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# Original Paper

# Beta Interferon Inhibits HIV-1 Tat-induced Angiogenesis: Synergism with 13-*cis* Retinoic Acid

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**Kaposi's sarcoma (KS) is a highly angiogenic lesion which frequently presents as an aggressive form in HIV-infected male patients. We have previously shown that the HIV-1 Tat protein induces endothelial cell migration and invasion *in vitro* and a rapid angiogenic response *in vivo*, suggesting that it acts as a cofactor in epidemic KS. In this study we tested beta interferon (IFNβ) and retinoic acid (RA) for the inhibition of Tat-induced angiogenesis using *in vivo* and *in vitro* models. IFNβ, at a concentration above 2500 U/ml, was an effective inhibitor of Tat-stimulated growth, migration and morphogenesis of an endothelial cell line *in vitro* and of angiogenesis *in vivo*. A strong reduction of properties associated with neovascularisation was induced by 10 000 U/ml. *In vivo*, RA alone was on ineffective inhibitor of angiogenesis, and *in vitro* gave only a limited inhibition of endothelial cell growth. However, 13-*cis* RA used in combination with IFNβ impressively potentiated its effects. A combination of lower doses of IFNβ (2500 U/ml) and 13-*cis* RA induced a virtually complete inhibition of the Tat-related angiogenic phenotype both *in vivo* and *in vitro*. The potentiation of the anti-angiogenic activity of IFNβ by 13-*cis* RA suggests that this combination could be a useful approach for the therapy of epidemic KS.** **© 1998 Elsevier Science Ltd. All rights reserved.**

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## INTRODUCTION

TUMOUR NEOVASCULARISATION is necessary for tumour growth and metastatic dissemination of tumour cells [1]. The first step in the multistep process of neovascularisation is stimulation of the endothelial cells by the cytokines produced by inflammatory and tumour cells. The endothelial cells then lose their contact inhibition, migrate and breach the basement membrane using specific proteolytic enzymes (MMP-2, MMP-9, u-PA) [2], proliferate and differentiate to organise new vessels.

Kaposi's sarcoma (KS) is a mesenchymal neoplasm involving the skin, in some cases the lymph nodes and the mucosae, particularly of the digestive tract. The classic form occurs in the skin of elderly men, but a more virulent and invasive form frequently occurs in homosexual male AIDS patients [3–6]. Histologically, KS is characterised by an inflammatory infiltrate and spindle-shaped cells, in addition to endothelial cells [5, 6]. One of the most peculiar features of KS is a prominent but poorly organised angiogenesis. The KS spindle cells are considered the neoplastic cells in the KS lesion [7]. The origin of these cells is debated, as they co-express mesenchymal, endothelial and macrophage markers [8, 9]. In most KS cases, the spindle cells have a normal karyotype [10], although some chromosomal alterations have been reported [11–13].

KS appears to occur in patients with an immunological imbalance, found both in the elderly and immunodeficiency conditions, including AIDS and post-transplant immunosuppression. The aetiopathogenesis of AIDS-related KS is still controversial, the factors probably involved are activation of a latent HHV-8 infection (a new member of the family of the herpes viruses [14]), a local cytokine imbalance [15] and, in the case of the aggressive epidemic form, the HIV-1 *tat* gene product [16–19].

HIV-Tat is an angiogenic factor which stimulates growth and migration of vascular cells and promotes endothelial cell morphogenesis [20]. Tat is able to bind heparin and it

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mimics cellular heparin binding angiogenic factors [21], activating the vascular endothelial growth factor (VEGF) receptor KDR through its basic Arg–Lys rich domain [22]. These data suggest that Tat is involved in the highly aggressive KS forms found in AIDS patients. The inhibitor of gelatinase A, TIMP-2, has previously been shown to block effectively basic fibroblast growth factor (bFGF)-induced angiogenesis [16, 23], but had no effect on that induced by Tat [16]. Therefore, we have searched for novel pharmacological approaches to interfere with Tat-induced angiogenesis.

Interferon (IFNs) have been found to be effective in the anti-angiogenic therapy of tumours such as haemangiomas [24]. IFNα, whose antiretroviral properties are well known [25], is already used in the therapy of AIDS-KS [26, 27]. IFNs have been found to have antiproliferative and anti-invasive activity in various malignancies, both *in vitro* and *in vivo* [28–30]. The systemic administration of IFNα and β can produce the regression of vascularised tumours through a mechanism associated with endothelial cell damage which leads to necrosis [30]. IFNs are able to downregulate bFGF mRNA expression and protein production in human carcinomas [31]. Several years ago, we were the first group to demonstrate the anti-invasive properties of fibroblast interferon [32]. Recent studies have shown that IFNβ decreases 72 KDa gelatinase production and the type IV collagenolysis in human carcinoma cells [33], while production of MMP-2 in normal cells was not affected, suggesting that its action may be specific for transformed cell types. IFNβ is poorly expressed in the normal epithelium surrounding invasive tumours [34].

Retinoids, a group of vitamin A metabolites and synthetic analogues, can suppress the transformed phenotype and enhance the differentiation of many types of tumour cells *in vitro* and *in vivo*. Some of these compounds have been evaluated for potential use in the prevention and treatment of cancer in humans [35]. Several retinoids decrease the activity of type IV collagenase in various transformed and normal cells [36]. The inhibition of angiogenesis by retinoids is probably linked to their antiproliferative effect which downregulates the expression and release of angiogenic factors, including bFGF, tumour necrosis factor (TNFα), epidermal growth factor (EGF), and VEGF by tumour cells. Retinoids could also regulate metalloprotease activity directly in endothelial cells [37]. In this study, we tested the ability of IFNβ and 13-*cis* retinoic acid (RA) alone and in combination to inhibit Tat stimulation of endothelial cells *in vivo* and *in vitro*.

## MATERIALS AND METHODS

### *Materials*

Heparin (Clarisco, Schwarz Pharma S.p.A.) was used *in vitro* at a concentration of 0.01 U/ml and *in vivo* at 24 U/ml. Human synthetic HIV-1 Tat protein (Tecnogen) was dissolved in phosphate buffered saline (PBS) with 0.1% bovine serum albumin (BSA), aliquoted and stored at −80°C. The Tat purity was tested in Western blot analysis (Silver-staining) and Tat was used in the dark at 4°C to avoid degradation. Human IFNβ (Frone 3 000 000, Serono, a preparation for clinical use containing no preservatives) was dissolved in PBS, aliquoted, stored at −80°C and used at a concentration of 2500 U/ml. *All-trans* and 13-*cis* RA were purchased from Sigma, dissolved in ethanol, stored at −20°C and used at the concentration of 10 μM. Matrigel was purified from the EHS tumour as previously described [38] and is commercially available from Collaborative Biomedical Products (Bedford, Massachusetts, U.S.A.).

### *Cells*

The human endothelial-like immortalised cell-line EAhy926, derived from the fusion of human umbilical vein endothelial (HUVE) cells with the A549 lung carcinoma cell line, has been shown to have an endothelial-like phenotype [39]. These cells were maintained in Dulbecco's modified essential medium (DMEM) with 10% fetal calf serum (FCS) heat inactivated (Seromed), supplemented with glutamine (300 μg/ml), penicillin (100 U/ml) and streptomycin (50 μg/ml). Primary cultures of KS spindle cells [40] were grown in 50% DMEM and 50% RPMI 1640, 10% FCS supplemented with glutamine (300 μg/ml). HUVE cells were obtained from the ATCC and cultivated on gelatin-coated plates (1.5% in PBS) in M199 containing 10% heat inactivated FCS, 100 μg/ml heparin, 60 μg/ml endothelial cell growth factor (ECGS; crude extract isolated from bovine brain), at 37°C in a 5% $CO_2$.

### In vivo *angiogenesis*

We utilised the matrigel sponge model of angiogenesis introduced by Passaniti and colleagues [41] and modified by Albini and associates [16]. IFNβ (250, 1000, 2500, 5000 and 10 000 U/ml), *all-trans* and 13-*cis* RA (15 μg/gel) singly or together were mixed with Tat and heparin and added to unpolymerised liquid matrigel at 4°C to a final volume of 600 μl. The matrigel suspension was slowly injected subcutaneously into the flank of C57/bl6 male mice using a cold syringe. *In vivo* the gel polymerises quickly to form a solid gel. Between four and 11 mice were used for each treatment and experiments were performed four times. Matrigel with buffer alone was used as the negative control. After 4 days, the gels were collected and weighed. Samples were either minced and diluted in water to measure the haemoglobin content, utilising a Drabkin reagent kit (Sigma, St Louis, Missouri, U.S.A.), with normalisation to 100 mg of recovered gel and a standard curve of mouse blood haemoglobin, or some samples fixed in formalin, embedded in paraffin and sections stained with haematoxylin and eosin for histological analysis.

### *Chemotaxis assay*

These assays were carried out in Boyden chambers as described by Postlethwaite and colleagues [42] and modified by Albini and associates [43]. EAhy926 cells were harvested in trypsin/ethylenediaminetetraacetic acid (EDTA) solution (0.05/0.02% in PBS, Seromed), collected by centrifugation and resuspended in DMEM with 0.1% BSA. The lower compartment of Boyden chambers (200 ml) was filled with the chemoattractant: Tat protein (10 ng/ml) with heparin (0.01 U/ml) diluted in DMEM with 0.1% BSA. DMEM with 0.1% BSA alone (serum-free medium (SFM)) was used as a negative control to evaluate random migration. EAhy926 cells ($1.2 \times 10^5$/400 μl per chamber) were placed in the upper compartment. The cells (except the controls) were pretreated for 24 h with IFNβ and with 13-*cis* RA either separately or together. The drugs were maintained in the cell culture during the experiments. The two compartments were separated by a polycarbonate filter (12 mm pore/size; Nucleopore) coated with gelatin to allow for cell adhesion. The chambers were incubated for 6 h at 37°C in a humidified atmosphere containing 5% $CO_2$. After incubation, cells on the upper side

of the filter were removed. The cells which had migrated to the lower side of the filter were fixed in 100% ethanol, stained with toluidine blue and between five and eight unit fields per filter were counted at 160 × magnification with a microscope (Zeiss). Tests were run in triplicate and repeated six times.

*Growth assays*

EAhy926 cells were counted and plated at the appropriate cell number (20 000 cells/1000 µl/well) into 24-well microtitre plates. After 6 h, Tat-stimulated cells were treated with drugs at the final concentration of 2500 U/ml IFNβ and/or 10 µM 13-*cis* RA, each treatment made in triplicate. SFM was used as the negative control. The cultures were incubated for 48 h at 37°C. The cells were collected by trypsinisation, fixed with formalin, and counted in a Burkitt haemocytometer.

*Matrigel morphogenesis assay*

Three hundred microlitres of matrigel/well was polymerised into a 24-well microtitre plate for 1 h at 37°C. A suspension of HUVE cells was carefully layered on top of the polymerised gel and incubated at 37°C as previously described [44, 45]. Cells were plated in DMEM with 10% FCS containing Tat (10 ng/ml) and heparin (0.01 U/ml), negative controls were plated in DMEM with 10% FCS alone. IFNβ (2500 U/ml) and RA (10 µM) were used either alone or in combination. The effects of the treatments on the growth and morphogenesis of Tat-treated endothelial cells could be observed microscopically after as little as 6 h [20]. After 18 h the networks were photographed with a Leika DM-IRB microscope.

## RESULTS

In vivo *angiogenesis*

Tat and heparin together induced a very strong, haemorrhagic angiogenic response (Figure 1) as previously reported [16], while untreated control gels were not vascularised. Inclusion of IFNβ with Tat in the matrigel dose-dependently inhibited the vascularisation of the gel beginning at concentrations of 2500 U/ml (Figure 1), inhibition was virtually complete at 10 000 U/ml IFNβ (Table 1). However, low-doses of IFNβ (250–1000 U/ml) produced an apparent increase in Tat-induced angiogenesis. 13-*cis* RA alone (15 µg/gel) did not inhibit Tat-induced vascularisation but appeared to sustain Tat-induced angiogenesis (Figure 1 and Table 1). However, 13-*cis* RA potentiated the inhibitory effect of IFNβ (Figure 1 and Table 1), giving maximal inhibition with much lower doses of IFNβ. *All-trans* RA (ATRA) appeared to exert a slight increase of Tat-induced angiogenesis either alone or in combination with IFNβ.

*Chemotaxis*

The EAhy926 immortalised endothelial cell line was used to assess the inhibitory effect of IFNβ and 13-*cis* RA (separately

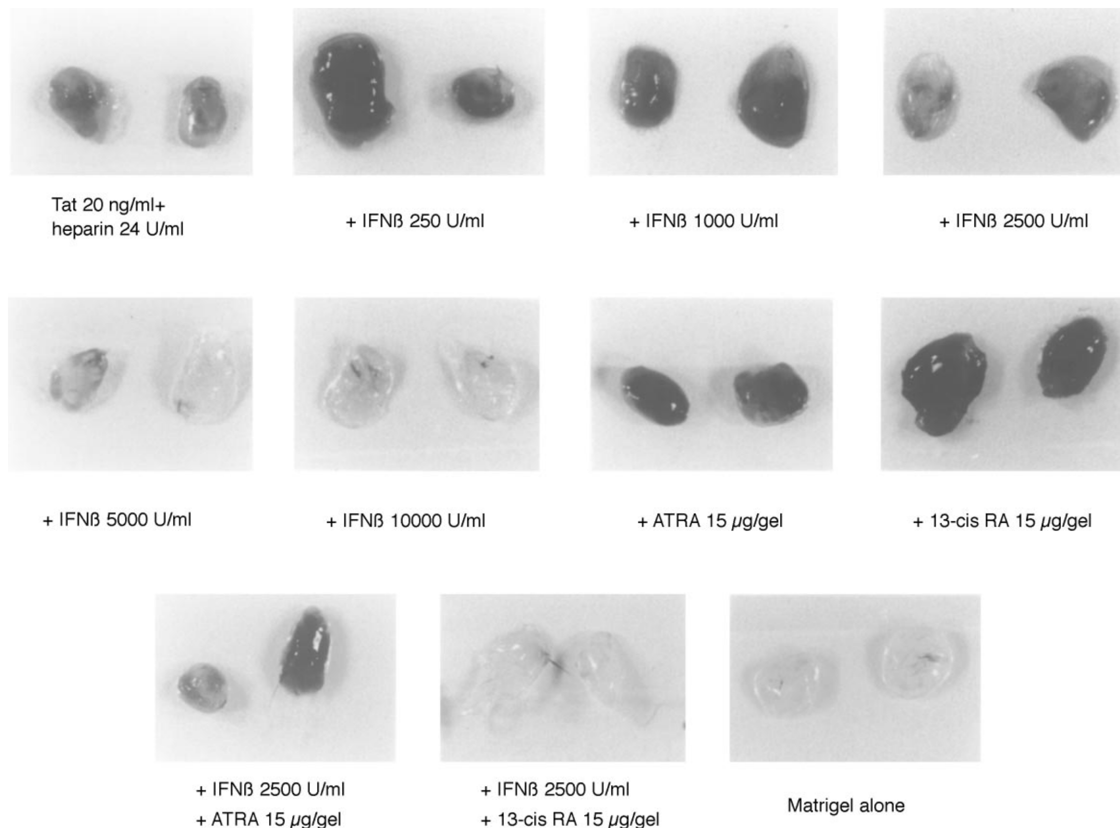


**Figure 1. Dose-dependent effects of beta interferon (IFNβ) and synergistic inhibitory effects of low-dose IFNβ and 13-*cis* retinoic acid (RA) on Tat-induced angiogenesis. Matrigel pellets were recovered 4 days after the inoculation and photographed. The inhibitory effect of IFNβ was conspicuous only at elevated doses (5000 and 10 000 U/ml). Both IFNβ (2 500 U/ml) and RAs (15 µg/gel) used alone did not show any anti-angiogenic properties. In addition, the combination of IFNβ and *all-trans* retinoic acid (ATRA) failed to give any inhibitory effect. In contrast, the addition of IFNβ and 13-*cis* RA together results in total inhibition of Tat-induced angiogenesis with a median haemoglobin (see Table 1) content comparable to the negative control (matrigel alone).**

*Table 1. Angiogenic response in matrigel sponges measured as haemoglobin content*

| Factors added to matrigel | Haemoglobin g/dl (median) | Range | No matrigel sponges |
|---|---|---|---|
| Matrigel alone | 0.015 | 0–0.069 | 8 |
| Tat+hep | 0.274 | 0.035–4.260 | 22 |
| Tat+hep+IFNβ250U | 0.648 | 0.119–1.324 | 4 |
| Tat+hep+IFNβ1000U | 0.632 | 0.014–1.293 | 8 |
| Tat+hep+IFNβ2500U | 0.284 | 0.025–1.835 | 19 |
| Tat+hep+IFNβ5000U | 0.101 | 0.005–2.683 | 8 |
| Tat+hep+IFNβ10000U | 0.086 | 0.006–1.664 | 4 |
| Tat+hep+ATRA15µg | 0.501 | 0.013–1.416 | 9 |
| Tat+hep+*cis*RA15µg | 0.364 | 0.115–2.065 | 8 |
| Tat+hep+IFNβ2500U+*cis*RA15µg | 0.024 | 0.006–0.615 | 9 |
| Tat+hep+IFNβ2500+ATRA15µg | 0.545 | 0.088–1.806 | 8 |

Hep, heparin; IFNβ, beta interferon, ATRA, *all-trans* retinoic acid; *cis*RA, 13-*cis* retinoic acid. Inhibition of Tat-induced angiogenesis in matrigel pellets by IFNβ. Tat-induced antiogenesis *in vivo* is measured as g/dl of haemoglobin in matrigel sponges. Median value, range and number of sponges are given. Downregulation of angiogenesis with IFNβ was dose-dependent starting from 2500 U/ml. Low-doses of IFNβ, *all-trans* and 13-*cis* RA appeared to increase the angiogenic response. Inhibition of Tat–heparin-induced angiogenesis in matrigel pellets by IFNβ (2500 U/ml) was strongly enhanced by the association with 13-*cis* RA (15 µg/gel). This combination completely abolished the angiogenic effect of the Tat protein, while the association of IFNβ with ATRA had no inhibitory effect.

and combined) on Tat-induced migration. Tat, in the presence of heparin in the lower compartment of Boyden chambers, induced migration of EAhy926 cells. Pretreatment for 24 h with IFNβ gave dose-dependent inhibition of Tat-induced migration (Table 2), with 70% inhibition at 10 000 U IFNβ. 13-*cis* RA or IFNβ 2500 used alone (10 µM) showed limited inhibitory effect on Tat-induced endothelial cell migration (Table 2), but a combination of IFNβ (2500 U/ml) with 13-*cis* RA (10 µM) gave 50% inhibition with respect to controls (Table 2).

*Growth*

The effect of IFNβ and 13-*cis* RA on the growth of Tat-stimulated endothelial cells was also analysed. Both 2500 U/ml IFNβ and 10 µM 13-*cis* RA alone reduced Tat-stimulated growth, with IFNβ alone reducing growth to nearly that seen with SFM alone. A combination of IFNβ and 13-*cis* RA completely blocked Tat-stimulated endothelial cell growth (Figure 2).

*Growth on matrigel*

Endothelial cells (EAhy926 and HUVE) form capillary-like structures when cultured on polymerised matrigel at 37°C [45]. Tat increases the formation of this anastomosed cellular network as compared with untreated cells [20], similar to that produced by addition of ECGS. Treatment with 2500 U/ml IFNβ or 10 µM 13-*cis* RA inhibited the effect of Tat and only incomplete structures were formed after 18 h. Complete inhibition was induced by the combination of the two molecules: HUVE cells were unable to form anastomosed capillary structures in the presence of both IFNβ and 13-*cis* RA (Figure 3).

## DISCUSSION

KS is a form of malignancy which occurs in elderly or immunosuppressed patients, and it is often associated with AIDS [4–6]. It has some features that are not typical of tumours: for example, KS may regress, it can appear as a multicentric lesion, and its cells usually do not show abnormal karyotypes, although some immortal cases have been shown to have abnormal karyotypes [11–13]. KS is composed of different cells, an inflammatory infiltrate, endothelial cells and spindle-shaped cells which are considered to be the true neoplastic cells [4–6].

The aetiopathogenesis of KS is still controversial—a new herpesvirus, KSHV/HHV-8 is implicated in KS [14]. The aggressiveness of the AIDS-associated form of KS can be partially explained by the angiogenic effect of the HIV-1 Tat protein [16], which can be considered a cofactor. The *tat* gene product could stimulate the spindle-shaped KS cells initially and act to maintain the lesion once induced, through recruitment of endothelial cells [20] and monocytes [46]. The angiogenic activity of Tat is strongly enhanced by heparin [16, 21]. Tat contains a basic sequence, common to other heparin binding angiogenic growth factors [21] which is involved in Tat binding and activation of the VEGF KDR receptor [22].

*Table 2. Migrated cells/field*

| Chemoattractant | EAhy926 | +IFNβ (2500 U/ml) | +IFNβ (10 000 U/ml) | +*cis*RA (10 µM) | +IFNβ (2500 U/ml) + *cis*RA (10 µM) |
|---|---|---|---|---|---|
| Serum-free medium | 27 ± 6 | – | – | – | – |
| Tat+heparin | 39 ± 6 | 25 ± 7 | 14 ± 2 | 27 ± 4 | 19 ± 7 |

IFNβ, beta interferon; *cis*RA, 13-cis retinoic acid. Dose-dependent inhibition of Tat-induced migration of endothelial cells (EAhy926), by IFNβ. Serum-free medium alone was used as a negative control and Tat (10 ng/ml) plus heparin (0.01 U/ml) as the chemoattractant. The inhibition exerted by 2500 U/ml IFNβ on Tat-induced chemotaxis caused a significant decrease of cell migration comparable to unstimulated basal levels ($P < 0.006$ using Student $t$ test for Tat+hep+IFNβ2500 versus Tat+hep). A 4-fold higher dose (10 000 U/ml) of IFNβ showed potent inhibition of Tat-induced endothelial cell migration, with a cell count below the value of unstimulated cell migration (using Student $t$ test Tat+hep+IFNβ10000 versus Tat+hep $P < 0.0001$). While IFNβ 2500 U/ml was partially effective in inhibiting endothelial cell growth and 13-*cis* RA alone shows a weak inhibition; these drugs used together gave a significant synergistic inhibition of Tat-induced endothelial cell migration (Student $t$ test, $P < 0.001$ when compared with Tat+hep).

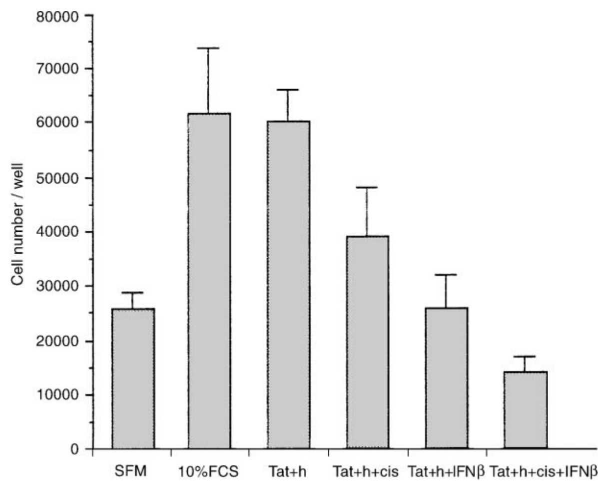


**Figure 2. Growth assay. The EAhy926 endothelial cell line (20 000 cells/ml) was incubated with Tat (10 ng/ml) plus heparin (h) (0.01 U/ml) as a growth stimulus, while serum-free medium (SFM) was used as a negative control. Beta interferon (IFNβ) was used at a concentration of 2500 U/ml and/or 13-*cis* retinoic acid (*cis*) at 10 μM. Both drugs used singly showed inhibition of Tat-induced cell growth; when used together, the inhibition observed was greater than that with SFM alone.**

Angiogenesis inhibitors act on different steps of tumour angiogenesis: endothelial cell motility, basement membrane degradation, cell growth and cell organisation into the new, often incomplete, vessel network [1]. The anti-angiogenic activity of IFNα and IFNβ are already known, and IFNα is currently employed in the therapy of haemangiomas [24] and of KS itself [27]. IFN-CON 1, a derivative of IFNα widely employed in the therapy of AIDS-KS, downregulates the elevated c-myc expression which stimulates the proliferation and the migration of cultivated AIDS-KS spindle cells [47]. IFNβ is able to modulate growth factor production and function, blocking colony stimulating factor (CSF)-1-induced monocycte replication [48], inhibiting platelet derived growth factor (PDGF)-induced signal transduction in fibroblasts [49], downregulating bFGF mRNA in renal carcinoma cells [31] and reducing EGF receptor expression in breast cancer cells [50]. From these observations, IFNβ appears to exert its inhibitory effect on growth factors acting directly on their expression or on receptor expression and function. IFNβ has also been employed in combination with a derivative of fumagillin (AGM1470) as an anti-angiogenic drug against solid tumours [51]. We have shown that IFNβ can inhibit tumour cell invasion [32]. IFNβ has also been demonstrated to downregulate 72 kDa type IV collagenase

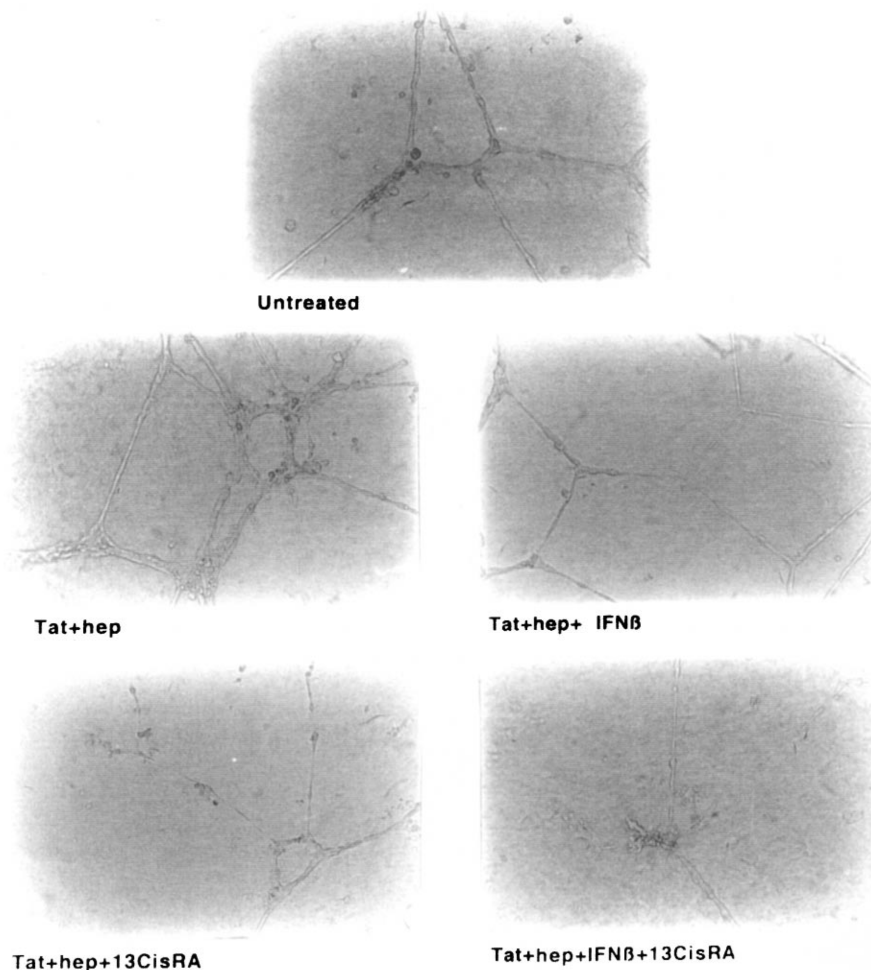


**Figure 3. Inhibition of Tat-induced formation of a capillary-like network on a matrigel substrate (*in vitro* morphogenesis). Cells were photographed after 24 h of incubation. Cells stimulated with Tat (10 ng/ml) plus heparin (0.01 U/ml) were treated with beta interferon (IFNβ) (2500 U/ml) and/or 13-*cis* retinoic acid (RA) (10 μM). The combination of the two drugs prevented human umbilical vein endothelial (HUVE) cell organisation into a 'vascular' network.**

(MMP-2) production in renal carcinoma and choriocarcinoma cells, thus reducing their invasiveness [33, 52].

Retinoids are a class of molecules well known for their effects on cellular differentiation, and they are considered an adjuvant treatment for many tumours. Retinoids down-regulate the production of MMP-2 and plasminogen activator (both uPA and tPA) in melanoma [53] and also the production of growth factors (Oncostatin M and TNFα) in KS [54].

Here we have shown that IFNβ used at 2500 U/ml is able to inhibit partially endothelial cell growth and also partially inhibits the organisation in capillary-like structures. IFNβ inhibits endothelial cell migration *in vitro* and angiogenesis *in vivo* at 10 000 U/ml. Lower doses of IFNβ (2500 and 5000 U/ml) were less effective in inhibition of migration and angiogenesis, while 250 or 1000 U/ml IFNβ appear to cause, *in vivo*, a potentiation of the angiogenic response; a similar observation was made regarding the effect of IFNα on Tat-induced angiogenesis [55]. While retinoids alone appear to support *in vivo* Tat angiogenesis, the combination of 13-*cis* RA potentiated the effects of IFNβ. This effect appears to be specific for the 13-*cis* isomer, as treatment with ATRA had no inhibitory effect when combined with IFNβ.

Here we have demonstrated that association of 13-*cis* RA with IFNβ permits the use of IFNβ at lower doses, by-passing the toxicity often observed in clinical use at the therapeutic dose (3 000 000 U/day). These studies indicate that low dose treatment with both IFNβ and 13-*cis* RA could be an effective alternative for treatment of epidemic KS. In contrast, a potential increase of Tat angiogenic response by retinoids alone or low doses of IFNβ suggests that single RA applications or low doses of IFNβ should be avoided. Direct intralesional application of a combination of the two therapeutics, or the use of highly expressing IFNβ constructs for local gene therapy, will most likely be the most effective approaches.


1. Folkman J. The role of angiogenesis in tumor growth. *Semin Cancer Biol* 1992, **3**, 65–71.
2. Liotta L, Steeg P, Stetler-Stevenson W. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991, **64**, 327–336.
3. Kaposi M. Idiopathisches multiples pigment Sarcom der Haut. *Arch Dermatol Syph* 1872, **4**, 265–273.
4. Friedman-Kien AE, Laubenstein W, Rubenstein PLJ. *et al.* Disseminated Kaposi's sarcoma in homosexual men. *Ann Intern Med* 1982, **96**, 693–700.
5. Roth WK, Brandstetter H, Stürzl M. Cellular and molecular features of HIV-associated Kaposi's sarcoma. *AIDS* 1992, **6**, 895–913.
6. Armes J. A review of Kaposi's sarcoma. *Adv Cancer Res* 1989, **53**, 73–87.
7. Nakamura S, Salahuddin SZ, Biberfeld P, *et al.* Kaposi's sarcoma cells: long-term culture with growth factor from retrovirus-infected CD4+T cells. *Science* 1988, **242**, 426–429.
8. Uccini S, Ruco L, Monardo F, *et al.* Co-expression of endothelial cell and macrophage antigens in Kaposi's sarcoma cells. *J Pathol* 1994, **173**, 23–31.
9. Kaaya E, Parravicini C, Ordonez C, *et al.* Heterogeneity of spindle cells in Kaposi's sarcoma: comparison of cells in lesions and in culture. *J Acq Immun Def Synd Hum Retrovirol* 1995, **10**, 295–305.
10. Nickoloff BJ, Griffiths CEM. The spindle-shaped cells in cutaneous Kaposi's sarcoma. *Am J Pathol* 1989, **135**, 793–800.
11. Popescu NC, Zimonjic DB, Leventon KS, Bryant JL, Lunardi IY, Gallo RC. Deletion and translocation involving chromosome 3 (p14) in two tumorigenic Kaposi's sarcoma cell lines. *J Natl Cancer Inst* 1996, **88**, 450–455.
12. Lunardi-Iskandar Y, Gill P, Lam V, *et al.* Isolation and characterization of an immortal neoplastic cell line (KSY-1) from AIDS-associated Kaposi's Sarcoma. *J Natl Cancer Inst* 1995, **87**, 974–981.
13. Albini A, Paglieri I, Orengo G, *et al.* The β-core fragment of human Chorionic Gonadotropin (hCG) inhibits growth of Kaposi's sarcoma-derived cells and a new immortalized Kaposi's sarcoma cell line. *AIDS* 1997, **11**, 713–721.
14. Chang Y, Cesarman E, Pessin MS, *et al.* Identification of Herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994, **266**, 1865–1869.
15. Ensoli B, Nakamura S, Salahuddin SZ, *et al.* AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. *Science* 1989, **243**, 223–226.
16. Albini A, Fontanini G, Masiello L, *et al.* Angiogenic potential *in vivo* by Kaposi sarcoma cell-free supernatants and HIV1-tat product: inhibition of KS-like lesions by TIMP-2. *AIDS* 1994, **8**, 1237–1244.
17. Vogel J, Hinrichs SH, Reynolds RK, Luciw PA, Jay G. The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature* 1988, **335**, 606–611.
18. Corallini A, Altavilla G, Pozzi L, *et al.* Systemic expression of HIV1 *tat* gene in transgenic mice induces endothelial proliferation and tumors of different histotypes. *Cancer Res* 1993, **53**, 5569–5575.
19. Ensoli B, Barillari G, Salahuddin SZ, Gallo R, Wong-Staal F. Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. *Nature* 1990, **345**, 84–86.
20. Albini A, Barillari G, Benelli R, Gallo RC, Ensoli B. Angiogenic properties of human immunodeficiency virus type 1 Tat protein. *Proc Natl Acad Sci USA* 1995, **92**, 4838–4842.
21. Albini A, Benelli R, Presta M, *et al.* HIV-tat protein is a heparin-binding angiogenic growth factor. *Oncogene* 1996, **12**, 289–297.
22. Albini A, Soldi R, Giunciuglio D, *et al.* HIV-1 Tat induced angiogenesis is mediated by activation of the flk-1/KDR tyrosine kinase receptor on vascular endothelial cells. *Nature Med* 1996, **2**, 1371–1375.
23. Murphy AN, Unsworth EJ, Stetler-Stevenson WG. Tissue inhibitor of metalloproteinases-2 inhibits bFGF-induced human microvascular endothelial cell proliferation. *J Cell Physiol* 1993, **157**, 351–358.
24. Folkman J, Ingber D. Inhibition of angiogenesis. *Semin Cancer Biol* 1992, **3**, 89–96.
25. Gutterman JU. Cytokine therapeutics: lessons from interferon alpha. *Proc Natl Acad Sci USA* 1994, **91**, 1198–1205.
26. Lilenbaum RC, Ratner L. Systemic treatment of Kaposi's sarcoma: current status and future directions. *AIDS* 1994, **8**, 141–151.
27. Sulis E, Floris C, Sulis ML, *et al.* Interferon administered intralesionally in skin and oral cavity lesions in heterosexual drug addicted patients with AIDS-related kaposi's sarcoma. *Eur J Cancer Clin Oncol* 1989, **25**, 759–761.
28. Nishimura J, Mitsui K, Ishikawa T, *et al.* Antitumor and antimetastatic activities of human recombinant interferon alpha. *Clin Exp Metast* 1985, **3**, 295–304.
29. Hujanen ES, Turpeenniemi-Hujanen TM. Recombinant interferon-alpha modulates the invasive potential of human melanoma *in-vitro*. *Anticancer Res* 1988, **8**, 1066.
30. Wadler S, Schwartz E. Antineoplastic activity of the combination of interferon and cytotoxic agents against experimental and human malignancies: a review. *Cancer Res* 1990, **50**, 3473–3486.
31. Singh RK, Gutman M, Bucana CD, Sanchez R, Llansa N, Fidler IJ. Interferons alpha and beta down-regulate the expression of basic fibroblast growth factor in human carcinomas. *Proc Natl Acad Sci USA* 1995, **92**, 4562–4566.
32. Melchiori A, Allavena G, Bohm J, *et al.* Interferons inhibit chemotaxis of transformed cells and their invasion of a reconstituted basement membrane. *Anticancer Res* 1987, **7**, 475–480.
33. Gohji K, Fidler IJ, Tsan RJ, *et al.* Human recombinant interferons-beta and -gamma decrease gelatinase production and invasion by human KG-2 renal-carcinoma cells. *Int J Cancer* 1994, **58**, 380–384.
34. Fidler IJ. Angiogenesis and metastasis growth factors. *Clin Exp Metast* 1996, **14**, 30.

35. Hong WK, Tri LM. Retinoids in human cancer. In Sporn, Roberts, Goodman, eds. *The Retinoids: Biology, Chemistry and Medicine*, 1994.
36. Hendrix M, Wood W, Seftor E, *et al.* Retinoic acid inhibition of human melanoma cell invasion through a reconstituted basement membrane and its relation to decreases in the expression of proteolytic enzymes and motility factor receptor. *Cancer Res* 1990, **50**, 4121–4130.
37. Majewski S, Marczak M, Szmurlo A, Jablonska S, Bollag W. Retinoids, interferon alpha, 1,25-dihydroxyvitamin D3 and their combination inhibit angiogenesis induced by non-HPV-harboring tumor cell lines. RARa mediates the antiangiogenic effect of retinoids. *Cancer Lett* 1995, **89**, 117–124.
38. Kleinman HK, McGarvey ML, Hassell JR, *et al.* Basement membrane complexes with biological activity. *Biochemistry* 1986, **25**, 312–318.
39. Edgell CJ, McDonald CC, Graham JB. Permanent cell line expressing human factor VIII-related antigen established by hybridization. *Proc Natl Acad Sci USA* 1983, **80**, 3734–3737.
40. Benelli R, Repetto L, Carlone S, Parravicini C, Albini A. Establishment and characterization of two new Kaposi's sarcoma cell cultures from an AIDS and a non-AIDS patient. *Res Virol* 1994, **145**, 251–259.
41. Passaniti A, Taylor RM, Pili R, *et al.* A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. *Lab Invest* 1992, **67**, 519–528.
42. Postlethwaite AE, Snyderman R, Kang AH. The chemotactic attraction of human fibroblasts to a lymphocyte-derived factor. *J Exp Med* 1976, **144**, 1188.
43. Albini A, Allavena G, Melchiori A, *et al.* Chemotaxis of 3T3 and SV3T3 cells to fibronectin is mediated through the cell attachment site in fibronectin and a fibronectin cell surface receptor. *J Cell Biol* 1987, **105**, 1867–1872.
44. Albini A, Iwamoto Y, Kleinman HK, *et al.* A rapid *in vitro* assay for quantitating the invasive potential of tumor cells. *Cancer Res* 1987, **47**, 3239–3245.
45. Grant DS, Tashiro K, Segui-Real B, Yamada Y, Martin GR, Kleinman HK. Two different laminin domains mediate the differentiation of human endothelial cells into capillary-like structures in vitro. *Cell* 1989, **58**, 933–943.
46. Lafrenie RM, Wahl LM, Epstein JS, Hewlett IK, Yamada KM, Dhawan S. HIV-1-Tat protein promotes chemotaxis and invasive behavior by monocytes. *J Immunol* 1996, **157**, 974–977.
47. Köster RBL, M SM, Zietz C, Hermeking H, Brysch W, Stürzle M. Consensus-interferon and platelet-derived growth factor adversely regulate proliferation and migration of Kaposi's sarcoma cells by control of c-myc expression. *Am J Pathol* 1996, **149**, 1871–1885.
48. Hamilton JA, Whitty GA, Kola I, Hertzog PJ. Endogenous IFN-alpha beta suppresses colony-stimulating factor (CSF)-1-stimulated macrophage DNA synthesis and mediates inhibitory effects of lipopolysaccharide and TNF-alpha. *J Immunol* 1996, **156**, 2553–2557.
49. Tamm I, Kikuchi T, Kreutter D, Pledger WJ, Pfeffer LM. Selective interferon-alpha/beta effects on platelet-derived growth factor-stimulated processes in quiescent BALB/c-3T3 fibroblasts. *J Interferon Res* 1994, **14**, 265–273.
50. Iacopino F, Ferrandina G, Scambia G, Benedetti PP, Mancuso S, Sica G. Interferons inhibit EGF-stimulated cell growth and reduce EGF binding in human breast cancer cells. *Anticancer Res* 1996, **16**, 1919–1924.
51. Brem H, Gresser I, Grosfeld J, Folkman J. The combination of antiangiogenic agents to inhibit primary tumor growth and metastasis. *J Pediatr Surg* 1993, **28**, 1253–1257.
52. Kato N, Nawa A, Tamakoshi K, *et al.* Suppression of gelatinase production with decreased invasiveness of choriocarcinoma cells by human recombinant interferon beta. *Am J Obstet Gynecol* 1995, **172**, 601–606.
53. Wood WR, Seftor EA, Lotan D, *et al.* Retinoic acid inhibits human melanoma tumor cell invasion. *Anticancer Res* 1990, **10**, 423–432.
54. Guo WX, Gill P, Antakly T. Inhibition of AIDS-Kaposi's Sarcoma proliferation following retinoic acid receptor activation. *Cancer Res* 1995, **55**, 823–829.
55. Benelli R, Masiello L, Paglialunga G, *et al.* IFN$\alpha$ inhibits Tat-induced angiogenesis. *Guenounou ed.* Paris, Inserm, 1997, 447–458.


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