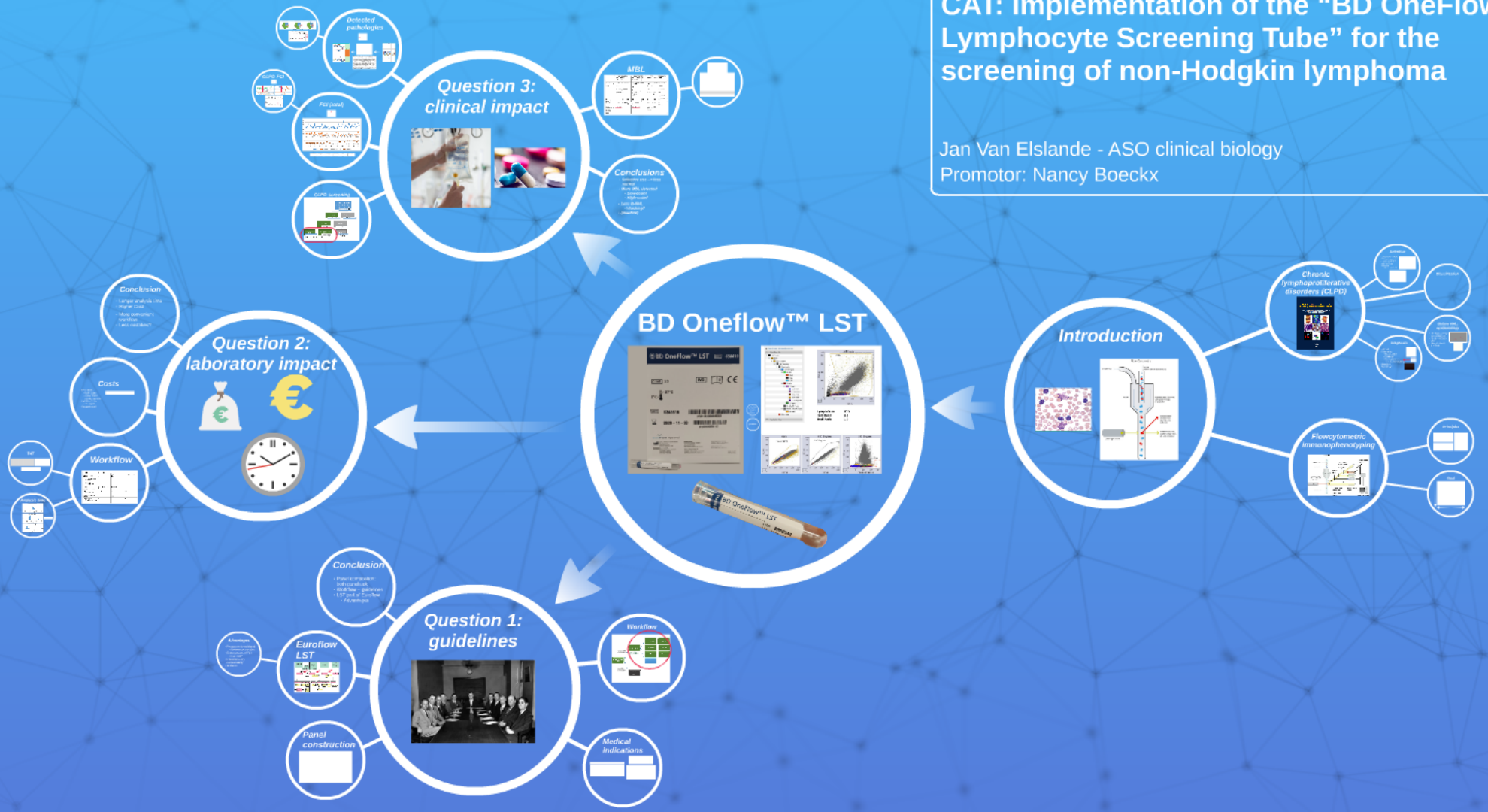
CAT: Implementation of the “BD OneFlow™ Lymphocyte Screening Tube” for the screening of non-Hodgkin lymphoma

Jan Van Elslande - ASO clinical biology
Promotor: Nancy Boeckx



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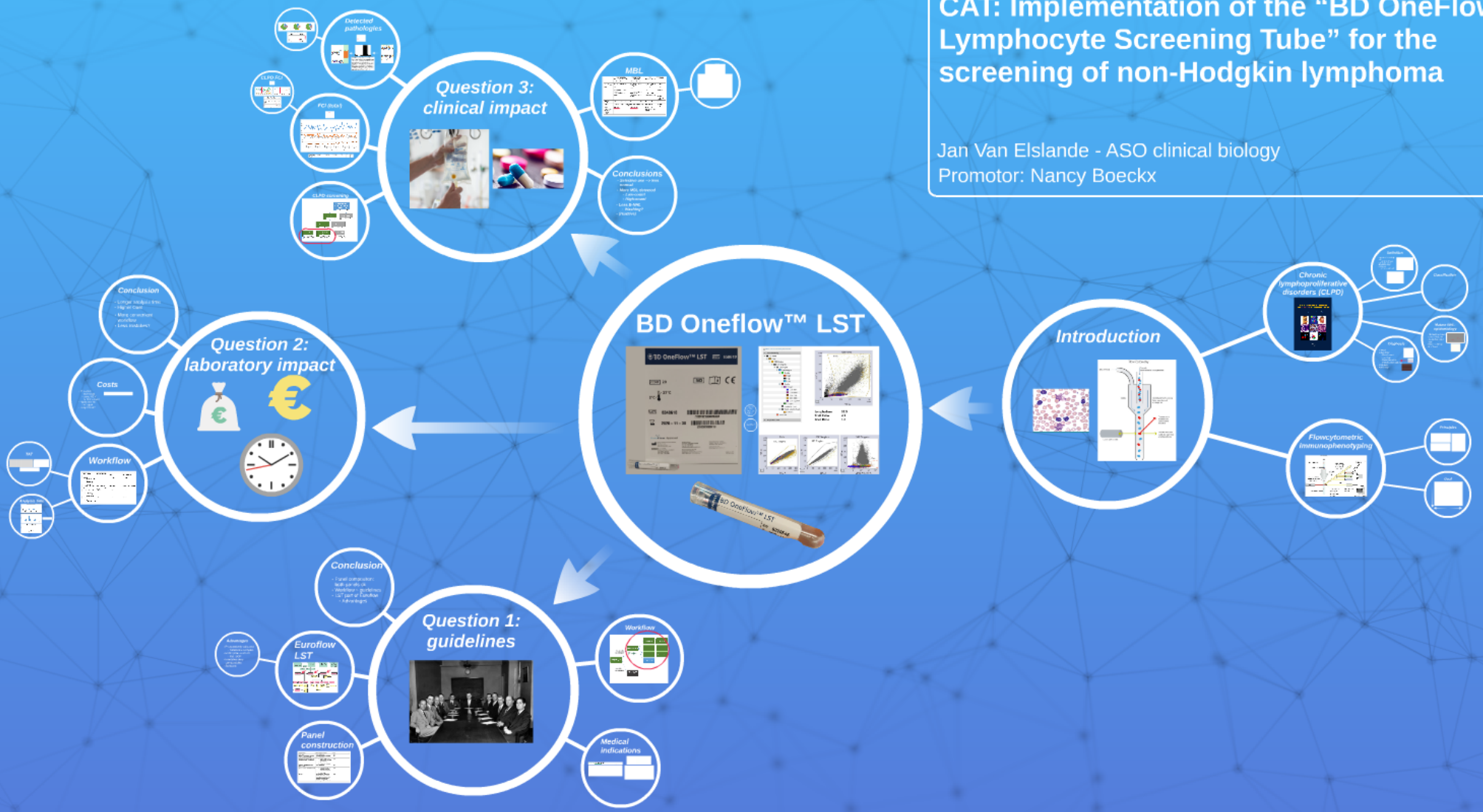

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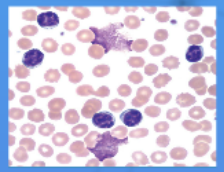
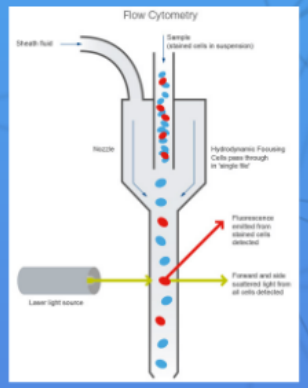
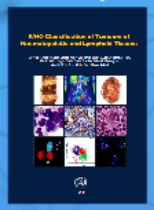
Introduction





Chronic lymphoproliferative disorders (CLPD)



Definition

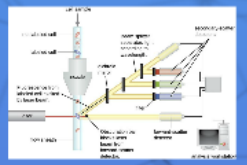
Classification

Mature NHL: epidemiology

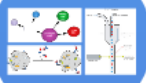
Diagnosis

- Clinical
- Laboratory
  - Blood count
  - Cytology
  - Flowcytometric immunophenotyping
- Histology
- Radiology

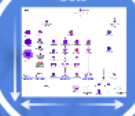
Flowcytometric immunophenotyping



Principles



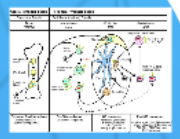
Goal

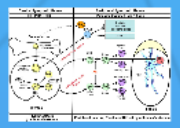


Chronic lymphoproliferative disorders (CLPD)

Definition

- Cancers of hematologic cells
  - Lymphoid lineage
- Clonogenic events
- Maturation stage / sublineage
  - tumour classification





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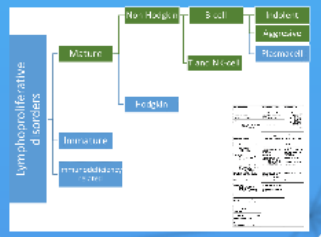
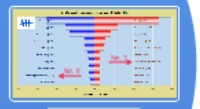


Mature NHL: epidemiology

- 7th most common cancer Belgium
- 20-30 /100.000 / year
- More in elderly
- B > T-cell



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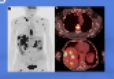

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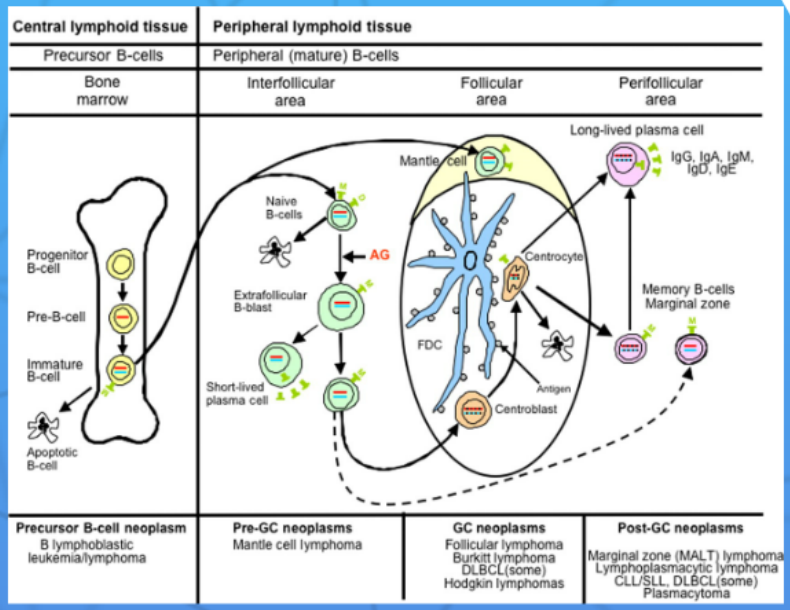
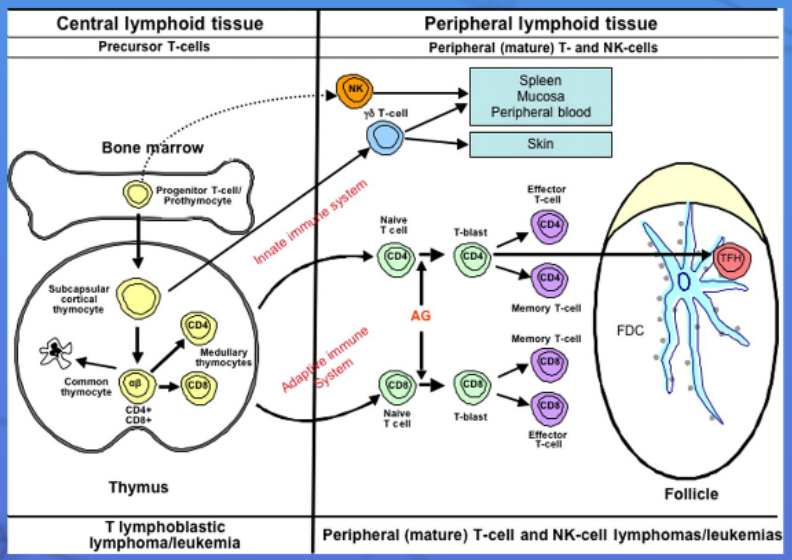
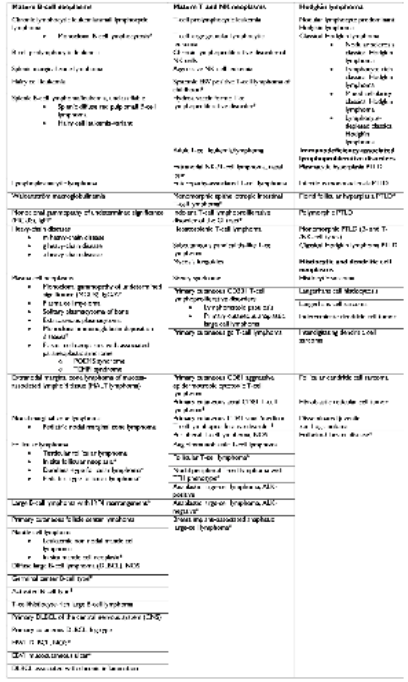




Classification

- Lymphoproliferative disorders
  - Mature
    - Non-Hodgkin
      - B-cell
        - Indolent
        - Aggresive
        - Plasmacell
      - T and NK-cell
    - Hodgkin
  - Immature
  - Immunodeficiency related



Mature B-cell neoplasms	**Mature T and NK neoplasms**	**Hodgkin lymphoma**
Chronic lymphocytic leukemia/small lymphocytic lymphoma • Monoclonal B-cell lymphocytosis*	T-cell prolymphocytic leukemia	Nodular lymphocyte predominant Hodgkin lymphoma
	T-cell large granular lymphocytic leukemia	Classical Hodgkin lymphoma • Nodular sclerosis classical Hodgkin lymphoma • Lymphocyte-rich classical Hodgkin lymphoma • Mixed cellularity classical Hodgkin lymphoma • Lymphocyte-depleted classical Hodgkin lymphoma
B-cell prolymphocytic leukemia	Chronic lymphoproliferative disorder of NK cells	
Splenic marginal zone lymphoma	Aggressive NK-cell leukemia	
Hairy cell leukemia	Systemic EBV positive T-cell lymphoma of childhood*	
Splenic B-cell lymphoma/leukemia, unclassifiable • Splenic diffuse red pulp small B-cell lymphoma • Hairy cell leukemia-variant	Hydroa vacciniforme–like lymphoproliferative disorder*	
	Adult T-cell leukemia/lymphoma	**Immunodeficiency-associated lymphoproliferative disorders**
	Extranodal NK-/T-cell lymphoma, nasal type	Plasmacytic hyperplasia PTLD
Lymphoplasmacytic lymphoma	Enteropathy-associated T-cell lymphoma	Infectious mononucleosis PTLD
Waldenström macroglobulinemia	Monomorphic epitheliotropic intestinal T-cell lymphoma*	Florid follicular hyperplasia PTLD*
Monoclonal gammopathy of undetermined significance (MGUS), IgM*	Indolent T-cell lymphoproliferative disorder of the GI tract*	Polymorphic PTLD
Heavy-chain diseases • m heavy-chain disease • g heavy-chain disease • a heavy-chain disease	Hepatosplenic T-cell lymphoma	Monomorphic PTLD (B- and T-/NK-cell types)
	Subcutaneous panniculitis-like T-cell lymphoma	Classical Hodgkin lymphoma PTLD
	Mycosis fungoides	**Histiocytic and dendritic cell neoplasms**
Plasma cell neoplasms • Monoclonal gammopathy of undetermined significance (MGUS), IgG/A* • Plasma cell myeloma • Solitary plasmacytoma of bone • Extraosseous plasmacytoma	Sézary syndrome	Histiocytic sarcoma
	Primary cutaneous CD30+ T-cell lymphoproliferative disorders • Lymphomatoid papulosis • Primary cutaneous anaplastic large cell lymphoma	Langerhans cell histiocytosis
		Langerhans cell sarcoma
		Indeterminate dendritic cell tumor

• Solitary plasmacytoma of bone
• Extraosseous plasmacytoma
• Monoclonal immunoglobulin deposition diseases*
• Plasma cell neoplasms with associated paraneoplastic syndrome
  ○ POEMS syndrome
  ○ TEMPI syndrome
	• Primary cutaneous anaplastic large cell lymphoma	Indeterminate dendritic cell tumor

	Primary cutaneous gd T-cell lymphoma	Interdigitating dendritic cell sarcoma
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)	Primary cutaneous CD8+ aggressive epidermotropic cytotoxic T-cell lymphoma	Follicular dendritic cell sarcoma
	Primary cutaneous acral CD8+ T-cell lymphoma*	Fibroblastic reticular cell tumor
Nodal marginal zone lymphoma • Pediatric nodal marginal zone lymphoma	Primary cutaneous CD4+ small/medium T-cell lymphoproliferative disorder*	Disseminated juvenile xanthogranuloma
	Peripheral T-cell lymphoma, NOS	Erdheim-Chester disease*
Follicular lymphoma • Testicular follicular lymphoma • In situ follicular neoplasia* • Duodenal-type follicular lymphoma* • Pediatric-type follicular lymphoma*	Angioimmunoblastic T-cell lymphoma	
	Follicular T-cell lymphoma*	
	Nodal peripheral T-cell lymphoma with TFH phenotype*	
	Anaplastic large-cell lymphoma, ALK+	
Large B-cell lymphoma with IRF4 rearrangement*	Anaplastic large-cell lymphoma, ALK-	
Primary cutaneous follicle center lymphoma	Breast implant–associated anaplastic large-cell lymphoma*	
Mantle cell lymphoma • Leukaemic non-nodal mantle cell lymphoma • In situ mantle cell neoplasia*		
Diffuse large B-cell lymphoma (DLBCL), NOS		
Germinal center B-cell type*		
Activated B-cell type*		
T-cell/histiocyte-rich large B-cell lymphoma		
Primary DLBCL of the central nervous system (CNS)		
Primary cutaneous DLBCL, leg type		
EBV+ DLBCL, NOS*		
EBV+ mucocutaneous ulcer*		
DLBCL associated with chronic inflammation		

Classification

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  - Mature
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      - B-cell
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        - Plasmacell
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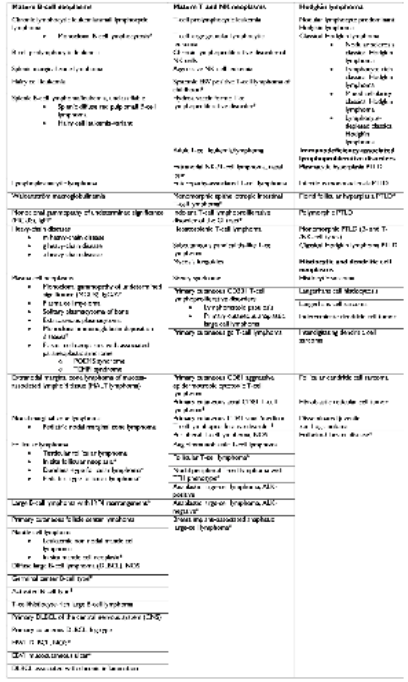

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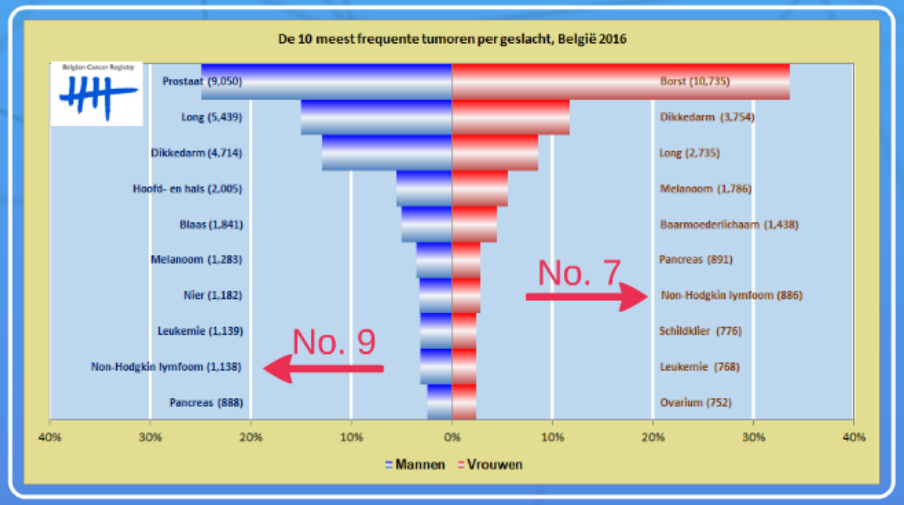

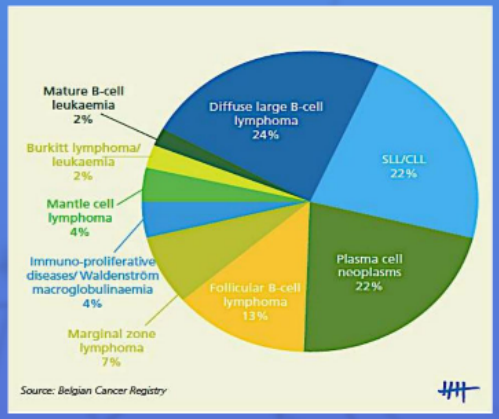

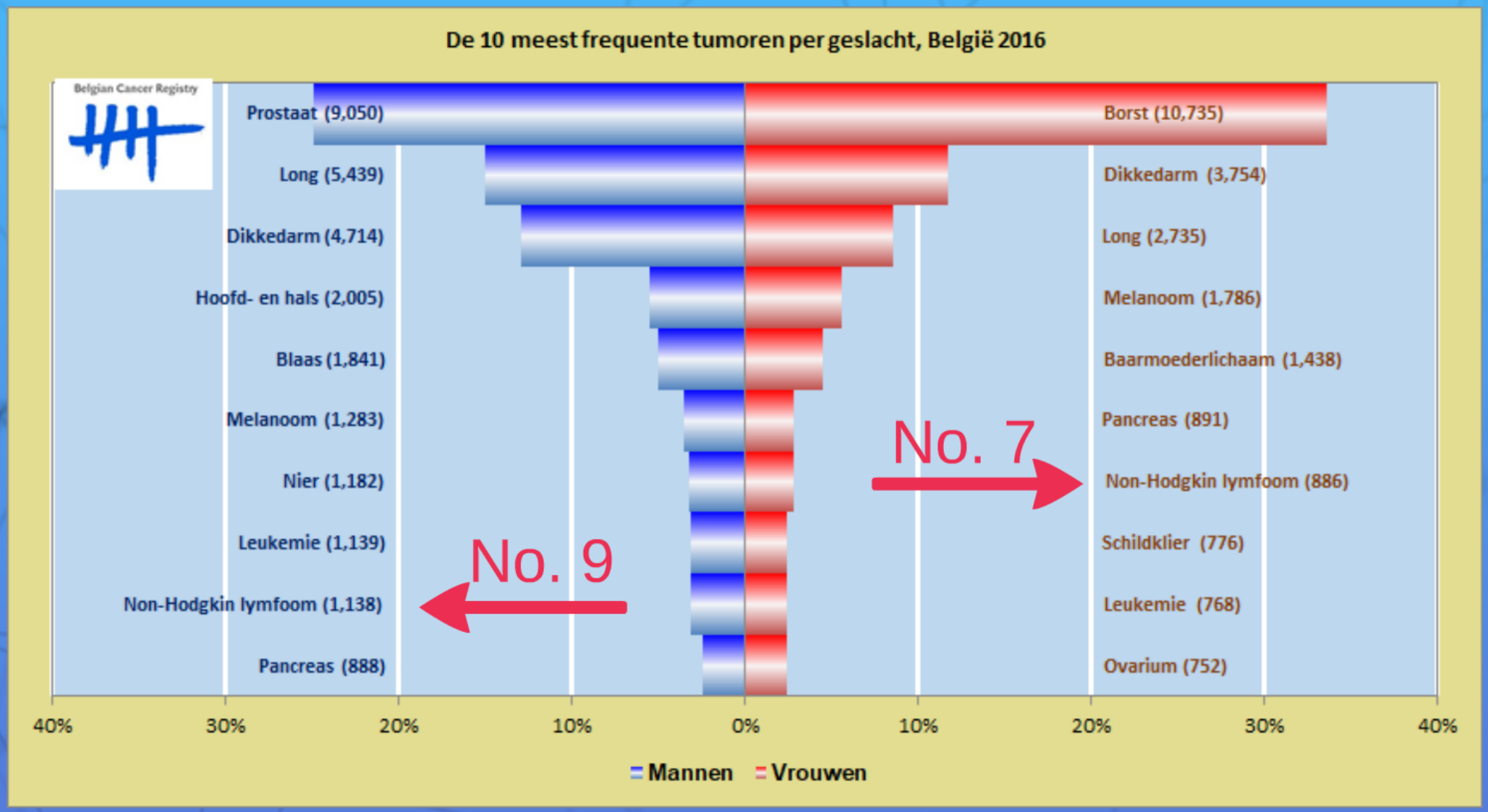
De 10 meest frequente tumoren per geslacht, België 2016
Belgian Cancer Registry
Prostaat (9,050)
Long (5,439)
Dikkedarm (4,714)
Hoofd- en hals (2,005)
Blaas (1,841)
Melanoom (1,283)
Nier (1,182)
Leukemie (1,139)
Non-Hodgkin lymfoom (1,138)
Pancreas (888)
Borst (10,735)
Dikkedarm (3,754)
Long (2,735)
Melanoom (1,786)
Baarmoederlichaam (1,438)
Pancreas (891)
Non-Hodgkin lymfoom (886)
Schildklier (776)
Leukemie (768)
Ovarium (752)
No. 7
No. 9
40%
30%
20%
10%
0%
10%
20%
30%
40%
Mannen
Vrouwen

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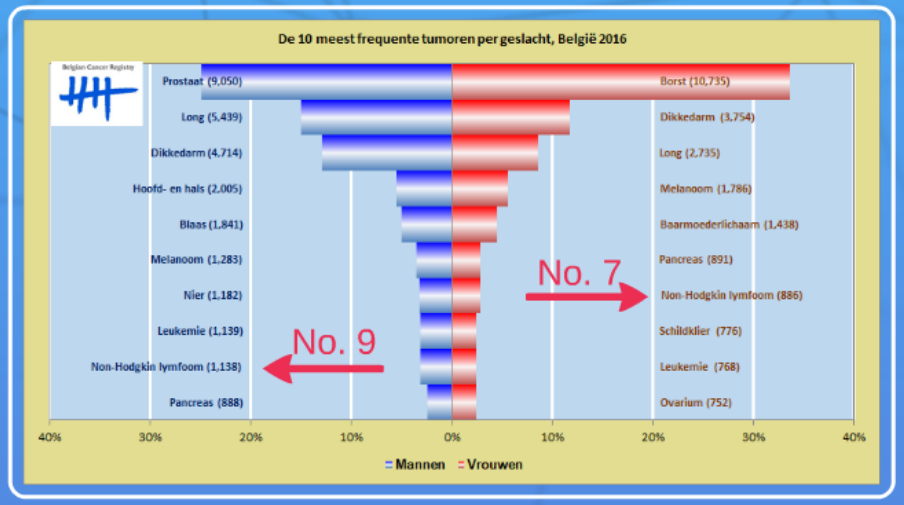

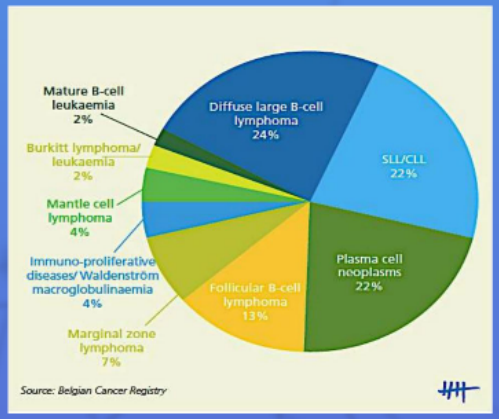

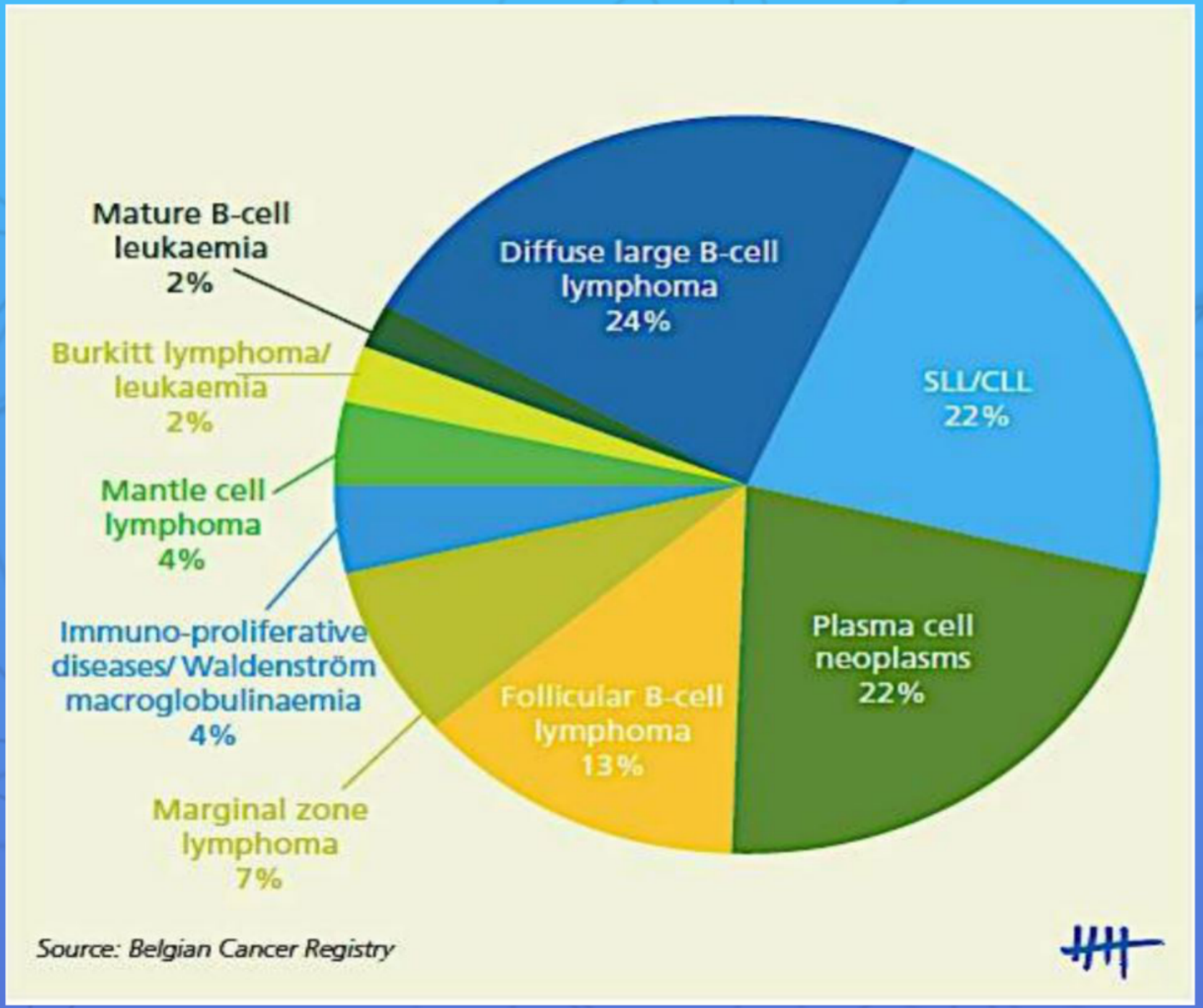
Mature B-cell leukaemia 2%
Diffuse large B-cell lymphoma 24%
Burkitt lymphoma/ leukaemia 2%
SLL/CLL 22%
Mantle cell lymphoma 4%
Immuno-proliferative diseases/ Waldenström macroglobulinaemia 4%
Plasma cell neoplasms 22%
Follicular B-cell lymphoma 13%
Marginal zone lymphoma 7%
Source: Belgian Cancer Registry

Diagnosis

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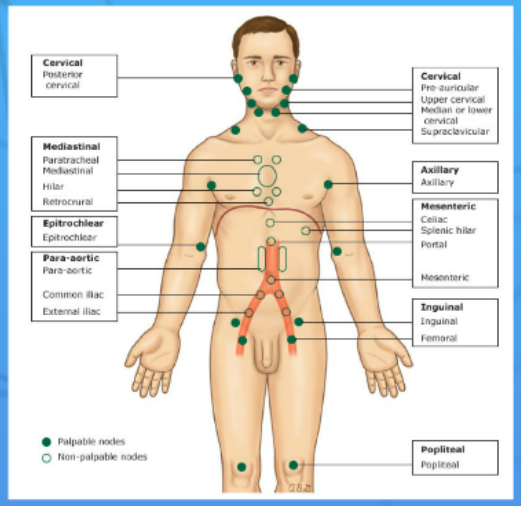

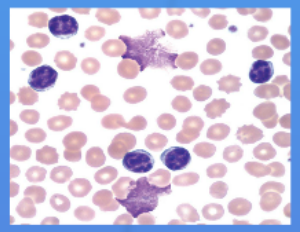

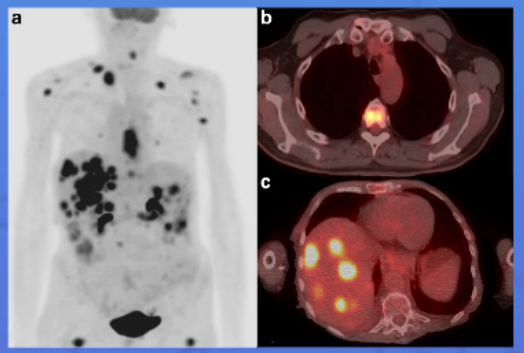

Chronic lymphoproliferative disorders (CLPD)

Definition

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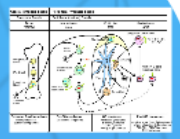

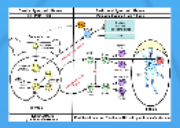

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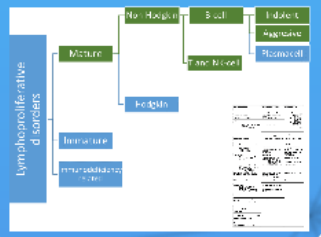

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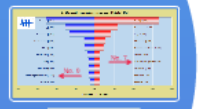

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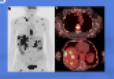

Promotor: Nancy Boeckx

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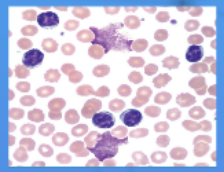

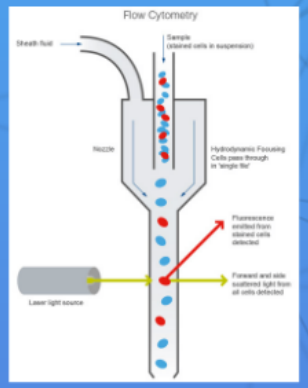

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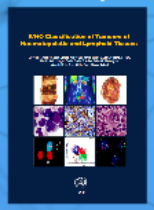

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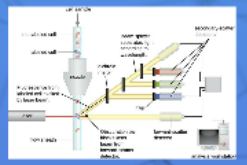
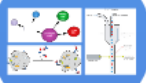
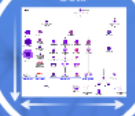


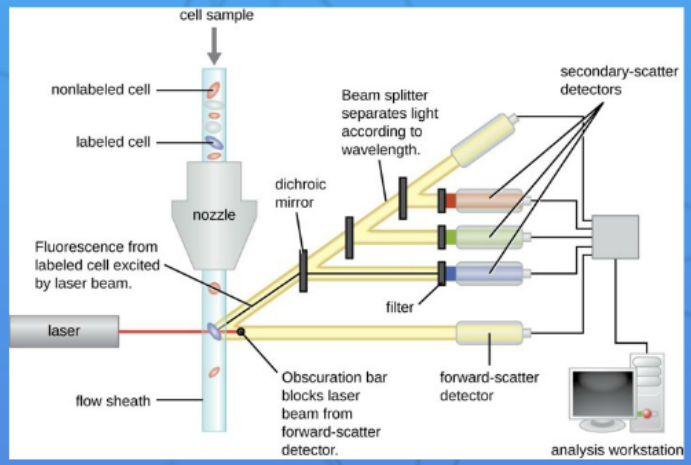
Principles



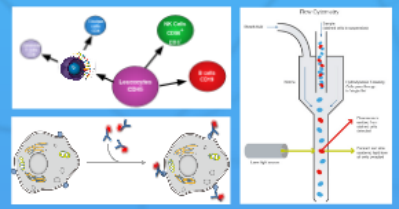
Goal



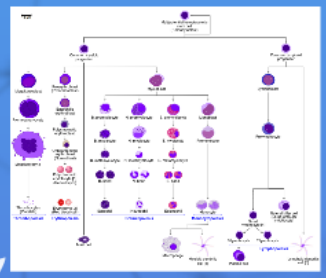
Flowcytometric immunophenotyping



Principles



Goal



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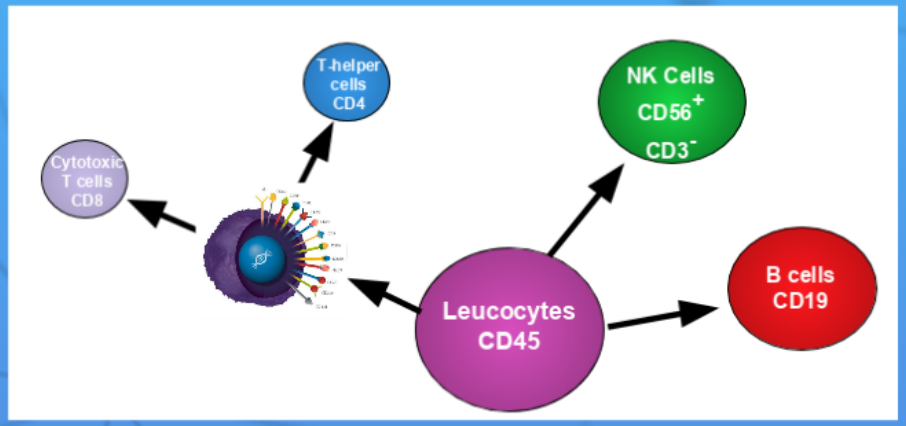
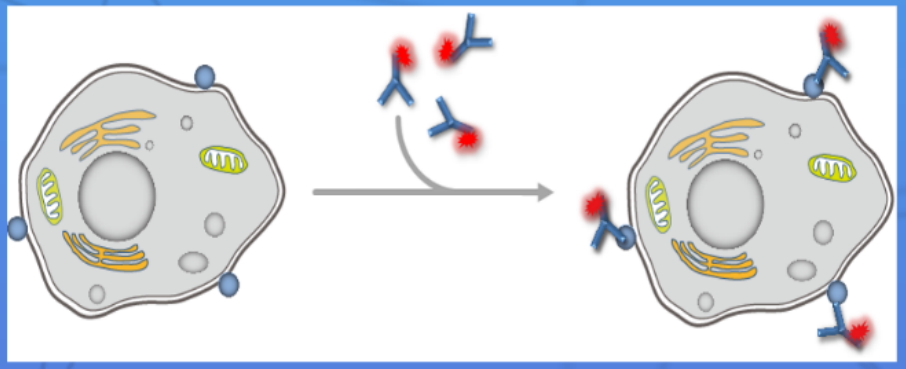
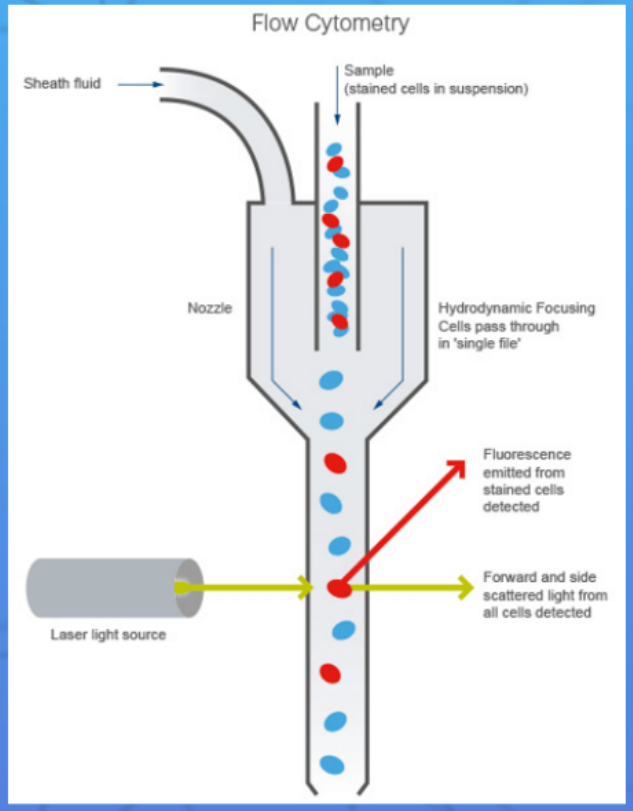
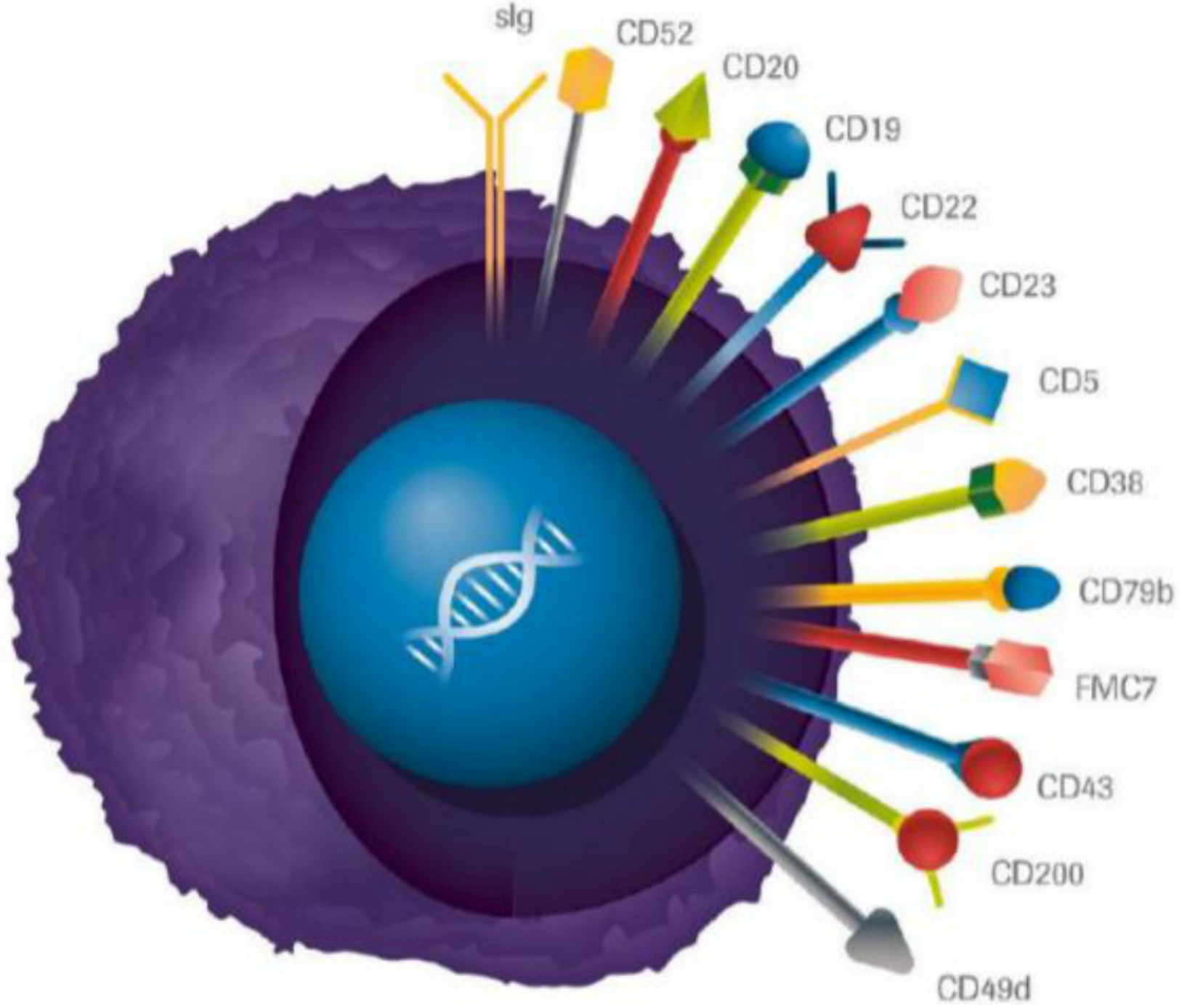
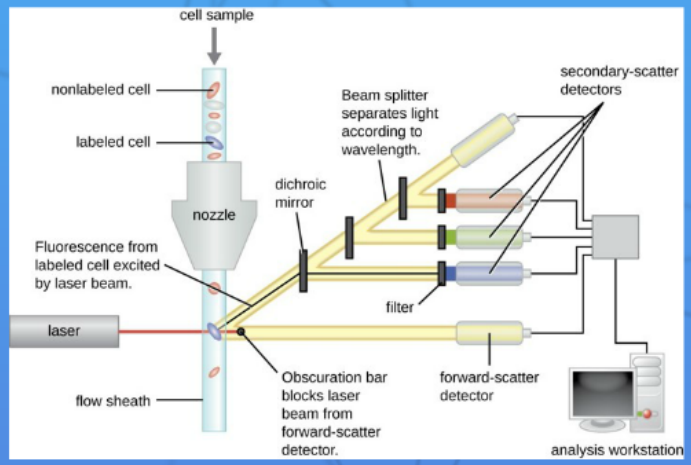

T-helper cells CD4
Cytotoxic T cells CD8
NK Cells CD56+ CD3-
B cells CD19
Leucocytes CD45



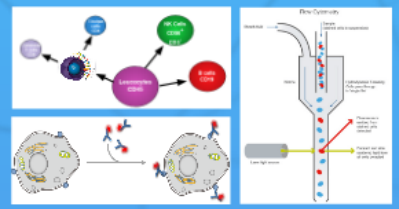

Flow Cytometry
Sheath fluid
Sample (stained cells in suspension)
Nozzle
Hydrodynamic Focusing Cells pass through in 'single file'
Fluorescence emitted from stained cells detected
Forward and side scattered light from all cells detected
Laser light source


slg
CD52
CD20
CD19
CD22
CD23
CD5
CD38
CD79b
FMC7
CD43
CD200
CD49d

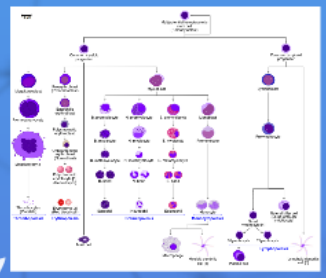
Flowcytometric immunophenotyping

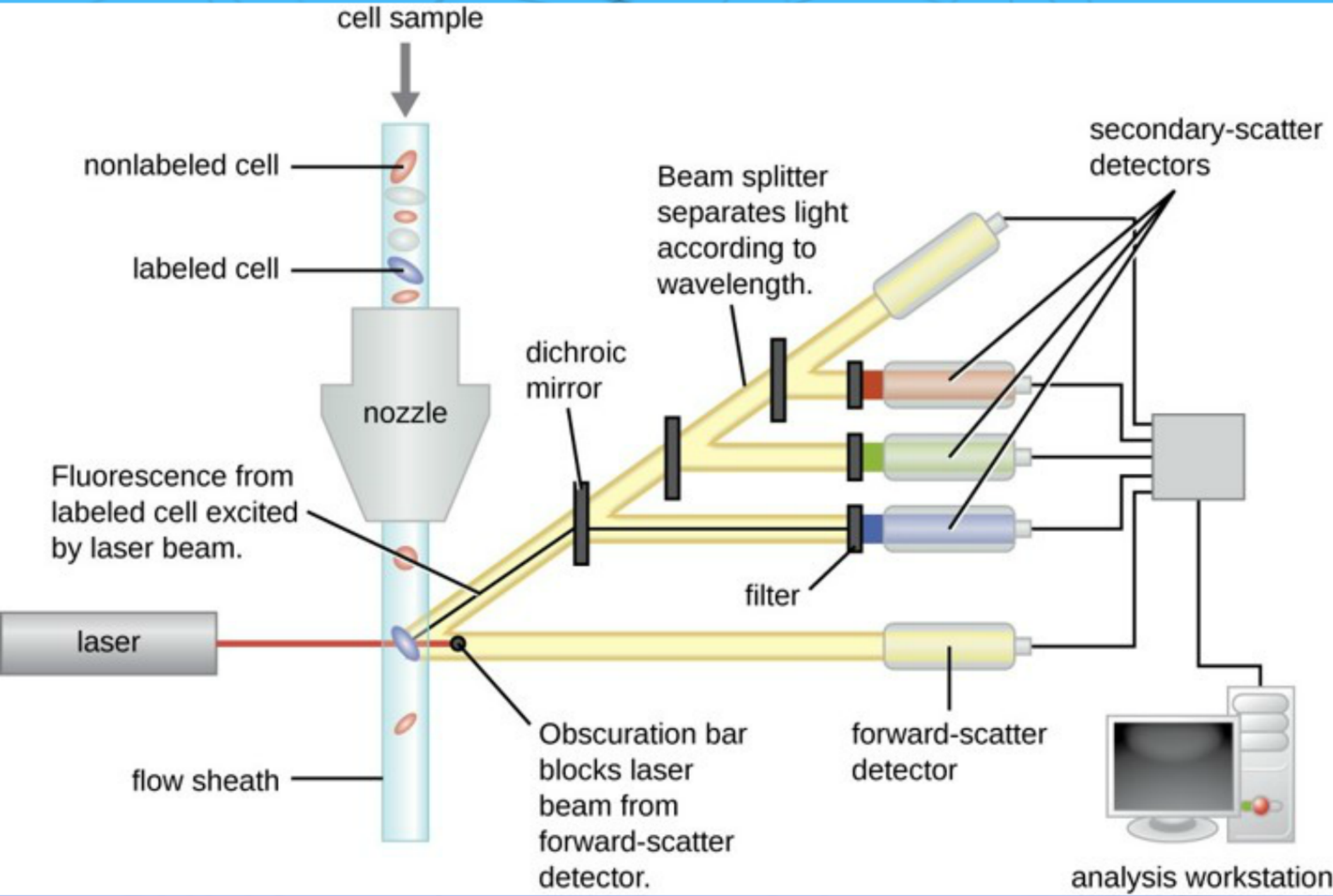


Principles

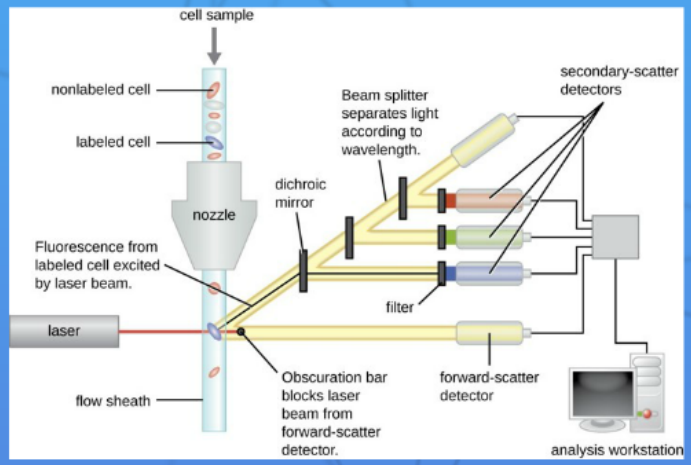


Goal

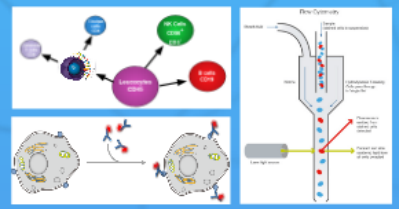



cell sample
nonlabeled cell
labeled cell
nozzle
Fluorescence from labeled cell excited by laser beam.
laser
flow sheath
Obscuration bar blocks laser beam from forward-scatter detector.
dichroic mirror
Beam splitter separates light according to wavelength.
filter
secondary-scatter detectors
forward-scatter detector
analysis workstation

Flowcytometric immunophenotyping



Principles



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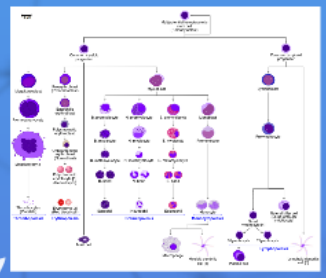

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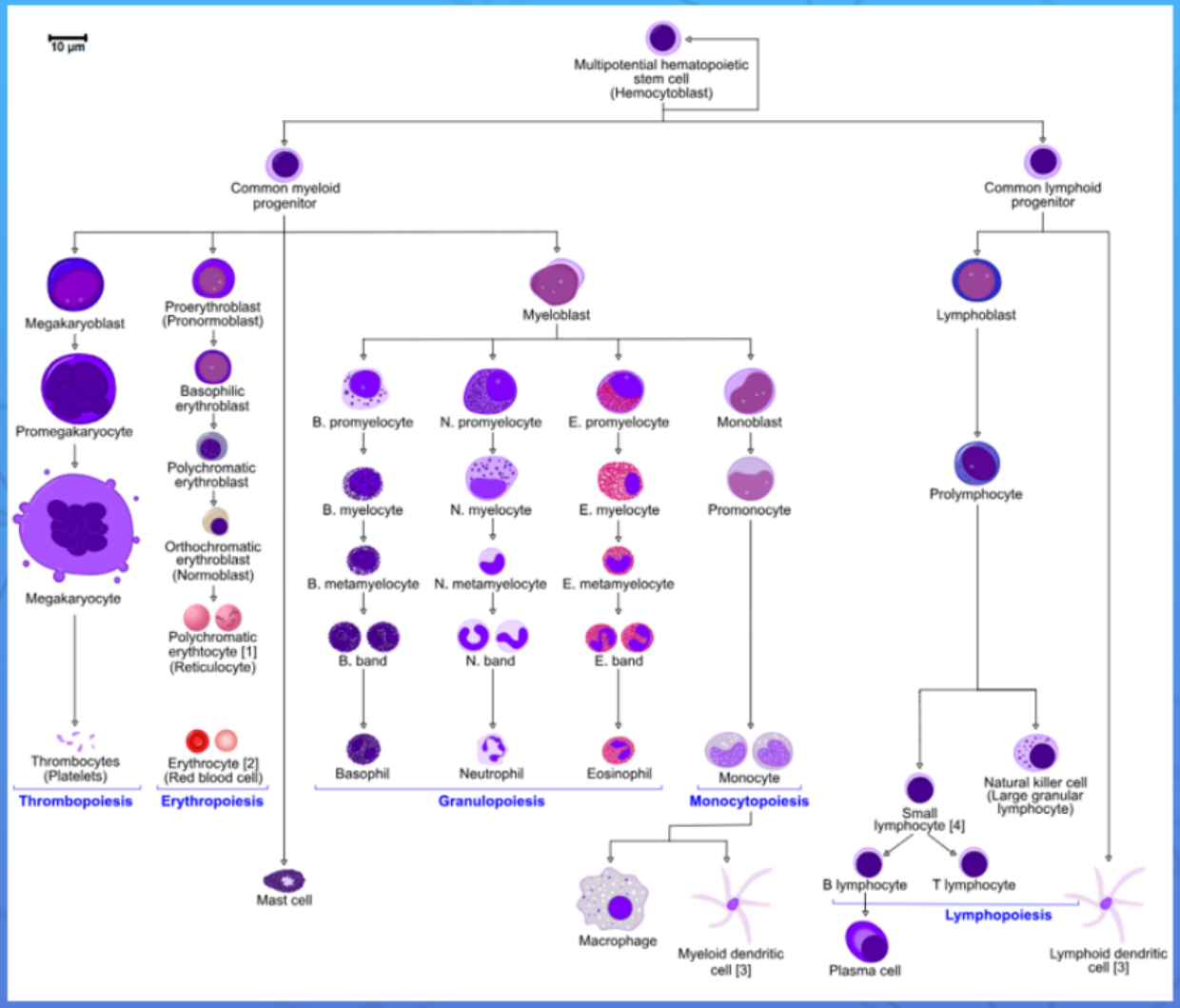
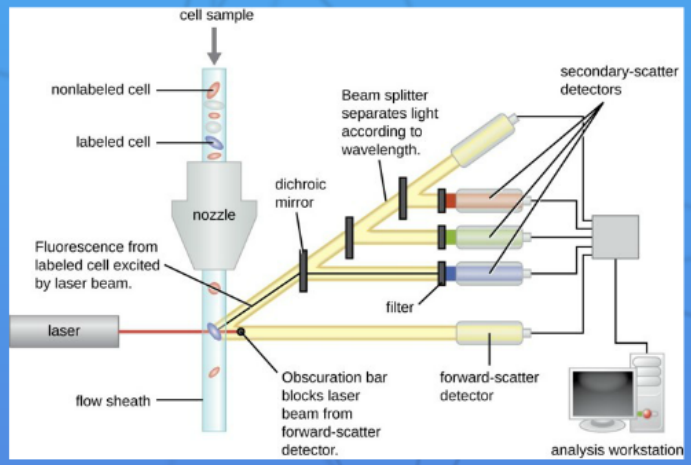
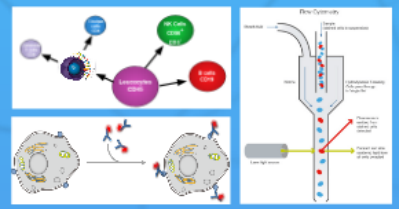

10 µm
Multipotential hematopoietic stem cell (Hemocytoblast)
Common myeloid progenitor
Common lymphoid progenitor
Megakaryoblast
Promegakaryocyte
Megakaryocyte
Thrombocytes (Platelets)
Thrombopoiesis
Proerythroblast (Pronormoblast)
Basophilic erythroblast
Polychromatic erythroblast
Orthochromatic erythroblast (Normoblast)
Polychromatic erythtocyte [1] (Reticulocyte)
Erythrocyte [2] (Red blood cell)
Erythropoiesis
Mast cell
Myeloblast
B. promyelocyte
B. myelocyte
B. metamyelocyte
B. band
Basophil
N. promyelocyte
N. myelocyte
N. metamyelocyte
N. band
Neutrophil
E. promyelocyte
E. myelocyte
E. metamyelocyte
E. band
Eosinophil
Granulopoiesis
Monoblast
Promonocyte
Monocyte
Monocytopoiesis
Macrophage
Myeloid dendritic cell [3]
Lymphoblast
Prolymphocyte
Small lymphocyte [4]
B lymphocyte
T lymphocyte
Lymphopoiesis
Plasma cell
Natural killer cell (Large granular lymphocyte)
Lymphoid dendritic cell [3]

Flowcytometric immunophenotyping



Principles



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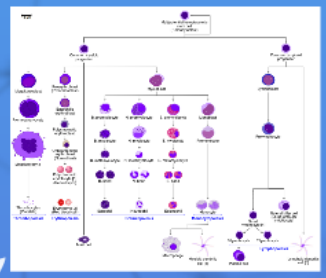
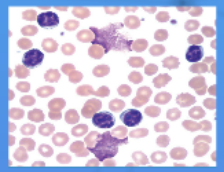
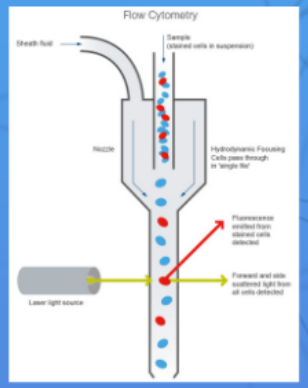


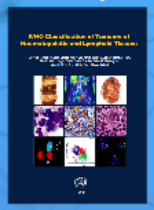
Promotor: Nancy Boeckx

Introduction





Chronic lymphoproliferative disorders (CLPD)



Definition

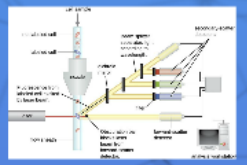
Classification

Mature NHL: epidemiology

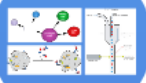
Diagnosis

- Clinical
- Laboratory
  - Blood count
  - Cytology
  - Flowcytometric immunophenotyping
- Histology
- Radiology

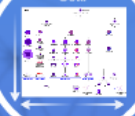
Flowcytometric immunophenotyping



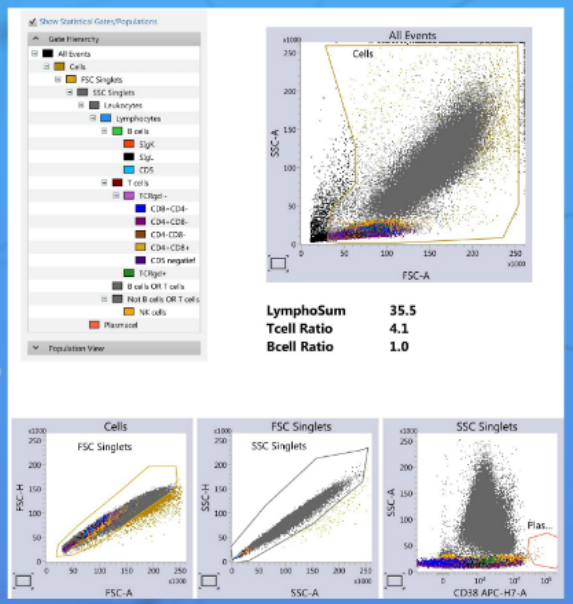
Principles

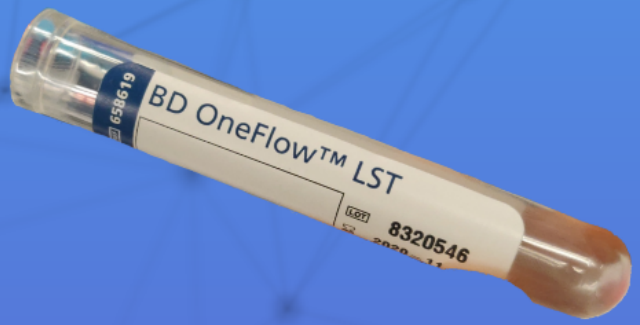


Goal



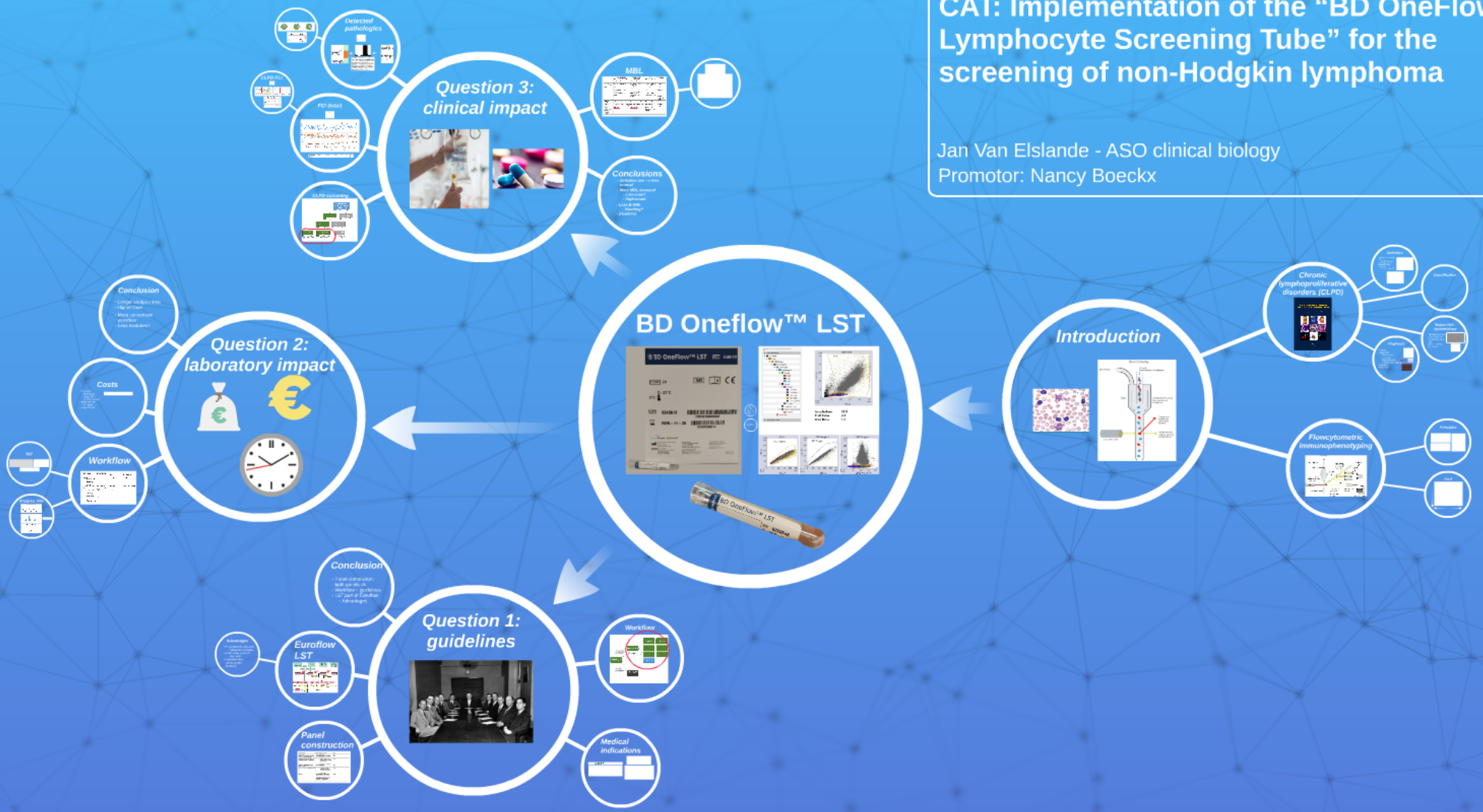
BD Oneflow™ LST





CAT: Implementation of the “BD OneFlow™ Lymphocyte Screening Tube” for the screening of non-Hodgkin lymphoma

Jan Van Elslande - ASO clinical biology
Promotor: Nancy Boeckx



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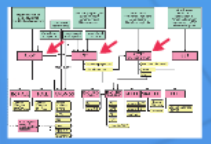
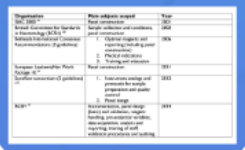
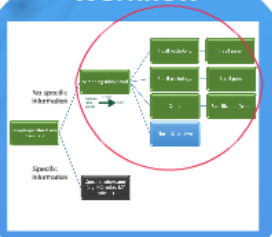
Euroflow LST



Panel construction

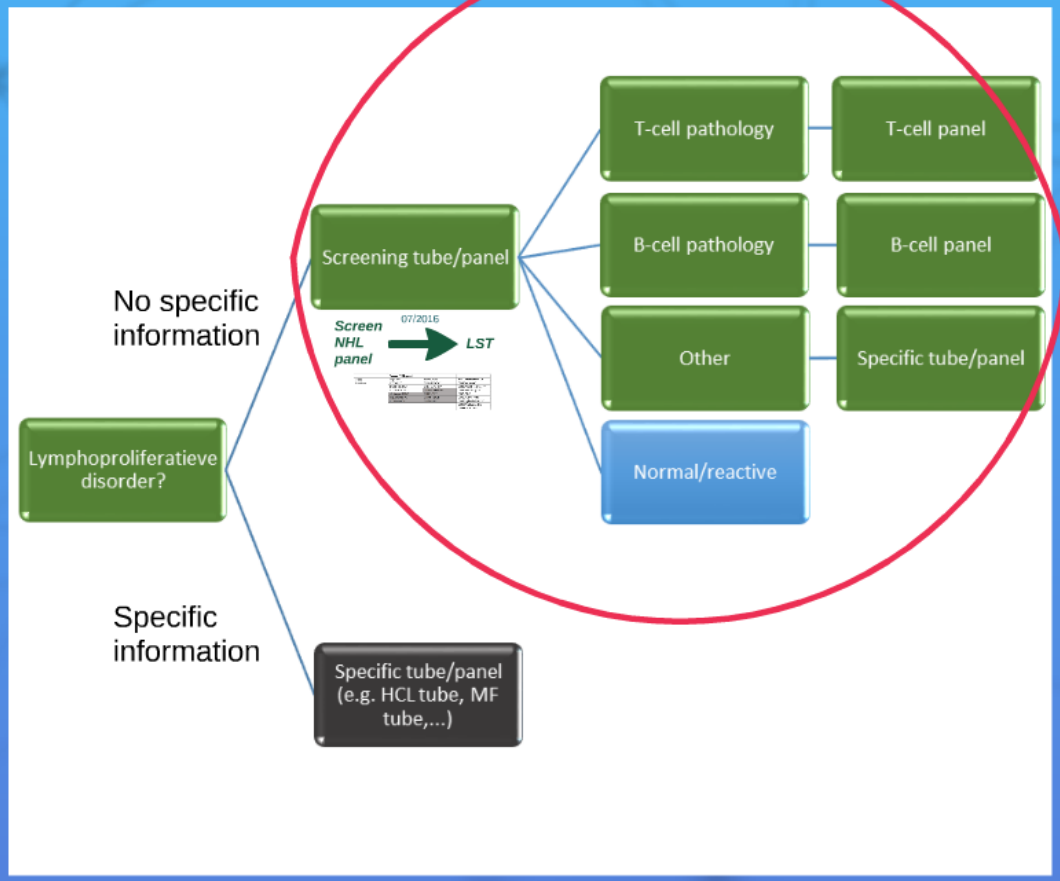


Workflow



Medical indications

Workflow


Lymphoproliferatieve disorder?
No specific information
Specific information
Screening tube/panel
Screen NHL panel
07/2016
LST
Specific tube/panel (e.g. HCL tube, MF tube,...)
T-cell pathology
T-cell panel
B-cell pathology
B-cell panel
Other
Specific tube/panel
Normal/reactive

07/2016

Screen NHL panel → *LST*

	Screen NHL panel		
Tube	sIgB tube	TBNK tube	**BD OneFlow™ LST**
Markers	CD5-APC	CD4–PE-CY7	CD45-V500-C
	CD10-PE-CY7	CD8–APC-H7	CD19/TCR-γδ-PE-cy7
	CD20-APC-H7	CD16/CD56-PE	CD5-PerCP-Cy5.5
	sIgKappa-FITC	CD19-APC	CD3-APC
	sIgLambda-PE	CD45-PerCP	CD20/CD4-V450
	CD19-PerCP	CD3-FITC	CD8/sIglambda-FITC
			CD56/sIgkappa-PE
			CD38–APC-H7

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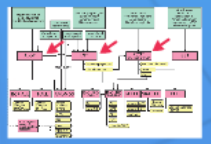
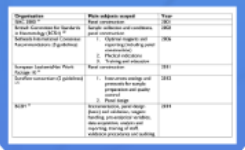


Panel construction



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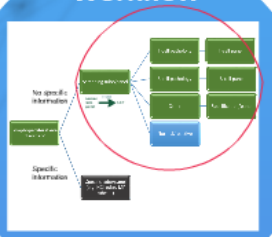

Medical indications

Medical indications

Urgent indications	Less urgent indications
Blasts in the peripheral blood (precursor lymphoid neoplasm?)	ALC >5.000/µL, unless recent viral infection, asplenia, medication that can explain this finding
Absolute lymphocyte count (ALC) >30.000/µL, without a known diagnosis	Unexplained ALC >4.000/µL for >1 month
	Rising ALC
	Atypical lymphocytes on peripheral blood smear suggestive for malignancy
	Cytopenias
	Hepatosplenomegaly
	Lymphadenopathy

2006 Bethesda International Consensus recommendations on the flow cytometric immunophenotypic analysis of hematolymphoid neoplasia: Medical indications†

B. H. Davis✉, J.T. Holden, M.C. Bene, M.J. Borowitz, R.C. Braylan, D. Cornfield, W. Gorczyca, R. Lee, R. Mallese, A. Orfao, D. Wells, B.L. Wood, M. Stetler-Stevenson

First published: 05 September 2007 | https://doi.org/10.1002/cyto.b.20365 | Cited by: 53

Indications	No indications
Lymphadenopathy, organomegaly (especially hepatosplenomegaly), tissue infiltrates (especially skin, mucosa and bone)	Isolated anemia
Bi or pancytopenia without clear explanation	Isolated thrombocytosis, neutrophilia or basophilia
Unexplained lymphocytosis, monocytosis, eosinophilia	Polyclonal hypergammaglobulinemia
Atypical cells / blasts observed by morphology (absolute indication!)	
Paraprotein and/or plasmacytosis in blood and/or bone marrow	
Monitoring • Staging • Prognostication • MRD • Follow-up (progression? Relapse? Disease acceleration?)	

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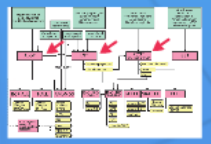

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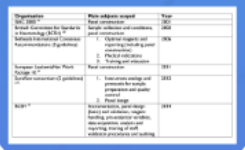
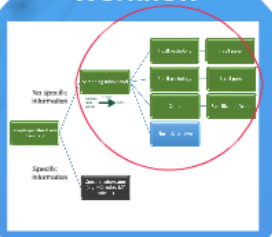


Workflow



Medical indications

Panel construction

Organisation	Main subjects scoped	Year
ISAC 2000 [19]	Panel construction	2001
Britisch Committee for Standards in Haematology (BCSH) [20]	Sample collection and conditions, panel construction	2002
Bethesda International Consensus Recommendations (3 guidelines)	1. Optimal reagents and reporting (including panel construction) 2. Medical indications 3. Training and education	2006
European LeukemiaNet Work Package 10 [10]	Panel construction	2011
Euroflow consortium (2 guidelines) [2,12]	1. Instrument settings and protocols for sample preparation and quality control 2. Panel design	2012
BCSH [21]	Instrumentation, panel design (basic) and validation, reagent handling, pre-analytical variables, data acquisition, analysis and reporting, training of staff, validation procedures and auditing.	2014

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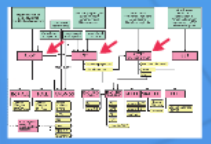

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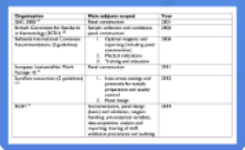
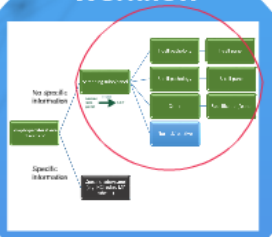


Workflow



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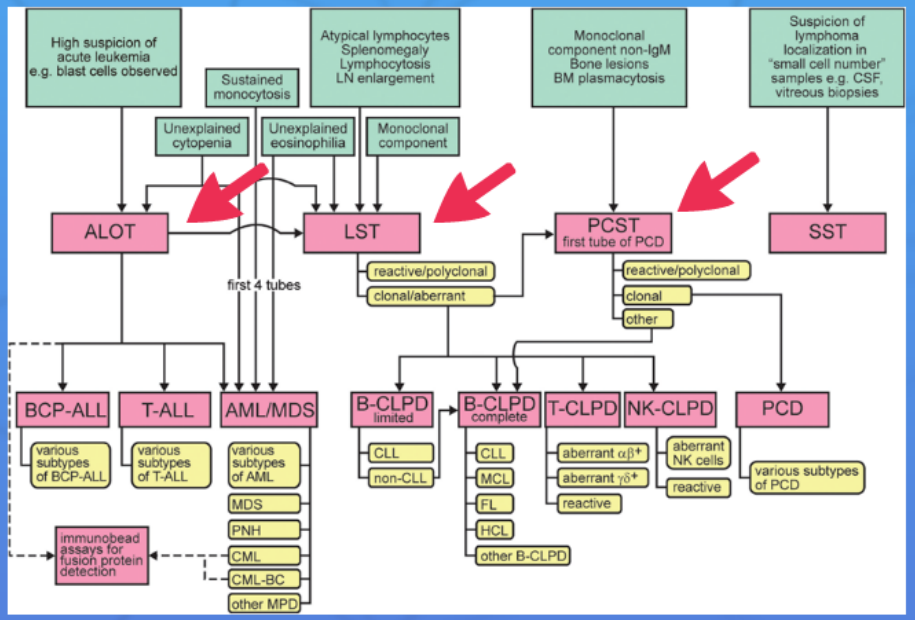

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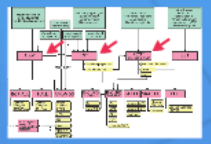
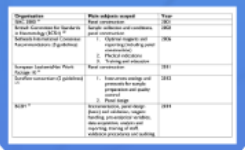
- Software

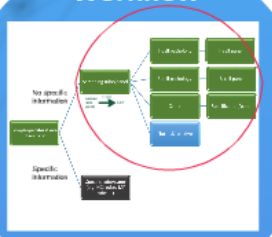
Euroflow LST



Panel construction



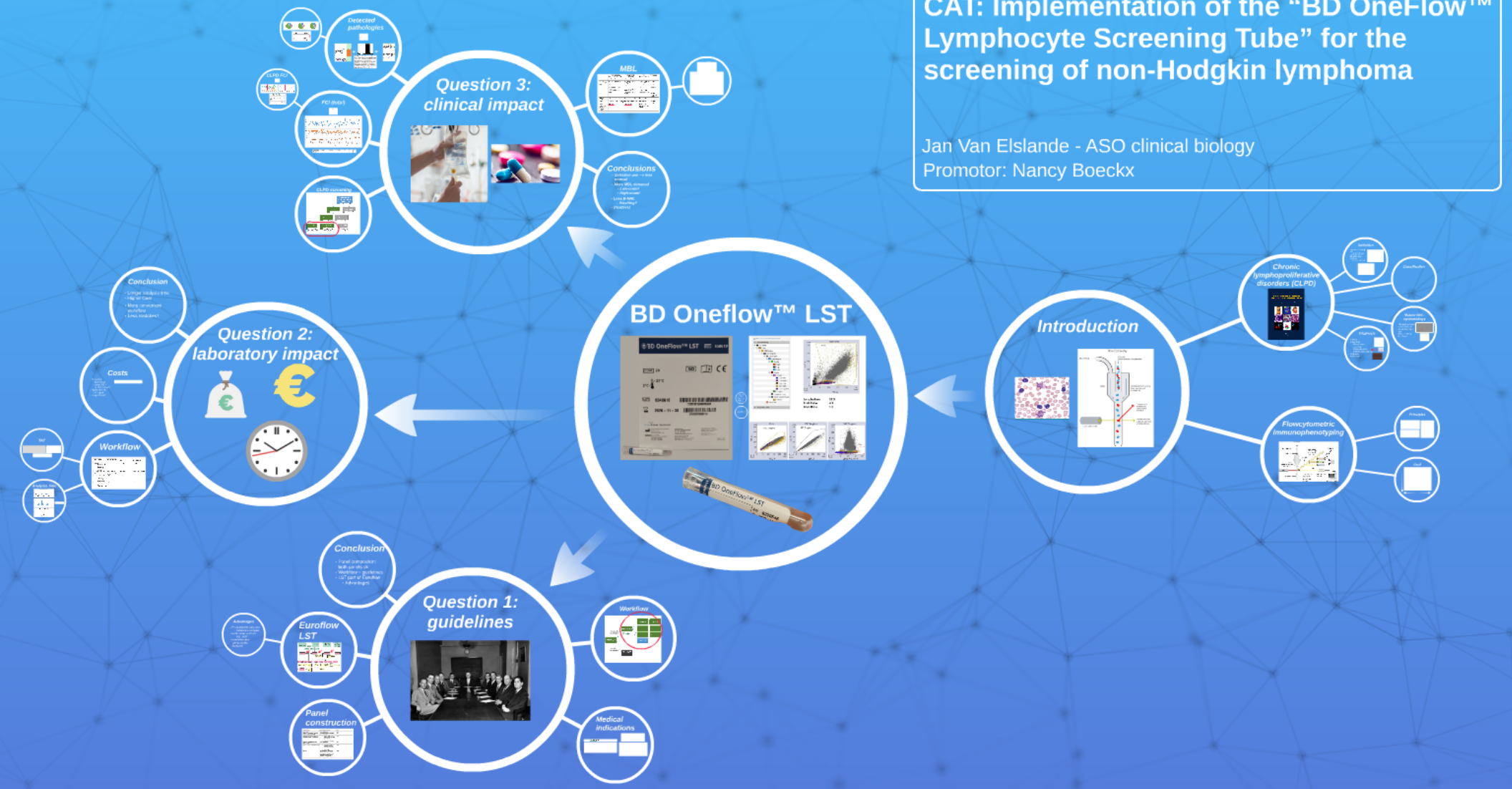
Workflow



Medical indications

CAT: Implementation of the “BD OneFlow™ Lymphocyte Screening Tube” for the screening of non-Hodgkin lymphoma

Jan Van Elslande - ASO clinical biology
Promotor: Nancy Boeckx



Question 2: laboratory impact

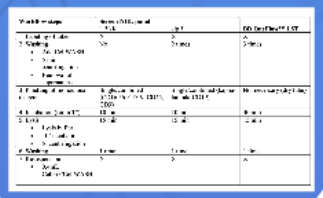
Conclusion

- Longer analysis time
- Higher Cost
- More convenient workflow
- Less mistakes?

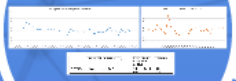
Costs

- 12 markers
  - Both tubes
  - same RIZIV reimbursement
- Hands-on time
  - +/- equal
- Reagent cost?

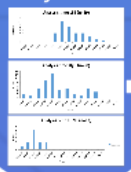
Workflow



TAT



Analysis time



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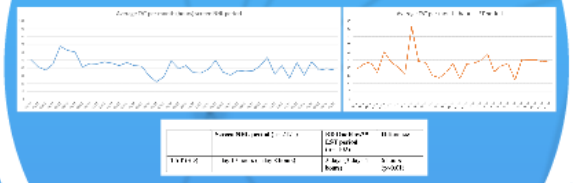
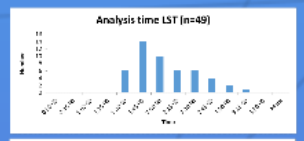
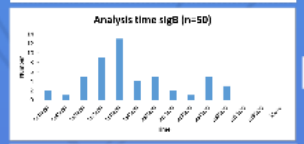
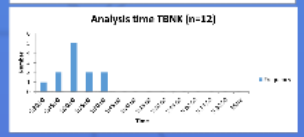


Analysis time

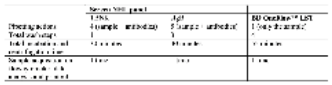






Workflow

Workflow steps	Screen NHL panel		
	TBNK	sIgB	**BD OneFlow™ LST**
1. Labeling of tubes	X	X	X
2. Washing • Add CellWASH • 5 min centrifugation • Removal of supernatans	No	2 times	3 times
3. Pipetting of monoclonal reagents	Single/combined (CD16.56, CD19, CD45, CD3)	Single/combined (kappa-lambda-CD19)	Not necessary (dry tube)
4. Incubation (room T°)	10 min	10 min	30 min
5. Lysis • Lysis buffer • 10' incubation • 5' centrifugation	15 min	15 min	15 min
6. Washing	1 time	1 time	1 time
7. Resuspension • 0,4mL CellFix/CellWASH	X	X	X

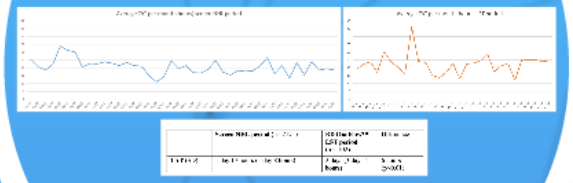
Workflow steps	**Screen NHL panel**		
	TBNK	sIgB	**BD OneFlow™ LST**
1. Labeling of tubes	X	X	X
2. Washing • Add CellWASH • 5 min centrifugation • Removal of supernatans	No	2 times 	3 times
3. Pipetting of monoclonal reagents	Single/combined (CD16.56, CD19, CD45, CD3)	Single/combined (kappa-lambda-CD19)	Not necessary (dry tube)
4. Incubation (room T°)	10 min	10 min	30 min
5. Lysis • Lysis buffer • 10’ incubation • 5’ centrifugation	15 min	15 min	15 min
6. Washing	1 time	1 time	1 time
7. Resuspension • 0,4mL CellFix/CellWASH	X	X	X

2 times

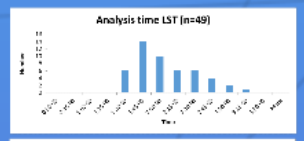
	Screen NHL panel		
	TBNK	sIgB	**BD OneFlow™ LST**
Pipetting actions	4 (sample + antibodies)	5 (sample + antibodies)	1 (only the sample)
Total wash steps	1	3	4
Total incubation and centrifugation time	30 minutes	40 minutes	55 minutes
Sample acquisition on flowcytometer, data analysis and printout	1 time	1 time	1 time

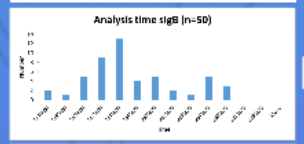
Single/combined (kappa

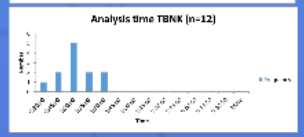
TAT



Analysis time







Workflow

Workflow steps	Screen NHL panel		
	TBNK	sIgB	**BD OneFlow™ LST**
1. Labeling of tubes	X	X	X
2. Washing • Add CellWASH • 5 min centrifugation • Removal of supernatans	No	2 times	3 times
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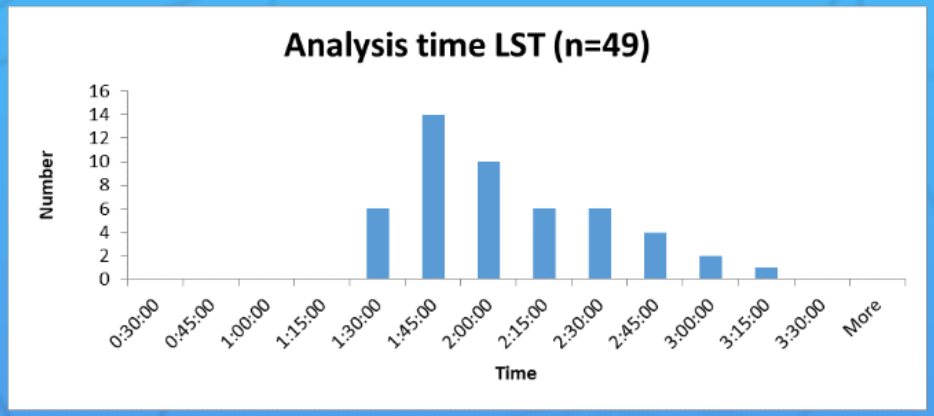
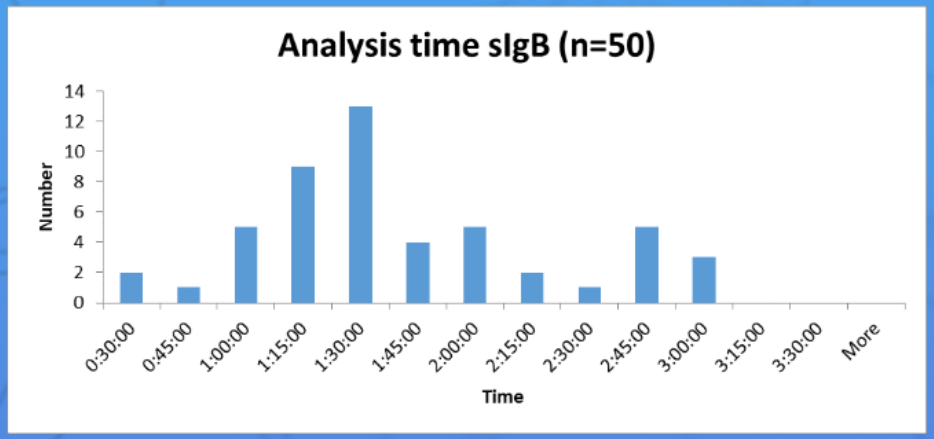
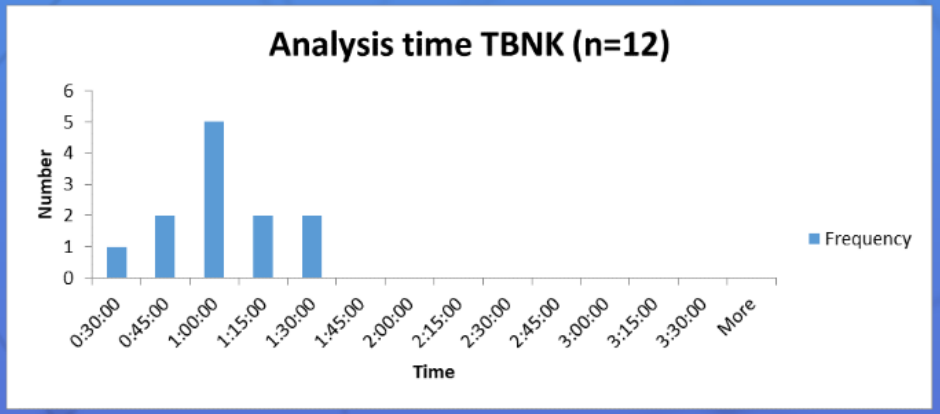
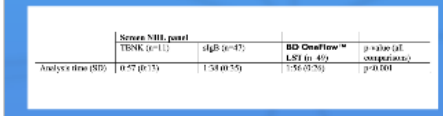
5. Lysis • Lysis buffer • 10' incubation • 5' centrifugation	15 min	15 min	15 min
6. Washing	1 time	1 time	1 time
7. Resuspension • 0,4mL CellFix/CellWASH	X	X	X

Analysis time



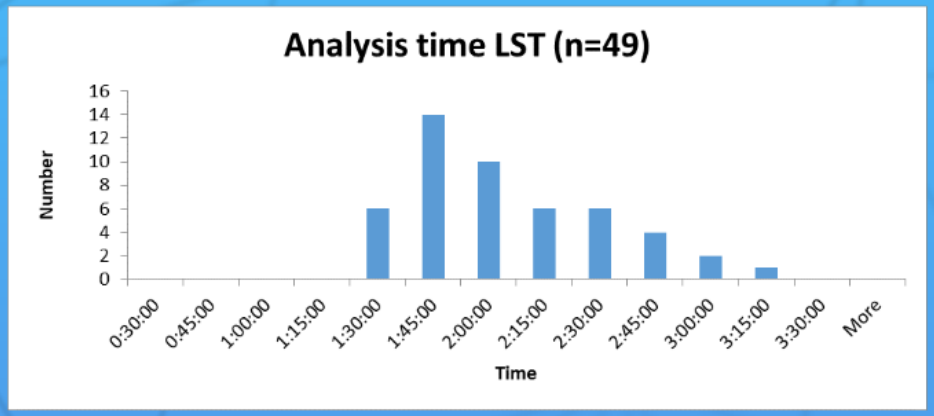


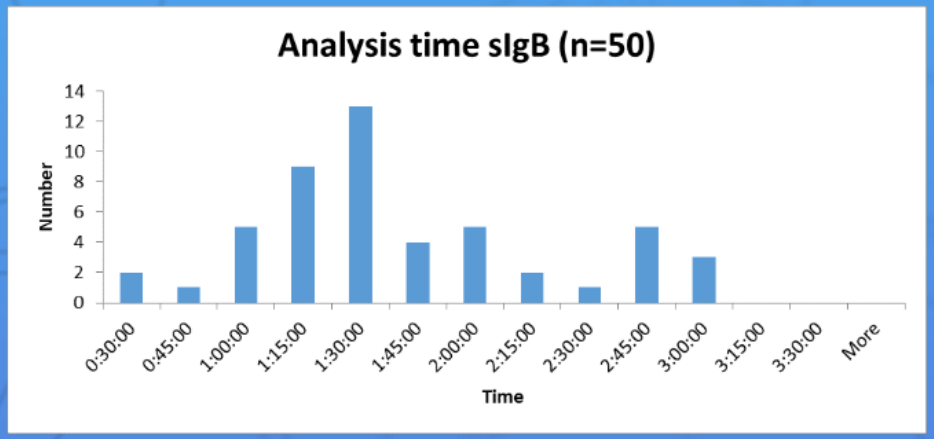


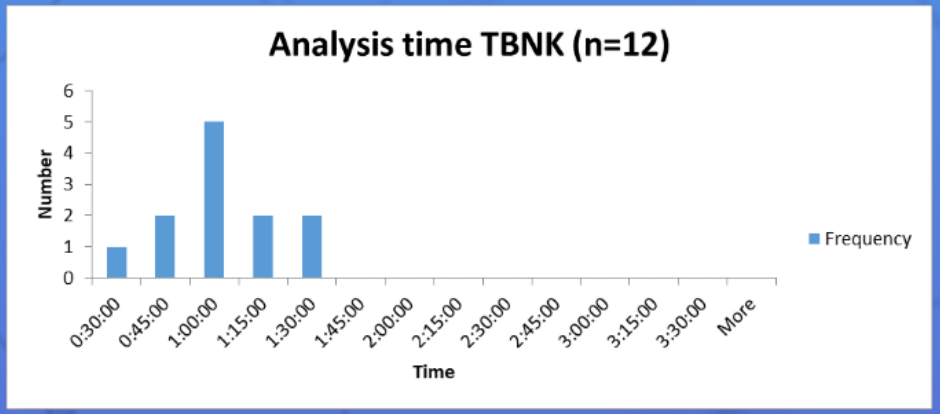


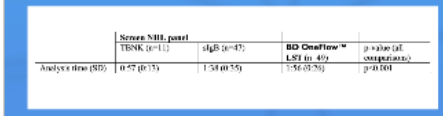
	Screen NHL panel			
	TBNK (n=11)	sIgB (n=47)	**BD OneFlow™ LST** (n=49)	p-value (all comparisons)
Analysis time (SD)	0:57 (0:13)	1:38 (0:35)	1:56 (0:26)	p<0.001

Analysis time

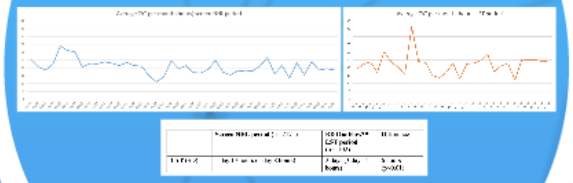




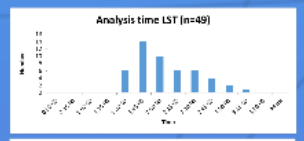


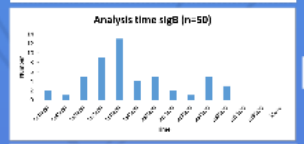


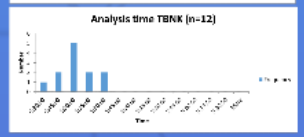
TAT



Analysis time







Workflow

Workflow steps	Screen NHL panel		
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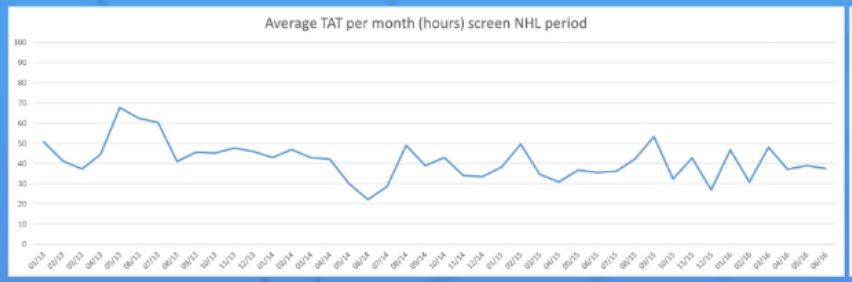
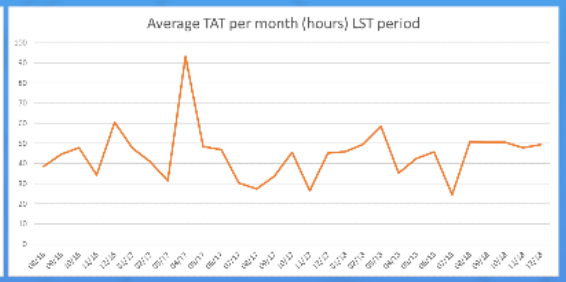
6. Washing	1 time	1 time	1 time
7. Resuspension • 0,4mL CellFix/CellWASH	X	X	X

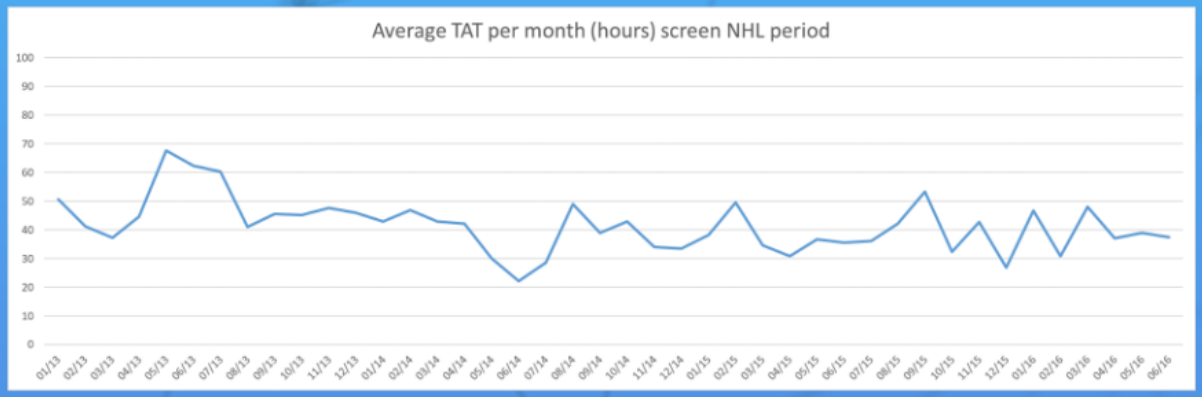
TAT

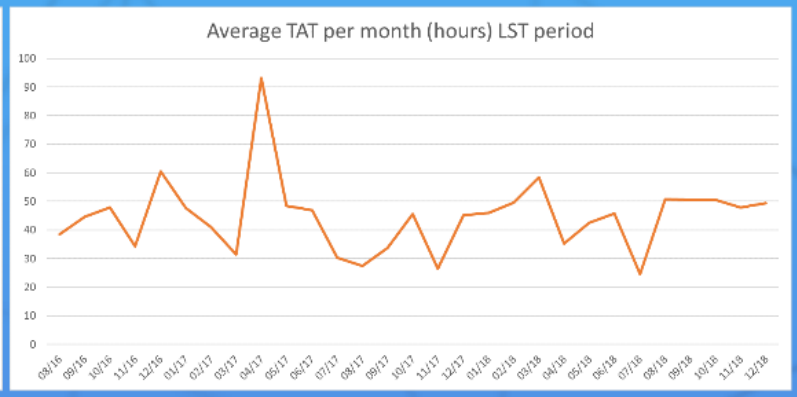




	Screen NHL period (n= 2171)	**BD OneFlow™ LST period** (n=1102)	Difference
TAT (SD)	1 day 17 hours (1 day 8 hours)	2 days (3 days 4 hours)	6 hours ($p<0.01$)

TAT





	Screen NHL period (n= 2171)	**BD OneFlow™ LST period** (n=1102)	Difference
TAT (SD)	1 day 17 hours (1 day 8 hours)	2 days (3 days 4 hours)	6 hours ($p<0.01$)

Question 2: laboratory impact

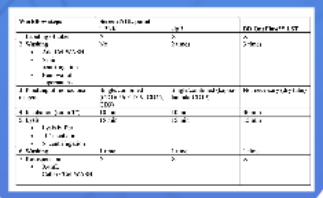
Conclusion

- Longer analysis time
- Higher Cost
- More convenient workflow
- Less mistakes?

Costs

- 12 markers
  - Both tubes
  - same RIZIV reimbursement
- Hands-on time
  - +/- equal
- Reagent cost?

Workflow



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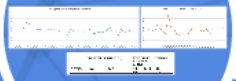
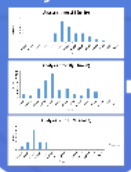


Analysis time



Costs

- 12 markers
  - Both tubes
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- Hands-on time
  - +/- equal
- Reagent cost?

	Screen NHL panel		
	sIgB	TBNK	**BD Oneflow™ LST**
Reagent cost per analysis (excl. tax)	+/- 20 euros	+/- 20 euros	+/- 60 euros

Question 2: laboratory impact

Conclusion

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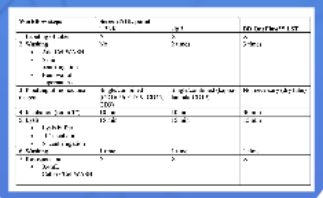
Workflow



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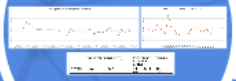
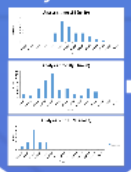


Analysis time



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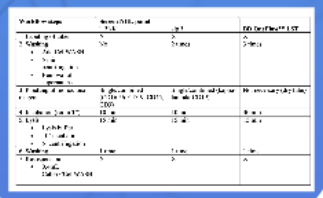
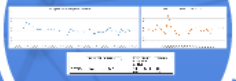
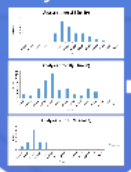
Workflow



TAT

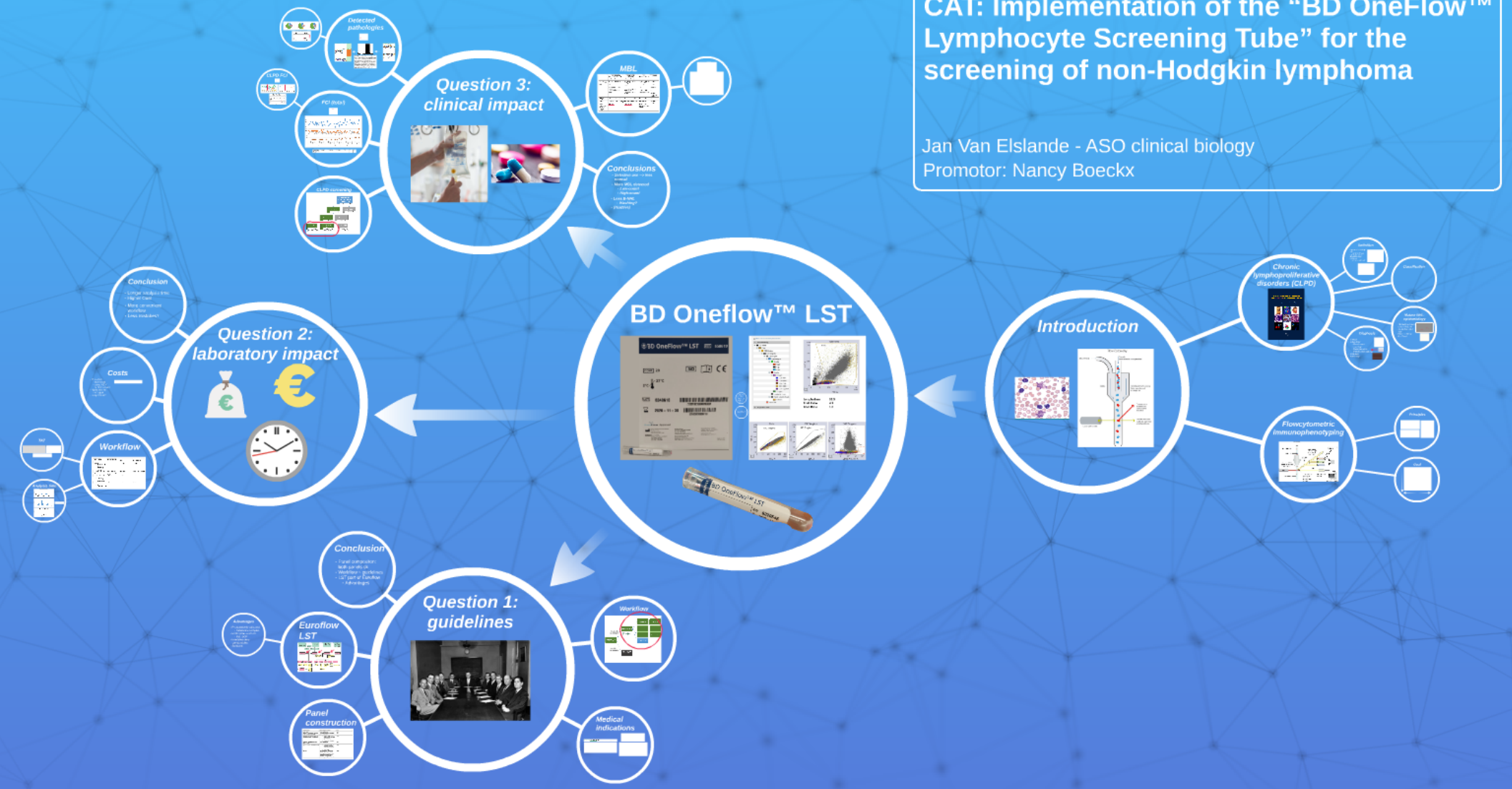


Analysis time



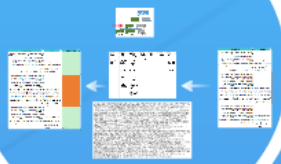
CAT: Implementation of the “BD OneFlow™ Lymphocyte Screening Tube” for the screening of non-Hodgkin lymphoma

Jan Van Elslande - ASO clinical biology
Promotor: Nancy Boeckx



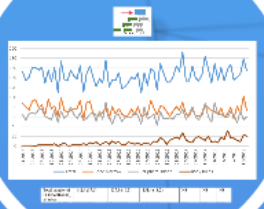
Question 3: clinical impact

Detected pathologies

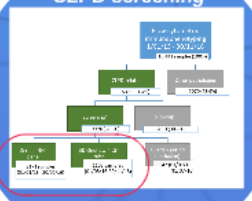


CLPD FCI

FCI (total)



CLPD screening

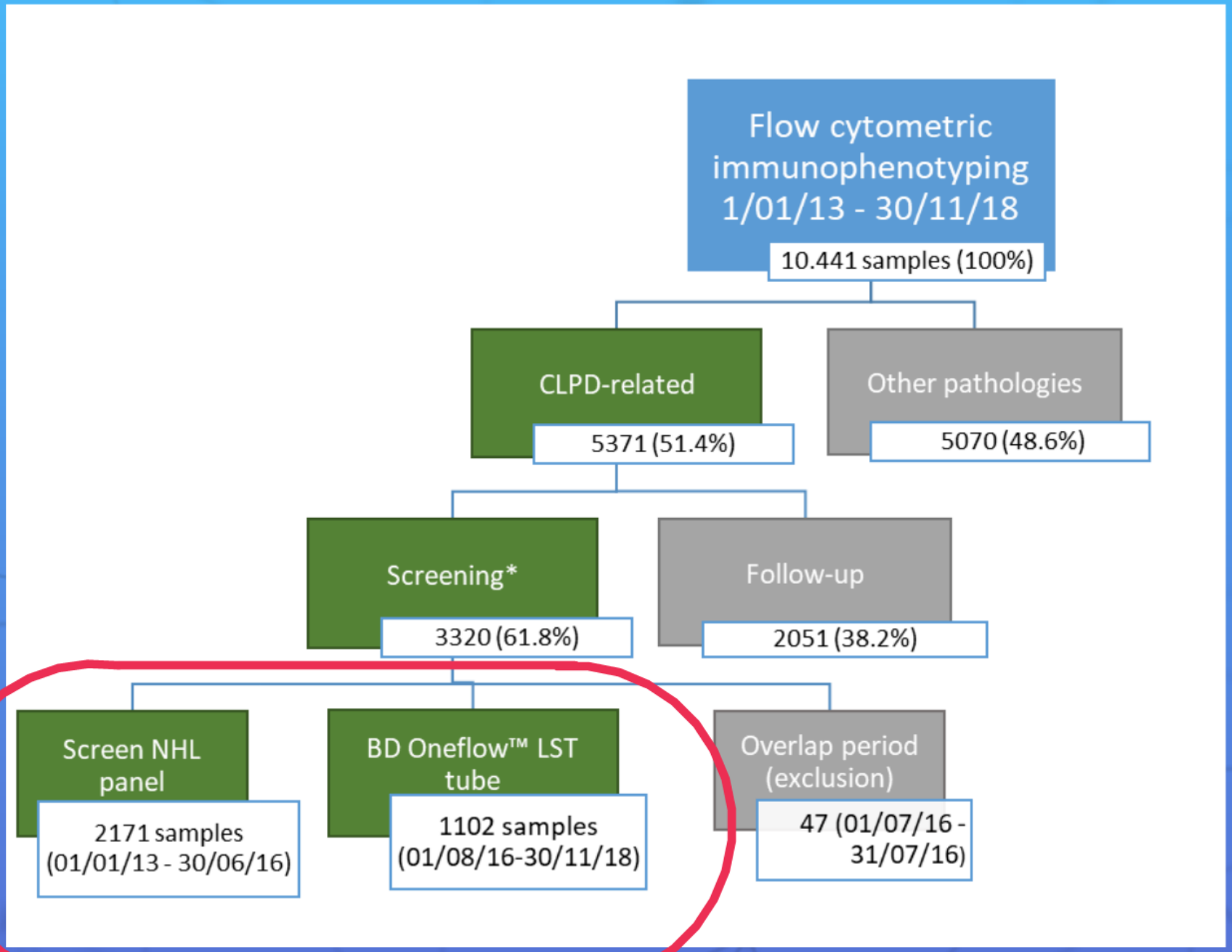


MBL

Conclusions

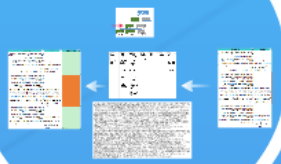
- Selective use --> less normal
- More MBL detected
  - Low-count
  - High-count
- Less B-NHL
  - Washing?
- (reactive)

CLPD screening



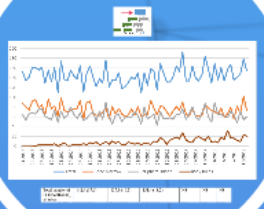
Question 3: clinical impact

Detected pathologies

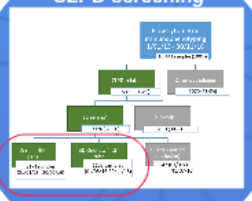


CLPD FCI

FCI (total)



CLPD screening

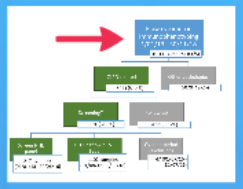


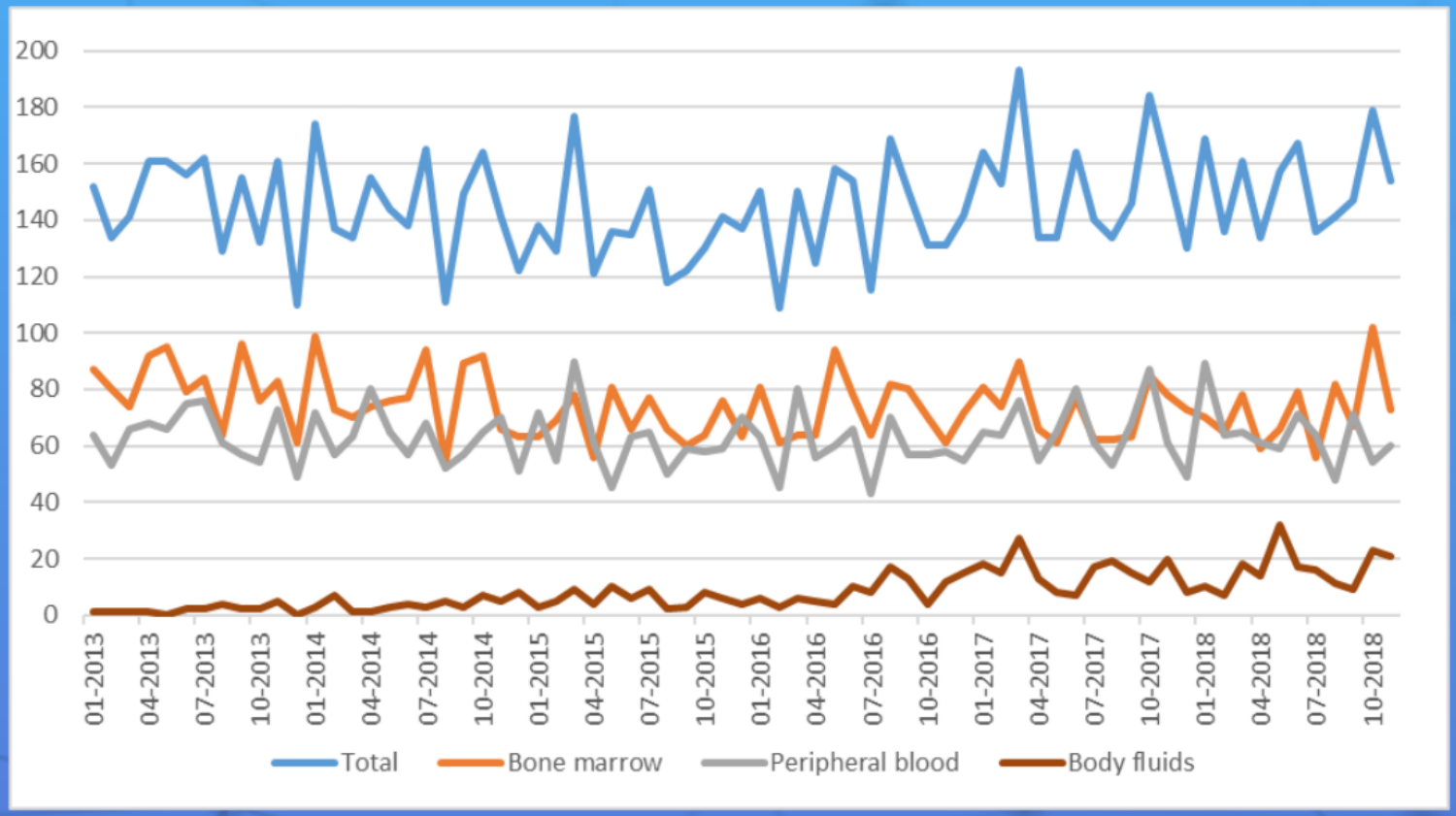
MBL

Conclusions

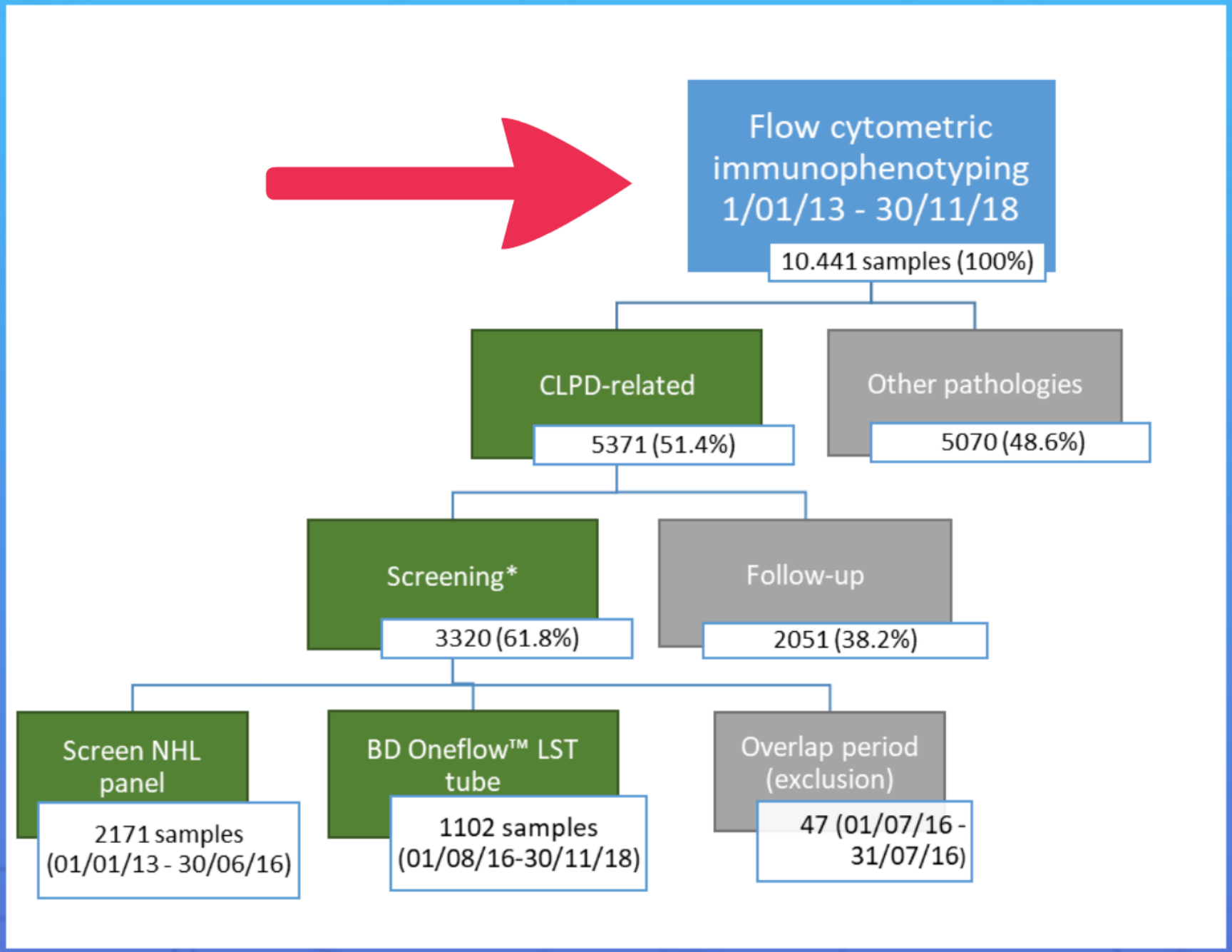
- Selective use --> less normal
- More MBL detected
  - Low-count
  - High-count
- Less B-NHL
  - Washing?
- (reactive)

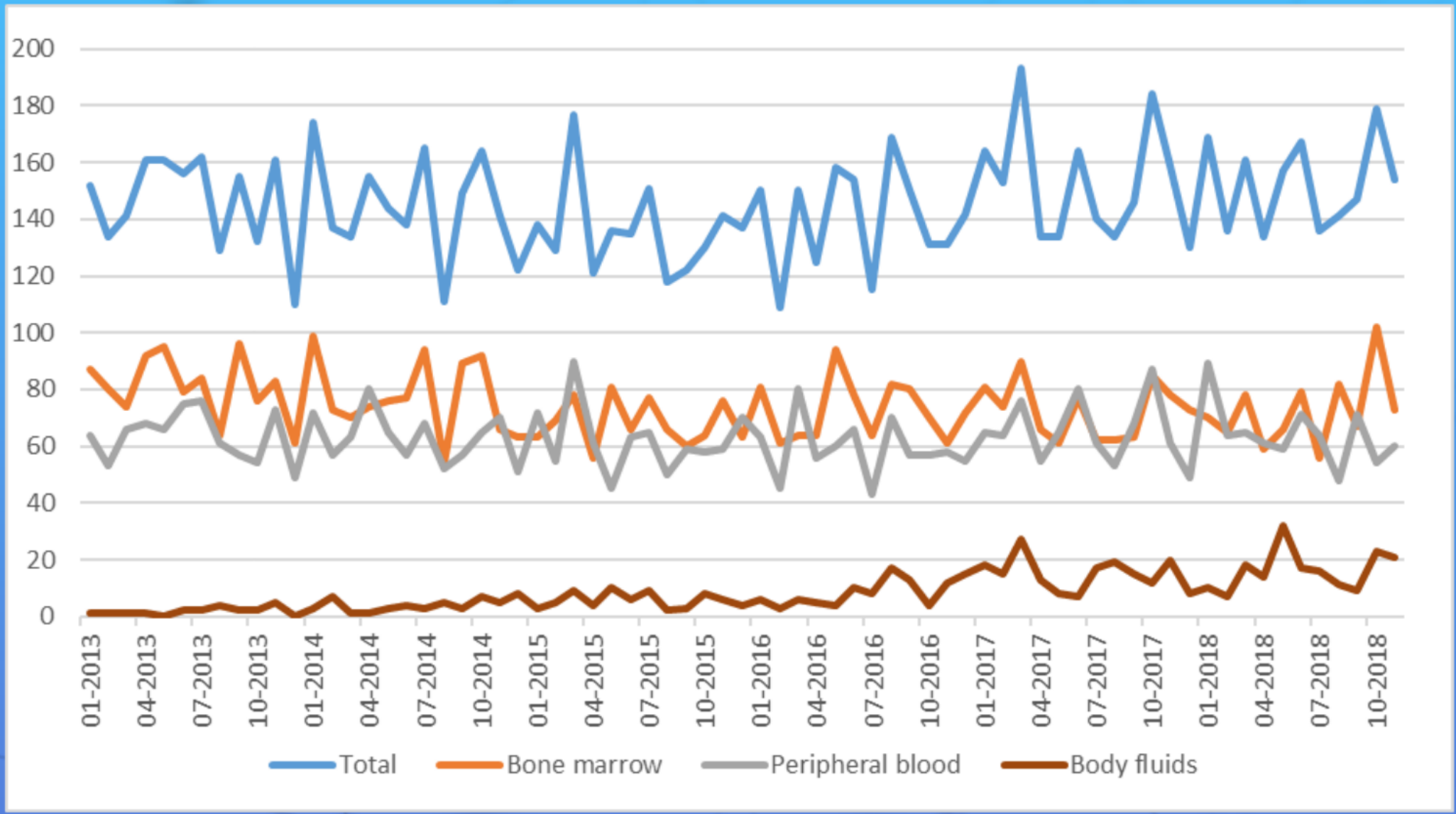
FCI (total)





Total number of FCI/month (SD), all tubes	142.4 (17.6)	137.8 (16.3)	151.4 (18.2)	NS	NS	NS


Flow cytometric immunophenotyping 1/01/13 - 30/11/18
10.441 samples (100%)
CLPD-related
5371 (51.4%)
Other pathologies
5070 (48.6%)
Screening*
3320 (61.8%)
Follow-up
2051 (38.2%)
Screen NHL panel
2171 samples (01/01/13 - 30/06/16)
BD Oneflow™ LST tube
1102 samples (01/08/16-30/11/18)
Overlap period (exclusion)
47 (01/07/16 - 31/07/16)



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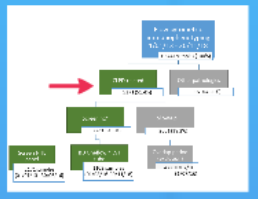
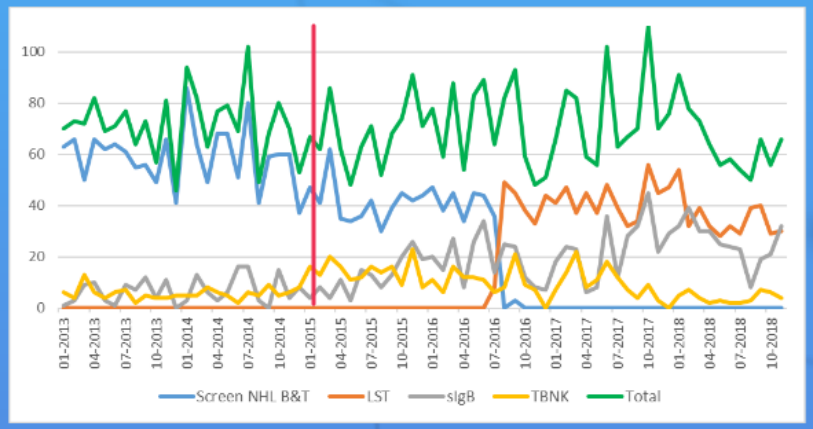
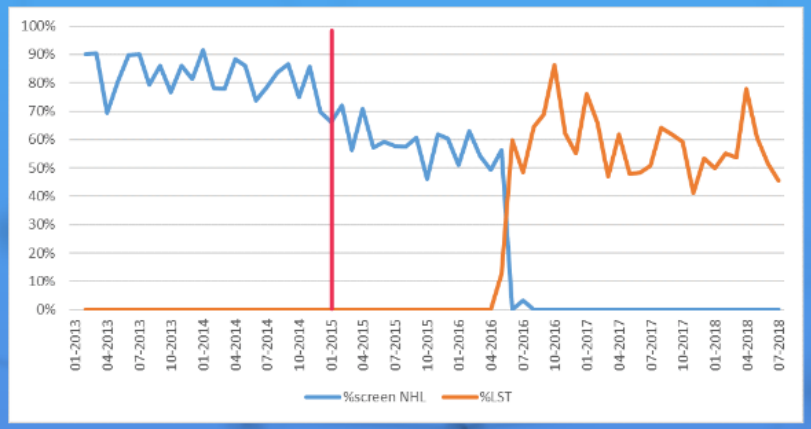
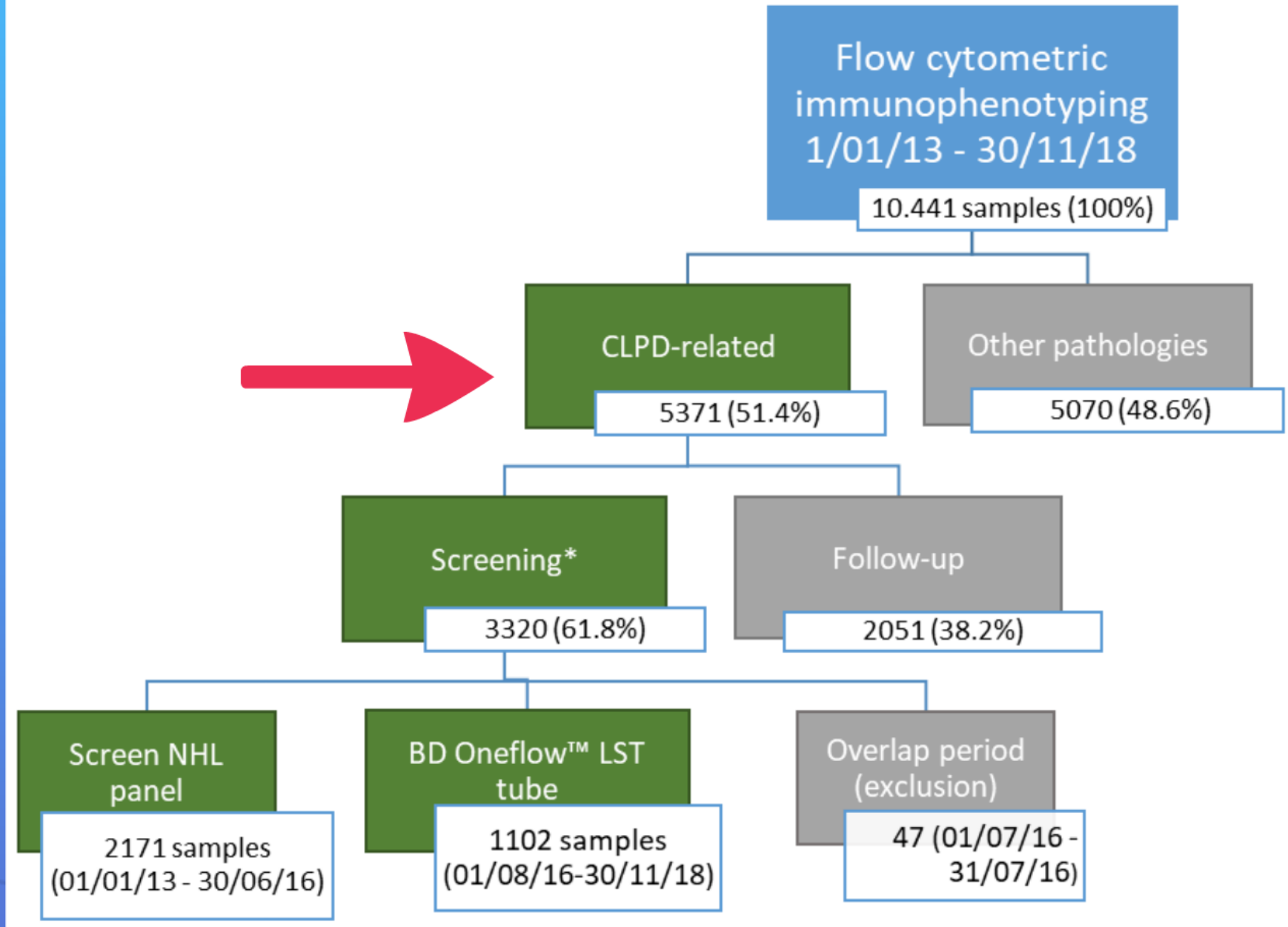
CLPD FCI







	Screen NHL period 1	Screen NHL period 2	BD OneFlow™ LST period	Difference (99% CI)		
Time window	01/01/2013 - 31/12/2014 (24 months)	01/01/2015 – 30/06/2016 (18 months	01/08/2016- 30/11/2018 (28 months)	Screen NHL 1 vs 2	Screen NHL 1 vs LST	Screen NHL 2 vs LST
n (screening tubes)	1421	750	1102	N/A	N/A	N/A
Age (SD)	60.1 (18.4)	61.8 (18.2)	61.8 (18.4)	NS	NS	NS
Male / female	54.1% / 45.9%	54.4% / 45.6%	50.1% / 49.9%	NS	NS	NS
Total number of FCI/month (SD), all tubes	142.4 (17.6)	137.8 (16.3)	151.4 (18.2)	NS	NS	NS
Average CLPD tubes / month (SD)	71.7 (12.9)	70.3 (13.3)	69.7 (15.9)	NS	NS	NS
Average CLPD screening tubes per month	59.3 (11.5)	41.7 (6.9)	39.3 (7.8)	17.6 (10.2 – 25.0)	20 (12.9-27.1)	NS
Proportion of CLPD tubes	82.7%	59.9%	57.6%	23% (19.0-27.6)	26% (22.4-29.8)	NS
Proportion external analyses	39.8%	45.1%	49.8%	NS	10% (5,0 – 15,2)	NS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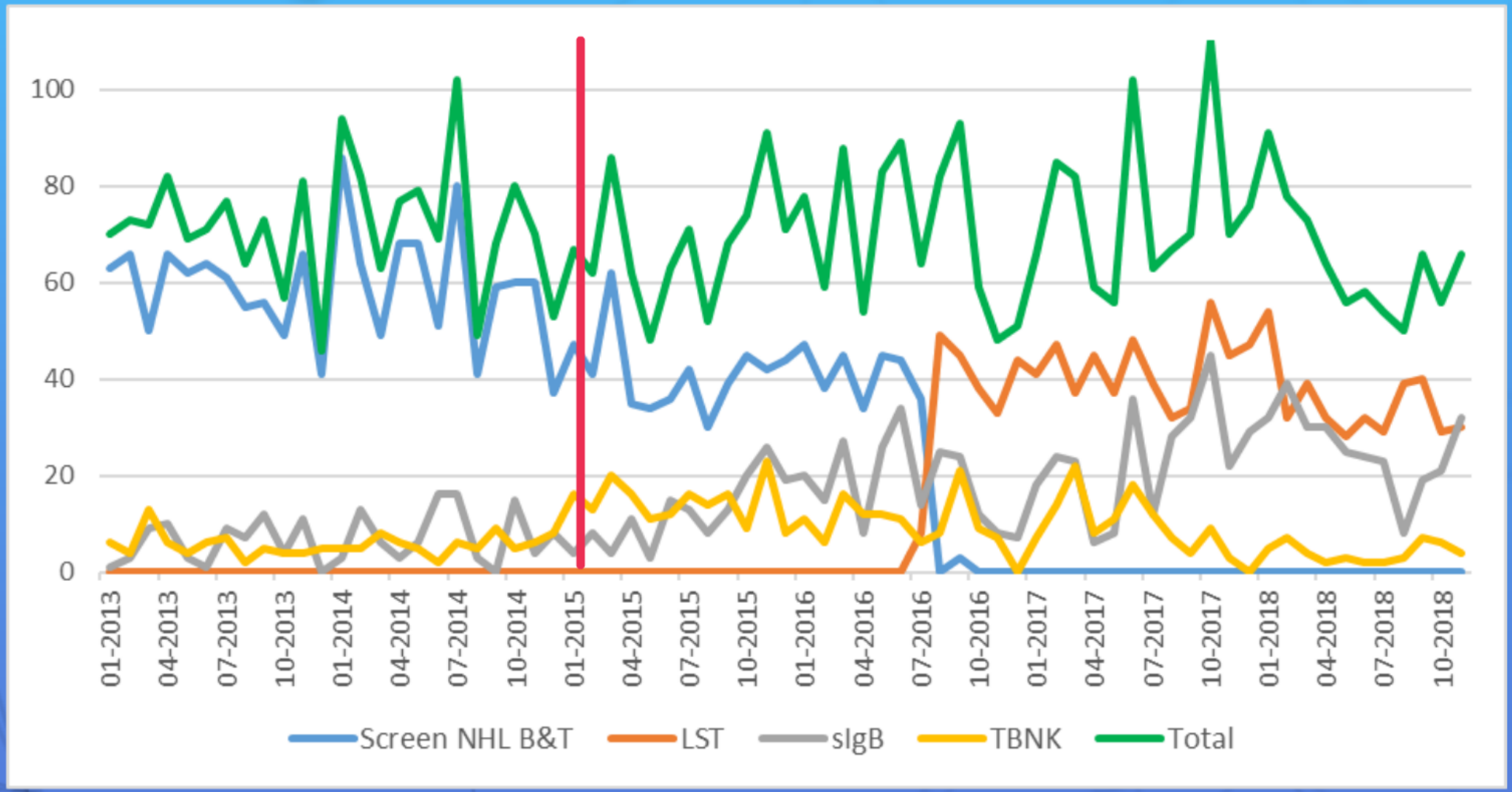
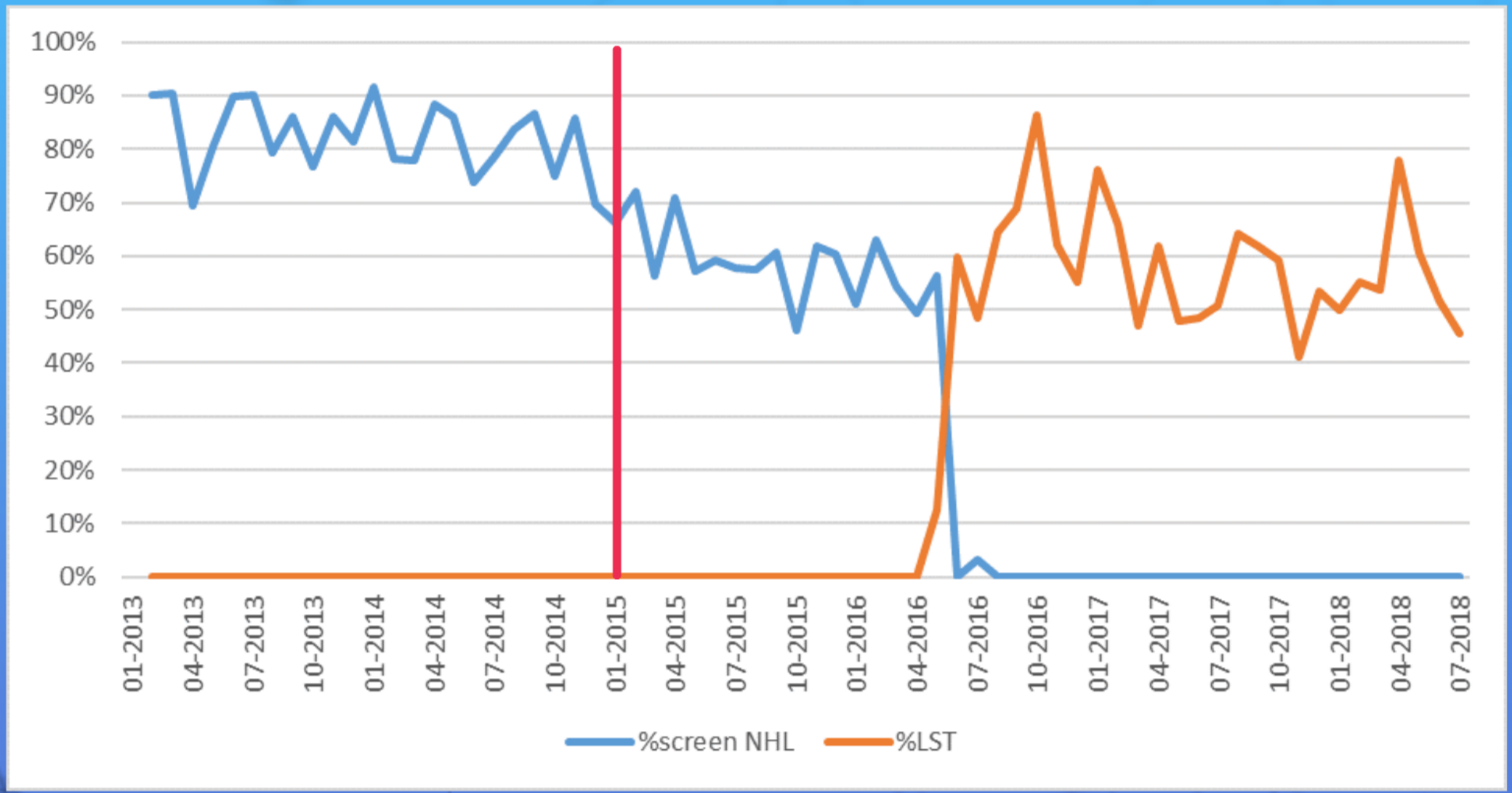
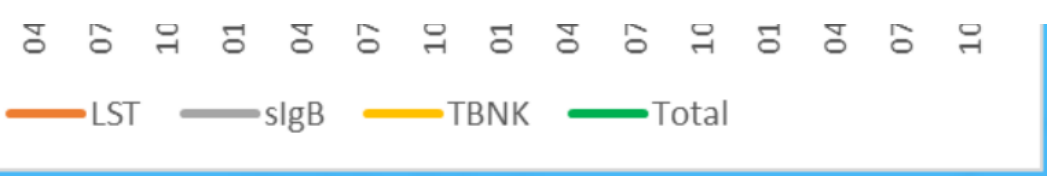


	Screen NHL period 1	Screen NHL period 2
Time window	01/01/2013 -	01/01/2015 –

47 (01/07/16 - 31/07/16)



	BD OneFlow™ LST period	Difference (99% CI)		
	01/08/2016-	Screen	Screen	Screen



	Screen NHL period 1	Screen NHL period 2	BD OneFlow™ LST period	Difference (99% CI)		
Time window	01/01/2013 - 31/12/2014 (24 months)	01/01/2015 – 30/06/2016 (18 months	01/08/2016- 30/11/2018 (28 months)	Screen NHL 1 vs 2	Screen NHL 1 vs LST	Screen NHL 2 vs LST
n (screening tubes)	1421	750	1102	N/A	N/A	N/A
Age (SD)	60.1 (18.4)	61.8 (18.2)	61.8 (18.4)	NS	NS	NS
Male / female	54.1% / 45.9%	54.4% / 45.6%	50.1% / 49.9%	NS	NS	NS
Total number of FCI/month (SD), all tubes	142.4 (17.6)	137.8 (16.3)	151.4 (18.2)	NS	NS	NS
Average CLPD tubes / month (SD)	71.7 (12.9)	70.3 (13.3)	69.7 (15.9)	NS	NS	NS
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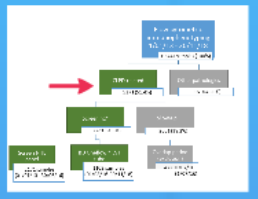
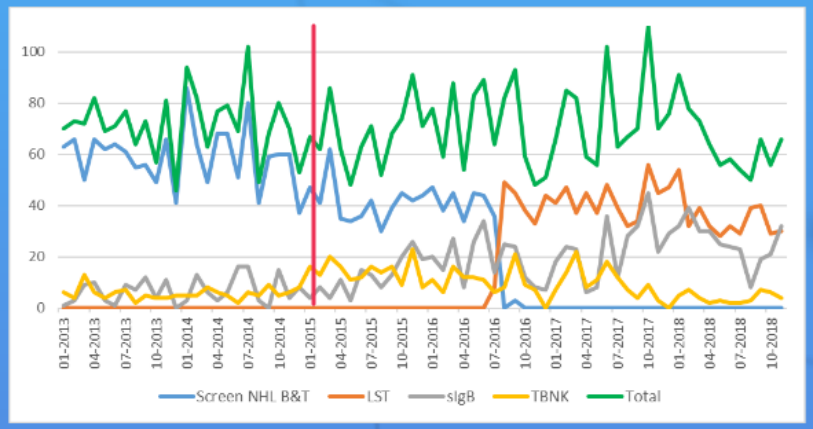
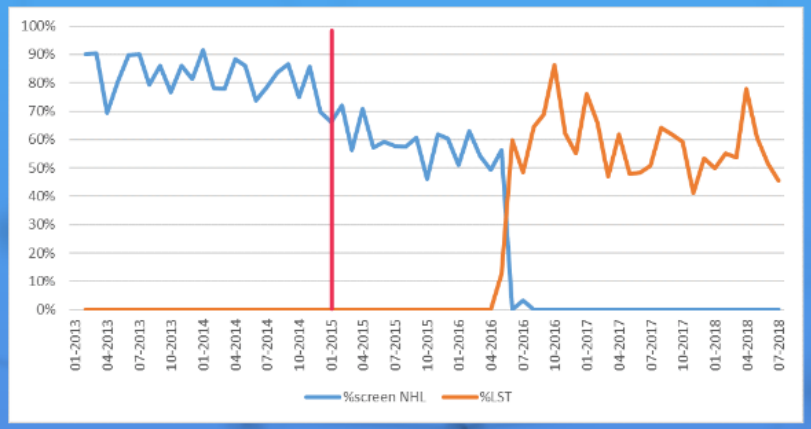
CLPD FCI







	Screen NHL period 1	Screen NHL period 2	BD OneFlow™ LST period	Difference (99% CI)		
Time window	01/01/2013 - 31/12/2014 (24 months)	01/01/2015 – 30/06/2016 (18 months	01/08/2016- 30/11/2018 (28 months)	Screen NHL 1 vs 2	Screen NHL 1 vs LST	Screen NHL 2 vs LST
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Total number of FCI/month (SD), all tubes	142.4 (17.6)	137.8 (16.3)	151.4 (18.2)	NS	NS	NS
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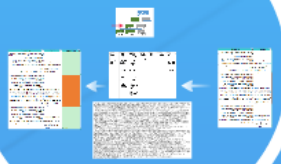
Proportion external analyses	39.8%	45.1%	49.8%	NS	10% (5,0 – 15,2)	NS

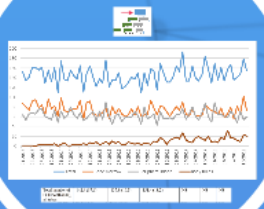
Question 3: clinical impact

Detected pathologies

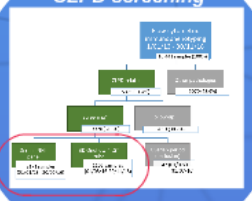


CLPD FCI

FCI (total)



CLPD screening

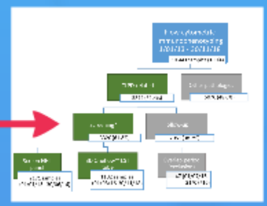


MBL

Conclusions

- Selective use --> less normal
- More MBL detected
  - Low-count
  - High-count
- Less B-NHL
  - Washing?
- (reactive)

Detected pathologies



Bespreking	Besluit	Interpretatie
De relevante populatie bevindt zich in de gate bij de lymfocyten. Immunofenotypering toont 2% lymfocyten (obv CD45-SSC). Binnen de lymfogate zijn er 2% B-lymfocyten (CD19+) zonder lichte ketenrestrictie, 58% T-lymfocyten (CD3+) met een normale CD4/CD8-verhouding en 40% NK-cellen. Normaal aantal T-LGL's. Enkele jongere B-lymfoide cellen.	Immunofenotypering toont geen argumenten voor de aanwezigheid van een residuele monoclonale B-celpopulatie.	Normaal
De relevante populatie bevindt zich in de gate bij de lymfocyten. Immunofenotypering toont 55% lymfocyten (obv CD45-SSC). Binnen de lymfogate zijn er 79% B-lymfocyten (CD19+) met lichte ketenrestrictie, 19% T-lymfocyten (CD3+) met een normale CD4/CD8-verhouding en 2% NK-cellen. Normaal aantal T-LGL's (0.4% binnen de lymfogate, 0.2% op ANC). Het immunofenotype van de aberrante / pathologische populatie is als volgt: positief voor CD19, CD20, CD38, FMC7, sKappa (zwak), zwak/partieel CD5 en negatief voor CD10, CD23, CD79b.	Immunofenotypering toont de aanwezigheid van een monoclonale B-celpopulatie (absolute 5,4.10^9/L): passend bij een B-NHL, niet nader te typeren (catovsky <3/5).	B-NHL
De relevante populatie bevindt zich in de gate bij de lymfocyten. Immunofenotypering toont 11% lymfocyten (obv CD45-SSC). Binnen de lymfogate zijn er 12% B-lymfocyten (CD19+) zonder lichte ketenrestrictie, 83% T-lymfocyten (CD3+) met een normale CD4/CD8-verhouding en 7% NK-cellen. Normaal aantal T-LGL's (13% binnen de lymfogate, 1% op ANC).	Immunofenotypering toont onvoldoende argumenten voor de aanwezigheid van een monoclonaal B- of T-celproces.	Normaal

Normal	Reactive	Dubious	B-NHL	MBL	T-NHL	Other
No evidence for monoclonal B-, T- or NK-pathology.	Disturbed CD4/CD8 ratio without significant aberrant phenotype AND/OR Increased number of NK cells AND/OR Weaker expression CD7 on T-cells without abnormalities of other markers	Disturbed kappa/lambda ratio AND/OR Markedly disturbed CD4/CD8 ratio AND/OR Minimal invasion not excludable AND/OR Weaker expression of CD5 on T-cells without abnormalities of other markers (except weaker CD7 expression)	Monoclonal B-cells >5.000/µL	Monoclonal B-cells <5.000/µL	Evidence for the presence of a T-NHL	Blasts OR Non reliable interpretation OR Plasmacells

Bespreking	Besluit
De relevante populatie bevindt zich in de gate bij de lymfocyten. Immunofenotypering toont 2% lymfocyten (obv CD45-SSC). Binnen de lymfogate zijn er 2% B-lymfocyten (CD19+) zonder lichte ketenrestrictie, 58% T-lymfocyten (CD3+) met een normale CD4/CD8-verhouding en 40% NK-cellen. Normaal aantal T-LGL's. Enkele jongere B-lymfoide cellen.	Immunofenotypering toont geen argumenten voor de aanwezigheid van een residuele monoclonale B-celpopulatie.
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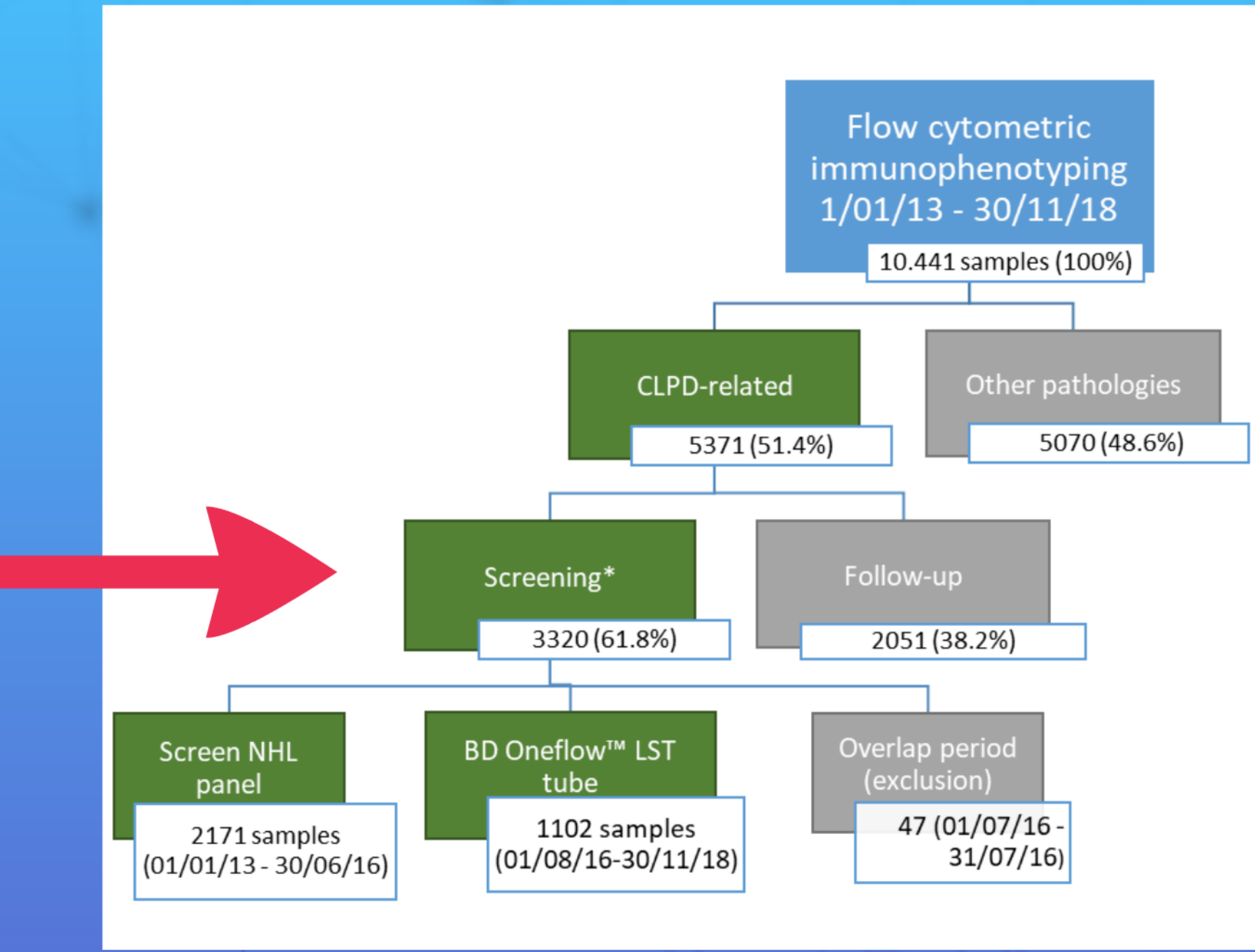
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BD Oneflow™ LST tube
1102 samples (01/08/16-30/11/18)
Overlap period (exclusion)
47 (01/07/16 - 31/07/16)

Bespreking	Besluit
De relevante populatie bevindt zich in de gate bij de lymfocyten. Immunofenotypering toont 2% lymfocyten (obv CD45-SSC). Binnen de lymfogate zijn er 2% B-lymfocyten (CD19+) zonder lichte ketenrestrictie, 58% T-lymfocyten (CD3+) met een normale CD4/CD8-verhouding en 40% NK-cellen. Normaal aantal T-LGL's. Enkele jongere B-lymfoide cellen.	Immunofenotypering toont geen argumenten voor de aanwezigheid van een residuele monoclonale B-celpopulatie.
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Normal	Reactive	Dubious	B-NHL	MBL	T-NHL	Other
No evidence for monoclonal B- ,T- , or NK-pathology.	Disturbed CD4/CD8 ratio without significant aberrant phenotype AND/OR Increased number of NK cells AND/OR Weaker expression CD7 on T-cells without abnormalities of other markers	Disturbed kappa/lambda ratio AND/OR Markedly disturbed CD4/CD8 ratio AND/OR Minimal invasion not excludable AND/OR Weaker expression of CD5 on T-cells without abnormalities of other markers (except weaker CD7 expression)	Monoclonal B-cells >5.000/µL	Monoclonal B-cells <5.000/µL	Evidence for the presence of a T-NHL	Blasts OR Non reliable interpretation OR Plasmacells

		other markers (except weaker CD7 expression)				

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Bespreking	Besluit	Interpretatie
De relevante populatie bevindt zich in de gate bij de lymfocyten. Immunofenotypering toont 2% lymfocyten (obv CD45-SSC). Binnen de lymfogate zijn er 2% B-lymfocyten (CD19+) zonder lichte ketenrestrictie, 58% T-lymfocyten (CD3+) met een normale CD4/CD8-verhouding en 40% NK-cellen. Normaal aantal T-LGL's. Enkele jongere B-lymfoide cellen.	Immunofenotypering toont geen argumenten voor de aanwezigheid van een residuele monoclonale B-celpopulatie.	Normaal
De relevante populatie bevindt zich in de gate bij de lymfocyten. Immunofenotypering toont 55% lymfocyten (obv CD45-SSC). Binnen de lymfogate zijn er 79% B-lymfocyten (CD19+) met lichte ketenrestrictie, 19% T-lymfocyten (CD3+) met een normale CD4/CD8-verhouding en 2% NK-cellen. Normaal aantal T-LGL's (0.4% binnen de lymfogate, 0.2% op ANC). Het immunofenotype van de aberrante / pathologische populatie is als volgt: positief voor CD19, CD20, CD38, FMC7, sKappa (zwak), zwak/partieel CD5 en negatief voor CD10, CD23, CD79b.	Immunofenotypering toont de aanwezigheid van een monoclonale B-celpopulatie (absolute 5,4.10^9/L): passend bij een B-NHL, niet nader te typeren (catovsky <3/5).	B-NHL
De relevante populatie bevindt zich in de gate bij de lymfocyten. Immunofenotypering toont 11% lymfocyten (obv CD45-SSC). Binnen de lymfogate zijn er 12% B-lymfocyten (CD19+) zonder lichte ketenrestrictie, 83% T-lymfocyten (CD3+) met een normale CD4/CD8-verhouding en 7% NK-cellen. Normaal aantal T-LGL's (13% binnen de lymfogate, 1% op ANC).	Immunofenotypering toont onvoldoende argumenten voor de aanwezigheid van een monoclonaal B- of T-celproces.	Normaal

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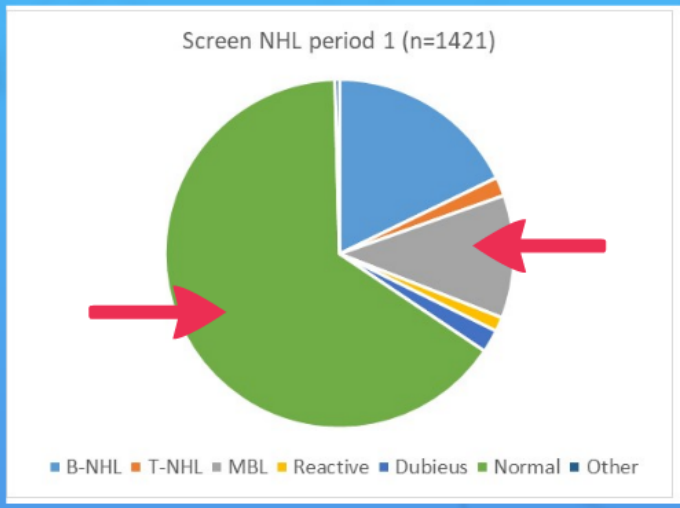
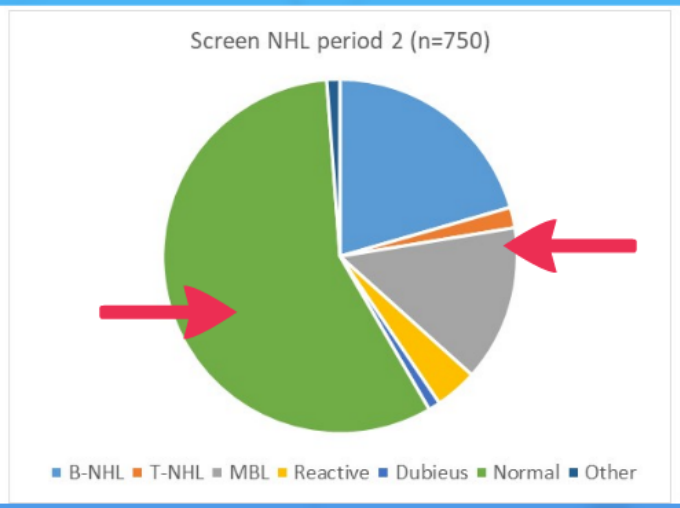
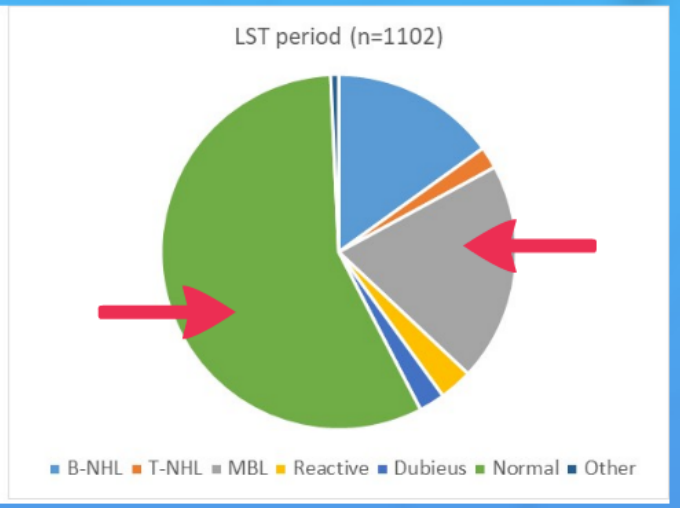
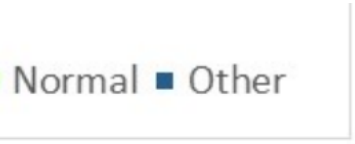
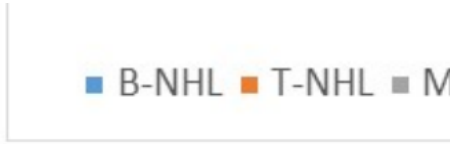






	Screen NHL period 1	**Screen NHL period 2**	**BD OneFlow™ LST period**	**Difference (99% CI)**		
Time window	01/01/2013 - 31/12/2014 (24 months)	01/01/2015 – 30/06/2016 (18 months	01/08/2016-30/11/2018 (28 months)	Screen NHL 1 vs 2	Screen NHL 1 vs LST	Screen NHL 2 vs LST
Normal	65.2%	57.2%	56.9%	8.0% (2.3-13.6)	8.3% (3.2-13.3)	NS
B-NHL	17.8%	20.5%	15.1%	NS	NS	5.5% (0.7-10.2)
MBL	11.4%	14.3%	20.0%	NS	8.6% (4.8-12.4)	5.7% (1.2-10.2)
Reactive	1.3%	3.9%	3.0%	1.9% (0.6-4.5)	1,7% (0.1-3.2)	NS
Dubious	2.1%	1.1%	2.4%	NS	NS	NS
T-NHL	1.8%	1.9%	2.0%	NS	NS	NS
Other	0.4%	1.2%	0.7%	NS	NS	NS

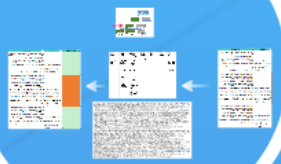




	Screen NHL period 1	**Screen NHL period 2**	**BD OneFlow™ LST period**	**Difference (99% CI)**		
Time window	01/01/2013 - 31/12/2014 (24 months)	01/01/2015 – 30/06/2016 (18 months	01/08/2016-30/11/2018 (28 months)	Screen NHL 1 vs 2	Screen NHL 1 vs LST	Screen NHL 2 vs LST
Normal	65.2%	57.2%	56.9%	8.0% (2.3-13.6)	8.3% (3.2-13.3)	NS
B-NHL	17.8%	20.5%	15.1%	NS	NS	5.5% (0.7-10.2)
MBL	11.4%	14.3%	20.0%	NS	8.6% (4.8-12.4)	5.7% (1.2-10.2)
Reactive	1.3%	3.9%	3.0%	1.9% (0.6-4.5)	1,7% (0.1-3.2)	NS
Dubious	2.1%	1.1%	2.4%	NS	NS	NS
T-NHL	1.8%	1.9%	2.0%	NS	NS	NS
Other	0.4%	1.2%	0.7%	NS	NS	NS

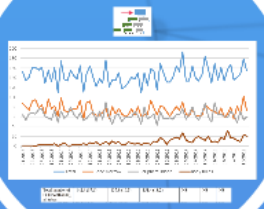
Question 3: clinical impact

Detected pathologies

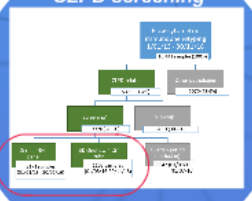


CLPD FCI

FCI (total)



CLPD screening



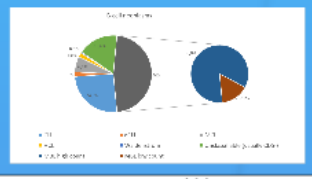
MBL

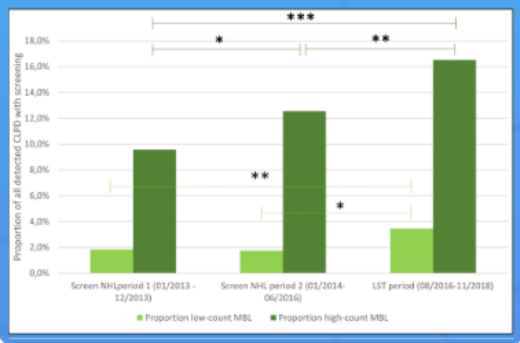
Conclusions

- Selective use --> less normal
- More MBL detected
  - Low-count
  - High-count
- Less B-NHL
  - Washing?
- (reactive)

MBL

	Low-count MBL			High-count MBL			CLL		Non-CLL B-NHL
	Non-CLL	Atypical CLL	CLL	Non-CLL	Atypical CLL	CLL	Atypical	Typical	
Number of monoclonal B-cells	<500/µL (Median 1/µL[28]) AND No clinical symptoms indicative for lymphoproliferative disorder			500-5.000/µL (Median 2.900/µL[28]) AND No clinical symptoms indicative for lymphoproliferative disorder			>5.000/µL with or without clinical symptoms OR <5.000/µL with clinical symptoms		>5.000/µL with or without clinical symptoms OR <5.000/µL with clinical symptoms
Catovsky score*	0-2	3	4-5	0-2	3	4-5	3	4-5	0-2
Prevalence	3-12% (>20% if >60 years, up to 50-75% if >90 years[29])						0.04 - 0.05% [30]		Depends on subtype
Risk of progression to treatment requiring disease	Very rare			1-2% per year			Rai A: 5-70%, depending on risk factors		





MBL

	Low-count MBL			High-count MBL			CLL		Non-CLL B-NHL
	Non-CLL	Atypical CLL	CLL	Non-CLL	Atypical CLL	CLL	Atypical	Typical	
Number of monoclonal B-cells	<500/µL (Median 1/µL[28]) AND No clinical symptoms indicative for lymphoproliferative disorder			500-5.000/µL (Median 2.900/µL[28]) AND No clinical symptoms indicative for lymphoproliferative disorder			>5.000/µL with or without clinical symptoms OR <5.000/µL with clinical symptoms		>5.000/µL with or without clinical symptoms OR <5.000/µL with clinical symptoms
Catovsky score*	0-2	3	4-5	0-2	3	4-5	3	4-5	0-2
Prevalence	3-12% (>20% if >60 years, up to 50-75% if >90 years[29])						0.04 - 0.05% [30]		Depends on subtype
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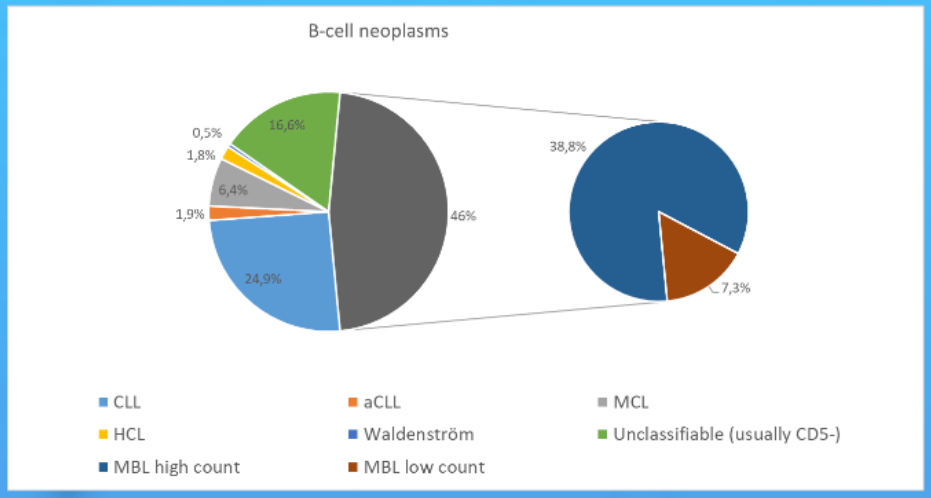
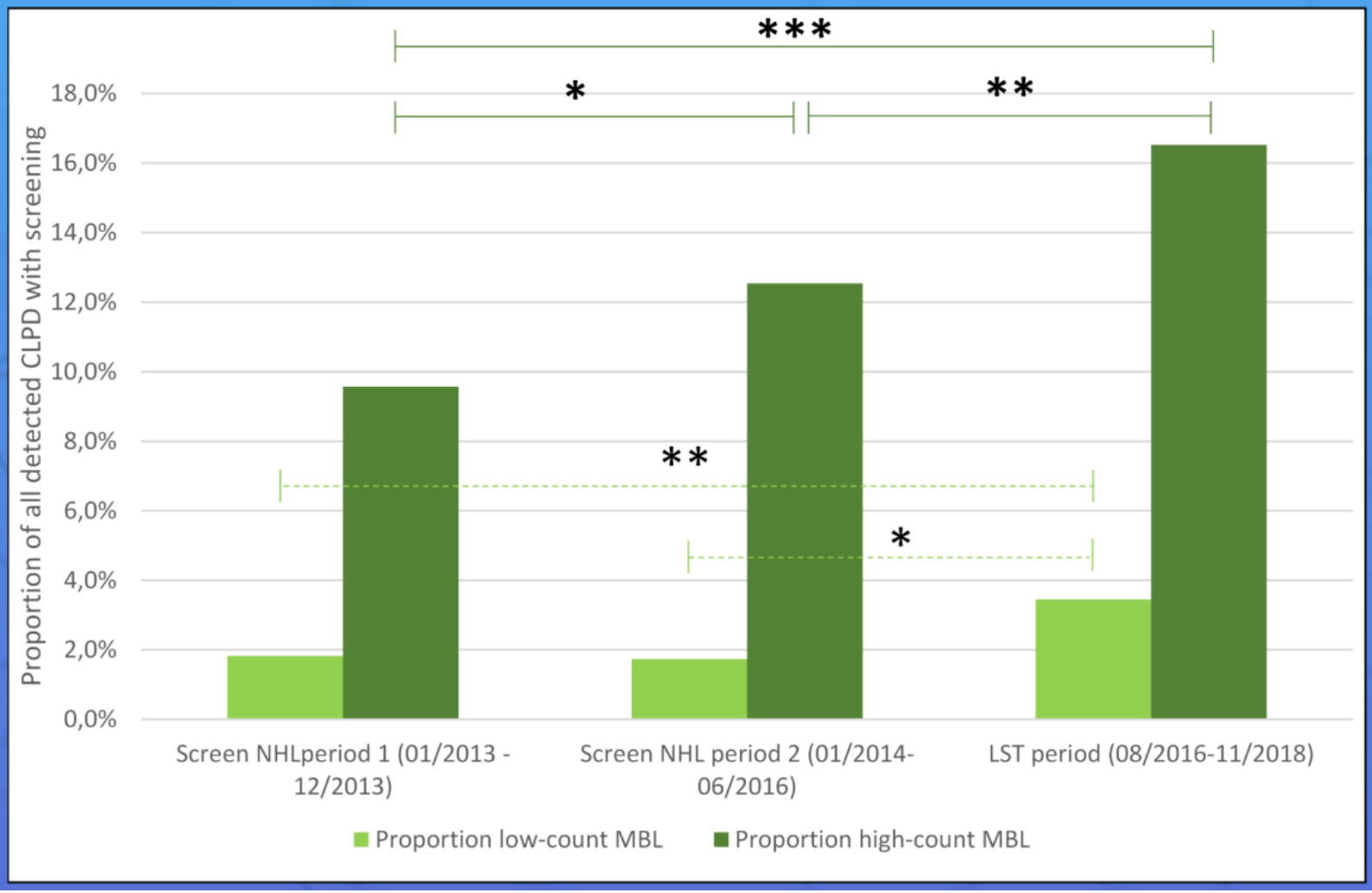

B-cell neoplasms
0,5%
1,8%
6,4%
1,9%
16,6%
24,9%
46%
38,8%
7,3%
CLL
aCLL
MCL
HCL
Waldenström
Unclassifiable (usually CD5-)
MBL high count
MBL low count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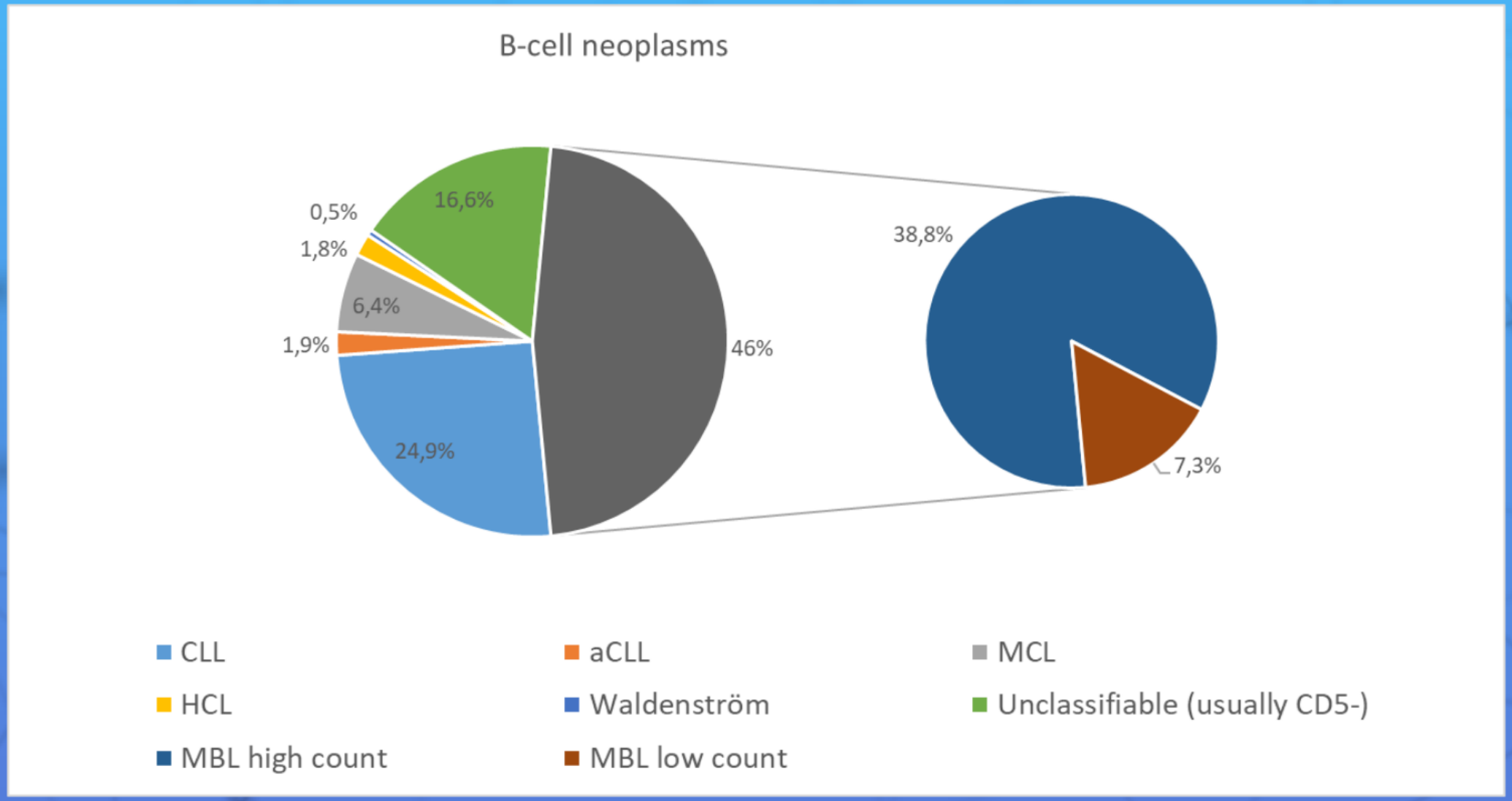
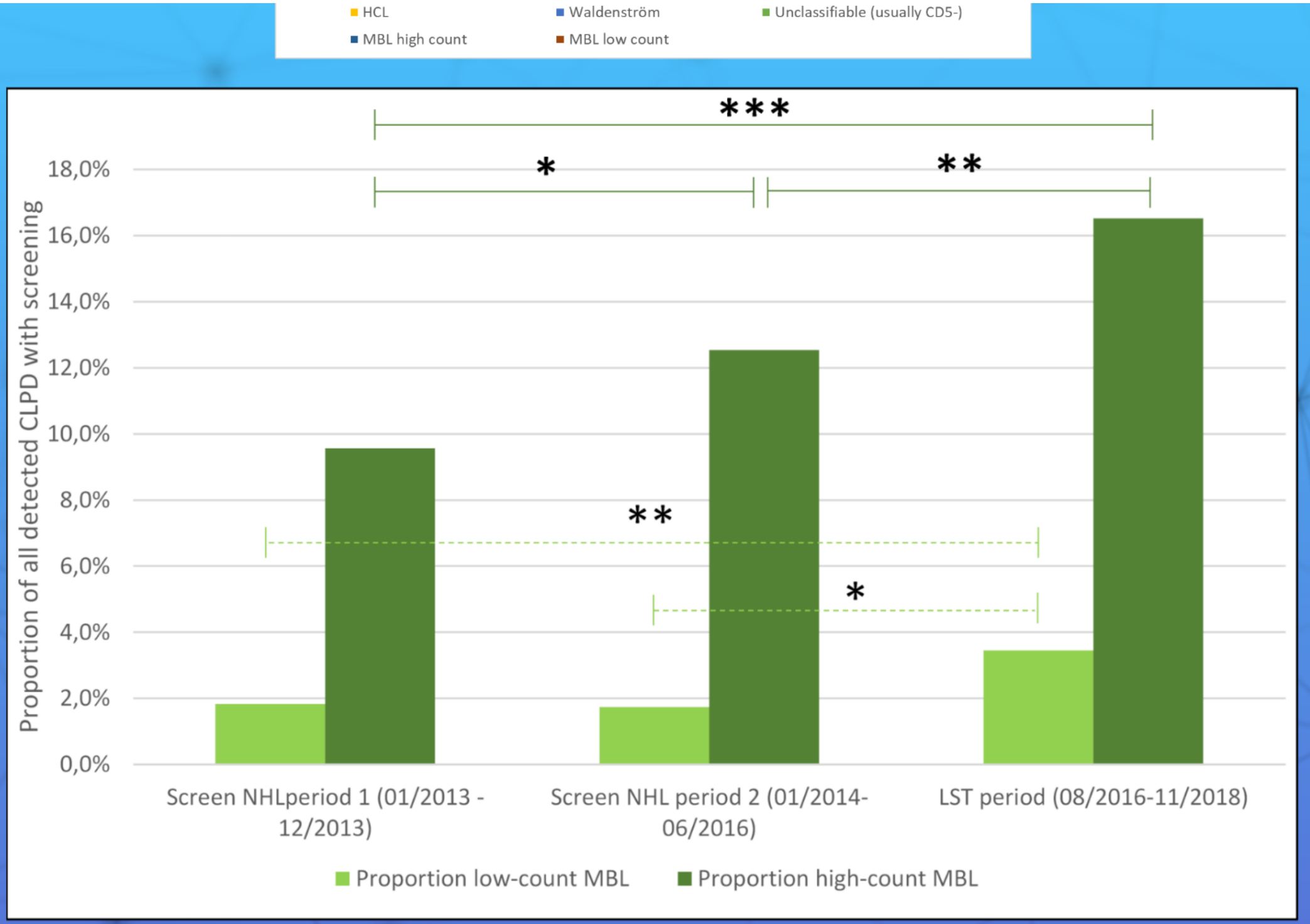
Proportion of all detected CLPD with screening
18,0%
16,0%
14,0%
12,0%
10,0%
8,0%
6,0%
4,0%
2,0%
0,0%
Screen NHLperiod 1 (01/2013 - 12/2013)
Screen NHL period 2 (01/2014-06/2016)
LST period (08/2016-11/2018)
Proportion low-count MBL
Proportion high-count MBL


B-cell neoplasms
0,5%
16,6%
1,8%
6,4%
1,9%
46%
24,9%
38,8%
7,3%
CLL
aCLL
MCL
HCL
Waldenström
Unclassifiable (usually CD5-)
MBL high count
MBL low count

***


HCL
Waldenström
Unclassifiable (usually CD5-)
MBL high count
MBL low count
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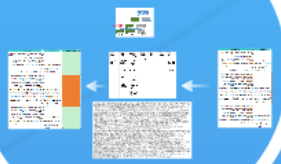
18,0%
16,0%
14,0%
12,0%
10,0%
8,0%
6,0%
4,0%
2,0%
0,0%
Proportion of all detected CLPD with screening
Screen NHLperiod 1 (01/2013 - 12/2013)
Screen NHL period 2 (01/2014-06/2016)
LST period (08/2016-11/2018)
Proportion low-count MBL
Proportion high-count MBL

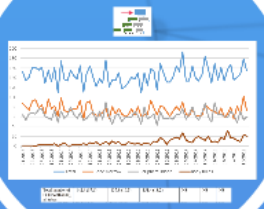
Question 3: clinical impact

Detected pathologies

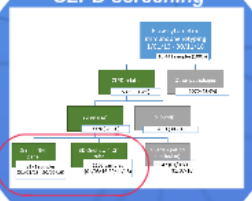


CLPD FCI

FCI (total)



CLPD screening



MBL

Conclusions

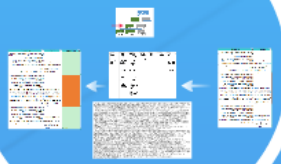
- Selective use --> less normal
- More MBL detected
  - Low-count
  - High-count
- Less B-NHL
  - Washing?
- (reactive)

Conclusions

- Selective use --> less normal
- More MBL detected
  - Low-count
  - High-count
- Less B-NHL
  - Washing?
- (reactive)

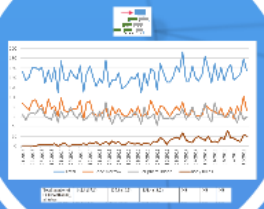
Question 3: clinical impact

Detected pathologies

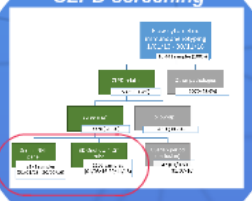


CLPD FCI

FCI (total)



CLPD screening



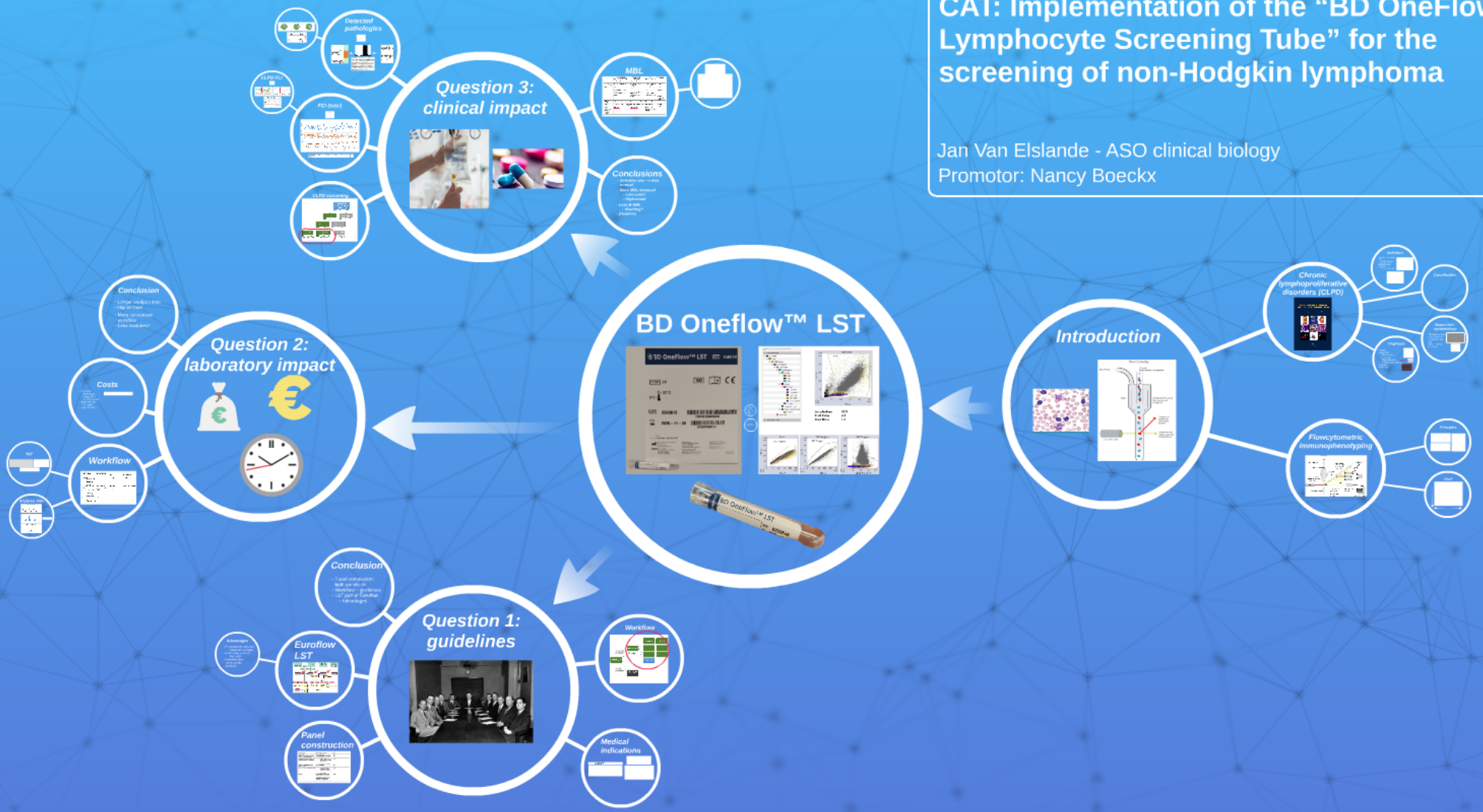
MBL

Conclusions

- Selective use --> less normal
- More MBL detected
  - Low-count
  - High-count
- Less B-NHL
  - Washing?
- (reactive)

CAT: Implementation of the “BD OneFlow™ Lymphocyte Screening Tube” for the screening of non-Hodgkin lymphoma

Jan Van Elslande - ASO clinical biology
Promotor: Nancy Boeckx



Take home

- FCI not always indicated
- First screening panel
- LST
  - Increase cost, analysis time (TAT?)
  - Less mistakes (?) --> quality
- Higher detection MBL
  - Clinical impact?
  - B-NHL?

Questions?

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