Aus der Medizinischen Klinik mit Schwerpunkt
Rheumatologie und klinische Imunologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Bortezomib plus continuous B cell depletion results in sustained plasma cell depletion and amelioration of lupus nephritis in NZB/W F1 mice

zur Erlangung des akademischen Grades
Doctor of Philosophy (PhD)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

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Datum der Promotion: 09.09.2016

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Abstrakt

Einleitung: Langlebige Gedächtnis-Plasmazellen haben sich in den letzten Jahren als ein wichtiges therapeutisches Ziel bei Autoantikörper-vermittelten Erkrankungen wie dem systemischen Lupus erythematodes (SLE) herausgestellt, da sie resistent auf die herkömmlichen immunsuppressiven Medikamente sind. Hauptsächlich aus Mausexperimenten ist bekannt, dass Gedächtnis-Plasmazellen mit Hilfe des Proteasominhibitors Bortezomib sowie durch die Blockade der Integrine LFA-1 und VLA-4 depletiert werden können. Diese Depletion ist jedoch transient, da sich das Gedächtnis-Plasmazellkompartiment bei Vorliegen einer B-Zellhyperaktivität sehr schnell regeneriert.
Aus diesem Grund wurden in dieser Arbeit unterschiedliche Therapiestrategien, die zur Elimination von Gedächtnis-Plasmazellen führen, im Mausmodell des SLE untersucht. Dabei wurde die selektive Plasmazelldepletion mit Ansätzen, die zusätzlich auch ihre Vorläuferzellen (B-Zellen) depletieren, verglichen, um eine wirksame Strategie für eine anhaltende Eliminierung von autoreaktiven Gedächtnis-Plsmazellen zu entwickeln.

Methoden: NZB/W F1 Mäuse wurden behandelt mit 1) Anti-CD20-Antikörpern, 2) Kombination von Anti-CD20 und Bortezomib 3) Kombination von Anti-CD20 und Anti-LFA-1/VLA-4 blockierenden Antikörpern 4) Kombination von Anti-CD20 plus Bortezomib und LFA-1/VLA4 blockierenden Antikörpern. 7 Tage nach der Behandlung wurden sowohl die kurzlebigen als auch die langlebigen Plasmazellen (einschließlich der autoreaktiven Zellen) mittels Durchflusszytometrie und ELISPOT in Milz und Knochenmark analysiert. Basierend auf diesen Ergebnissen wurden NZB/W F1 Mäuse in einem Folgeexperiment zunächst mit einem Zyklus Anti-CD20 in Kombination mit Bortezomib behandelt und danach wurden die B-Zellen im Abstand von jeweils 10 Tagen mit 4 weiteren Zyklen von Anti-CD20-Injektionen depletiert, um die Regeneration von Gedächtnis-Plasmazellen zu blockieren. Die Effekte auf das Plasmazell-Kompartiment und den Krankheitsverlauf wurden analysiert.

Ergebnisse: Kurzlebige Plasmablasten und Plasmazellen wurden durch alle angewandten Therapieverfahren in Milz und Knochenmark effektiv eliminiert. Im Gegensatz hierzu ließen sich die langlebigen Gedächtnis-Plasmazellen sowie die autoreaktiven, dsDNA-spezifischen Plasmazellen deutlich schlechter reduzieren. Nur die Kombination von Anti-CD20 mit Bortezomib ergab eine deutliche Depletion dieser Zellen. Die effiziente Depletion der Plasmazellen durch Bortezomib in Kombination mit einer kontinuierlichen Eliminierung der

Plasmazell-Vorläufer durch Anti-CD20-Antikörper führte zu einer anhaltenden Unterdrückung der IgG Anti-dsDNA-Autoantikörper, einem verzögerten Auftreten der Nephritis und einem verlängerten Überleben der NZB/W F1-Mäuse.

Schlußfolgerung: Die Ergebnisse unterstreichen, dass die effiziente Depletion der Gedächtnis-Plasmazellen in Kombination mit einer kontinuierlichen Blockade ihrer Regeneration aus Vorläuferzellen eine vielversprechende Therapieastrategie bei SLE und anderen Antikörper-vermittelter Erkrankungen sein kann.

Abstract

Introduction: Long-lived plasma cells (LLPCs) are an unmet therapeutic challenge, and developing strategies for their targeting is an emerging goal of autoantibody-mediated diseases such as systemic lupus erythematosus (SLE). It was previously shown that plasma cells can be depleted by agents such as bortezomib (Bz) or by blocking LFA-1 and VLA-4 integrins. However, they regenerate quickly after depletion due to B cell hyperactivity in autoimmune conditions. Therefore, we compared different therapies for the elimination of LLPCs combined with selective B-cell targeting in order to identify the most effective treatment to eliminate LLPCs and prevent their regeneration in lupus-prone NZB/W F1 mice.

Methods: NZB/W F1 mice were treated with: 1) anti-CD20, 2) anti-CD20 plus bortezomib, 3) anti-CD20 plus anti-LFA-1/anti-VLA-4 blocking antibodies, 4) anti-CD20 plus bortezomib and anti-LFA-1/anti-VLA4 blocking antibodies. Short- and long-lived plasma cells including autoreactive cells in the bone marrow and spleen were enumerated by flow cytometry and ELISPOT seven days after treatment. Based on these data in another experiment, mice received one cycle of anti-CD20 plus bortezomib followed by four cycles of anti-CD20 therapy every 10 days and were monitored for its effect on plasma cells and disease.

Results: Short-lived plasma cells in bone marrow and spleen were efficiently depleted by all regimens targeting plasma cells. Conversely, LLPCs and anti-dsDNA-secreting plasma cells in bone marrow and spleen showed resistance to depletion but were strongly reduced by bortezomib plus anti-CD20. The effective depletion of plasma cells by bortezomib complemented by the continuous depletion of their precursor B cells using anti-CD20 promoted the persistent reduction of IgG anti-dsDNA antibodies, delayed nephritis and prolonged survival in NZB/W F1 mice.

Conclusions: These findings suggest that the effective depletion of LLPCs using bortezomib in combination with a therapy that continuously targeting B cells as their precursors may prevent the regeneration of autoreactive LLPCs and, thus, might represent a promising treatment strategy for SLE and other (auto)antibody-mediated diseases.

Affidavit

I, Laleh Khodadadi certify under penalty of perjury by my own signature that I have submitted the thesis on the topic "**Bortezomib plus continuous B cell depletion results in sustained plasma cell depletion and amelioration of lupus nephritis in NZB/W F1 mice"**
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 07.08.2015 Signature

Detailed Declaration of Contribution

Laleh Khodadadi had the following share in the following publication:

Khodadadi L, Cheng Q, Alexander T, Sercan-Alp Ö, Klotsche J, Radbruch A, Hiepe F, Hoyer BF, Taddeo A. *"Bortezomib plus continuous B cell depletion results in sustained plasma cell depletion and amelioration of lupus nephritis in NZB/W F1 mice".* PLoS One. **2015** Aug 7;10(8):e0135081

Contribution in detail:

Laleh Khodadadi was substantially involved in designing and planning the experiments, and she independently performed all experiments. In large experiments, she was sometimes assisted by Qingyu Cheng to ensure timely completion and cell survival. She thoroughly discussed the results with the co-authors. Laleh Khodadadi wrote and revised the manuscript after discussion with the co-authors. Consequently, she meets the criteria for being firs author of this publication

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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☐	1	NATURE	0028-0836	617363	41.456	41.296	9.585	862	>10.0	1.50140	21.947
☐	2	SCIENCE	0036-8075	557558	33.611	35.263	7.221	828	>10.0	1.22419	17.874
☐	3	NAT COMMUN	2041-1723	39396	11.470	11.904	1.706	2788	1.9	0.26374	5.720
☐	4	P NATL ACAD SCI USA	0027-8424	586144	9.674	10.563	1.967	3579	8.4	1.41892	4.730
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☐	7	J R SOC INTERFACE	1742-5689	7717	3.917	4.650	0.680	344	4.4	0.03090	1.699
☐	8	PLOS ONE	1932-6203	332716	3.234	3.702	0.489	30040	2.7	1.53547	1.208
☐	9	P JPN ACAD B-PHYS	0386-2208	1080	2.652	3.096	0.182	33	5.2	0.00350	1.008
☐	10	P ROY SOC A-MATH PHY	1364-5021	17231	2.192	2.372	0.405	220	>10.0	0.01869	1.214
☐	11	PHILOS T R SOC A	1364-503X	13391	2.147	3.038	1.287	293	8.4	0.03101	1.287
☐	12	PEERJ	2167-8359	655	2.112	2.112	0.350	471	1.3	0.00277	0.751
☐	13	NATURWISSENSCHAFTEN	0028-1042	6500	2.098	2.076	0.361	122	>10.0	0.00748	0.730
☐	14	P ROMANIAN ACAD A	1454-9069	256	1.658	0.927	0.102	49	2.2	0.00078	0.198
☐	15	CHINESE SCI BULL	1001-6538	10429	1.579	1.519	0.285	632	6.6	0.01998	0.414
☐	16	JOVE-J VIS EXP	1940-087X	4551	1.325		0.201	941	3.3	0.02284	
☐	17	FRACTALS	0218-348X	650	1.220	0.702	0.444	27	>10.0	0.00068	0.226
☐	18	INT J BIFURCAT CHAOS	0218-1274	4576	1.078	1.113	0.233	219	8.5	0.00851	0.389
☐	19	SCI AM	0036-8733	4738	1.070	1.373	0.231	108	>10.0	0.00512	0.664
☐	20	COMPLEXITY	1076-2787	608	1.041	1.342	0.514	37	8.4	0.00104	0.430

Printed copy of the selected publication

Laleh Khodadadi, Qingyu Cheng, Tobias Alexander, Özen Sercan-Alp, Jens Klotsche, Andreas Radbruch, Falk Hiepe, Bimba F. Hoyer, Adriano Taddeo

Bortezomib Plus Continuous B Cell Depletion Results in Sustained Plasma Cell Depletion and Amelioration of Lupus Nephritis in NZB/WF1 Mice

PLOS ONE |August 7, 2015-DOI:
http://dx.doi.org/10.1371/journal.pone.0135081

Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

Complete List of Publications

1. **Khodadadi L**, Cheng Q, Alexander T, Sercan-Alp Ö, Klotsche J, Radbruch A, Hiepe F, Hoyer BF, Taddeo A. "Bortezomib plus continuous B cell depletion results in sustained plasma cell depletion and amelioration of lupus nephritis in NZB/W F1 mice". PLoS One. **2015** Aug 7;10(8):e0135081. **Eigenfactor score: 1.53** and **Impact factor: 3.23**

2. Taddeo A, **Khodadadi L,** Voigt C, Mumtaz IM, Cheng Q, Moser k, Alexander T, Manz RA, Radbruch A, Hiepe F, Hoyer BF. "Long-lived plasma cells are early and constantly generated in New Zealand Black/New Zealand White F1 mice and their therapeutic depletion requires a combined targeting of autoreactive plasma cells and their precursors". Arthritis Res Ther. **2015** Mar 2;17(1):39. **Eigenfactor score: 0.02** and **Impact factor: 3.75**

3. Cheng Q, Mumtaz IM, **Khodadadi L**, Radbruch A, Hoyer BF, Hiepe F. "Autoantibodies from long-lived "memory" plasma cells of NZB/W mice drive immune complex nephritis". Ann Rheum Dis. **2013** Dec; 72(12):2011-7. **Eigenfactor Score: 0.07** and **Impact factor: 10.37**

4. Winter O, Musiol S, Cheng Q, Schablowsky M, **Khodadadi L,** Hiepe F. "Analyzing pathogenic (double-stranded (ds) DNA-specific plasma cells via immunofluorescence microscopy". Arthritis Res Ther. **2015** Oct 21;17:293. **Eigenfactor score: 0.02** and **Impact factor: 3.75**

Acknowledgement:

When I was 13-year-old girl-a spoiled one-my brother gave me a book and asked me to read!
" Leukemia is a very killing disease…" was the first sentence. I didn't understand it at all! One month later when my mom passed away, I did!

It was terrible for me but something more horrible happened. I heard if she had bone marrow transplantation, she could survive! I couldn't bare it. Is it true? If she had bone marrow transplantation, she could survive? It became the biggest question in my life!! I tried to find the answer and started to collect any information about BMT. Finally, when I entered the faculty of medicine and found out that my mom had AML type M3, I realized that it was not possible at least that time!

I couldn't do anything for mom so I decided to do something to help the others moms, dads or beloved ones. This research as a step in science would not be done without my advisor Prof. Dr. med. Falk Hiepe. I am so deeply grateful to Prof. Hiepe for his help, patience, professionalism, valuable guidance, and most importantly his very nice character. Dear Prof. Hiepe, I thank you for everything you have done for me.

I am also grateful of the Berlin-Brandenburg School for Regenerative Therapies (BSRT) for financial support that allowed me to pursue my goals. I acknowledge especially the other two members of my mentoring committee "Prof. Dr. med. Gerd-Rüdiger Burmester and Prof. DR. rer. nat. Andreas Radbruch" for their input and valuable discussions. I also would like to express my special thank to the administrative coordinator of BSRT, Dr. Sabine Bartosch who was always willing to help. I extend my gratitude to all friends, colleagues in the past and now at Deutsches Rheuma-Forschungszentrum Berlin (DRFZ) and whoever helped me to stay determined on my decision and move forward.

Finally, I must express my deepest feeling to my late dad "Azar Khodadadi" and mom "Zhaleh Sadeghi" but I do not have enough words to do it. I just say, "You will be forever loved and will never be forgotten". I owe a big thank to the most important people in my life, my brothers and sisters "Afshin, Mozhgan, Babak and Arezou". I thank you for your unconditional love, support and continuous encouragement throughout my life, my studies and the process of researching. This accomplishment would not have been possible without you. No matter where you are around the world, you are always with me!

Thank you