Aus der Klinik für Dermatologie, Venerologie und Allergologie der
Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Oncogenic Signaling Cascades in Cutaneous T Cell Lymphomas: Implications for Pathogenesis, Diagnosis and Targeted Therapies.

Doctor rerum medicinalium
(Dr. rer. medic.)

der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

vorgelegt von

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aus Albaidha, Jemen

Datum der Promotion: 11.12.2015

1 Table of Contents

2.1 Zusammenfassung

Einleitung: Die Kutanen T-Zell-Lymphome (KTZL) bilden eine heterogene Gruppe innerhalb der Non-Hodgkin-Lymphome. Sie entstehen durch eine klonale Expansion neoplastischer TZellen in der Haut und zeigen zum Zeitpunkt der Diagnose per Definition keinen weiteren systemischen Befall. Trotz erheblicher Fortschritte bei der Identifizierung neuer Gene und Signalwege, welche in der Pathogenese Kutaner Lymphome involviert sind, ist die Prognose für Patienten im fortgeschrittenen Stadium der Erkrankung weiterhin schlecht. Zwar befinden sich unter den bisher bekannten Genen eine Reihe bekannter Onkogene und Tumorsuppressoren, mit wichtigen Funktionen innerhalb des Zellzyklusses oder innerhalb vonSignalwegen, welche das Überleben der Zelle oder die Apoptose regulieren, ein direkter therapeutischer Nutzen dieser Erkenntnisse konnte daraus bisher jedoch in vielen Fällen nicht gewonnen werden. Es besteht daher weiterhin ein dringender Bedarf an neuen Therapiemöglichkeiten für späte Stadien Kutaner Lymphome.
Beim Menschen existieren drei Aurorakinasen (AURK), AURK-A, AURK-B und AURK-C mit hoher Sequenze-Homologität. AURK-A wird in den meisten Zellen gebildet, für eine Reihe an Tumoren wurde jedoch eine deutliche Überexpression beschrieben. Folglich kam es zur Entwicklung verschiedener AURK-Inhibitoren, die zur Zeit in ersten klinischen Studien getestet werden und die eine vielversprechende Option für die Krebstherapie darstellen. Für Kutane Lymphome existieren bisher nur wenige Daten zur Rolle der AURK-A und einer möglichen Bedeutung ihrer Inhibition zu therapeutischen Zwecken.
Methodik: Zur genaueren Charakterisierung von krankheitsrelevanten Signaltransduktionswegen und zur Identifizierung neuer potentieller Zielmoleküle für eine therapeutische Intervention wurden mittels Genexpressions-Arrays und Reverser-Transkriptase-Polymerase-Kettenreaktion Hautproben unterschiedlicher KTZL-Stadien miteinander und mit gesunder Haut verglichen. Der Fokus lag dabei auf Genen, welche für Serin/Threonin-Kinasen bzw. Rezeptor-Serin/Threonin/Tyrosin-Kinasen kodieren und welche mittels chemischer Inhibitoren beeinflusst werden können. Nach Behandlung mehrerer KTZL-Zelllinien und primärer T-Zellen gesunder Spender mit einem für AURK-A spezifischen Inhibitor wurden im Anschluss Einflüsse auf die Zellviabilität, den Zellzyklus und die Apoptose untersucht.
Ergebnisse und Schlussfolgerungen: Wir konnten zeigen, dass AURK-A in Hautproben von KTZL-Patienten und primären Tumorzellen von Patienten mit Sézary Syndrom zu den am stärksten überexprimierten Genen der Familie der Serin/Threonin-Kinasen gehört .Die Hemmung mit einem spezifischen AURK-A Inhibitor führt in KTZL-Zelllinien und stimulierten primären T-

Zellen zu einem Zellzyklus-Arrest in der G2-Phase und löst in einer von vier KTZLLinien zusätzlich Apoptose aus. Auf unstimulierte primäre T-Zelle konnte kein negativer Effekt beobachtet werden. Diese Daten bilden eine vielversprechende Grundlage zur Verwendung von AURK-A-Inhibitoren als Therapieoption bei KTZL.

2.2 Abstract

Introduction: Cutaneous T-cell lymphomas (CTCL) forms a heterogeneous group of nonHodgkin lymphomas which by definition originates through clonal expansion of neoplastic Tcells homing to the skin without further systemic involvement at the time of diagnosis. Despite significant progress made in the identification of novel genes and pathways involved in the pathogenesis of cutaneous lymphoma, the prognosis especially in advanced stage disease is still poor. The so far identified genes enclose several known oncogenes and tumorsuppressor genes, which are involved in major pathways regulating the cell cycle, cell survival or apoptosis but the therapeutic value of these findings has still to be proven and there remains a particular need for treatments of patients with advanced stage CTCL. There are three human homologues of Aurora kinases, A, B, C, which share a high degree of sequence homology. Aurora kinase A is in principle expressed in most normal cells, but its overexpression has been reported in several tumors. This resulted in the development of several AURK inhibitors (AURK-I), which are currently tested in first clinical trials and have become a promising therapeutic option in cancer therapy. In cutaneous lymphoma, the status of Aurora kinase A is still poorly studied and the role of Aurora kinase A inhibitor has still to be proven.

Methodology: In an approach to further define pathways and to identify novel potential targets for therapeutic intervention, we have investigated gene expression profiles of CTCL samples of different stages compared to normal skin by microarray and real time reverse transcriptase polymerase chain reaction (RT-PCR) analysis, focusing on genes encoding serine/threonine kinases and serine/threonine/tyrosine receptor kinases which can be targeted with small molecule inhibitors. After incubation of several CTCL cell lines and primary T-cells of healthy controls with a specific AURKA inhibitor cell viability, cell cycle inhibition and apoptosis was assessed. **Results and conclusions:** We could demonstrate that aurora kinase A is one of highly overexpressed serine/threonine kinases in CTCL skin samples and primary tumor cells from patients with Sézary Syndrome. Moreover, treatment with a specific aurora kinase A inhibitor blocks cell proliferation by inducing cell cycle arrest in G2 phase in CTCL cell lines as well as stimulated primary T-cells as well as apoptosis in one of 4 CTCL cell lines, but had no negative effect on unstimulated primary T-cells. These data provide a promising rationale for using aurora kinase A inhibition as a therapeutic modality of CTCL.

3 Affidavit

I, Ahmed Haider, certify under penalty of perjury by my own signature that I have submitted the thesis on the topic "Oncogenic Signaling Cascades in Cutaneous T Cell Lymphomas: Implications for Pathogenesis, Diagnosis and Targeted Therapies". I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The section on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) corresponds to the URM (s.o) and are answered by me. My contribution in the selected publication for this dissertation corresponds to those that are specified in the following joint declaration with the responsible person and supervisor.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date 23.4.2015 Signature

Detailed Declaration of Contribution

Ahmed Ali Ali Haider had the following share in the following publication:

> Humme D, Haider A, Möbs M, Mitsui H, Suárez-Fariñas M, Ohmatsu H, Geilen CI, Eberle J, Krueger JG, Beyer M, Hummel M, Anagnostopoulos I, Sterry W, Assaf C. Aurora Kinase A is Upregulated in Cutaneous T-cell Lymphoma and Represents a Potential Therapeutic Target.
> J Invest Dermatol. 2015 Apr 7. doi: 10.1038/jid.2015.139. [Epub ahead of print]
>
> Contribution in detail (please explain in detail):

Conceptual design of the study: 40%

> After the initial identification of AURKA overexpression in CTCL skin samples, Mr. Haider substantially contributed to the design of the verification in CTCL cell lines and primary tumor cells of Sézary Syndrome patients and the functional characterisation of AURKA inhibition.

Planing and conception of experiments: 50%

> Mr. Haider almost completely independently planed and designed the experiments for confirming AURKA overexpression in CTCL cell lines and primary tumor cells from Sézary Syndrome patients and for analyzing the effects of AURKA inhibition.

Realization of experiments **60%**

Mr. Haider independently performed:

- Sorting of primary tumor cells from Sézary Syndrome patients and controles 100%
- Culturing of CTCL cell lines 90%
- Gene expression profiling of AURKA in tumor cells and controls 100%
- AURKA inhibition of CTCL cell lines and primary T-cells (including stimulation) 80%
- Western blot analysis of AURKA expression and caspase.cleavage 50%
- Functional analyses after AURKA inhibition (cell viability, apoptosis, cell cycle analysis) 90%

Data Analysis **50%**

Mr. Haider almost completely independently analysed the data of his experiments

Writing of the manuscript **30%**

Mr. Haider substantially contributed to the conception of the manuscript and wrote the first draft. He reviewed changes made by the other authors and helped to revise the manuscript according to the suggestions of the reviewers.

Preparation of figures and tables **40%**

Mr. Haidar made the draft versions of most figures/tables which later have been finalized by the co-authors

Signature, date and stamp of the supervising University teacher

23/04/2015

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☐	14	DERMATOLOGY	1018-8665	4646	1.685	2.192	0.168	125	>10.0	0.00685	0.670
☐	15	J CUTAN PATHOL	0303-6987	3358	1.560	1.552	0.257	140	6.8	0.00662	0.423
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☐	20	EUR J DERMATOL	1167-1122	2338	1.953	2.272	0.311	106	6.1	0.00521	0.630

5 Printed Copy of the Selected Publication

“Aurora kinase A is upregulated in cutaneous T-cell lymphoma and represents a potential therapeutic target”

by

Daniel Humme*, Ahmed Haider*, Markus Möbs, Hiroshi Mitsui, Mayte Suárez-Fariñas, Hanako Ohmatsu, Cyprienne Isabell Geilen, Jürgen Eberle, James G. Krueger, Marc Beyer, Michael Hummel, Ioannis Anagnostopoulos, Wolfram Sterry, and Chalid Assaf

*D.H. and A.H. contributed equally as first authors

http://dx.doi.org/10.1038/jid.2015.139

6 Curriculum vitae

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7 Publication

Humme D, **Haider A**, Möbs M, Hiroshi Mitsui, Mayte Suárez-Fariñas, Hanako Ohmatsu, Cyprienne Isabell Geilen, Eberle J, James G. Krueger, Beyer M, Hummel M, Ioannis Anagnostopoulos, Sterry M, Assaf C., **Aurora kinase A is upregulated in cutaneous T-cell lymphoma and represents a potential therapeutic target.** J Invest Dermatol. 7 April 2015; doi:10.1038.139.

Möbs M, Gryzik S, **Haidar A**, Humme D, Beyer M., Vandersee S. **Analysis of the IL-31 pathway in Mycosis fungoides and Sézary syndrome.** Arch Dermatol Res. 2014 Dec 7.

Humme D, Lukowsky A, Gierisch M, **Haider A**, Vandersee S, Assaf C, Sterry W, Möbs M, Beyer M., **T-cell receptor gene rearrangement analysis of sequential biopsies in cutaneous T-cell lymphomas with the Biomed-2 PCR reveals transient T-cell clones in addition to the tumor clone**. Exp Dermatol. 2014 Jul;23(7):504-8.;

Humme D, Möbs M, Pullmann S, **Haidar A**, Beyer M, Sterry W, Assaf C., **Cutaneous malignant lymphomas. Update on diagnosis and therapy of cutaneous T-cell lymphomas,** Hautarzt, 2012 May;63(5):423-35;

Asem A. Shehabi, **Ahmed. A. Haider** & Manar K. Fyyad, **Frequency of antimicrobial resistance markers among *Pseudomonas aeruginosa* and *Escherichia coli* isolates from municipal sewage effluent water and patients in Jordan,** The International Arabic Journal of Antimicrobial Agents, 2011

8 Acknowledgements

This work would not have been possible without the guidance and the help of several individuals who contributed and extended their valuable assistance in the preparation and completion of this study.

At the outset, I would like to thank my supervisor PD. Dr. med. Chalid Assaf for giving me the opportunity to conduct my PhD in this interesting subject, his continuous advice and guidance, his unlimited support, his helpful discussions through frequent meetings, his good ideas, his help in reading and writing the paper and for providing assistance whenever I needed it.

I would also like to thank my practical supervisor, Dr. Markus Möbs for creating enjoyable working atmosphere. I would like also to thank him for his help in planning and analyzing the experiments, in writing the paper and for providing assistance whenever I needed it. His continuous help and advice in the work facilitated the difficulties in the lab. Dr. Möbs, I would also like to thank you for the financial support and for helping me with administration and translating German letters.

Dr. Assaf and Dr. Möbs, it has been a real pleasure to work with you. You helped me to achieve my hope. I will not forget your help forever and it is not enough to say thank you.

I would also like to thank all members of our group, especially Dr. Marc Beyer, Dr. Staffan Vandersee and Dr. Daniel Humme for their support and for providing a nice atmosphere during my work.

My thanks also go to Prof. Michael Hummel for his help in immunohistochemistry experiments and for the financial support.

I wish to further extend my thanks to both past and present members of Dr. Eberle's and Prof. Walden's workgroups, especially to Katja Steinhorst.

Finally, my parents, my wive, my four children (Aisha, Asmhan, Ali and Amal), my siblings, and my friend Mohammed Otaif deserve special mention here. My deep and lasting gratitude to them for their praying, patience, faith, and support. They have been a constant source of inspiration and this work is especially dedicated to them.

Erratum

1. Despite stated in the manuscript, we haven't been the first to report a strong expression of AURKA in cutaneous T-cell lymphoma. In 2013, Kanagal-Shamanna et al. reported in an immunhistological study, covering primary systemic lymphoma and in addition 19 cases of CTCL, AURKA expression in 9/13 cutaneous anaplastic large cell lymphoma (cALCL) and 4/6 cases of Mycosis fungoides (MF) with modest to strong expression in 5 of the 9 positiv cALCL and one of the 4 positive MF cases.

Kanagal-Shamanna R1, Lehman NL, O'Donnell JP, Lim MS, Schultz DS, Chitale DA, BuesoRamos CE, Medeiros LJ, Inamdar KV. Differential expression of aurora-A kinase in T-cell lymphomas. *Mod Pathol*. 2013 May;26(5):640-7. doi: 10.1038/modpathol.2012.211.

2. In the discussion it is mentioned that "…our immunohistochemistry results demonstrate that the proliferation marker Mib-1 is only weakly exhibited in patch and plaque MF samples in comparison with AURKA". This part should have been changed in the final manuscript as we decided not to include this primary data in the manuscript. Instead it should be stated as: "…immunohistochemistry results demonstrate that proliferation marker Mib-1 is only weakly exhibited in patch and plaque MF samples in comparison with our strong AURKA stainings" and following publications should have been cited:

Kanavaros P, Bai M, Stefanaki K, Poussias G, Rontogianni D, Zioga E, Gorgoulis V, Agnantis NJ. Immunohistochemical expression of the p53, mdm2, p21/Waf-1, Rb, p16, Ki67, cyclin D1, cyclin A and cyclin B1 proteins and apoptotic index in T-cell lymphomas. *Histol Histopathol*. 2001 Apr;16(2):377-86.

3. About why not AURKA shows overexpression in all CTCL? We think that AURKA is one of several factors responsible for the pathogenesis of CTCL This also applies for the other genes (e.g. cMYC) that participated in the pathogenesis of CTCL or in other tumors. Therefore we do not expect that the participated genes are detected in 100% of the same tumor samples.