DETECTION OF ABERRANT INTRA-EPITHELIAL LYMPHOCYTES IN REFRACTORY CELIAC DISEASE

BSAC, 21-10-2016

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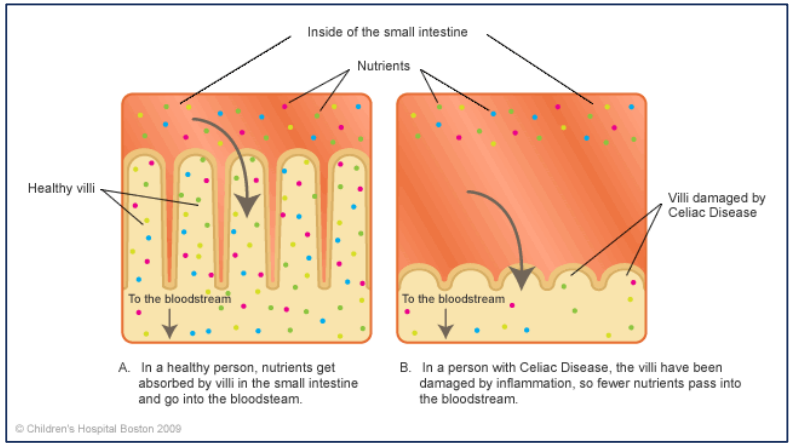
CELIAC DISEASE: WHAT?

- **Auto-immune** disorder that chronically affects **the small intestine**
- Induced by **dietary gluten** in genetically predisposed individuals (alleles encoding HLA-DQ2 or DQ8)
- Worldwide **prevalence ~1%**

CELIAC DISEASE: CLINICAL FEATURES

- **GASTRO-INTESTINAL** signs and symptoms
  - chronic diarrhea and abdominal pain
  - steatorrhea
  - weight loss, failure to thrive, growth failure, anorexia
  - bloating
  - vomiting, …



- **EXTRA-INTESTINAL** signs and symptoms
  - iron-deficiency anemia and other nutritional deficiencies (vitamin B12, vitamin D, folate, zinc, vitamin B6)
  - fatigue, …

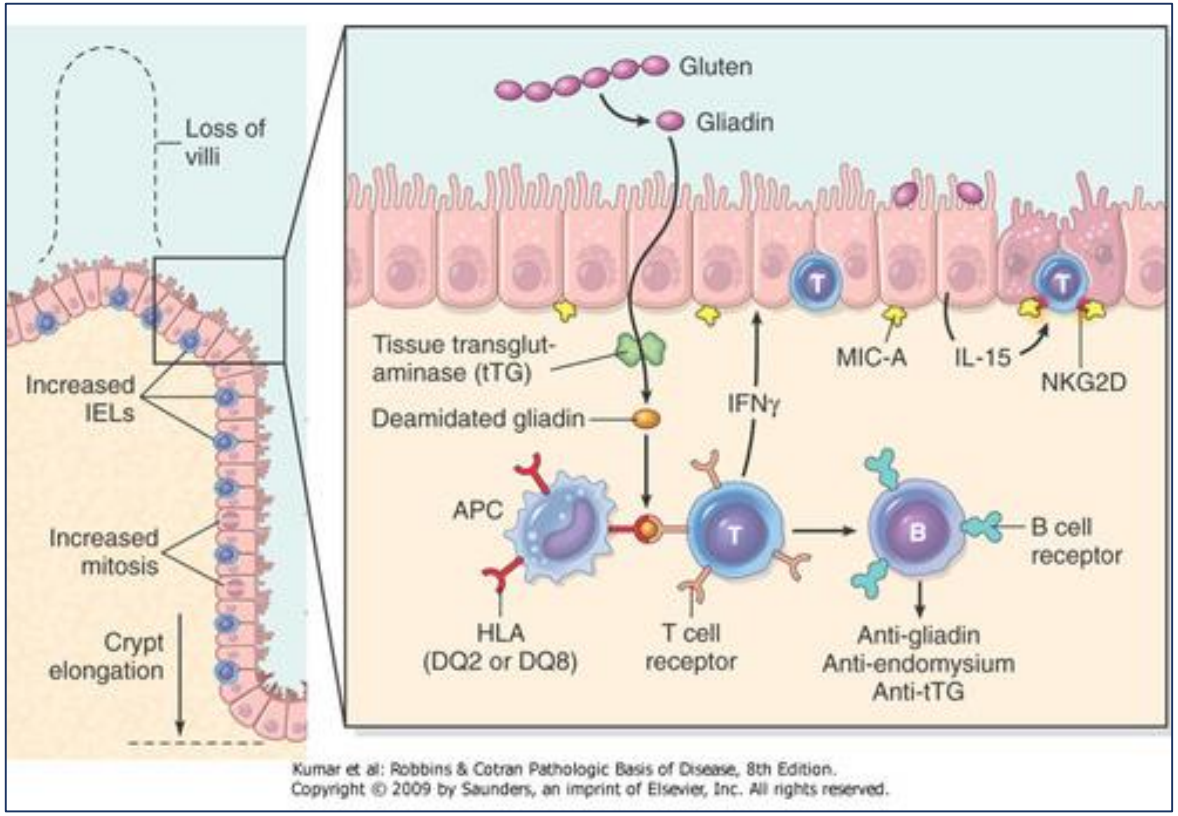
- **ASSOCIATED (AUTOIMMUNE) CONDITIONS**
  - type I diabetes
  - autoimmune thyroid / liver disease
  - Sjögren syndrome, ….

} all associated with HLA risk alleles (HLA haplotypes DQ2 and/or DQ8)

Kelly CP. *et al.* Gastroenterology 2015, 148:1175-86.

CELIAC DISEASE: DIAGNOSIS

1. Serologic markers of celiac disease
   - IgA/IgG against tissue transglutaminase (tTG)
   - Endomysial antibody (IgA)
   - IgA/IgG against deamidated gliadin peptide (DGD)



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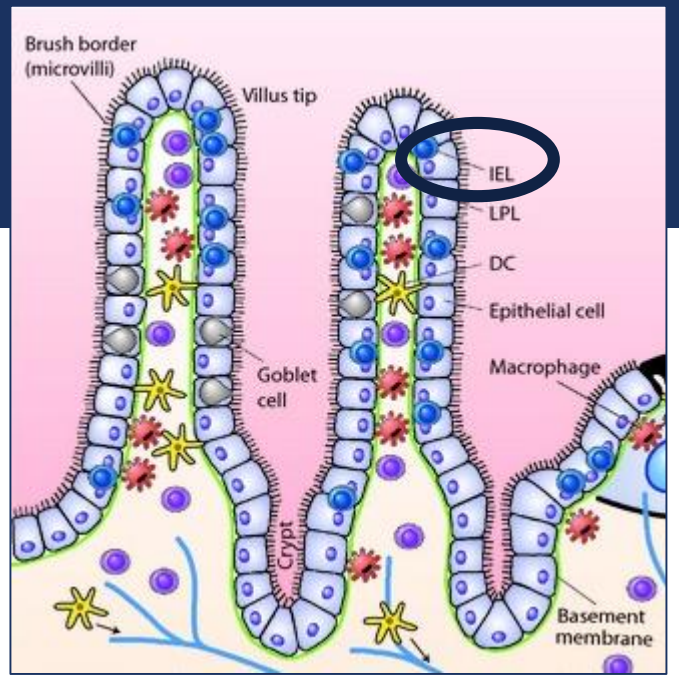

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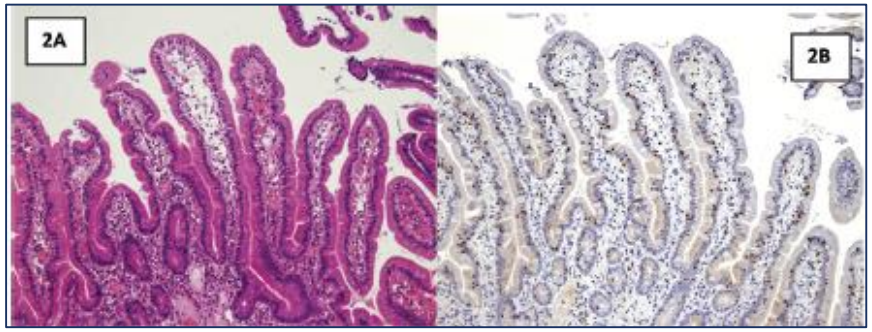
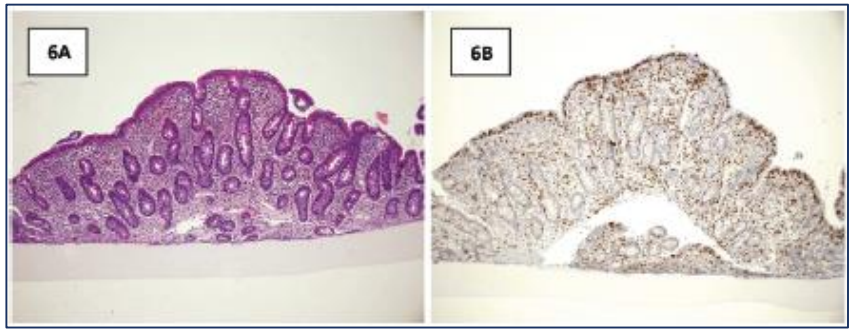
   - Microscopic findings: atrophic villi, crypt hyperplasia, increase in number of intra-epithelial lymphocytes (IEL) (NOT specific for CD)



normal duodenal mucosa



celiac disease

Villanacci V *et al.* Digestive and liver disease, 2011: 385-95.

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3. Genetics
   - Class II HLA types DQ2 and DQ8 (in almost all CD patients, but also in 30-40% of Western Caucasian population; only 3% of individuals with these haplotypes develop CD)

CELIAC DISEASE: TREATMENT

- the only treatment for celiac disease is a **strict gluten-free diet**
  - reduces symptoms, mortality and risk for malignancy
  - lifelong diet (expensive, socially isolating)
  - avoiding
    - wheat (‘*tarwe*’)
    - rye (‘*rogge*’)
    - barley (‘*gerst*’)

OBVIOUS SOURCES OF GLUTEN:

bread, bagels, cakes, cereal, cookies, pasta, noodles, pastries, pies, rolls

REFRACTORY CELIAC DISEASE (RCD)

- persisting or recurring symptoms despite strict adherence to gluten-free diet
  - diarrhea, abdominal pain, involuntary weight loss, …
  - severe malnutrition, protein-losing enteropathy, ulcerative jejunitis, ….
- patients are nearly always adults (50 years or thereafter)
- affects less than 1% of CD patients, but significant morbidity and mortality
- subdivided into 2 types of RCD
  - **RCD type I**
  - **RCD type II**

RCD TYPE I AND II

RCD type I	RCD type II
no increased risk for enteropathy-associated T-cell lymphoma (EATL)	increased risk to develop EATL risk for other gastro-intestinal cancers is not substantially increased

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low numbers of aberrant intra-epithelial lymphocytes (IELs)	high(er) numbers of aberrant IEL
BENIGN => often responds to treatment with eg. topical steroids	**PRE-MALIGNANT** (indolent lymphoma (pre-EATL)) => requires cytotoxic chemotherapeutic therapy, eg. 2-CDA)

PHENOTYPE OF IELs

Normal IELs

- Majority (>70%) of IELs are sCD3+ T-cells
  - TCRab (80%)
    - >85% CD8+
    - only ~10% CD4+
  - TCRgd (5-15%) with variable expression of CD8 (40-80%)
- 10-20% of IELs are CD3- cells

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Aberrant IELs

- T-cells
  - surface CD3-
  - surface CD8-
  - cytoplasmatic CD3+

Clonal expansion of this population is only found in a subgroup of RCD patients and EATL patients

- RCD type I: <20% aberrant IELs
- RCD type II: 20-100% aberrant IELs

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METHODS TO IDENTIFY ABERRANT IELS

1. Immunohistochemistry : CD3 and CD8 staining

2. TCR gene rearrangement studies (γ, ß, δ)

3. Flowcytometric immunophenotyping

METHODS TO IDENTIFY ABERRANT IELS

		
Immunohistochemistry CD3 and CD8 staining	IHC and TCR-clonality studies: • reliable tools to identify dominant aberrant IEL populations • BUT fails to identify a moderate increase of these cells	no differentiation between cyCD3 and sCD3 lower sensitivity: high cut-off (>50% CD3+CD8- of CD3+ IELs) high interobserver variability
TCR gene rearrangement studies (γ, ß, δ)		fails to identify clonal IELs in patients with 20-25% aberrant IELs

van Wanrooij RLJ. *et al.* J. Clin. Imm. 2014, 34: 828-35.
Verbeek WHM. *et al.* Clin. Imm. 2008, 126: 48-56.
Tack GJ. *et al.* Mol. Imm. 2012, 50: 262-70.

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Flowcytometric immunophenotyping **GOLDEN STANDARD**	• can differentiate between cyCD3 and sCD3 • can also identify patients with only a moderate increase in aberrant IELs **(sCD3-CD8-CD7+cyCD3+)**	in 95% of non-refractory CD and control patients, the highest % aberrant T-cells in duodenal biopsy specimens is **20%**

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T-CELL CLONALITY ANALYSIS VERSUS FCM ANALYSIS

	RCD evolving to EATL, N = 10	RCD without EATL, N = 13
Detection of aberrant IELs		
>20% aberrant IELs	10	7
<20% aberrant IELs	0	6
T-cell clonality analysis		
Monoclonal	7*	7
Polyclonal	2	6

* Poor quality DNA, clonality analysis inconclusive

	FCM	Molecular
Sensitivity	100%	78%
Specificity	46%	46%
NPV	100%	75%
PPV	59%	50%

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LYMPHOCYTE SUBSETS IN DUODENAL BIOPSY SPECIMENS AS % OF INTESTINAL LYMPHOCYTES (BY FCM)

Subset	Controls without CD n= 49	Untreated CD n = 17	CD on GFD n = 60	RCD I n = 16	RCD II n = 17	Primary EATL n = 8
CD3+ T-cells						
median (10^{th}-90^{th} percentile)	86 (78-93)	94 (80-97)	90 (78-97)	93 (81-98)	43 (16-63)	90 (55-94)
CD4+ T-cells						
median (10^{th}-90^{th} percentile)	24 (10-44)	19 (9-32)	18 (7-38)	13 (5-29)	13 (3-17)	19 (11-21)
CD8+ T-cells						
median (10^{th}-90^{th} percentile)	56 (39-76)	67 (41-81)	61 (42-79)	70 (52-88)	20 (2-31)	63 (25-64)
CD7+ lymphocytes						
median (10^{th}-90^{th} percentile)	96 (88-98)	95 (85-99)	96 (88-98)	95 (91-99)	96 (90-98)	94 (58-96)
CD16/56+ NK cells						
median (10^{th}-90^{th} percentile)	7 (3-14)	3 (1-7)	5 (1-12)	3 (1-10)	5 (1-17)	4 (0.4-5)
CD19+ B-cells						
median (10^{th}-90^{th} percentile)	0.5 (0.1-3)	2 (0.4-12)	1 (0.1-6)	1 (0.01-3)	1 (0.2-8)	2 (0.01-13)
CD7+CD3-cyCD3+ aberrant T						
median (10^{th}-90^{th} percentile)	4 (1-9)	1 (0.07-4)	2 (0-5)	2 (0.5-10)	52 (34-89)	2 (0.4-7)

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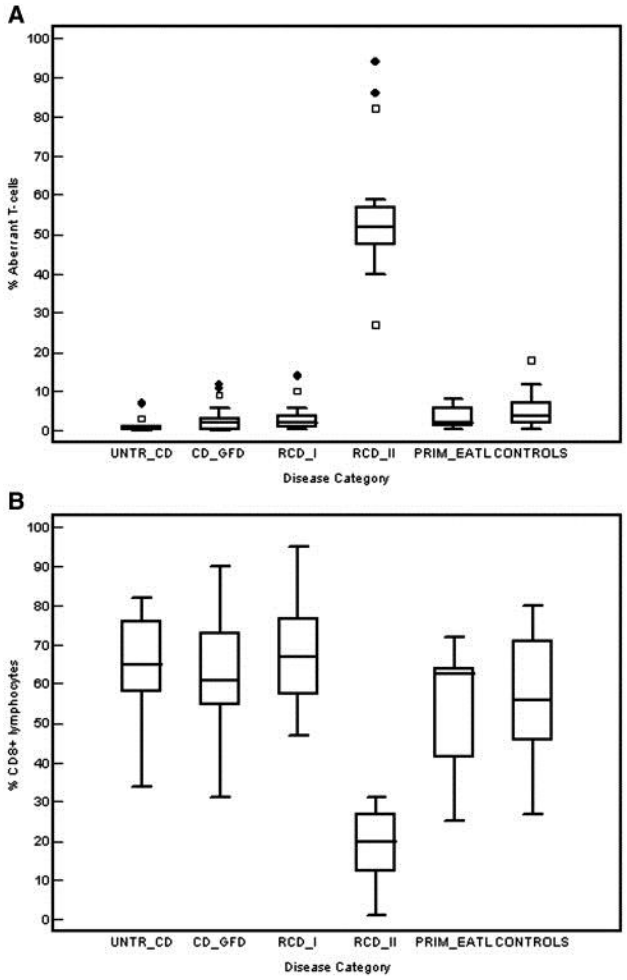

⇒ Percentage aberrant T-cells (CD7+ surface CD3− cytoplasmic CD3+) in duodenal biopsy specimens of each disease category. There were **significantly more aberrant T-cells in the RCD II group** as compared to all other groups, in all cases $p < 0.0001$.

⇒ Percentage CD8+ lymphocytes in duodenal biopsy specimens of each disease category. There were **significantly less CD8+ T-cells in RCD II** as compared to all other groups, in all cases $p < 0.0001$.

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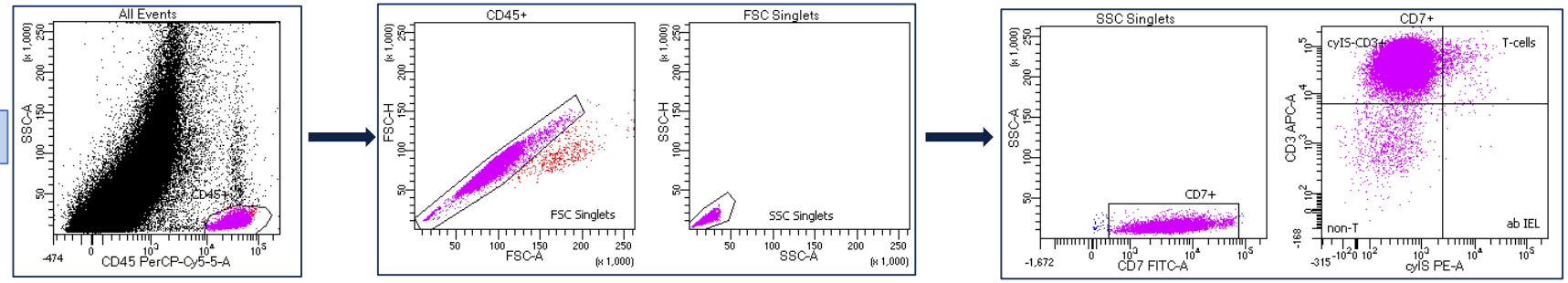
FCM ANALYSIS: ISOLATION AND STAINING OF IELS

- 4 – 8 biopsies (stored in PBS at 0-4°C)
- isolation of IELs from intestinal biopsies
  - no chemical or enzymatic treatment
  - done by vigorous shaking : 60 min at 37°C (can also be done at room temperature)
    - calcium chelants (DTT, EDTA): induces the disassembly of inter-epithelial junctions and the release of epithelial cells and IELs
    - ~100.000 IELs per cubic millimeter small bowel biopsies (1 x 1 x 1 mm): enough for staining of IELs required for diagnosis and monitoring of CD (IELs will constitute ~5% (1-10% range) of the released cells)
    - **IELs in supernantant**

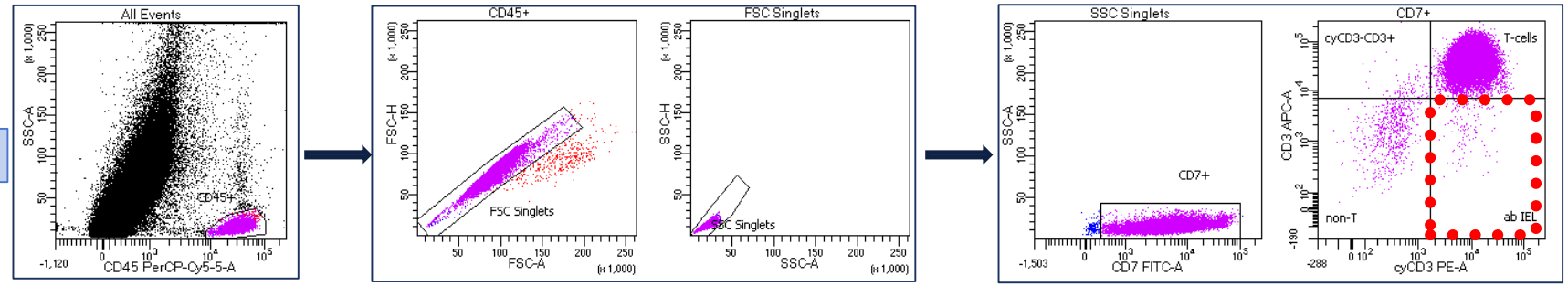
FCM ANALYSIS: PANEL AND GATING STRATEGY

- CD3 – CD16/56 – CD45 – CD19 – CD4 – CD8
- CD7 – cy isotype – CD45 – sCD3
- CD7 – cy CD3 – CD45 – sCD3

CD7/cy isotype/CD45/sCD3



CD7/cyCD3/CD45/sCD3



CLINICAL CASE 1

	Case 1 **M, 57y**
Main clinical problem(s)	2011: celiac disease, R/ GFD 6-2015: vomiting, anorexia, weight loss, diarrhea, …. => **RCD**
Pre treatment FCM % aberrant IEL	11-2015: FCM on intestinal biopsy
RCD: type I or II?	
Post Cladribine/ Everolimus FCM % aberrant IEL	

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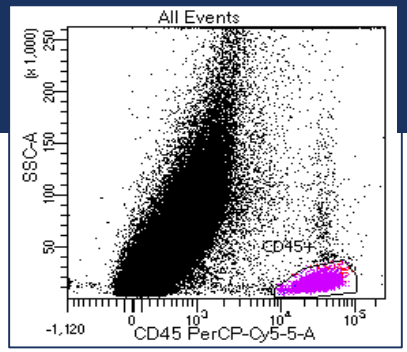
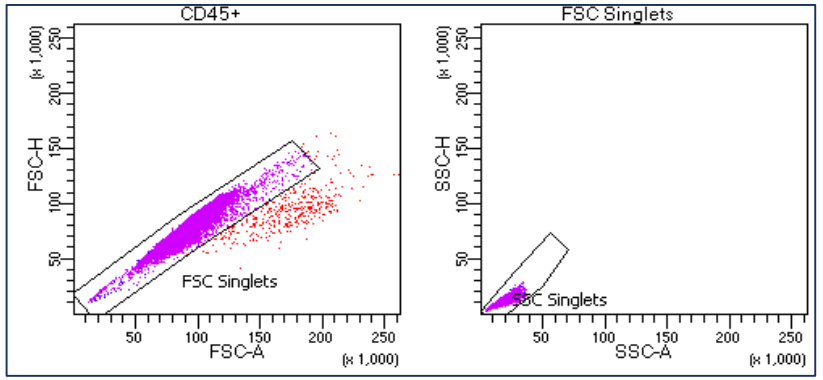
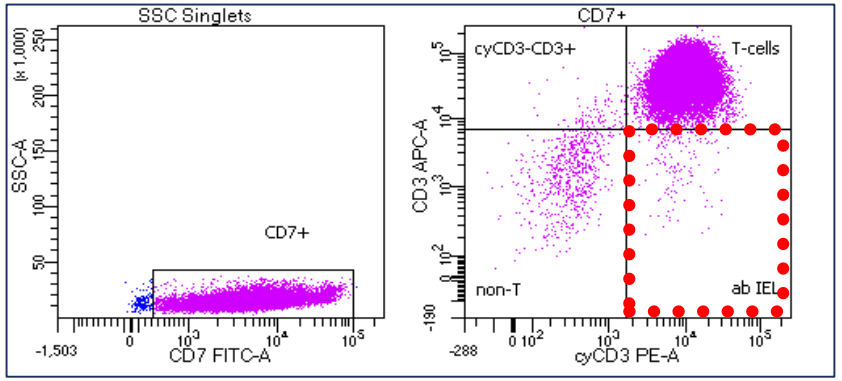
Main clinical problem(s)	2011: celiac disease, R/ GFD 6-2015: vomiting, anorexia, weight loss, diarrhea, …. => **RCD**
Pre treatment FCM % aberrant IEL	11-2015: FCM on intestinal biopsy **<1%**
RCD: type I or II?	**type I**
Post Cladribine/ Everolimus FCM % aberrant IEL	NA







CLINICAL CASE 2

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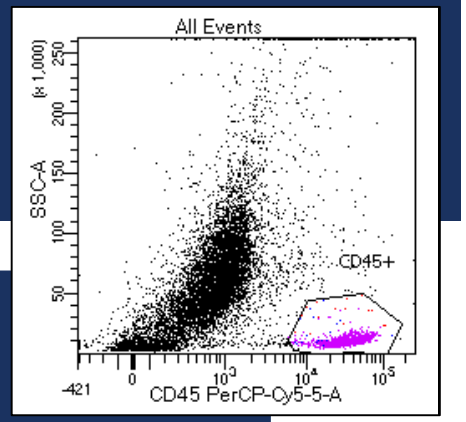
Main clinical problem(s)	2002: celiac disease, R/ GFD 2013: dysphagia, weight loss, vomiting, …despite GFD => **RCD** 2013-2015: multiple gastroscopies: no macro- / microscopic evidence of progression towards lymphoma
Pre treatment FCM % aberrant IEL	7-2015: FCM on intestinal biopsy **96%**
RCD: type I or II?	**type II** => R/ Cladribine + Everolimus

CLINICAL CASE 2



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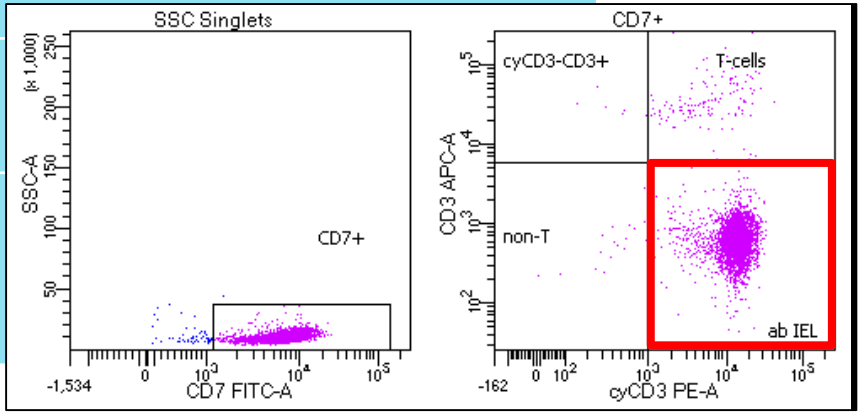
Post Cladribine / Everolimus FCM % aberrant IEL	3-2016: FCM on intestinal biopsy **94%**



CLINICAL CASE 3

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Main clinical problem(s)	2002: celiac disease, R/ GFD 11-2014: weigth loss, diarrhea, … despite strict GFD => **RCD**
Pre treatment FCM % aberrant IEL	1-2015: FCM on intestinal biopsy **73%**
RCD: type I or II?	**type II** => R/ Cladribine

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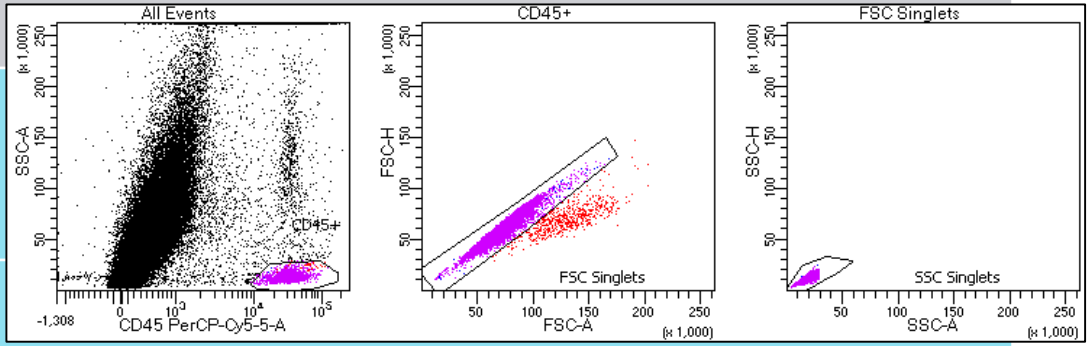
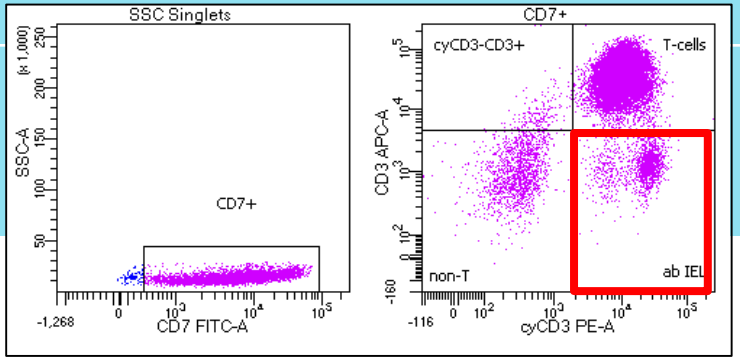
Pre treatment FCM % aberrant IEL	1-2015: FCM on intestinal biopsy **73%**
RCD: type I or II?	**type II** => R/ Cladribine
Post Cladribine FCM % aberrant IEL	3-2016: FCM on intestinal biopsy **9%**





TAKE HOME MESSAGES

- RCD type II patients are at risk for development of EATL
- FCM is well suited for the identification of RCD type II patients
- A cut-off value off 20% aberrant IELs appears reliable for early risk stratification and targeted therapeutic options in RCD patients
- Quantification of aberrant IELs is usefull for subsequent follow-up of treated RCD II patients

ACKNOWLEDGEMENTS

Dept. of Laboratory Medicine

S. Govers

N. Van den Panhuyzen

Dept. of Gastroenterology

M. Hiele

T. Vanuytsel

Dept. of Hematology

P. Vandenberghe

G. Verhoef

Dept. of Gastroenterology

K. Nys

J. Cremer

S. Vermeire

T. van Gils

HJ. Bontkes

CJJ. Mulder

G. Bouma