ESMO Research Research Fellowship

(02/2020-06/2021)

Gabor Dobos

FINAL REPORT

Host Institute: **Hôpital Saint-Louis, AP-HP, Paris**
Mentor: **Prof. Martine Bagot**
Project title: **Evaluation of a combination of biomarkers for an early diagnosis of mycosis fungoides**

Home Institute: **Klinik für Dermatologie, Venerologie und Allergologie, Charité-Universitätsmedizin Berlin**

Introduction

Cutaneous lymphomas are frequent form of extranodal lymphomas. The majority of them are cutaneous T-cell lymphomas (CTCL), with an incidence of approximately 1/100000[1]. Mycosis fungoides (MF) is the most frequent form, representing 50-72% of the cases[1-3]. This disease shows an age-dependent increase with a median age of 57 years and a male/female ratio of 2/1 [4]. MF is characterized by red patches and plaques on the skin with severe itching and may lead to a decrease in the quality of life. We currently have only palliative treatment options, with no definitive cure. Approximately 20% of cases may progress to tumour stage or erythroderma with a reduced overall survival [1, 4, 5]. The diagnosis of MF is difficult and frequently delayed, in particular in the absence of real clinical distinction from other benign inflammatory dermatoses. MF histology is characterized by atypical subepidermal and epidermotropic lymphocytes. Immunohistochemistry is used to confirm the cutaneous T-cell infiltration and the progressive loss of lymphocyte epitopes (*e.g.* CD7, CD26), but the histological pattern analysis in early stages is quite difficult to clearly differentiate MF from benign skin diseases [1, 4]. Thus, there is an unmet need to identify specific and sensitive MF markers to favour an early diagnosis.

Previous studies of the host institution investigated numerous biomarkers of Sézary syndrome and advanced MF *in situ* or in peripheral blood samples [6-9]. A combination of five blood markers (T-plastine, Twist, Tox, NKp64 and KIR3DL2) were identified for the diagnosis of advanced stage CTCLs using q-PCR [9]. This study also showed a promising 10-30% expression of these specific blood features in patients with early forms of MF.

Rationale and Aim

The aim of this project was to identify the diagnostic and possibly prognostic value of circulating tumour markers in the blood of patients with a diagnosed early form of MF. An array of 10 markers will be measured by using flow cytometry and transcriptomic analysis using microfluidic qRT-PCR on purified circulating $CD4^+$ T-cells.

Experimental design

A French national cohort of blood samples from 620 patients diagnosed as having either a suspected / confirmed MF or an inflammatory skin dermatosis is currently collected by 27 dermatological centres over the country. After isolation from blood, peripheral mononuclear cells were cryopreserved in liquid nitrogen for future analysis. Each patient enrolled in the cohort will then be clinically investigated in order to evaluate the clinical outcome one year after collection of the blood sample. The first patient of the cohort was included in January 2018, almost 550 patients have been included and the study is going on until 620 patients are recruited before the end of Sept. 2019. All patients have given their written informed consent. The study was registered, authorized by the institutional review boards and conducted in accordance with the current version of the declaration of Helsinki.
For each collected blood sample, cryopreserved lymphocytes will be assessed for T-cell phenotype by flow cytometry, including CD3, CD4 and CD8 staining, and expression of selected CTCL markers (*e.g.* KIR3DL2). Isolation of $CD4^+$ T-cells will be performed by magnetic cell sorting before processed for mRNA extraction in order to quantify the expression

of the ten selected tumour markers using microfluidic q-PCR. Statistical analysis will be conducted in SPSS. The endpoint of the project will be the comparison of marker expression between patients identified as confirmed MF and patients presenting a benign inflammatory dermatoses. The sensitivity and specificity of the biomarkers will be quantitatively determined by calculating the areas under the curve (AUC).

Results, Conclusions and Future Perspectives

Due to the pandemy a part of the laboratory analyses and the data analysis was impaired and delayed. The last patient was included on the 28.01.2020 in the study. The follow up of the patients ended already. The data management and resolution of queries is ongoing. The scripts for the statistical analysis are finished. The analysis and interpretation of the results is ongoing.

Nevertheless, the first part of the study was successfully published in high-impact dermatological journal. Future publications are currently under review.

During the prolongation of the fellowship three additional manuscripts were finished. All of them were submitted to oncologic or dermatologic journals, all are currently under review. The ESMO Fellowship was acknowledged in the funding section.

Additionally, the results of three of our projects were presented at the annual meeting of the European Organization for research and Treatment of Cancer – Cutaneous Lymphoma Task Force (EORTC-CLTF) in 2021 in Marseille, France. Altogether during the ESMO Translational Fellowship four first-authored original articles were accepted. Additionally, four case reports and a review article were published and research results were presented at various conferences.

List of Publications and Presentations Resulting from the Translational Research Project "*Evaluation of a combination of biomarkers for an early diagnosis of mycosis fungoides*"

Dobos G, De Cevins C, Ly Ka So S, Jean-Louis F, Mathieu S, Ram-Wolff C, Resche-Rigon M, Bensussan A, Bagot M, Michel L. The value of five blood markers in differentiating mycosis fungoides and Sézary syndrome: a validation cohort. Br J Dermatol. 2020 Dec 14.
PMID: 33314029
doi: 10.1111/bjd.19719.

List of Publications and Presentations resulting from other projects during the fellowship period (if applicable)

Dobos G, de Masson A, Ram-Wolff C, Beylot-Barry M, Pham-Ledard A, Ortonne N, Ingen-Housz-Oro S, Battistella M, D Incan M, Rouanet J, Franck F, Vignon-Pennamen MD, Franck N, Carlotti A, Boulinguez S, Lamant L, Petrella T, Dalac S, Joly P, Courville P, Rivet J, Dereure O, Amatore F, Taix S, Grange F, Durlach A, Quéreux G, Josselin N, Moulonguet I, Mortier L, Dubois R, Maubec E, Laroche L, Michel L, Templier I, Barete S, Nardin C, Augereau O, Vergier B, Bagot M. Epidemiological changes in cutaneous lymphomas: an analysis of 8,593 patients from the French Cutaneous Lymphoma Registry. Br J Dermatol.
PMID: 33131055
DOI: 10.1111/bjd.19644.

Dobos G, Pohrt A, Ram-Wolff C, Lebbé C, Bouaziz JD, Battistella M, Bagot M, Masson A. Epidemiology of Cutaneous T-Cell Lymphomas: A Systematic Review and Meta-Analysis of 16,953 Patients. Cancers (Basel). 11;12(10):2921.
PMID: 33050643
DOI: 10.3390/cancers12102921.

Trager M, Farmer K, Ulrich C, Basset-Seguin N, Herms F, Geskin LJ, Bouaziz JD, Lebbé C, de Masson A, Bagot M*, **Dobos G***. Actinic Cheilitis: A Systematic Review of Treatment Options. J Eur Acad Dermatol Venereol. in press, ms no. JEADV-2020-1417.R2. (shared last authorship)
PMID: n.a.
DOI: 10.1111/jdv.16995

Oral presentations:
- Global epidemiology of primary cutaneous lymphomas: a systematic review of relative frequencies and meta-analysis (February 2020), Presentation at the World Congress of Cutaneous Lymphomas, Barcelona, Spain
- Genic signature of cutaneous T-cell lymphoma-associated fibroblasts from mycosis fungoides and Sézary syndrome (February 2020), Presentation at the World Congress of Cutaneous Lymphomas, Barcelona, Spain
- Die weltweite Epidemiologie der kutanen T-Zell Lymphome: eine systematische Literaturübersichtsarbeit und Meta-Analyse von 16953 Patienten (September 2020), Presentation at the Annual Conference of the Skin Cancer Group of the German Cancer Society, Online, Germany
- Changements dans l´épidémiologie des lymphomes cutanés primitifs en France : une analyse de 8593 patients du registre du Groupe Français d'Etude des Lymphomes Cutanés (GFELC) (December 2020) Presentation at the Journées Dermatologiques de Paris, Annual Conference of the French Dermatology Society, Online
- Analyse transcriptomique des fibroblastes dermiques associés aux lymphomes T cutanés : démonstration de leur rôle support (December 2020) Presentation at the Journées Dermatologiques de Paris, Annual Conference of the French Dermatology Society, Online
- Epidemiological changes in cutaneous lymphomas (October 2021), European Academy of Dermatology and Venerology, Online
- Änderungen in der Epidemiologie von primär kutanen Lymphomen (September 2021), Presentation at the Annual Conference of the Skin Cancer Group of the German Cancer Society, Online, Germany
- Quantifying response to various treatments using the revisited blood staging of mycosis fungoides and Sézary syndrome with the KIR3DL2 marker. (October 2021) Presentation at the EORTC-Cutaneous Lymphoma Task Force Conference, Marseille, France
- Exploring the role of the skin microenvironment in cutaneous T-cell lymphoma using single cell RNA-sequencing. (October 2021) Presentation at the EORTC-Cutaneous Lymphoma Task Force Conference, Marseille, France

Selection of Courses and Workshops Attended During the Fellowship

- 2020: Leadership training for postdoctoral researchers (EMBO Solutions)
- 2020: European Society for Dermatological Research: Future Leaders Academy (ESDR)
- 2021: European Society for Dermatological Research: Leadership Training

Acknowledgements

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References

1. Willemze, R., et al., *The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas.* Blood, 2019. **133**(16): p. 1703-1714.
2. Olsen, E.A., *The United States Cutaneous Lymphoma Consortium (USCLC).* Clin Lymphoma Myeloma Leuk, 2010. **10 Suppl 2**: p. S88-9.
3. Olsen, E.A., et al., *Clinical end points and response criteria in mycosis fungoides and Sezary syndrome: a consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer.* J Clin Oncol, 2011. **29**(18): p. 2598-607.
4. Scarisbrick, J.J., et al., *The PROCLIPI international registry of early-stage mycosis fungoides identifies substantial diagnostic delay in most patients.* Br J Dermatol, 2018.
5. Scarisbrick, J.J., et al., *Developments in the understanding of blood involvement and stage in mycosis fungoides/Sezary syndrome.* Eur J Cancer, 2018. **101**: p. 278-280.
6. Hurabielle, C., et al., *Expression of Sézary Biomarkers in the Blood of Patients with Erythrodermic Mycosis*

Fungoides. Journal of Investigative Dermatology, 2016. **136**(1): p. 317-320.
7. Moins-Teisserenc, H., et al., *CD158k Is a Reliable Marker for Diagnosis of Sézary Syndrome and Reveals an Unprecedented Heterogeneity of Circulating Malignant Cells.* Journal of Investigative Dermatology, 2015. **135**(1): p. 247-257.
8. Hurabielle, C., et al., *Frequency and prognostic value of cutaneous molecular residual disease in mycosis fungoides: a prospective multicentre trial of the Cutaneous Lymphoma French Study Group.* Br J Dermatol, 2015. **173**(4): p. 1015-23.
9. Michel, L., et al., *Use of PLS3, Twist, CD158k/KIR3DL2, and NKp46 gene expression combination for reliable Sezary syndrome diagnosis.* Blood, 2013. **121**(8): p. 1477-8.

SIGNATURES	
Award Recipient full name	***Signature and Date***
Dr. Gabor Dobos	15.01.2022

Research Mentor full name	***Signature and Date***
Prof. Martine Bagot	15.01.2022

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