

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Cool CD, Rai PR, Yeager ME, et al. Expression of Human Herpesvirus 8 in Primary Pulmonary Hypertension. *N Engl J Med* 2003;349:1113-22.

Supplementary Appendix 1. Treatment and Hemodynamic Characteristics of the Patients.*

Patient No.	Prostacyclin Therapy	Mean PAP <i>mm Hg</i>	Cardiac Output <i>liters/min</i>	PVR <i>Wood units</i>
1	Yes	99	NA	NA
2	Yes	49	2.9	NA
3	Yes	55	3.9	14.2
4	Yes	64	2.1	27.6
5	No	NA	NA	NA
6	No	112	2.0	49.2
7	No	NA	NA	NA
8	Yes	58	3.6	13.9
9	Yes	NA	NA	NA
10	Yes	44	4.5	9.8
11	Yes	70	2.3	23.4
12	No	NA	NA	NA
13	Yes	NA	NA	NA
14	No	86	3.4	25.1
15	No	NA	NA	NA
16	Yes	NA	NA	NA
17	NA	NA	NA	NA
18	NA	NA	NA	NA
19	NA	NA	NA	NA
20	NA	NA	NA	NA
21	NA	46	2.6	13.9
22	Yes	52	4.83	8.5
23	NA	NA	NA	NA
24	NA	48	3.15	15.3
25	NA	58	3.0	17.8
26	Yes	NA	NA	NA
27	Yes	71	NA	NA
28	Yes	NA	NA	NA
29	Yes	70	NA	NA
30	Yes	104	NA	NA
31	NA	NA	NA	NA

* PAP denotes pulmonary-artery pressure, PVR peripheral vascular resistance, and NA not available. The workup of patients with pulmonary hypertension begins with a screening echocardiogram and includes a battery of tests for pulmonary embolic disease (ventilation–perfusion lung scanning or helical computed tomography [CT] of the chest and, in rare cases, pulmonary angiography — for example, in Patient 14), intracardiac shunts (determined during routine right heart catheterization), collagen vascular diseases (tests for anti-nuclear antibodies and antiphospholipid antibodies and erythrocyte sedimentation rate and measurement of C-reactive protein), history of drug use (amphetamines, anorexigens, or injection drugs), sleep apnea (nocturnal oximetry followed by formal sleep-apnea monitoring), lung diseases (complete lung-function study and high-resolution chest CT), and more recently, human immunodeficiency virus infection and liver and thyroid function.

SUPPLEMENTARY APPENDIX 2

TESTS FOR MUTATIONS IN *BMPR2*

The various exons of the gene for bone morphogenetic protein receptor 2 (*BMPR2*) were amplified with use of the polymerase chain reaction (PCR) from 100 ng of genomic DNA purified from paraffin-embedded tissue (Epicentre Technologies) with use of the proofreading Herculase polymerase (Stratagene) and 100 ng of each oligonucleotide primer (Deng). The PCR programs were optimized for each amplicon. Restriction-fragment-length polymorphism (RFLP) analysis was performed by digesting the various amplicons with their specific restriction enzyme (New England Biolabs) and subjected to electrophoresis in NuSieve agarose at different concentrations in TBE buffer (TRIS base, boric acid, and EDTA) at 70 V. The gels were stained with ethidium bromide, and the images were analyzed with Kodak version 3.6 software to determine the molecular weights of the products of digestion. The editing of sequences and the construction of restriction maps were done with use of DNASTAR version 4.0 software.

Exons 3, 4, 8, 12b, and 12d were amplified by two rounds of touchdown PCR programs (programs in which the annealing temperature is gradually reduced in order to ensure amplification) as follows: a first round of 20 cycles at 94°C for 15 seconds and then 65°C for 30 seconds, with the temperature decreased by 2°C for each cycle, followed by 10 cycles at 94°C for 15 seconds and then 55°C for 30 seconds, and a second round of 20 cycles at 94°C for 15 seconds and then 60°C for 30 seconds, with the temperature decreased by 2°C for each cycle, followed by 25 cycles at 94°C for 15 seconds and then 60°C for 30 seconds. Exons 9, 6, and 12 were amplified with PCR consisting of denaturing at 92°C for 2 minutes and 35 cycles at 92°C for 30 seconds; amplification at 60.2°C, 61.9°C, and 59°C, respectively, for 30 seconds and at 72°C for 30 seconds; and finally 10 minutes of extension at 72°C.