

Sézary Syndrome (and mycosis fungoides)

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- Programme section / Accreditation page

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Cutaneous T-cell Lymphomas (CTCLs)

- CTCLs are T-cell lymphomas largely confined or predominantly involving the skin at diagnosis.
- 9 WHO disease categories.
- CTCL is also commonly used to refer to MF and SS.
- Other T-cell lymphomas can frequently present with or develop skin involvement.

Mycosis fungoides/Sézary

Mycosis fungoides
Sézary syndrome

CD30-positive primary cutaneous lymphoproliferative disorders

Lymphomatoid papulosis
Primary cutaneous anaplastic large cell lymphoma

Variably aggressive cutaneous T-cell lymphomas

Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma
Primary cutaneous gamma/delta T-cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma

Typically indolent cutaneous T-cell lymphomas


Primary cutaneous CD4 positive small/medium T-cell lymphoproliferative disorder.
Primary cutaneous acral CD8-positive T-cell lymphoma.

Other T-cell lymphomas with frequent skin involvement

Extranodal NK/T-cell lymphoma, nasal type
Adult T-cell leukemia/lymphoma
T-cell prolymphocytic leukemia
EBV+ TNK lymphoproliferative disorders of childhood

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Sézary syndrome




- Rare, aggressive type of CTCL.
- Diagnostic Criteria**
 - Erythroderma
 - >1000 Sézary cells/ μ L of blood.

Jawed SI, et al. J Am Acad Dermatol 2014;70:205.e1-16

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Mycosis fungoides



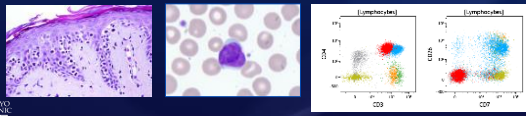
- Most common CTCL.
- Slowly progressive / protean clinical course.

Jawed SI, et al. J Am Acad Dermatol 2014;70:205.e1-16

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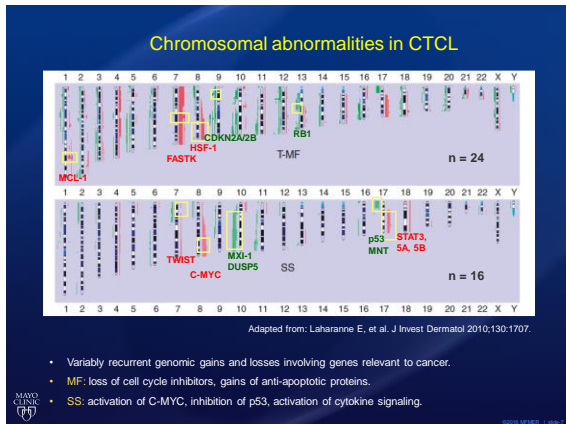
Mycosis Fungoides and Sézary syndrome

- Morphologically indistinguishable skin, blood and lymph node involvement.
- The immunophenotype of SS and most MFs is essentially identical.
- MF can progress to an erythrodermic phase identical to SS.
- MF and SS share the same staging system.
- Both entities are commonly accepted in the same clinical trials.

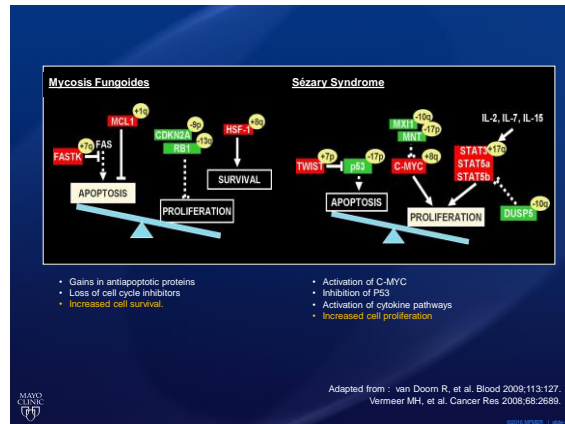


Jawed SI, et al. J Am Acad Dermatol 2014;70:205.e1-16

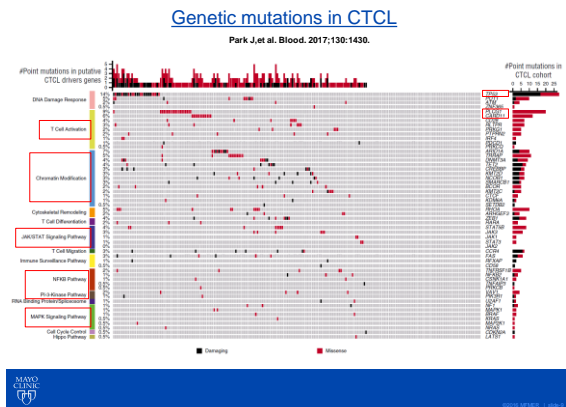
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Blood Staging in Sézary Syndrome and Mycosis Fungoides

- Currently, no widely utilized phenotypic, genetic or molecular biomarkers for prognosis.
- Prognosis in CTCL relies largely on tumor burden.
- Quantitative assessment of **peripheral blood tumor burden** is essential for staging and prognosis.

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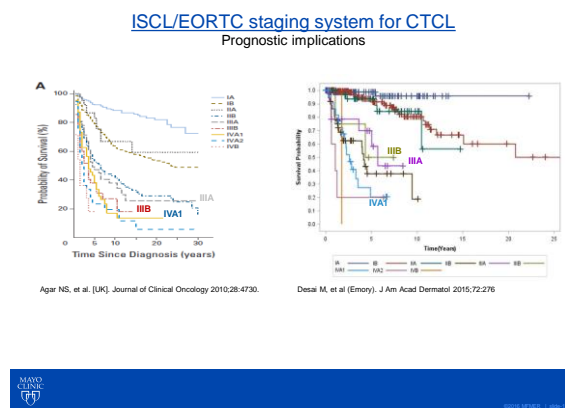
ISCL/EORTC staging system for CTCL

STAGE I	IA: <10% BSA IB: >10% BSA
STAGE II	IIA: Lymphadenopathy (non-effaced) IIB: Skin tumors
STAGE III	IIIA: Sézary cell count < 250/μL IIIB: Sézary cell count ≥ 250/μL
STAGE IV	IVA1: Sézary cell count ≥ 1000/μL IVA2: lymphadenopathy (effaced) IVB: Visceral Involvement

- Most stage IV disease defined by blood tumor burden.
 - Excisional lymph node biopsies rarely performed.
 - Visceral involvement is rare in CTCL.
- Flow cytometry is the recommended method to assess peripheral blood tumor burden.
 - Morphometry has large been discontinued in most centers.

Olsen E, et al. Blood 2007;110(6):1713. Scarisbrick JJ, et al. Eur J Cancer 2018; 93:47

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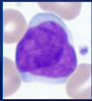
Basic immunophenotypic features

Mycosis Fungoides

- Most cases are CD4+
 - Well described CD8+ cases
 - Few CD4-/CD8-
- Mostly cases are TCRαβ+
 - Well described TCRγδ+ subset
- Almost all cases that progress to peripheral blood involvement on CD4+ and TCRαβ+

Sézary Syndrome

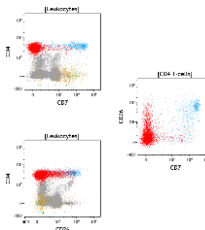
- CD4+
 - Rare CD4-/CD8-
- TCRαβ+



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Basic immunophenotypic features of Sézary cells

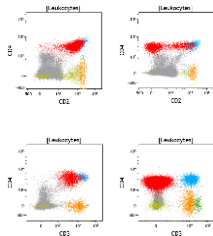
- CD7** Partially or completely negative in 50-80% of cases. Frequent variable loss in reactive CD4 T-cell subsets.
- CD26** Partially or completely negative in 80-100% of cases. Frequent variable loss in reactive CD4 T-cell subsets.



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Basic immunophenotypic features of Sézary cells

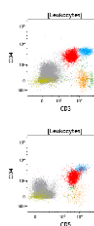
- CD2** Positive. Slight dim expression in 40-70% of cases. Occasional partial or complete negativity.
- CD3** Positive. Slight dim expression in 40-80% of cases. Rare partial or complete negativity.



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Basic immunophenotypic features of Sézary cells

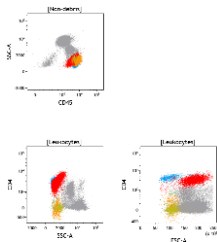
- CD4** Positive. Slight dim expression in 30-50% of cases. Rare partial or complete negativity.
- CD5** Slightly dim expression inconsistently reported in 10-30% of cases. Rare partial or complete negativity.



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Basic immunophenotypic features of Sézary cells

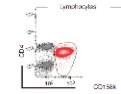
- CD45** Bright positive, occasional slightly dim expression.
- FSC/SSC** Frequent slight increase in FSC and SSC. Occasional marked increase in FSC (large cells).



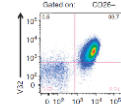
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Other useful immunophenotypic features of Sézary cells

- CD158k (KIR3DL2)** Positive on 20% to 80% of cases, might depend on the antibody utilized.
- CD164** Variably overexpressed in most cases.



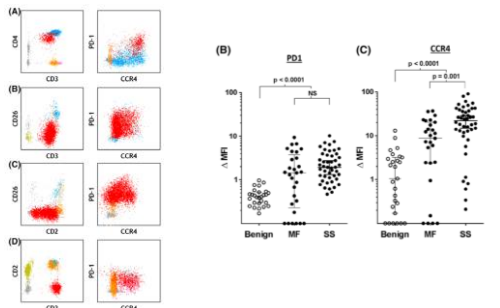
Mons-Telserenc H, et al. J Invest Dermatol 2015;135:247.



Wysocka M, et al. J Invest Dermatol 2014;134:229.

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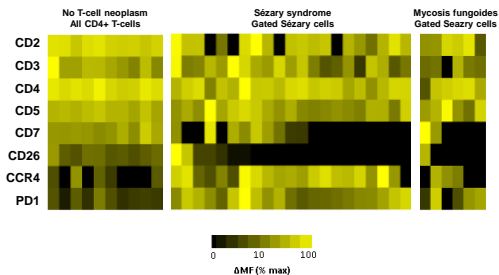
Other useful immunophenotypic features of Sézary cells
CCR4 and PD-1



Horna P, et al. Cytometry B Clin Cytom. 2019 May;96(3):234.

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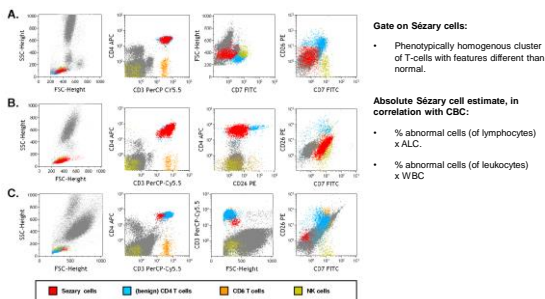
Immunophenotypic features of Sézary cells



Horna P, et al. Cytometry B Clin Cytom. 2019 May;96(3):234.

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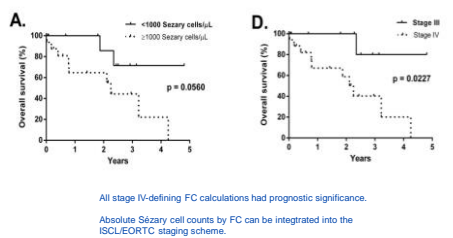
Identification and quantitation of Sézary cells by flow cytometry



Horna et al. J Clin Pathol 2014;67:431

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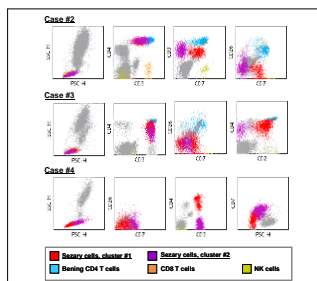
Retrospective study on 28 patients with erythrodermic CTCL



Horna et al. J Clin Pathol 2014;67:431

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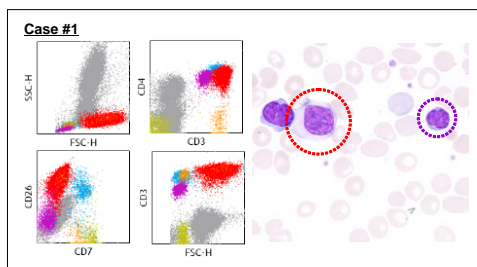
Sézary cells often show two phenotypically distinct subsets



Kirees A, Sokol L, Horna P. Poster at: International Clinical Cytometry Society Meeting 2013, Ft Lauderdale, FL.

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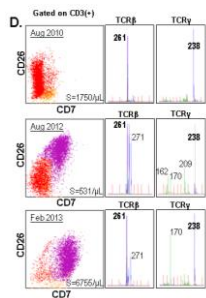
Sézary cells with two phenotypically distinct subsets



Kirees A, Sokol L, Horna P. Poster at: International Clinical Cytometry Society Meeting 2013, Ft Lauderdale, FL.

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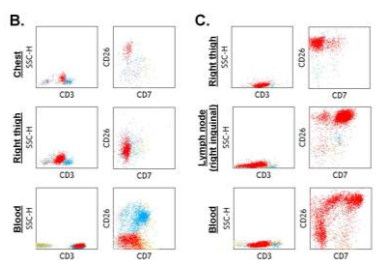
Sézary cells with two phenotypically distinct subsets



Horna et al. J Clin Pathol 2014;67:431

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The immunophenotype of Sézary cells can vary depending on the anatomic location

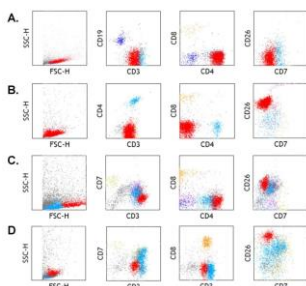
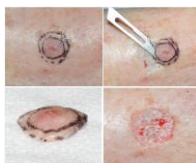


Horna et al. AJCP 2015;143:785

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Patch/plaque MF skin lesions often show bright CD26 and/or occasional CD4(-)/CD8(-) phenotype

33 shave biopsies from 31 MF patients



- CD4(-)/CD8(-): 3 cases, 16%
- Bright CD26: 11 cases, 58%

Horna et al. AJCP 2015;143:785

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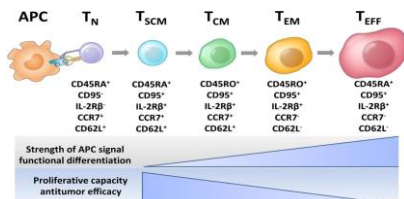
Immunophenotypic features of Sézary cells

- More complex and varied than originally appreciated.
- Most have loss of CD7 and CD26 (overlaps with reactive).
- Most have slight decreased expression CD2, CD3, CD4 and/or CD5.
- Many have increased light scatter.
- The immunophenotype can vary through time and on different anatomic locations.
- Two immunophenotypically distinct tumor clusters is not a rare finding.

Horna et al. AJCP 2015;143:785

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Naïve/memory phenotype of Sézary cells



Horna et al. AJCP 2015;143:785

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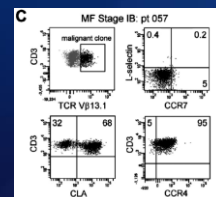
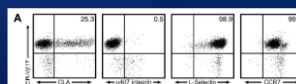
Naïve/memory phenotype of MF and SS
Different cell of origin?

Sézary Syndrome

- Central memory phenotype
- CD62L(+), CCR7(+)
- CLA(-)

Mycosis Fungoides

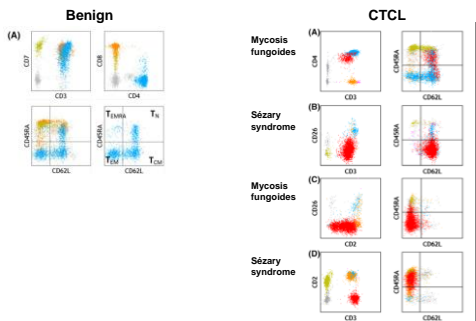
- Effector memory phenotype
- CD62L(-), CCR7(-)
- CLA(+)



Campbell JJ, et al. Blood. 2010;116:767.

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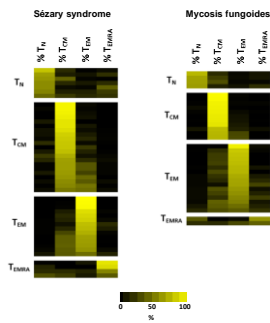
Naïve/memory phenotype of peripheral blood Sézary cells



Horna P, et al. Cytometry B Clin Cytom. 2019 May;96(3):234.

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Naïve/memory T-cell phenotype of Sézary cells

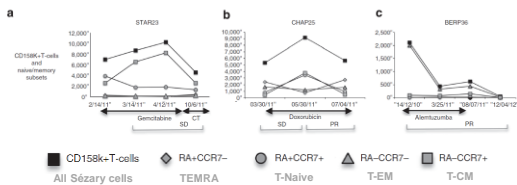


- 79 blood or marrow samples from 33 SS and 19 MF patients.
- Naive/memory phenotype is broadly heterogeneous both for SS and MF.
- No difference in naive/memory phenotype between MF and SS.

Horna P, et al. Cytometry B Clin Cytom. 2019 May;96(3):234.

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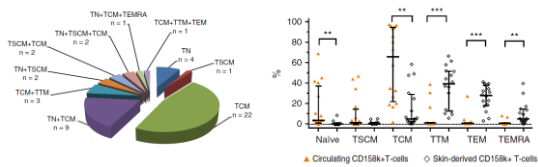
Naïve/memory phenotype of Sézary cells over time



Moins-Teisserenc H, et al. J Invest Dermatol 2015;135:247

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Naïve/memory phenotypic plasticity of Sézary cells



In many cases, Sézary cells show a mix of naive and memory phenotypes.

Sézary cells in blood tend to have a less activated phenotype that Sézary cells in skin.

Roelens M, et al. Blood 2017;130:1468

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Naïve/memory phenotype of Sézary cells

- Is highly variable.
- No difference between MF and SS.
- Can vary with time and depending on anatomic site.
- Likely indicative of a functional/activation state, rather than a "cell or origin".

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Flow cytometric assessment of T-cell clonality for the identification of Sézary cells

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Assessment of T-cell clonality by TCR-Vβ repertoire analysis

Most T-cells have a surface T-cell receptor consisting of an α and a β chain.

Each chain is the product of the genetic rearrangement of a constant region with a random selection of variable, D and J regions.

Responsible for the large repertoire of antigen specificities.

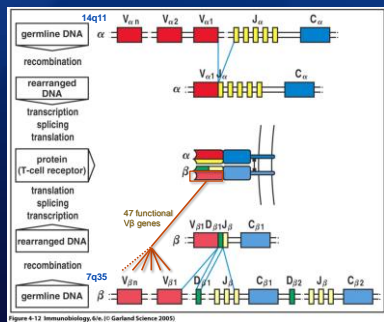


Figure 4-12 Immunobiology, 6/e. © Garland Science 2005

Demonstration of clonality of Sézary cells by TCR-Vβ analysis

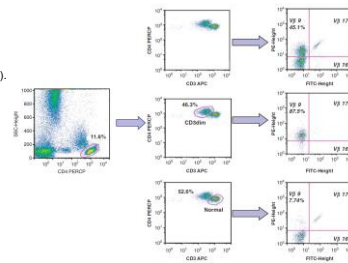
IOtest Beta Mark (Beckman Coulter).

- 24 Vβ-specific antibodies in 8 tubes (FITC, PE, and FITC + PE).
- Recognize 70% of Vβ classes.

Combined with 2 or more informative T-cell antigens.

Clonal T-cells:

- >50% positive for a single Vβ class.
- >70% negative for all classes



Feng B, et al. Mod Pathol. 2010;23:284-295.

Limitations of TCR-Vβ repertoire analysis by flow cytometry

- Technically demanding and time consuming.
 - Set up of 8 additional tubes after initial T-cell analysis.
- Not a simple analysis, requires expertise.
- Significant increase in costs.
 - 24 additional antibodies.
- Limited ability to analyze phenotypically distinct T-cell subsets.
- 30% of the TCR-Vβ repertoire not covered by the analysis.

Assessment of T-cell clonality by TCR Cβ restriction

2 TCR-Cβ genes: TRBC1 and TRBC2.

Their selection is random and mutually exclusive.

Similar to kappa and lambda.

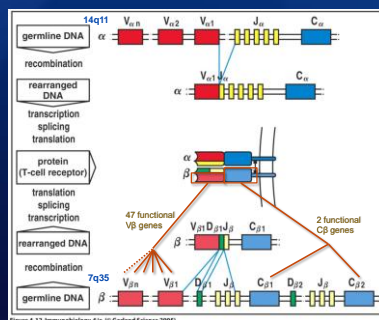


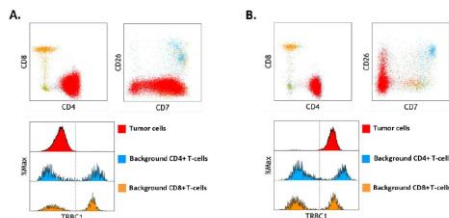
Figure 4-12 Immunobiology, 6/e. © Garland Science 2005

Assessment of T-cell clonality by TCR Cβ restriction

- Antibody clone JOVI-1:
 - Specific for T-cell receptor β constant region 1 (TRBC1).
 - Maccioia PM et al. Nat Med 2018; 23:1416.
 - Available from various vendors.
- Currently, no anti-TRBC2 antibody available for flow cytometry.
- TCR Cβ restriction can be inferred by pattern of staining for TRBC1.
- A single antibody could routinely included in comprehensive T-cell panel.

Assessment of T-cell clonality by TCR Cβ restriction

Examples of Sézary syndrome

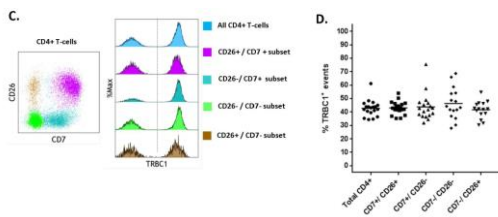


Clonal T-cells defined by:

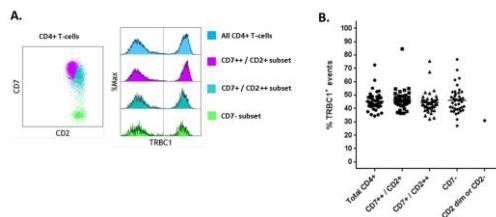
- >85% TRBC1-positive (TRBC1-restricted).
- <15% TRBC1-positive (TRBC2-restricted).
- Dominant TRBC1-intermediate population.

Shi M, et al. Cytometry B Clin Cytom. 2019 [Epub ahead of print]

Assessment of T-cell clonality by TCR C β restriction
CD4+ T-cells subsets from patients with no T-cell malignancy



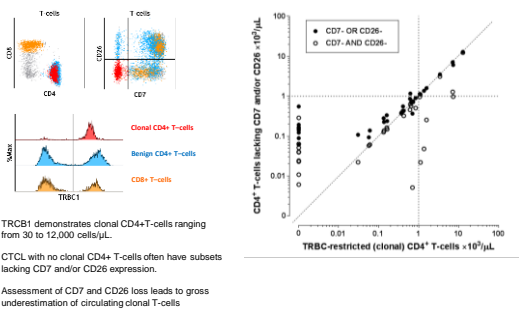
Assessment of T-cell clonality by TCR C β restriction
CD4+ T-cells subsets from patients with no T-cell malignancy



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Quantitation of clonal CD4 T-cells by TCR C β restriction in patients with CTCL
33 peripheral bloods from 24 CTCL patients

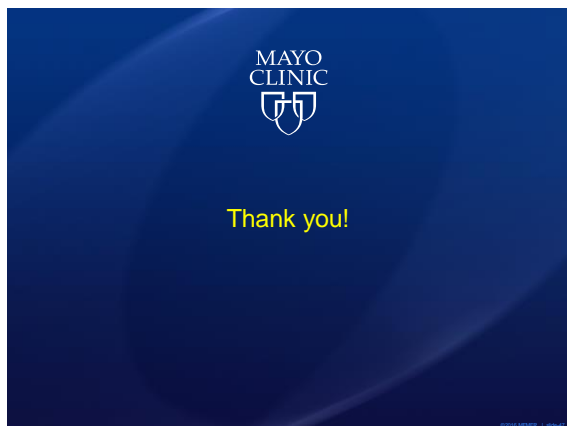


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CONCLUSIONS

- Sézary syndrome (SS) and mycosis fungoides (MF) are two clinically distinct entities.
- The tumor cells (Sézary cells) in MF and SS show remarkable similarities in terms of morphology, infiltration pattern, immunophenotype.
- Detection and quantitation of Sézary cells by flow cytometry is essential for staging and prognosis in SS and MF.
- The phenotype of Sézary cells is complex, variable and prone to be unstable, requiring comprehensive flow cytometric evaluation of several T-cell antigens for accurate identification.
- Routine single-antibody assessment of T-cell clonality with a T-cell flow cytometry panel might resolve many of the diagnostic uncertainties in the detection of Sézary cells.

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