analysis. *K-ras* mutations were found in 14 (25%) cases of adenocarcinoma; 13 were detected by direct sequencing and the remaining mutation was only detected by pyrosequencing analysis. Detected mutations were G12C (eight cases), G12V (four cases), G12D (one case) and G12A (the case identified by pyrosequencing). This group comprised eight females and six males, of whom 11 were current smokers, two were former smokers and one had never smoked. Two had stage II, seven had stage III and five had stage IV disease. None of the patients showed both *EGFR* and *K-ras* mutations.

In five patients with gene mutations, two *EGFR* and three *K-ras*, we performed the analysis on a scalar number of cells isolated from cytologic samples by pyrosequencing (from 100 to 20 cells) to verify whether mutations could be detected in a small number of cells. *EGFR* and *K-ras* mutations were detectable in samples with as few as 20 cells. In 32 patients for whom cell blocks (20 samples) and/or histologic material (19 samples) were available, comparative analyses confirmed results obtained from cytologic smears (table 1).

In conclusion, we demonstrated that cytologic specimens from archival material are adequate for *EGFR* and *K-ras* molecular analyses and that results are concordant with those obtained from histologic material. We also showed that very few cells are required for mutation detection, thus enabling molecular analyses to be performed on patients for whom very little biological material is available, and leaving open the possibility of using different methodologies to analyse other potentially interesting molecular targets.

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Ambulatory oxygen in idiopathic pulmonary fibrosis: of what benefit?

To the Editors:

We read with interest the Letter by VISCA *et al.* [1], recently published in the *European Respiratory Journal*, which described improvement in 6-min walk distance with ambulatory oxygen in patients with interstitial lung disease (ILD). We have looked specifically at the effects of ambulatory oxygen on walk distance in patients with idiopathic pulmonary fibrosis (IPF), and here describe a practical way of ensuring patients are prescribed an optimum flow rate of ambulatory oxygen.

Between 2004 and 2007, we conducted a retrospective review of anonymised data, studying the effect of ambulatory oxygen on the distance walked in patients with IPF in the ILD clinic of the University Hospital of South Manchester (Manchester, UK). 70 patients performed an adapted 6-min walk test (6MWT) on air or their usual flow rate of oxygen. If their oxygen saturation fell to <90%, the test was terminated and repeated with a 2 $L \cdot min^{-1}$ increase in oxygen flow rate. This continued until patients did not desaturate to <90% or reached a 6 $L \cdot min^{-1}$ flow rate. Diagnosis of IPF was based on the

TABLE 1 Results of adapted 6-min walk test in patients previously on and not on oxygen

	Patients on oxygen prior to walk test[#]			Patients not on oxygen prior to walk test[¶]		
	Baseline walk test	Optimal O_2 walk test	p-value	Baseline walk test	Optimal O_2 walk test	p-value
Distance walked m	76.5±66.5	93.4±66.6	0.02	135.0±108.8	216.2±115.0	<0.01
S_{p,O_2} %						
Pre-walk	94.4±2.1	96.6±1.9	<0.01	94.0±2.0	97.7±1.0	<0.01
Post-walk	77.3±7.6	83.0±6.9	<0.01	80.5±5.5	86.9±3.5	<0.01
Borg score						
Pre-walk	1.1±1.5	1.1±1.3	0.22	1.4±1.8	1.1±1.7	0.07
Post-walk	4.8±2.1	4.5±2.2	0.80	4.1±1.7	3.7±2.0	0.13

Data are presented as mean±SD, unless otherwise stated. S_{p,O_2}: arterial oxygen saturation measured by pulse oximetry. [#]: n=41; [¶]: n=29.

American Thoracic Society (ATS)/European Respiratory Society (ERS) consensus statement [2] and the 6MWT was conducted as per the ATS protocol [3]; however, once the patient had stopped, they were not allowed to continue. The distance patients walked was compared using the Wilcoxon signed-rank test. A paired t-test was used to compare oxygen saturations and Borg scores, and an unpaired t-test was used to compare characteristics of the patients who initially performed the walk test on air and those who performed it on their usual flow rate of oxygen.

Characteristics were as follows in patients who performed the walk test initially on their usual flow rate of oxygen *versus* those who performed it on air: 62.8 *versus* 61.5 yrs of age (p=0.62); 80.5 *versus* 65.5% males (p=0.16); 83 *versus* 65.5% ever-smokers (p=0.16); 51.2 *versus* 58.4 forced vital capacity % predicted (p=0.15); 27.7 *versus* 32.4 diffusion capacity of the lung for carbon monoxide % pred (p=0.05); 8.7 *versus* 8.5 kPa ear lobe gas partial oxygen pressure at rest (p=0.5).

The 29 patients not on oxygen therapy prior to testing walked a mean distance of 81.2 m further using optimal ambulatory oxygen (p<0.01). The 41 patients already on home oxygen walked a mean distance of 16.9 m extra with optimised flow rates (p=0.02) (table 1). Borg scores were not significantly different, suggesting that patients walked further with the same degree of breathlessness.

As well as its retrospective nature, limitations to this study are that patients who performed their baseline walk test on air did not carry a placebo air cylinder, and we did not document whether the increase in distance walked reflected improved quality of life.

Our study demonstrates that ambulatory oxygen significantly improves exercise capacity in IPF patients. In patients already using oxygen, titrating the flow rate of oxygen appropriately also significantly increased the distance they were able to walk. We have described a step-wise method of increasing ambulatory oxygen in 2 $L \cdot min^{-1}$ increments and suggest this is an objective method of ensuring patients are prescribed an optimum flow rate.

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