

**REPORT OF THE TUTOR ON PRAKASH SHANKARAN, MSc.
AND ON HIS DOCTORAL THESIS**

Prakash Shankaran, MSc. was officially accepted for PhD studies in Molecular biology, genetics and virology at the 1st Medical Faculty, Charles University since October 2007. Nevertheless, he joined our Virology lab only in May 2008 - because of his Indian citizenship, all the visa and other formalities took quite some time to arrange.

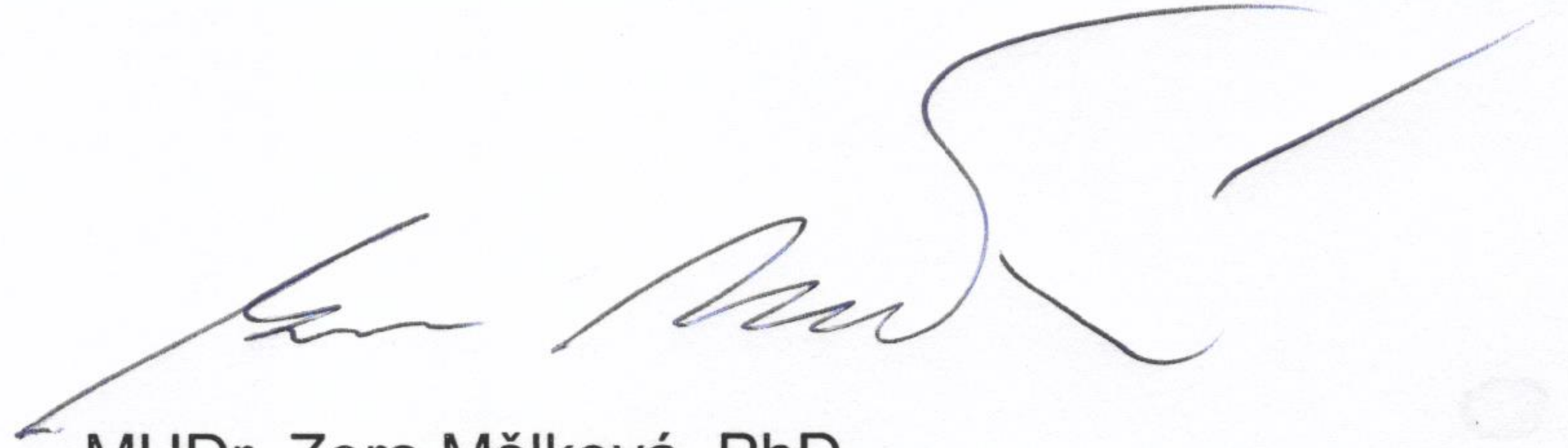
Engagement of Prakash was kind of adventurous from both his and my part, as we discussed about his scientific interests just by e-mail and on the phone, and then he applied for PhD studies at our faculty. However, the decision was fruitful in the end. Despite all different delays, obstacles, and back settings throughout the years, research performed by Prakash significantly contributed to the studies of the role of redox stress and heme arginate in HIV-1 acute infection and latency reversal. The first part of his results was finally published in 2011 in Antiviral Research after an almost 1 year of struggle to fulfill the requirements of the reviewers. In that time, we proposed that heme arginate might both reactivate the latent HIV-1 and prevent dissemination of the newly generated virions, the ideal combination for purging HIV-1 from the organism (the nowadays accepted "Shock and kill" strategy). Nevertheless, the way from the experiments in cell lines to primary HIV+ patients' cells was really painful and took several years. And only now, we are hopefully close to the tests in vivo, in human. During this time, Prakash significantly contributed to establishing the experiments in HIV+ patients' PBMCs ex vivo and to detecting HIV-1 transcripts using RT-qPCR and RT-ddPCR.

Lately, Prakash elaborated on the previous results with heme arginate and demonstrated the effects of individual heme degradation products, resulting in another research paper. In the meantime, he spent a lot of time and energy by performing experiments that are only marginally mentioned in the thesis. These experiments involved a screen of other potential latency reversing agents that resulted in a submission of a Czech and even international patent applications. Unfortunately, we had to retract the applications in order to be able to submit them successfully later.

During his PhD studies under my supervision, Prakash has got good experience with normal and HIV-infected tissue cultures, involving both cell lines and primary cells, with western blotting and its digital quantification, ELISA, flow cytometry, various PCR

techniques (namely RT-qPCR and RT-ddPCR). He passed all the required exams and fulfills the criteria for the PhD degree.

In conclusion, I fully recommend doctoral thesis of Prakash Shankaran, MSc. to be accepted and to award him the degree of PhD.



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