29 September 2004

Mr David Rudov,
Director,
Schumacher Pharmaceuticals Pty Ltd
252 Collins Street Melbourne
VIC 3000

Dear David,

I attach the results of virus inhibition assays for influenza A/Panama/2007/99 (H3N2), A/New Caledonia/20/99 (H1N1) and B/Shandong/7/97, which are the prototype viruses whose surface antigens are included in the current Australian influenza vaccine, and the "T" strain of avian infectious bronchitis virus. The latter is a coronavirus, which could be reasonably expected to be inhibited in the same manner as for the SARS virus.

For the influenza test system cells of the Madin-Darby canine kidney line were used and Oralmat was shown to be toxic at dilutions of 1:50-1:100 or lower. For influenza A/Panama/2007/99, 23.3-50% inhibition of infectious titre occurred at dilutions of 1:200-1:1000; for Influenza A/New Caledonia/20/99, these figures are 5.6-43.5% over the same drug concentration. Greatest inhibition was noted for influenza B/Shandong/7/97, which when tested at dilutions of 1:100-1:1000 showed inhibition of 2.0-70.8%.

The avian coronavirus was tested in primary chicken embryo kidney cultures over a dilution range of 1:100-1:1000 but inhibition was much less and ranged from 0-16.6%.

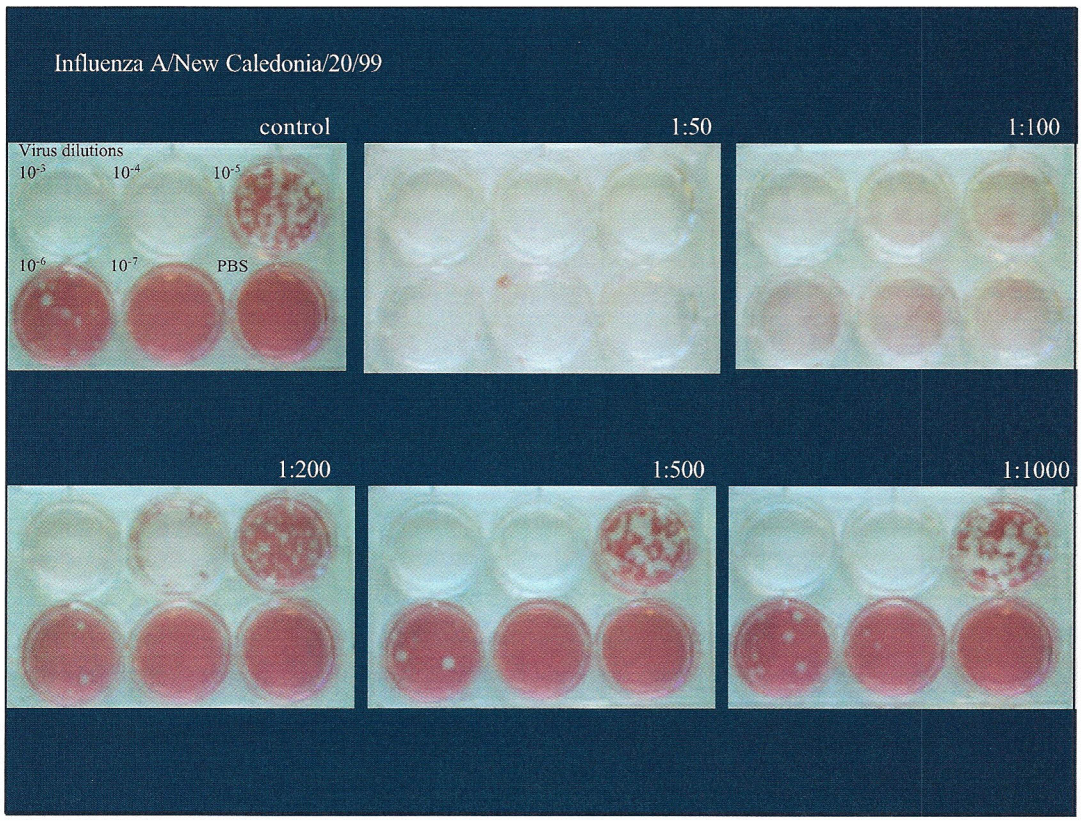
For influenza A/New Caledonia/20/99 and B/Shandong/7/97 measurements of plaque diameter were also made. The results indicate that, in addition to reducing infectious titre, the drug causes what appears to be a significant decrease in plaque diameter over the test dilutions. Plaque size reduction is probably a more sensitive test of inhibition and has been noted with some of the neuraminidase inhibitors that comprise the new generation of anti-influenza drugs now marketed by Roche and GSK.

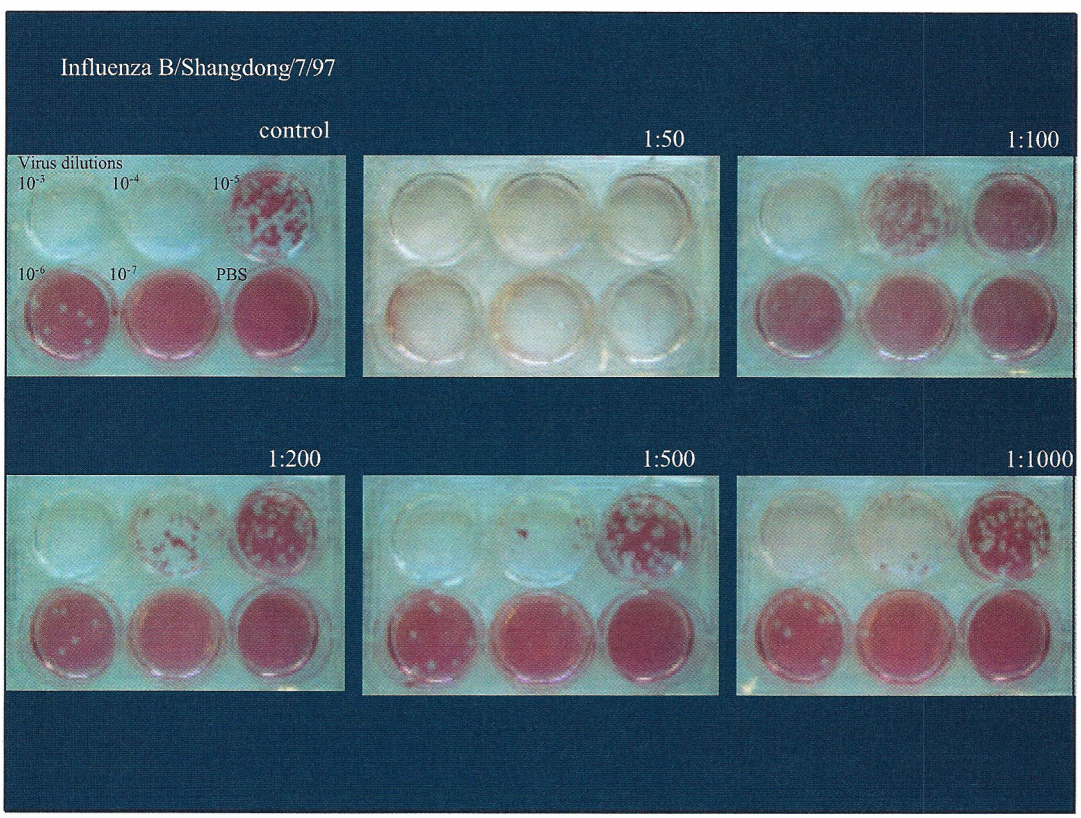
Overall, we have shown in our test systems that Oralmat does cause inhibition of two contemporary strains of influenza A and one of influenza B when tested at dilutions of 1:100-1:1000, but little effect against the coronavirus avian infectious bronchitis virus. I also enclose photographs which demonstrate our assay system for A/New Caledonia/20/99 and B/Shandong/7/97. I would be happy to discuss these results and any other work you may have in mind with you at any time. An invoice for $4,500 will be sent to you separately through the RMIT administration.

Yours sincerely,

G.A. Tannock

Professor of Virology


Influenza A/New Caledonia/20/99
control
1:50
1:100
Virus dilutions
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
PBS
1:200
1:500
1:1000


Influenza B/Shangdong/7/97
control
1:50
1:100
Virus dilutions
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}
PBS
1:200
1:500
1:1000

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Influenza A/Panama/2007/99 (H3N2) – assayed in MDCK[a] cell monolayers at 34°C

	Control	1:50	1:100	1:200	1:500	1:1000
Toxicity	-	++	++	-	-	-
Virus titre (PFU $mL^{-1}x10^7$)	6.82	ND[b]	ND	4.55	5.23	3.41
% reduction of titre		ND	ND	33.3	23.3	50

Influenza A/New Caledonia/20/99 (H1N1) – assayed in MDCK cell monolayers at 34°C

	Control	1:50	1:100	1:200	1:500	1:1000
Toxicity	-	++	++	-	-	-
Virus titre (PFU $mL^{-1}x10^8$)	1.61	ND	ND	0.91	1.52	1.43
% reduction of titre		ND	ND	43.5	5.6	11.2
Mean ± SD (Plaque diameter, mm)	1.92±1.15	ND	ND	0.83±0.89	1.3±0.98	2.1±1.02
% reduction of mean plaque diameter		ND	ND	56.8	32.3	-9.4

Influenza B/Shangdong/7/97 – assayed in MDCK cell monolayers at 34°C

	Control	1:50	1:100	1:200	1:500	1:1000
Toxicity	-	++	-	-	-	-
Virus titre (PFU $mL^{-1}x10^7$)	6	ND	1.75	3.40	5	4.09
% reduction of titre		ND	70.8	43.3	16.6	31.8
Mean ± SD (Plaque diameter, mm)	2.95±0.44	ND	0.86±0.24	1.65±0.41	2.89±0.33	2.34±0.51
% reduction of mean plaque diameter		ND	70.8	44.1	2.0	20.7

Avian Infectious Bronchitis Virus (IBV) – assayed in Chicken Embryo Kidney Primary cell monolayers at 37°C

	Control	1:100	1:200	1:500	1:1000
Toxicity	-	-	-	-	-
Virus titre (PFU $mL^{-1}x10^6$)	9	7.5	10.5	8	10.0
% reduction of titre		16.6	0	11.1	0

a: Cells of the Madin-Darby Canine Kidney line are commonly used for influenza virus assay.
b: Not Detected

Greg Tannock
Professor of Virology
Department of Biotechnology and Environmental Biology
RMIT University
PO Box 71, Bundoora Victoria 3083 Australia
E-mail: gtan@rmit.edu.au
http://www.life.rmit.edu.au/biotechnology
Tel +61 3 9925 7142 Fax +61 3 9925 7110