severe disease, and have a high acute mortality. This highlights the importance of ongoing efforts to develop and implement preventative strategies. The majority of these cases present within the first few months following ART initiation, and could potentially be prevented through cryptococcal antigen screening at ART initiation with pre-emptive therapy for those testing antigen positive.[11]

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References

1. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009 Feb 20;**23**(4):525–30.
2. Jarvis JN, Boulle A, Loyse A, Bicanic T, Rebe K, Williams A, et al. High ongoing burden of cryptococcal disease in Africa despite antiretroviral roll out. *AIDS* 2009;**23**:1181–5.
3. Lawn S, Harries A, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS* 2008 Oct 1;**22**(15):1897–908.
4. Lortholary O, Poizat G, Zeller V, Neuville S, Boibieux A, Alvarez M, et al. Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. *AIDS* 2006 Nov 14;**20**(17):2183–91.
5. Bicanic T, Meintjes G, Wood R, Hayes M, Rebe K, Bekker LG, et al. Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. *Clin Infect Dis* 2007 July 1;**45**(1):76–80.
6. Bisson GP, Nthobatsong R, Thakur R, Lesetedi G, Vinekar K, Tebas P, et al. The use of HAART is associated with decreased risk of death during initial treatment of cryptococcal meningitis in adults in botswana. *J Acquir Immune Defic Syndr*; Sep 2, 2008.
7. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis* 2010 Mar 15;**10**(1):67.
8. Jarvis JN, Meintjes G, Williams Z, Rebe K, Harrison T. Symptomatic relapse of HIV-associated cryptococcal meningitis in South Africa: the role of inadequate secondary prophylaxis. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde*, in press.
9. Bicanic T, Muzoora C, Brouwer AE, Meintjes G, Longley N, Taseera K, et al. Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. *Clin Infect Dis* 2009 Sep 1;**49**(5):702–9.
10. French MA, Price P, Stone SF. Immune restoration disease after antiretroviral therapy. *AIDS* 2004 Aug 20;**18**(12):1615–27.
11. Jarvis JN, Lawn SD, Vogt M, Bangani N, Wood R, Harrison TS. Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis* 2009 April;**48**(7):856–62.

Joseph N. Jarvis*
Infectious Diseases Unit, GF Jooste Hospital, Cape Town, South Africa
Desmond Tutu HIV Centre, Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Observatory 7925, South Africa
Centre for Infection, Department of Cellular and Molecular Medicine, St. George's University of London, Cranmer Terrace, London, SW17 0RE, UK
Division of Infectious Diseases and HIV Medicine, Department of Medicine, University of Cape Town, South Africa
*Desmond Tutu HIV Centre, Institute of Infectious Disease & Molecular Medicine, University of Cape Town, Faculty of Health Sciences, Anzio Road, Observatory 7925, South Africa.
Tel.: +44 27 21 650 6987; fax: +44 27 21 650 6963.
E-mail address: joejarvis@doctors.net.uk

Graeme Meintjes
Infectious Diseases Unit, GF Jooste Hospital, Cape Town, South Africa
Division of Infectious Diseases and HIV Medicine, Department of Medicine, University of Cape Town, South Africa
Institute of Infectious Diseases and Molecular Medicine, University of Cape Town, South Africa

Thomas S. Harrison
Centre for Infection, Department of Cellular and Molecular Medicine, St. George's University of London, Cranmer Terrace, London SW17 0RE, UK

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Therapeutic potential of peptide deformylase inhibitors against experimental tuberculosis

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Peptide deformylase;
Isoniazid;
rifampicin

Tuberculosis (TB) remains a leading cause of illness and death worldwide. The recent rise of TB is associated with the emergence of the human immunodeficiency virus (HIV) and rapid spread of multidrug-resistant and extensively

drug-resistant tuberculosis (XDR-TB). TB treatment presently requires 6 months of multidrug therapy. Although this chemotherapeutic regimen reduced the incidence of tuberculosis, the seemingly inevitable emergence of resistance of *Mycobacterium tuberculosis* to these antibiotics underline the need for improved treatment and the use of new antitubercular agents with novel mode of action.[1,2] In this respect, peptide deformylase (PDF) has been found to be an interesting potential candidate for discovering antimicrobial agents.[3] The unique difference between bacterial protein synthesis including mycobacteria and mammalian protein synthesis is the utilization of N-formylmethionine as the initiator for the bacterial process.[4] Whereas cytosolic protein synthesis in mammalian cells is initiated with methionine, protein synthesis in bacteria is initiated with N-formylmethionine.[4] The N-formylmethionine of the nascent bacterial proteins is subsequently removed by the sequential action of PDF and methionine amino peptidase (MAP) to form the mature functional protein. This formylation—deformylation cycle seems to be essential for bacterial growth, since it is conserved among all bacterial species studied and present in all sequenced pathogenic bacterial genomes.[5] Based on the structure of PDF, scientists have used either rational drug designing or high throughput studies to identify potent anti PDF drugs. Among these, actinonin and BB-3497 have been found to be efficacious PDF inhibitors against various gram-positive and gram-negative bacteria e.g. *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Escherichia coli*.[6] Also recently, the essentiality of PDF gene has been reported in *Mycobacterium bovis* BCG using PDF inhibitors under *in vitro* conditions.[7,8] However, complete *in vivo* validation of PDF inhibitors against experimental tuberculosis has not been worked out so far. Therefore, the present study was designed to evaluate the antitubercular potential of known PDF inhibitors e.g. actinonin and BB-3497 alone and in combination of antitubercular drugs (ATDs) against murine tuberculosis, to further validate future use of PDF inhibitors against tuberculosis. In our initial experiments, different known PDF inhibitors were evaluated for their potential against mycobacteria using short term chemotherapeutic regimen. The results of these experiments indicated BB-3497 and actinonin to be more potent antimycobacterial agents on the basis of cfu enumeration and BB-3497 was found to be comparable to isoniazid.[9] Both the compounds inhibited the mycobacterial growth in a concentration dependent manner and were found to be bacteriostatic in nature. Thus these two inhibitors were selected for further evaluation under 6 weeks chemotherapy following intranasal infection in murine model. Balb/c mice were infected intranasally with 10^5 CFU of *M. tuberculosis* $H_{37}R_v$ (*M.tb*) (grown in modified Youman's media at 37 °C) and kept for four weeks in order to establish the infection before the start of therapy. The establishment of infection was confirmed by sacrificing 5 mice followed by Ziehl—Neelsen staining of lung/spleen homogenates and basal bacterial load determination. The mice were grouped as follows with 6—8 animals in each group. The animals in each group were administered mentioned inhibitors and drugs through oral route and intraperitoneal (I.P.) route (for actinonin) daily at therapeutic doses required for 50 kg human being i.e., (INH — 10 mg/kg body weight, RIF-12 mg/kg body weight, PZA — 25 mg/kg body weight and actinonin — 25 mg/kg body weight through I.P route, and BB-3497 — 20 mg/kg body weight). The dosages used were determined on the basis of previous *in vivo* and safety data of these inhibitors in mice.[6,10,11]

After six weeks of chemotherapy, 6 animals from each group were sacrificed; lung and spleen homogenates (whole organs) were prepared under sterile conditions and homogenates (undiluted and 1:10 diluted) were plated on Middlebrook 7H11 agar plates for cfu enumeration after 28 days post-inoculation. The data were analyzed by analysis of variance (ANOVA), followed by Tukey test to compare the control and treated groups.

The actinonin treated mice have shown approximately 1.5 log cfu reduction in spleen and lungs as compared to untreated animals (Fig. 1) whereas the BB-3497 treated mice have shown almost 3 log reduction in cfu in lungs and spleen which was significantly lower as compared to control ($p < 0.001$) as well as to actinonin ($p < 0.01$) (Fig. 2). The efficacy of PDF inhibitors in combination with key frontline drugs replacing a bacteriostatic drug, PZA in murine model revealed encouraging results. When BB-3497 was replaced with PZA in three drug combination consisting of INH, RIF and PZA, the profile of cfu reduction for INH, RIF and BB-3497, was found to be similar to INH, RIF

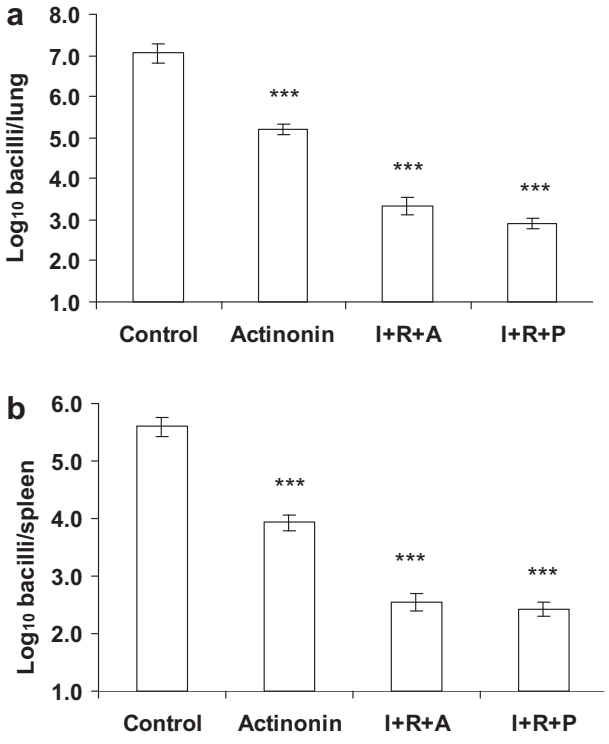

Figure 1 Chemotherapeutic efficacy of Actinonin (A) alone or in combination with INH (I), RIF (R) and PZA (P) against murine tuberculosis. (a) Log_{10} cfu in lung; (b) Log_{10} cfu in spleen. Results are expressed as the mean (log_{10} CFU) ± SD of 5—6 mice per group. The experiment was repeated two times with similar results. Significant difference determined by ANOVA followed by Tukey's test. ***$p \leq 0.001$ as compared to untreated group.