Brno, 26. 01. 2017

Application for junior group leader position at CEITEC MU

Mgr. Michal Šmída, Ph.D.

CONTENT

1. CURRICULUM VITAE

PERSONAL DETAILS

Date of birth:	01. 02. 1976
Place of birth:	Boskovice, Czech Republic
Gender:	male
Nationality:	Czech
Marital status:	married, 2 children
Work address:	Central European Institute of Technology – Masaryk University (CEITEC MU) Kamenice 753/5, building A35, CZ-62500 Brno, Czech Republic
Phone:	+ 420 549498194, + 420 736722290
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Researcher ID:	J-5046-2014

CURRENT POSITION

Senior researcher at CEITEC MU (1.0 FTE)

OTHER FUNCTIONS AND POSITIONS

- Deputy head of Medical Genomics research group, CEITEC MU
- Research assistant at the Department of Internal Medicine – Hematology and Oncology, University Hospital Brno
- PhD supervisor at Faculty of Medicine, Masaryk University, Brno
- Member of Economic committee at CEITEC MU
- GMO responsible person for Molecular Medicine, CEITEC MU

EDUCATION

2008	**Ph.D.** in Natural sciences, Thesis: „Biochemical and functional characterization of Fyn-PAG association and its role in T-cell anergy" Institute of Molecular and Clinical Immunology, Magdeburg, Germany
2002 – 2007	Doctoral studies at the Institute of Molecular and Clinical Immunology, Otto-von-Guericke University, Magdeburg, Germany
2002	**Mgr.** (~M.Sc.) in Molecular Biology and Genetics Faculty of Science, Masaryk University, Brno, Czech Republic
1997 – 2002	Faculty of Science, Masaryk University, Brno, Czech Republic
1994 – 1997	Faculty of Medicine, Masaryk University, Brno, Czech Republic

SCIENTIFIC BACKGROUND AND EXPERTISE

- Immunology
- Hematooncology
- T-cell signaling
- Cancer biology

- Functional genomics, synthetic lethality
- Molecular biology, CRISPR/Cas9 technology
- Lentiviral production and infection, Cell culture

CAREER-RELATED ACTIVITIES

- Supervisor of PhD students, diploma students and technician assistants
- Tutor at the practical courses and seminars for students
- Postdoctoral representative at CeMM, Vienna (2011-2014)
- Co-founding member of the "Vienna Area Postdoc Association" (VAPA)
- Co-organizer of the Postdoc Retreat for postdocs in Vienna area
- Associate member of AACR (American Association for Cancer Research)
- Member of EHA (European Hematology Association) and ERIC (European Research Initiative on CLL)
- Trainee at the Grant writing course, Leadership course and Mentoring program

OVERVIEW OF ORAL AND POSTER PRESENTATIONS

- 58th ASH annual meeting, San Diego, USA – poster presentation (2016)
- 2nd International Conference on New Concepts in B-Cell Malignancies, Estoril, Portugal – poster presentation (2016)
- AACR Precision Medicine Series "Synthetic lethal approaches to cancer vulnerabilities", Seattle, USA – poster presentation (2013)
- The 3rd EMBO meeting, Vienna, Austria – poster presentation (2011)
- EMBO Workshop on "Synthetic Lethality: From Yeast to Man", Vienna, Austria – poster presentation (2011)
- 13th Joint Meeting of the Signal Transduction Society (STS), Weimar, Germany – oral presentation (2009)
- 2nd European Congress of Immunology, Berlin, Germany – oral presentation (2009)
- 37th Annual Meeting of the German Society for Immunology, Heidelberg, Germany – poster presentation (2007)
- Joint meeting of European national society of immunology, Paris, France – poster presentation (2006)
- 9th Joint meeting in signal transduction – Receptors, mediators and genes, Weimar, Germany – oral presentation (2005)
- Joint 36th annual meetings of the German society of immunology and the Scandinavian society for immunology, Kiel, Germany – poster presentation (2005)
- Joint Annual Meeting of the German and Dutch Societies for Immunology, Maastricht, the Netherlands – poster presentation (2004)
- 6th EFIS Tatra Immunology Conference, Tatranske Zruby, Slovakia – poster presentation (2004)

PUBLICATIONS

Author of 15 publications, many of which were published in journals with impact factor >10. Currently 1 pending first-author publication and few co-author publications under writing.

Cumulative IF = 111.64
Average IF of top 5 publications = 14.15
Total sum of citations for all publications so far: 454
H-index: 10

(see part 4 for list of publications)

2. PROFESSIONAL EXPERIENCE

2015 - present	**Medical Faculty of Masaryk University and University Hospital Brno,** Department of Internal Medicine - Hematology and Oncology Research assistant
2014 - present	**Central European Institute of Technology – Masaryk University (CEITEC MU)**, Brno, Czech Republic Senior researcher, Molecular Medicine program
2010 – 2014	**Research Center for Molecular Medicine of the Austrian Academy of Sciences (CeMM)**, Vienna, Austria Senior post-doctoral fellow (Dr. S. Nijman)
2007 – 2010	**Institute of Molecular and Clinical Immunology**, Otto-von-Guericke University, Magdeburg, Germany Post-doctoral fellow (Dr. J. Lindquist)
2002 – 2007	**Institute of Molecular and Clinical Immunology**, Otto-von-Guericke University, Magdeburg, Germany Ph.D. student (Dr. J. Lindquist)
1997 – 2002	**Faculty of Science**, Masaryk University, Brno, Czech Republic Undergraduate student (diploma thesis with Dr. Vitek)
1994 – 1997	**Faculty of Medicine**, Masaryk University, Brno, Czech Republic Undergraduate student

Teaching activities

- **At CEITEC MU**

- Supervisor of 4 PhD students (1 at CEITEC PhD school, 3 at the Faculty of Medicine); 1 PhD student received PhD talent award.
- Supervisor of 2 diploma students (1 finished in 2016) and 1 medical student (within the scope of P-PooL program with extended research activities).

- **In the past**

- Supervisor of 3 PhD students and multiple diploma students (students of medicine, molecular biology and systems biology)
- Supervisor of several technician assistant candidates during their lab rotations
- Tutor at the practical courses for medical students, systems biology students and seminars for systems biology students

3. GRANTS

- „Resistance to monoclonal antibody therapy at B-CLL and B-lymphomas: its foundations and potential intervention strategies“, AZV (Agency for Biomedical Research, Ministry of Health of the Czech Republic) grant nr. 15-33561A (2015-2018), cca 10 mil. CZK
- „Chemical-genetics to uncover lung cancer vulnerabilities”, submitted for Stand-alone grant of the FWF Austrian Science Fund in 2013, applicant turned grant down due to accepted position at CEITEC MU, Brno, Czech Republic

Thanks to the received grant of AZV that is fully covering the PI´s salary and material expenses, the applicant could use the temporary freedom to attempt to obtain some of the prestigious mainly foreign grants:

- EMBO Installation grant (150.000 € total) – passed to the second round of selection (personal interview)
- Neuron Impuls (1.000.000 CZK total) – passed to the second round of selection (personal interview), finally ranked on the 2nd position
- EHA Non-clinical advanced research grant (160.000 € total) – first decision expected in March

The research project on functional CRISPR/Cas9 genomics submitted for EHA research grant will also be submitted for GAČR grant this year.

As part of our newly initiated collaboration, we are team members on the current AZV grant application of prof. Veselská (Department of Experimental Biology) (budget for expenses and part-time PhD student).

Within the collaborative project with prof. Mayer (IHOK, University Hospital Brno) on CAR-T cell therapy (see below), we have recently recruited a joint postdoc who applied with this project for Marie-Curie and SoMoPro fellowships. We are also currently debating with other CAR-T cell researchers in Germany to apply jointly for an H2020 grant in the future.

4. PUBLICATIONS

Author of 15 publications, many of which were published in journals with impact factor >10.
Currently 1 pending first-author publication and few co-author publications under writing.
Cumulative IF = 111.64
Average IF of top 5 publications = 14.15
Total sum of citations for all publications so far: 454
H-index: 10

Pending publications

1. Smida M, Kähne T, Merida I, Naumann M, Schraven B, Lindquist JA. IGAP, a novel Inducible Rac/Cdc42-GTPase-Activating Protein, regulates T-cell activation and migration. *In preparation*

Publications

1. Smida M, Fece de la Cruz F, Uras IZ, van Jaarsveld R, Konopka T, Nagy-Bojarszky K, Muellner MK, Bago-Horvath Z, Haura EB, Loizou JI, Nijman SMB. MEK inhibitors block growth of Ataxia Telangiectasia Mutated (ATM) mutant lung tumors. **Nature Communications**. 2016 Dec;7, 13701; *IF=11.329, times cited=0*
2. Smida M. Chimeric antigen receptor (CAR) T cells: gene therapy of the future for malignant diseases? **Klinická Onkologie**. 2015. Czech.; *no IF*
3. Doubek M, Smida M. Treatment of chronic lymphocytic leukemia with monoclonal antibodies, where are we heading? **Expert Rev Hematol**. 2015 Aug 26: 1-22; *IF=2.439, times cited=0*
4. Bürckstümmer T, Banning C, Hainzl P, Schobesberger R, Kerzendorfer C, Pauler FM, Chen D, Them N, Schischlik F, Rebsamen M, Smida M, Fece de la Cruz F, Lapao A, Liszt M, Eizinger B, Guenzl PM, Blomen VA, Konopka T, Gapp B, Parapatics K, Maier B, Stöckl J, Fischl W, Salic S, Casari MRT, Knapp S, Bennett KL, Bock Ch, Colinge J, Kralovics R, Ammerer G, Casari G, Brummelkamp TR, Superti-Furga G, Nijman SMB. A reversible gene trap collection empowers haploid genetics in human cells. **Nature Methods**. 2013 Oct;10(10):965-71; *IF=25.328, times cited=42*
5. Smida M, Cammann C, Gurbiel S, Kerstin N, Lingel H, Lindquist S, Simeoni L, Brunner-Weinzierl MC, Suchanek M, Schraven B, Lindquist JA. PAG/Cbp suppression reveals a contribution of CTLA-4 to setting the activation threshold in T cells. **Cell Commun Signal**. 2013 Apr 19;11(1):28; *IF=3.661, times cited=7*
6. Smida M and Nijman SM. Functional drug-gene interactions in lung cancer. **Expert Rev Mol Diagn**. 2012 Apr;12(3):291-302; *IF=3.333, times cited=5*
7. Muellner MK, Uras IZ, Gapp BV, Kerzendorfer C, Smida M, Lechtermann H, Craig-Mueller N, Colinge J, Duernberger G, Nijman SM. A chemical-genetic screen reveals a mechanism of resistance to PI3K inhibitors in cancer. **Nature Chemical Biology**. 2011 Sep 25;7(11):787-93; *IF=12.709, times cited=92*
8. Beyer T, Busse M, Hristov K, Gurbiel S, Smida M, Haus UU, Ballerstein K, Pfeuffer F, Weismantel R, Schraven B, Lindquist JA. Integrating signals from the T-cell receptor and the interleukin-2 receptor. **PLoS Comput Biol**. 2011 Aug;7(8):e1002121; *IF=4.587, times cited=11*

9. Börner C, Smida M, Höllt V, Schraven B, Kraus J. Cannabinoid receptor type 1 and 2-mediated increase in cyclic AMP inhibits T cell receptor-triggered signaling. **J Biol Chem**. 2009 Dec 18;284(51):35450-60; *IF=4.258, times cited=43*

10. Börner C, Warnick B, Smida M, Hartig R, Lindquist JA, Schraven B, Höllt V, Kraus J. Mechanisms of opioid-mediated inhibition of human T cell receptor signaling. **J Immunol**. 2009 Jul 15;183(2):882-9; *IF=4.985, times cited=63*

11. Simeoni L, Lindquist JA, Smida M, Witte V, Arndt B, Schraven B. Control of lymphocyte development and activation by negative regulatory transmembrane adapter proteins. **Immunol Rev**. 2008 Aug;224:215-28; *IF=9.542, times cited=25*

12. Posevitz-Fejfar A, Smida M, Kliche S, Hartig R, Schraven B, Lindquist JA. A displaced PAG enhances proximal signaling and SDF-1 induced T-cell migration. **Eur J Immunol**. 2008 Jan;38(1):250-9; *IF=4.179, times cited=24*

13. Smida M, Posevitz-Fejfar A, Horejsi V, Schraven B, Lindquist JA. A novel negative regulatory function of the phosphoprotein associated with glycosphingolipid-enriched microdomains: blocking Ras activation. **Blood**. 2007 Jul 15;110(2):596-615; *IF=11.847, times cited=61*

14. Filby A., Seddon B, Kleczkowska J, Salmond R, Tomlinson P, Smida M, Lindquist JA, Schraven B, Zamoyska R. Fyn regulates the duration of T cell receptor engagement needed for commitment to effector function. **J Immunol**. 2007 Oct 1;179(7):4635-44; *IF=4.985, times cited=55*

15. Simeoni L, Smida M, Posevitz V, Schraven B, and Lindquist JA. Right time, right place: the organization of membrane proximal signaling. **Semin Immunol**. 2005 Feb;17(1):35-49; *IF=8.461, times cited=26*

Abstracts in journals

1. Smida M, Kozlova V, Vakulova V, Ledererova A, Doubek M, Mayer J, Pospisilova S. Cellular Mechanisms Regulating CD20 As a Target of Monoclonal Antibody Therapy in B-Lymphoid Malignancies. **Blood** (2016);128:3968

2. Staňo Kozubík K., Pál K., Radová L., Šmída M., Réblová K., Plevová K., Pospíšilová Š., Doubek M. Využití celoexomového sekvenování. **Transfuze a hematologie dnes** (2016);22(s):89.

3. Smida M, Fece de la Cruz F, Uras I, Nijman SMB. Systematic analysis of gene-drug interactions in isogenic cells reveals novel unexpected dependencies in lung cancer cells. **Mol Cancer Ther** (2013);12(5 Suppl): A25

4. Fece de la Cruz F, Smida M, Nijman SMB. A Functional Pharmacogenetic Screen in Non-small Cell Lung Adenocarcinoma. **European Journal of Cancer** (2012);48: s234

5. RESEARCH CAREER

I obtained my PhD in the lab of Prof. Burkhart Schraven at the Institute of Molecular and Clinical Immunology in Magdeburg (Germany) under the direct supervision of Dr. Lindquist. Here, I received a deep insight into the field of T-cell immunity as well as a very broad knowledge of cellular signaling pathways and I successfully employed a broad range of cell biology, biochemistry and molecular biology techniques. I studied signaling pathways at the level of membrane proximal scaffolds and their impact upon Src kinases, but also elucidated novel modes of regulation of Ras-MAPK signaling. Thus, I focused on the regulatory pathways of two main oncogenes – Src and Ras, and investigated mechanisms of oncogene-induced senescence and transcription factor activation in the nucleus. During this project, I further managed a fruitful collaboration with Prof. Hořejší (Prague, CZ).

As a post-doc, I discovered the role of a previously uncharacterized protein as a GTPase-activating protein for Rho family GTPases involved in T-cell activation and cytoskeleton reorganization. We have generated knockout mouse in order to study the function *in vivo* (manuscript in preparation). To investigate a putative role of this protein in leukemia, I initiated a collaboration with the Hematology department at the Medical University Magdeburg to utilize bone marrow biopsies from patients.

My interdisciplinary and collaborative spirit also led to a cooperation with Dr Jürgen Kraus from the Department of Pharmacology and Toxicology at the Medical University Magdeburg in order to test the effects of neuromodulators like opioids and cannabinoids on the immune system. This project was established and performed independently of my PhD supervisor. I also participated in a SYBILLA consortium (FP7, coordinated by Dr. Schamel, Freiburg, Germany) to investigate T-cell activation in health and disease from a systems biology perspective, which gave me the opportunity to work together with highly recognized experts in the field.

I then took up a challenging senior post-doctoral position at the prestigious Centre for Molecular Medicine (CeMM) of the Austrian Academy of Sciences in Vienna, Austria. Here, I made my transition from basic research to a translational science and expanded my knowledge of intracellular signaling pathways with the focus on oncogenic transformation. I familiarized myself with the techniques required to study tumorigenesis and learned how to tackle drug sensitivities or resistances triggered by cellular mutations. I performed several high-throughput screens with small-molecule compounds, searching for functional gene-drug interactions primarily based on the principles of synthetic lethality. Thereby, I identified ATM mutation as mutation sensitizing lung tumors specifically to clinically interesting inhibitors of MEK kinases. As MEK inhibitors are currently in numerous clinical trials, we proposed to use ATM mutation status as the biomarker stratifying patients for the treatment with these drugs. These exciting results were recently published in Nature Communications. At CeMM, I learned many diverse state-of-the-art techniques, among others RNA-sequencing and CRISPR/Cas9 technology, which I successfully employed in my last project. I learned how to use the gene editing CRISPR/Cas9 system to generate gene knockouts or gene knock-ins at a specific residue and became familiar with diverse techniques used to analyze edited genes. Additionally, I gained experience with diverse mouse xenograft models and techniques associated with *in vivo* studies.

In June 2014, I accepted a position of an independent junior researcher at CEITEC MU in the Molecular Medicine program led by Prof. Š. Pospíšilová. Here, I started working in the field of

personalized medicine for B-cell malignancies. I started to investigate mechanisms of resistance to modern therapies as well as predicting novel targeted treatments. I launched and equipped BSL-2 (biosafety level 2) cell culture laboratory, where I established the technique of lentiviral production and lentiviral infection of mammalian cells. In addition, I established the technique of CRISPR/Cas9 gene editing in the laboratory of Medical Genomics and introduced this technology to the other labs within the Molecular Medicine program. I obtained a grant from the Ministry of Health of the Czech Republic to assess resistance mechanisms to monoclonal antibody therapy and to develop novel intervention strategies. I assembled my own group of four PhD students and two Master students and started several important collaborations (see below).

6. RESEARCH PLAN

My extensive international lab experience helped me to gain profound knowledge of diverse molecular biology techniques that I plan to establish in my own laboratory at CEITEC. At the moment, I am evolving my team into three major directions, which are distinct but their unifying theme is the use of the same high-tech techniques. My laboratory is thus predominantly technology driven, focusing on the use of lentiviral vectors, gene editing CRISPR/Cas9 technology, drug and CRISPR/Cas9 screens, synthetic lethality, next-generation sequencing and different functional analyses. Although many of the experiments are initially planned in B-lymphoid malignancies, all the techniques are very universal and may be applied to any other cellular system in the future. There are three main projects being developed that are planned to go on for (at least) next three years:

1) Mechanisms of resistance to monoclonal antibodies *(AZV grant)*

This project focuses on monoclonal antibody (mAb) therapy in B-lymphoid malignancies (B-cell lymphoma, B-cell chronic lymphocytic leukemia (CLL)). Therapy with mAbs is the gold standard in treatment of these diseases, however, it often fails due to the loss of the cognate antigen targeted by these mAbs. We are investigating the cellular mechanisms responsible for regulation of expression of these antigens. Better understanding of these mechanisms can help us to predict and/or counteract the disappearance of the antigens. We are planning to perform RNA sequencing in our generated mAb-resistant cell lines to identify changes in expression profiles imposed by the antigen loss. We will elucidate epigenetic changes (i.e. DNA methylation, histone acetylation and methylation) triggered in these cells. Techniques like bisulfite sequencing and chromatin immunoprecipitation will be implemented.

Furthermore, we aim to identify novel intervention strategies that would maintain the expression of the antigens, thereby improving the efficacy of the mAbs. We will make use of the CRISPR/Cas9 technology, which is simple, versatile, state-of-the-art technique developed to induce DNA modifications in any desired gene loci. We have obtained a collection of CRISPR guide RNAs targeted against almost the whole human genome and will apply this library on our resistant cell lines or cell lines with intrinsically low expression of the mAb antigens. Sorting the cells that will upregulate the antigens of interest and sequencing their edited loci will reveal the identity of genes that are able to regulate the expression of desired antigens. These identified genes will be validated, verified in primary cells and the underlying mechanisms elucidated. Drugs inhibiting the revealed gene(s) will be obtained and used to corroborate the findings also in an *in vivo* mouse model.

For proper implementation of this project, intense collaboration with the **Central Genomics** core facility will be crucial as well as cooperation with the **Animal facility** located within the university campus Bohunice. To accomplish the mechanistic studies, we envision the use of the **Cellular imaging core facility** and the **Proteomics core facility** to investigate subcellular localizations and protein-protein associations or protein phosphorylations.

2) Functional CRISPR/Cas9 genomics to reveal novel vulnerabilities as therapy targets

Here we aim to propose novel opportunities for targeted therapy by screening a panel of isogenic cell lines, i.e. differing in just one genetic aberration. Screening isogenic cell lines has the advantage that the observed phenotype can directly be attributed to the introduced genetic change. As a model disease, B-CLL was chosen as it was shown to be characterized by a variety of distinct somatic aberrations with the lack of any therapeutic biomarkers and limited targeted treatments. We have selected ten most prevalent aberrations recurrently found in CLL patients and will introduce these into a CLL cell line through specific CRISPR/Cas9 gene editing, thereby generating ten isogenic cell lines. These isogenic lines

will then be used to screen against whole-genome CRISPR/Cas9 knockout library. Next-generation sequencing (done jointly with **Central Genomics**) of the cell population after 14-day incubation period versus day 0 will reveal the identity of genes whose deletion leads to reduction in cell viability. Focusing on genes specific for single isogenic cell line will identify unique vulnerability explicitly for given genetic aberration. Identified genes will again be profoundly validated and confirmed on primary cells. Mechanisms underlying observed sensitivities will be investigated through a variety of molecular biology and cellular techniques. Here again we envision possible need for phosphoproteomic analysis or protein-protein interactions elucidation performed at the **Proteomics core facility**.
The main aim will be to identify a drug inhibiting the revealed gene product conferring the specific sensitivity. In this respect, we might team up with the lab of **Dr. Kubicek at CeMM** (Vienna, Austria) or **assoc. prof. Hajduch at IMTM** (Olomouc, CZ), who both possess large scale drug libraries and high-throughput drug screening facilities. Shall we identify only a tool compound, we may perform further drug optimization through medicinal chemistry approaches in cooperation with **Dr. Huber at the Nuffield Department of Medicine** (Oxford, UK). Finally, we will transfer our findings into an *in vivo* setting by performing the experiments in an appropriate CLL mouse model.

3) Chimeric antigen receptor T cells
Together with **prof. Mayer** (Department of Internal Medicine - Hematology and Oncology, Faculty of Medicine and University Hospital Brno) and **prof. Štěrba** (Clinic of Children Oncology) we have initiated a novel project to establish and optimize an innovative and exciting technology of Chimeric Antigen Receptor (CAR) T cells. These are patient´s autologous T cells that are genetically modified to carry a chimeric antigen receptor consisting of an extracellular antibody fragment (specific for a given antigen) fused to intracellular T-cell receptor signaling domains. Introduction of such a receptor reprograms patient´s T cells to specifically identify and kill only tumor cells expressing defined antigen. We plan to establish this technology in our laboratory, develop novel and improved designs of CAR constructs, lentivirally introduce them into T cells and exhaustively characterize generated products. In addition, we shall attempt to tackle some troubles of this therapy by producing allogeneic CAR-T cells, in which the endogenous TCR will be deleted by CRISPR/Cas9 system. The *in vitro* studies will again be followed by careful *in vivo* validations on mouse models. Finally, we will initiate intensive discussions with SÚKL (State Institute for Drug Control) to obtain permission for transferring this technology into clinic. We plan to establish CAR-T cells generation in our **Cleanroom facility at the University campus** or at the **ICRC at St Anna Hospital** (CTEU-cGMP facility) to be able to offer this to the patients at the University Hospital in the frame of a clinical trial.

Beside these projects, I am establishing several other important collaborations. As part of the cooperation with the team of **prof. Doubek** (IHOK, University Hospital Brno), we will be performing functional validations of diverse gene mutations that they identify in their thrombocytopenia patients. We have already initiated a collaboration with **prof. Veselská** (Department of Experimental Biology) to help her team with the use of lentiviral infection for knock-down and overexpression studies using specific transcription factors.
As I established the lentiviral technology in our cell culture laboratory, we have immediately introduced this technique to the members of research groups of **Mary O´Connell, Marek Mráz** and **Ondřej Slabý**. We have also trained them how to use the CRISPR/Cas9 technology and I am sure we will continue to join our efforts on this topic also in future. As it is very elegant technique, we will be happy to cooperate with any other labs and are very open for any new and exciting projects.

7. REFEREES

PD Dr. Jonathan A. Lindquist *(PhD supervisor)*
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