

Christopher S. Mulligan
Miriam E. Thomas, B.Sc.
Stephen P. Mulligan, M.B., B.S., Ph.D.

CLL Australian Research Consortium
Sydney, NSW 2113, Australia
mulligan@staff.usyd.edu.au

1. Rawstron AC, Bennett FL, O'Connor SJM, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. *N Engl J Med* 2008;359:575-83.
2. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood* 2008;111:5446-56.
3. French Cooperative Group on Chronic Lymphocytic Leukemia. Natural history of stage A chronic lymphocytic leukaemia untreated patients. *Br J Haematol* 1990;76:45-57.
4. Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood* 1996;87:4990-7.

THE AUTHORS REPLY: Our data are in agreement with those of Mulligan and colleagues, showing that persons with lymphocytosis and CLL-phenotype MBL are at risk for the development of progressive CLL even if the B-cell count at presentation is low. In our series, progressive lymphocytosis developed in 28% of patients (51 of 185); 3 of the 51 patients presented with a B-cell count below 1900 per cubic millimeter (as shown in Fig. 1B of our article), and 2 of these 3 patients eventually required treatment for progressive CLL. There is no cutoff point that absolutely discriminates the risk of progression. However, a B-cell count below 1900 per cubic millimeter was identified as the

optimal discriminator in our series on the basis of Youden's J statistic.

Both data sets confirm that few patients with a B-cell count below this level have disease progression. This level is close to the upper limit of CLL-phenotype MBL detectable in persons with normal blood counts.^{1,2} The use of the CLL cell count instead of the B-cell count makes little or no difference with respect to CLL and MBL classification because there are virtually no normal B cells remaining in cases in which the B-cell count is above 4000 per cubic millimeter. Cases with a low B-cell count that are investigated for lymphocytosis usually have increased T-cell numbers, which was a good prognostic factor for overall survival in univariate analysis and partly explains why the absolute lymphocyte count is relatively uninformative in MBL. Therefore, we agree with the use of the B-cell count to classify CLL and MBL and recommend that periodic blood-count monitoring be performed for all patients with CLL-type MBL presenting with lymphocytosis, independently of the B-cell count at presentation.

Andy C. Rawstron, Ph.D.

Peter Hillmen, Ph.D.

Leeds Teaching Hospitals
Leeds LS9 7TF, United Kingdom
andy.rawstron@hmds.org.uk

1. Rawstron AC, Green MJ, Kuzmicki A, et al. Monoclonal B lymphocytes with the characteristics of "indolent" chronic lymphocytic leukemia are present in 3.5% of adults with normal blood counts. *Blood* 2002;100:635-9.
2. Ghia P, Prato G, Scielzo C, et al. Monoclonal CD5+ and CD5- B-lymphocyte expansions are frequent in the peripheral blood of the elderly. *Blood* 2004;103:2337-42.

Cellulose Sulfate for Prevention of HIV Infection

TO THE EDITOR: The results of the trial of cellulose sulfate as a vaginal gel for the prevention of human immunodeficiency virus (HIV) infection, reported by Van Damme et al. (July 31 issue),¹ indicated that cellulose sulfate did not prevent sexual transmission of HIV and may have increased the risk of HIV acquisition, as compared with placebo. The cellulose sulfate and placebo gels had a pH of 7.5 and 4.4, respectively. The healthy human vagina provides a low pH, diminishing HIV infectivity and transmission of cell-associated HIV.^{2,3} Therefore, microbicides should have a pH of approximately 4.5.³ The apparently

increased risk of HIV acquisition among women using the cellulose sulfate gel might be due to the disparity in pH between the active-treatment and placebo gels.

The anti-HIV activity of anionic polymers (including cellulose sulfate) may be compromised by seminal fluid.⁴ This is expected to be overcome by inclusion of acidic pH buffering systems.⁴ Such combinations are currently in the microbicide pipeline. Langerhans' cells first encounter HIV during sexual transmission of the virus and bind HIV by means of langerin. Captured virus is internalized and degraded.⁵ It remains to be estab-

lished whether candidate microbicides interfere with this defense mechanism and possibly increase the probability of HIV acquisition.

A. Robert Neurath, Ph.D.

Virotech
New York, NY 10003
arneurath@att.net

1. Van Damme L, Govinden R, Mirembe FM, et al. Lack of effectiveness of cellulose sulfate gel for the prevention of vaginal HIV transmission. *N Engl J Med* 2008;359:463-72. [Erratum, *N Engl J Med* 2008;359:877.]
2. Olmsted SS, Khanna KV, Ng EM, et al. Low pH immobilizes and kills human leucocytes and prevents transmission of cell-associated HIV in a mouse model. *BMC Infect Dis* 2005;5:79.
3. Weber J, Desai K, Darbyshire J. The development of vaginal microbicides for the prevention of HIV transmission. *PLoS Med* 2005;2(5):e142.
4. Neurath AR, Strick N, Li Y-Y. Role of seminal plasma in the anti-HIV-1 activity of candidate microbicides. *BMC Infect Dis* 2006;6:150.
5. de Witte L, Nabatov A, Pion M, et al. Langerin is a natural barrier to HIV-1 transmission by Langerhans cells. *Nat Med* 2007;13:367-71.

TO THE EDITOR: The report that cellulose sulfate did not reduce the risk of HIV acquisition, and in fact increased it in women completing the protocol, is not surprising, given that clinically relevant concentrations of this compound reproducibly increase the in vitro infection rate of sexually transmissible R5-tropic strains of HIV.¹ Similar results were reported more than a decade ago for another sulfated polyanion, dextran sulfate, which increased the replication of primary isolates of HIV both in vivo² and in vitro.³ Unfortunately, detailed titrations, which are essential for detecting enhancement by biphasic compounds such as cellulose and dextran sulfate, have not been published for the viral strains that were prevalent at the trial sites.

Chris Richards, B.S.
Wang Tao, M.D., Ph.D.
Dean Hamer, Ph.D.
National Institutes of Health
Bethesda, MD 20892
deanh@helix.nih.gov

1. Tao W, Richards C, Hamer D. Enhancement of HIV infection by cellulose sulfate. *AIDS Res Hum Retroviruses* 2008;24:925-9.
2. Flexner C, Barditch-Crovo PA, Kornhauser DM, et al. Pharmacokinetics, toxicity, and activity of intravenous dextran sulfate in human immunodeficiency virus infection. *Antimicrob Agents Chemother* 1991;35:2544-50.
3. Meylan PRA, Kornbluth RS, Zbinden I, Richman DD. Influence of host cell type and V3 loop of the surface glycoprotein on susceptibility of human immunodeficiency virus type 1 to polyanion compounds. *Antimicrob Agents Chemother* 1994;38:2910-6.

THE AUTHORS REPLY: In response to Neurath: the low pH of a formulation is not enough to inactivate HIV transported by semen (pH of approximately 7.9) unless it is accompanied by a strong acid-buffering capacity. Although the cellulose sulfate and hydroxyethylcellulose-based placebo gels have different pH values, neither has significant buffering capacity. This is reinforced by the lack of anti-HIV activity of the hydroxyethylcellulose placebo (pH of approximately 4.5) in preclinical studies.¹ We agree that seminal plasma reduces the antiviral activity of anionic compounds; however, cellulose sulfate S was clinically administered at a concentration (60 mg per milliliter) that is orders of magnitude higher than the seminal plasma-increased median effective concentration reported by Neurath and colleagues.²

Regarding the comments by Richards et al., the statement that clinically relevant concentrations of cellulose sulfate reproducibly increase the in vitro infection rate of R5 tropic strains of HIV (R5-HIV) omits mention of the finding that all other tested concentrations in the data reported by Tao et al.³ were either not effective (<0.3 μg per milliliter) or highly inhibitory (>3 μg per milliliter). Although we do not dispute the reported spike in infectivity between 0.3 and 3.0 μg per milliliter, we question its clinical relevance, given that cellulose sulfate was applied intravaginally at 210 mg per dose. Furthermore, the CONRAD data presented by Tao et al.³ show a reduction in R5-HIV infection between 1.0 and 3.0 μg per milliliter, a disparity in results that cannot be explained by lack of statistical power. Although the results of the intravenous dextran sulfate study should be considered as a possible explanation for our findings, its experimental protocol is considerably different. In addition, cellulose sulfate did not induce a significant increase in macrophage infection in vitro.⁴

The potential increased risk of infection observed in our per-protocol analysis was driven by results from two sites (Benin and Uganda) where gel was reportedly used 20 times per week on average (9 infections with cellulose sulfate and 1 with placebo). This frequency of use was dramatically higher than the four-times-per-week use reported in South Africa, where there was essentially no evidence of an effect (12 infections with cellulose sulfate and 10 with placebo). Although not conclusive, these findings suggest that a mechanism related to very frequent exposure to

cellulose sulfate is a more likely explanation for our results.

Lut Van Damme, M.D.
Doug Taylor, Ph.D.
Family Health International
Arlington, VA 22203
lvandamme@fhi.org

1. Tien D, Schnaare RL, Kang F, et al. In vitro and in vivo characterization of a potential universal placebo designed for use in

vaginal microbicide clinical trials. *AIDS Res Hum Retroviruses* 2005;21:845-53.

2. Neurath AD, Strick N, Li Y-Y. Role of seminal plasma in the anti-HIV-1 activity of candidate microbicides. *BMC Infect Dis* 2006;6:150.

3. Tao W, Richards C, Hamer D. Enhancement of HIV infection by cellulose sulfate. *AIDS Res Hum Retroviruses* 2008;24:925-9.

4. Scordi-Bello IA, Mosoian A, He C, et al. Candidate sulfonated and sulfated topical microbicides: comparison of anti-human immunodeficiency virus activities and mechanisms of action. *Antimicrob Agents Chemother* 2005;49:3607-15.

Noninvasive Ventilation in Acute Cardiogenic Pulmonary Edema

TO THE EDITOR: Gray et al. (July 10 issue)¹ report that the Three Interventions in Cardiogenic Pulmonary Oedema (3CPO) trial showed no benefit from noninvasive ventilation for reducing the intubation rate or short-term mortality. These findings are of concern and appear to conflict with previous trial data.^{2,3}

This trial may be biased and lack generalizability for several reasons. First, sick patients, who required “lifesaving or emergency intervention” and might have benefited most, were excluded. Second, 19.4% of patients did not complete their assigned treatment. Third, there was considerable cross-contamination among the treatment groups.

Furthermore, there were no objective criteria for intubation. We are alarmed by the noticeably low intubation rate (3%) in the 3CPO trial, as compared with pooled data from both the noninvasive-ventilation groups (32%) and the control groups (12%) in previous studies.² Moreover, the observed mortality at 7 days (9.6%) greatly exceeded the intubation rate.

Why were these patients with cardiogenic pulmonary edema who did not undergo ventilation dying? It is uncertain whether decisions regarding end-of-life care or the use of life support in this elderly cohort may have confounded the observed outcome.

Robert C. McDermid, M.D.
Sean M. Bagshaw, M.D.

University of Alberta
Edmonton, AB T6G 2B7, Canada
robmcdermid@telus.net

1. Gray A, Goodacre S, Newby DE, Masson M, Sampson F, Nicholl J. Noninvasive ventilation in acute cardiogenic pulmonary edema. *N Engl J Med* 2008;359:142-51.

2. Masip J, Roque M, Sánchez B, Fernández R, Subirana M, Exósito JA. Noninvasive ventilation in acute cardiogenic pulmonary edema: systematic review and meta-analysis. *JAMA* 2005; 294:3124-30.

3. Peter JV, Moran JL, Phillips-Hughes J, Graham P, Bersten AD. Effect of non-invasive positive pressure ventilation (NIPPV) on mortality in patients with acute cardiogenic pulmonary oedema: a meta-analysis. *Lancet* 2006;367:1155-63.

TO THE EDITOR: The lack of a beneficial effect of noninvasive ventilation in the 3CPO trial contrasts with previous guidelines and meta-analyses.¹⁻⁴ First, both the mortality and intubation rates were much lower in the 3CPO trial than in the meta-analysis (Table 1), indicating that the populations were different. Second, patients in the 3CPO trial did not have hypoxemia (average baseline partial pressure of arterial oxygen, 98 to 101 mm Hg), and this fact might explain the low intubation rate in the study. Although it was not described, there was probably some delay in initiating the protocol. A delay of only 15 minutes markedly attenuates the beneficial effect of noninvasive ventilation.⁵ Third, progressive respiratory failure, treated by means of intubation in patients who received standard oxygen therapy in previous studies, was managed mainly with rescue noninvasive ventilation in the 3CPO study. This crossover did not alter the intubation rate, suggesting that noninvasive ventilation was effective in these patients but

Table 1. Comparison of Overall Mortality and Intubation Rates in the 3CPO Trial and a Previous Meta-Analysis.

Variable	3CPO, 7-Day Rate (N = 1069)	Meta-Analysis, In-Hospital Rate (N = 783)*
	% of patients	
Mortality	9.6	15.3
Intubation	2.9	21.9

* Data are from Masip et al.²