelated allogeneic marrow from a male [XY] donor.

Unfortunately, her presentation as depicted in this case study represents relapsed disease post-transplantation; the presence of two cells with a male karyotype reflects residual donor cells in a background of relapsed leukemia.

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e-CSI – Pediatric leukemia:

- Leukemias are the most common cancers affecting children, representing ~30% of all cancers in those under 15 years of age.

- In the United States, 75% of pediatric leukemias are lymphoblastic leukemia, 15-20% are acute myeloid leukemia (AML), and 5% are chronic myeloid leukemia.

- Infantile ALL (that diagnosed within the first 12 months of life) represents ~2.5-5.0% of pediatric ALL.

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e-CSI – Infantile ALL & MLL:

- Rearrangements in chromosomal band 11q23, involving the mixed lineage leukemia [MLL] gene, are common in infantile ALL, occurring in ~70% of cases.

- Its presence is inversely correlated with age:

  - >90% in those less than 6 months
  - ~50% in those 6 – 12 months
  - ~6-7% in those 12-24 months

- Abnormalities of MLL include deletions, inversions and unbalanced as well as reciprocal translocations. Many different translocation partners (> 70) have been described.

- The most common translocation is t(4;11)(q21;q23), occurring in 30-45% of infants.

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e-CSI – Infantile ALL with t(4;11)(q21;q23):

Clinical manifestations:

Hyperleukocytosis (median WBC > 150 $x10^9/L$)
Hepatosplenomegaly
CNS involvement

e-CSI – Infantile ALL with t(4;11)(q21;q23):

Immunophenotypic characteristics:

Represents an early precursor ("pro-B") cell

CD10 negative

CD10 (aka. *CALLA* = common acute lymphoblastic leukemia antigen) – was given this name because it is so common in B-ALL, but not in this particular subtype!)

Myeloid coexpression (typically CD15 positive)

Classic immunophenotypic profile:

$CD45^{dim}$, TdT^{pos}, $CD34^{pos}$, $CD19^{pos}$, $CD22^{neg/dim}$, $CD20^{neg}$, $CD10^{neg}$, cyt IgM^{neg}, $CD15^{dim}$, mostly $CD13^{neg}$, $CD33^{neg}$, $CD9^{pos}$

e-CSI – Infantile ALL with t(4;11)(q21;q23):

Prognosis:

Very poor

Long-term rates of event-free survival (EFS) of 28-45%.

This is much lower than EFS in older children with ALL, which is ~80%.

Relapses occur very early (typically within the first 2 years of diagnosis).

Therapeutic approaches are also controversial, but include intensified chemotherapy and hematopoietic stem cell transplantation.

Reasons for such a poor outcome are not well understood.

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e-CSI – A few words on MLL [11q23]:

- MLL = mixed lineage leukemia gene [11q23]
- Associated with ALL, AML and therapy-related myeloid neoplasms (as its name implies) in both the pediatric and adult populations.
- MLL rearrangements occur in 30-60% of infants with AML.
  - t(9;11)(p22;q23) is the most common translocation in this age group
  - t(11;19)(q23;q13.3) is the next most frequent
- MLL-associated AML is most commonly monoblastic and frequently presents with extramedullary infiltrates.

e-CSI – MLL-associated infant ALL: References

Rearrangement of the MLL gene confers a poor prognosis in childhood acute lymphoblastic leukemia, regardless of presenting age. Blood 1996;87(7):2870-2877 [Behm FG, Raimondi SC, Frestedt JL, et al].

Cytogenetic studies of infant acute lymphoblastic leukemia: poor prognosis of infants with t(4;11) – a report of the Children's Cancer Group. *Leukemia* 1999; 13:679-686 [Heerema NA, Sather HN, Ge J, et al].

Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. *Lancet* 2002;359(9321):1909-1915 [Pui CH, Gaynon PS, Boyett JM, et al]

Antigen expression patterns reflecting genotype of acute leukemias. *Leukemia* 2002;16(7):1233-1258 [Hrusak O and Porwitt-MacDonald A].

A treatment protocol for infants younger than 1 year with acute lymphoblastic leukemia (Interfant-99): an observational study and a multicentre randomised trial. *Lancet* 2007;370(9583):240-250 (Pieters R, Schrappe M, De Lorenzo P, et al).

Improved outcome with hematopoietic stem cell transplantation in a poor prognostic subgroup of infants with mixed-lineage-leukemia (MLL)-rearranged acute lymphoblastic leukemia: results from the Interfant-99 Study. Blood 2010;116(15):2644-2650 [Attarbaschi MG, Schrappe M, De Lorenzo P, et al].