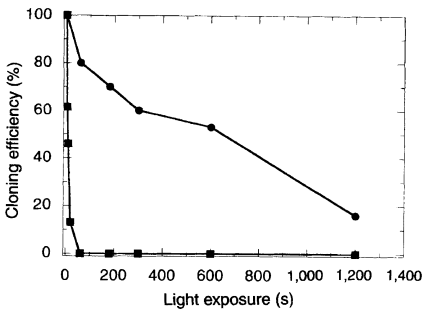

FIG. 2 Cloning efficiency against exposure time for CHO cells; 88 mW of 760-nm (■) and 800-nm (●) microirradiation.

and became red within 65±21 s (n=10). This shows two effects of the 760-nm trapping beam. First, two-photon-excited visible fluorescence is induced by the near-infrared trapping beam. Second, green to red fluorescence changes (coinciding with cessation of flagellar motion at 35±20 s (n=10)) indicate that the trap can cause lethal damage. In contrast, we were able to maintain most sperm (96±6%, n=50) in an 800-nm trap for 10 min without either loss in motility or intranuclear propidium iodide accumulation.

Unlabelled spermatozoa showed blue, two-photon-excited autofluorescence which became 10–100-fold brighter within 600 s of 760-nm trapping (n=10). Autofluorescence was also detected during 800-nm trapping, but the spot intensity did not change. As autofluorescence signals are probably due to reduced pyridine coenzymes (NAD(P)H), 760-nm alterations may be a consequence of UVA-like, two-photon-induced perturbations of the cellular redox state (that is, oxidative stress).

A final indicator of cellular damage was provided by evaluating the cloning efficiency of microirradiated Chinese hamster ovary (CHO) cells. Interphase cells exposed for up to 1,200 s (88 mW) and maintained in an incubator for 5–6 days were considered to be unaffected by light if clones consisting of ≥ 50 cells were produced. Cells irradiated with 760 nm were unable to form clones following light exposures of ≥60 s, whereas cloning efficiencies of 80% and 50% were measured following 60- and 600-s exposures at 800 nm (Fig. 2).

In conclusion, continuous-wave near-infrared microbeams can stimulate multiphoton processes in single living cells. The consequences of microirradiation include membrane permeability changes, alterations in cloning efficiency and UVA-like stress. These processes are clearly wavelength-dependent and should be considered during trapping experiments, particularly when using short-wavelength, near-infrared lasers.

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Kaposi's sarcoma in pregnant women

SIR — Lunardi-Iskandar *et al.*[1] demonstrated that the β-chain of human chorionic gonadotropin (hCG) kills Kaposi's sarcoma-derived cell lines in culture and inhibits tumour production by these cell lines in nude mice. They also observed regression of Kaposi's sarcoma in two women during pregnancy, when levels of this hormone are elevated[2]. Because of the potential aetiological and therapeutic significance of these results, we examined clinical data from the Dermatovenereology Clinic, University Teaching Hospital, Lusaka, Zambia. We postulated that if hCG protected against disease in humans, AIDS-related Kaposi's sarcoma would occur with lower frequency or severity during pregnancy.

We determined the pregnancy status of all female patients ≤ 40 years old who were newly diagnosed with AIDS-related Kaposi's sarcoma during 1994. For comparison, we used new cases of venereal ulcer or discharge presenting during 4 months (January, April, July and October) of that year. Non-pregnant women with Kaposi's sarcoma were further classified into those with and without a child under 2 years of age.

Thirteen (15%) of the 84 young women with Kaposi's sarcoma and 25 (19%) of the 133 young women with venereal disease were pregnant at the time of presentation. Adjusted for age, the odds ratio for the association of current pregnancy and Kaposi's sarcoma was 1.0 (95% confidence interval, 0.4–2.1). An additional 23 (27%) women with Kaposi's sarcoma had a child under 2 years of age. Thus, 36 (43%) of the Kaposi's sarcoma patients had been exposed to elevated levels of hCG within the past 2 years.

We also assessed the relationship of pregnancy status to extent of tumour at presentation, classified as localized against disseminated according to ref. 3. Seven (54%) of the 13 who were pregnant had disseminated disease, as compared with 12 (52%) of those with children under 2 years of age and 19 (40%) of the 48 who were neither pregnant nor had a child under 2. The age-adjusted odds ratio for the association of current pregnancy and disseminated sarcoma was 1.3 (95% confidence interval, 0.4–4.2).

These clinical data do not support the prediction from laboratory results that hCG is protective against Kaposi's sarcoma. Our data are not adjusted for possible fertility differences between these patient groups, nor for possible referral bias due to pregnancy and/or antenatal care. Furthermore, lack of effect at physiological levels does not exclude efficacy at higher pharmacological levels, for which the best test is a randomized, controlled clinical trial. Nevertheless, as pregnant and non-pregnant women appear to have a similar risk of this disorder, it is likely that factors other than hCG explain the difference between men and women in incidence of Kaposi's sarcoma.

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Pregnancy hormone and tumour marker

SIR — The classical pregnancy hormone and tumour marker human chorionic gonadotropin (hCG) has long been known for its ability to sustain pregnancy. In addition, it acts as a survival factor by suppressing apoptotic cell death in reproductive organs[4]. An entirely adverse effect of hCG, its blocking ability on tumorigenesis and metastasis of Kaposi's sarcoma, has recently been reported[1]. The authors' hypothesis that the trophic hormone hCG and its free β-subunit (βhCG) cause regression of Kaposi's sarcoma was tested in immunodeficient Bg-nude mice as well as *in vitro* with pregnancy sera of human and mouse origin, and with different preparations of hCG-derived molecules. Their premises run contrary to several long-held concepts and definitions of endocrinology concerning the evolutionary origin, biological function and nomenclature of hCG-derived molecules.

From an evolutionary point of view, hCG is a young hormone, as the genetic events generating its β-subunit took place long after the lineages of rodents and primates diverged[5]. Rodents possess neither a βCG gene nor a placental hormone similar to CG (refs 6, 7). In consequence, the mouse displays a gestational profile of hormone dependency entirely distinct from humans. Human gestation is dependent on placental hCG right from the beginning, whereas for the maintenance of pregnancy in rodents pituitary-derived luteinizing hormone (LH) — the cognate molecule of hCG — is an indispensable stimulus only in the second trimester[8]. The question remains, what is causing the

effects on Kaposi's sarcoma in the absence of a CG-like molecule in mice?

Of potentially more importance, the biological function of hCG is mediated by the αhCG/βhCG dimer and not by the free βhCG subunit, but the same function and receptors are being proposed for hCGβ as for hCG by Lunardi-Iskandar *et al.* This contradiction could be clarified by conventional radioreceptor assays or by direct demonstration of LH/hCG-receptor gene expression, but not by an immunohistochemical demonstration of receptors with a polyclonal antiserum against the ligand, as performed by Lunardi-Iskander *et al.* The hormonal profiles of pregnancy sera differ vastly from sera of non-pregnant humans and mice, not only with respect to hCG. The investigators should have included some conclusive specificity controls such as immunoneutralization by monoclonal antibodies against both hCG and its subunits[9,10]. The same applies to the crude hCG preparations used in their bioassays, especially as they did not rely on highly purified material (for example, hCG CR-127, 14,900 IU per mg), which is made available by their own research institution, NIH. Whether it is really CG, or even more surprisingly free βhCG, which exerts antioncogenic effects on Kaposi's sarcoma is therefore not resolved.

Finally, by definition, βhCG is not a pregnancy hormone, as claimed by Lunardi-Iskandar *et al.* It is at best a pregnancy marker whose serum levels are at least 100–1,000-fold less during gestation[11]. Furthermore, a "purified native hCG preparation containing 80% βhCG and 20% αhCG" is a contradiction in terms and certainly not a biologically active holo-hormone.

We believe that hCG-like molecules have a potential for additional functions, especially as βhCG and alternative messenger RNA splice products with open reading frames have been shown to be expressed, not only in the placenta, but also eutopically in the testis[12]. Moreover, the main metabolic product of hCG, the βhCG core fragment, has a striking structural similarity to nerve growth factor and other members of the superfamily of 'cystine-knot' growth factors, suggesting a distinct biological function[13]. The suggestions of Lunardi-Iskandar *et al.* are, however, not yet proven.

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LUNARDI-ISKANDAR *ET AL.* REPLY — Rabkin *et al.* state in their analyses the interesting observation that pregnancy does not protect against Kaposi's sarcoma (KS). We agree that it is likely that factors other than hCG explain the male/female differential in this disease. In pregnancy, hCG levels peak only for a short period, and even that peak would be insufficient for long-lasting effects. Rather, we propose LH, and not hCG, as a candidate for a hormonal contribution to the sex differential in Kaposi's sarcoma[1], because the LH β-chain is 85% homologous to the β-chain of hCG[14,15], and because LH is higher in women than in men and much higher at some stages of the menstrual cycle[16]. Moreover, we find that LH has some of the same effects we reported for some hCG preparations (unpublished results).

Berger and Dirnhofer are concerned that because mice do not have hCG, but do have LH-like molecules, the inhibitory factor in mouse sera cannot be the same as in humans, namely βhCG. Mice may not have the corresponding βCG gene nor a placental hormone similar to CG, but sera of pregnant mice and women have an anti-KS factor, especially in early pregnancy. This activity is present in some preparations of hCG; it resides in the β-chain, as demonstrated using purified native βhCG[1] and also purified βhCG recombinant peptides (our unpublished data). Berger and Dirnhofer note that LH carries a related activity. Because of the homology between βhCG and βLH, we assume that if mice do not have CG, the anti-KS activity in mouse sera may well be LH or LH-like.

They also state that the reproductive biological effects of hCG are associated with the dimer. This is indeed the case; however, several workers have shown that the β-chain alone can induce signal transduction[6,17–19] and also that βhCG competes with the dimer for receptor binding[13], so there is good evidence that the β-chain alone contains some biological activity.

With respect to the presence of hCG receptors, all our preliminary results indicate that the Kaposi sarcoma cells have hCG receptors (unfortunately, there was a mistake in our figure legend: the polyclonal sera were not to the ligand but to the receptor), but it is possible that the mechanism for the effect of βhCG could be independent of these. Berger and Dirnhofer note the similarity of βhCG to some growth factors[20], and we believe that one interpretation of our results (N. W. Isaacs, personal communication) is competition of βhCG for a growth factor needed by these tumour cells.

We disagree with Berger and Dirnhofer's contention that results with monoclonal antibodies to hCG would be more conclusive than those presented. We feel that the use of purified native βhCG and purified recombinant peptides of βhCG is sufficiently strong evidence to draw the conclusions made in our paper.

Finally, let us reiterate the novelty of our paper: Kaposi's sarcoma Y-1 cells are the first proven malignant cells obtained from HIV-associated Kaposi's sarcoma and they are killed by purified βhCG peptides. A possible additional novelty is the hypothesis of a gender susceptibility difference based on LH.

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Erratum

IN the Scientific Correspondence "Non-invasive bird tagging" by D. Michard, A Ancel, J-P Gendner, J Lage, Y Le Maho, T Zorn, L Gangloff, A Schierer, K Struyf and G Wey (*Nature* **376,** 649–650; 1995), the reference list was inadvertently omitted. It is given below. Also, the surname of Alfred Schierer was misspelt.

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