Cutaneous T-cell Lymphomas (CTCLs)

- CTCLs are T-cell lymphomas largely confined or predominately 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.

Sézary Syndrome

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

Mycosis fungoides

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

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.

Conflict of Interest Disclosure

In accordance with criterion 24 of document UEMS 2012/30 "Accreditation of Live Educational Events by the EACCME®" we herewith declare to have submitted a Conflict of Interest Disclosure Form to ESCCA.

This COI Disclosure Form can be viewed at the ESCCA 2019 Conference website www.escca.eu/norway2019 - Programme section / Accreditation page
Chromosomal abnormalities in CTCL

- Variable recurrent gains and losses involving genes relevant to cancer.
- MF: loss of cell cycle inhibitors, gain of anti-apoptotic proteins.
- SS: activation of C-MYC, inhibition of p53, activation of cytokine signaling.

Genetic mutations in CTCL


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.

ISCL/EORTC staging system for CTCL

Mycosis Fungoides

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

Sézary Syndrome

- CD4+
- Rare CD4-/CD8+
- TCRγδ+

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.

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.

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.

CD158k (KIR3DL2) Positive on 20% to 80% of cases, might depend on the antibody utilized.

CD164 Variably overexpressed in most cases.
Other useful immunophenotypic features of Sézary cells

CC4 and PD-1

Immunophenotypic features of Sézary cells

Identification and quantitation of Sézary cells by flow cytometry

Sézary cells often show two phenotypically distinct subsets

Retrospective study on 28 patients with erythrodermic CTCL

Sézary cells with two phenotypically distinct subsets
Sézary cells with two phenotypically distinct subsets

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

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

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

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.

Naïve/memory phenotype of MF and SS

Different cell of origin?

Naïve/memory phenotype of Sézary cells

Sézary Syndrome
Central memory phenotype
- CD62L(−), CCR7(−)
- CLA(−)

Mycosis Fungoides
Effector memory phenotype
- CD62L(−), CCR7(−)
- CLA(−)
Naïve/memory phenotype of peripheral blood Sézary cells

- Benign
- CTCL

Mycosis fungoides
Sézary syndrome
Mycosis fungoides
Sézary syndrome


Naïve/memory phenotype of Sézary cells over time


Naïve/memory phenotypic plasticity of Sézary cells

In many cases, Sézary cells show a mix of naïve and memory phenotypes.
Sézary cells in blood tend to have a less activated phenotype than Sézary cells in skin.


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".

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

Assessment of T-cell clonality by TCR Vβ repertoire analysis

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.
- 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

- Antibody clone JOVI-1:
  - Specific for T-cell receptor β constant region 1 (TRBC1).
  - 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.

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.

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.
Assessment of T-cell clonality by TCR Cβ restriction
CD4+ T-cell subsets from patients with no T-cell malignancy

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, and 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.