Inhibitors of angiogenesis can exhibit bell-shaped and U-shaped dose-response curves: relevance for cancer therapy

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breast cancer

The Institute
of Cancer Research

Summary

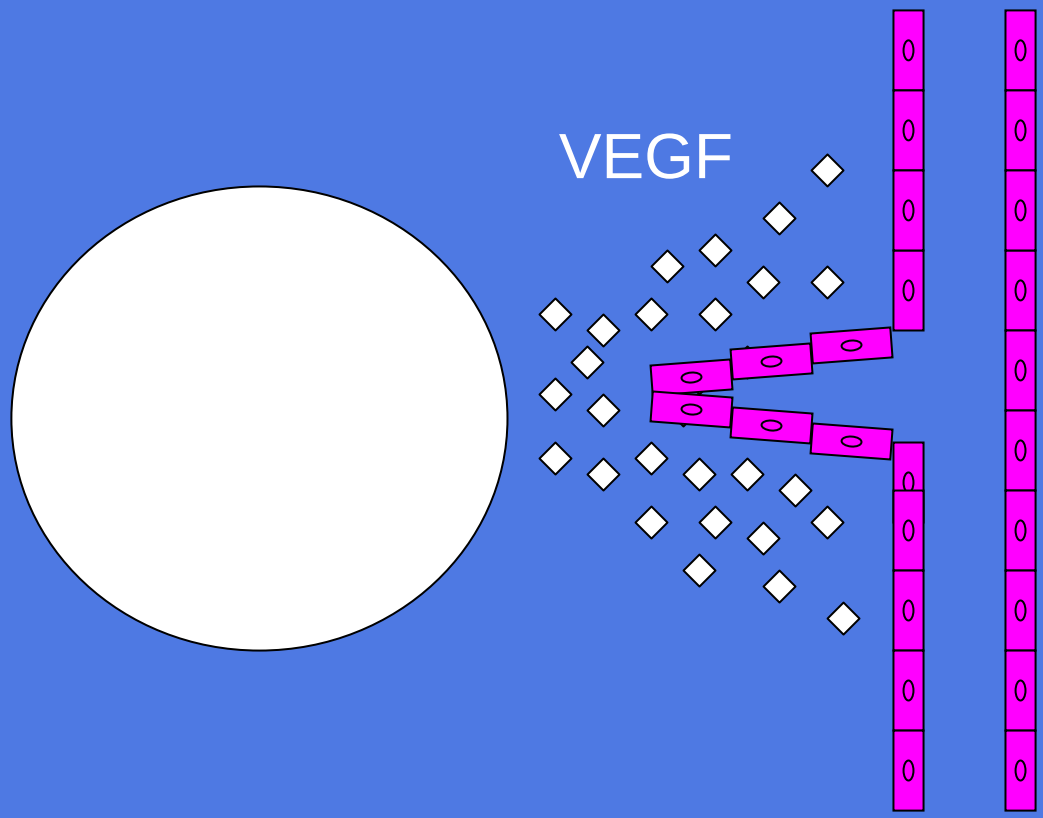
Tumor angiogenesis and anti-angiogenic therapy

Low doses of integrin inhibitors can stimulate tumour growth and angiogenesis

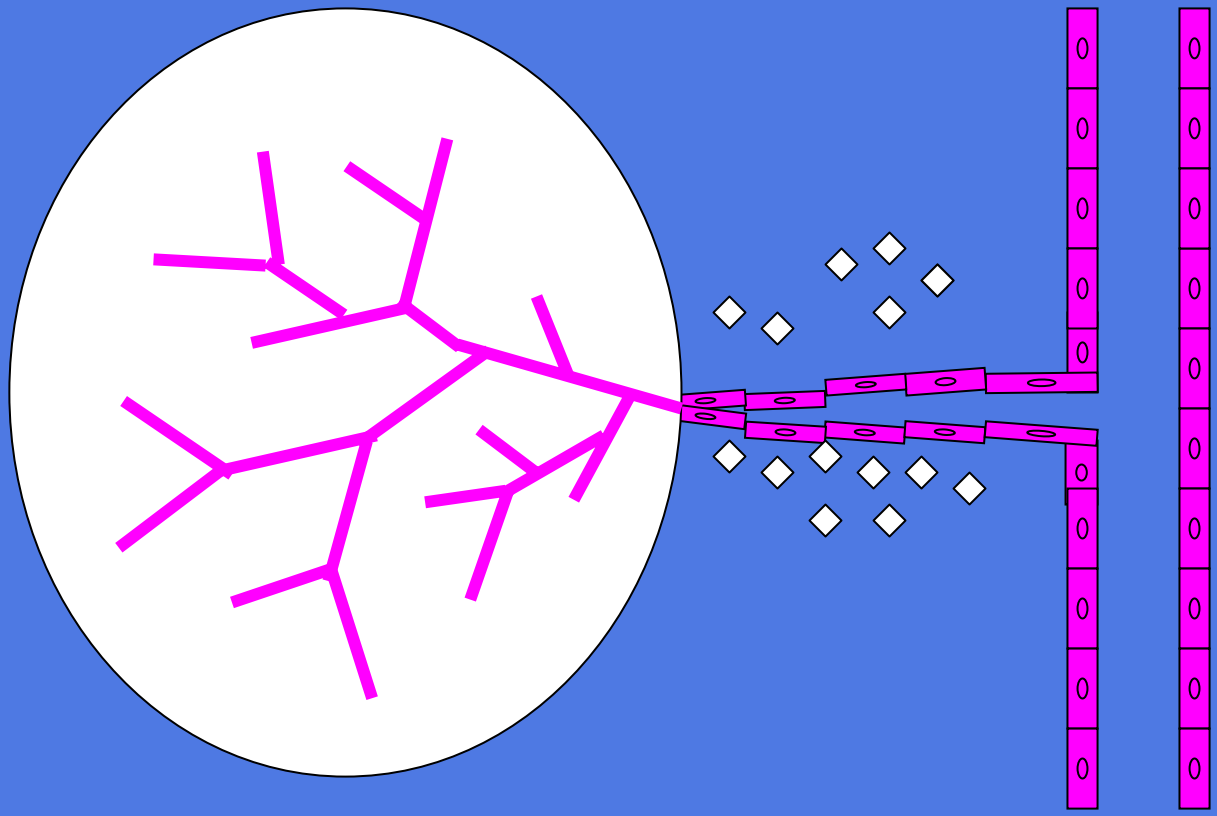
Consequences of non-linear dose-responses for anti-angiogenic therapy

Cancer therapy and tumour progression

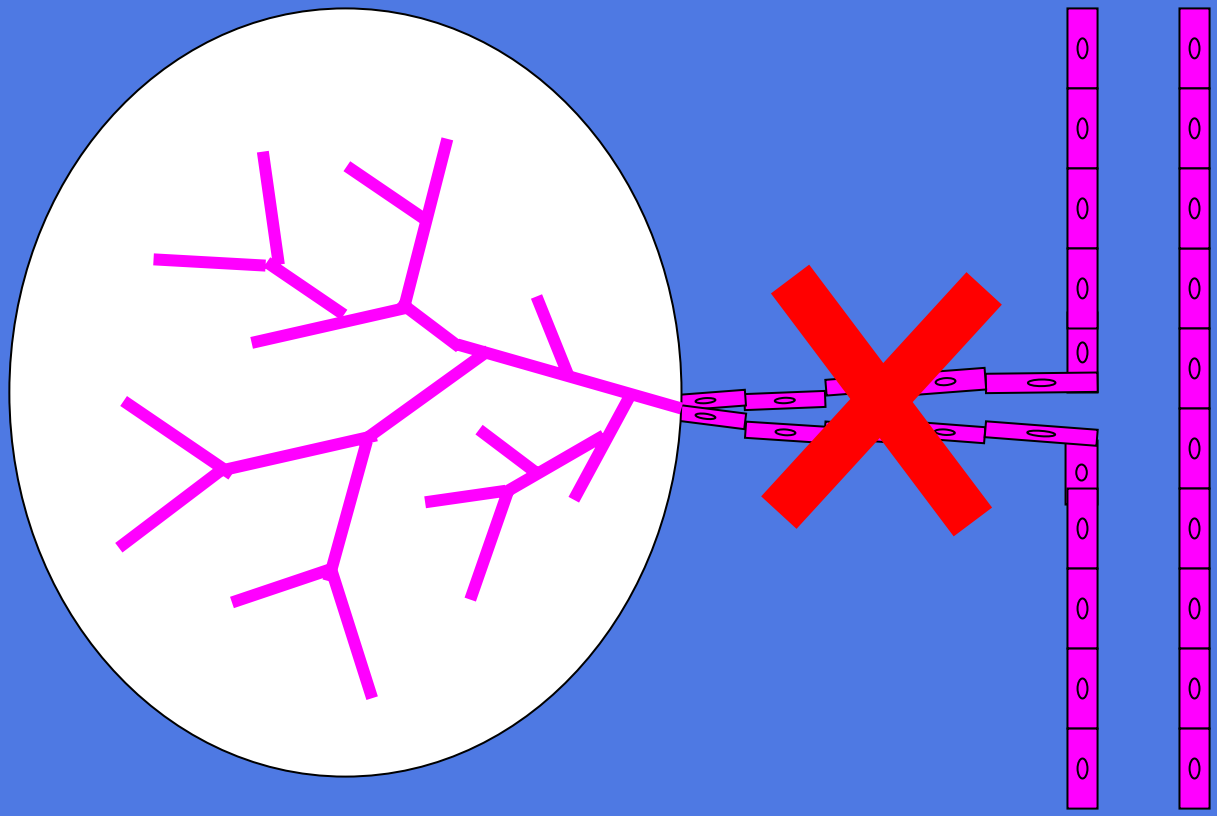
Tumor angiogenesis



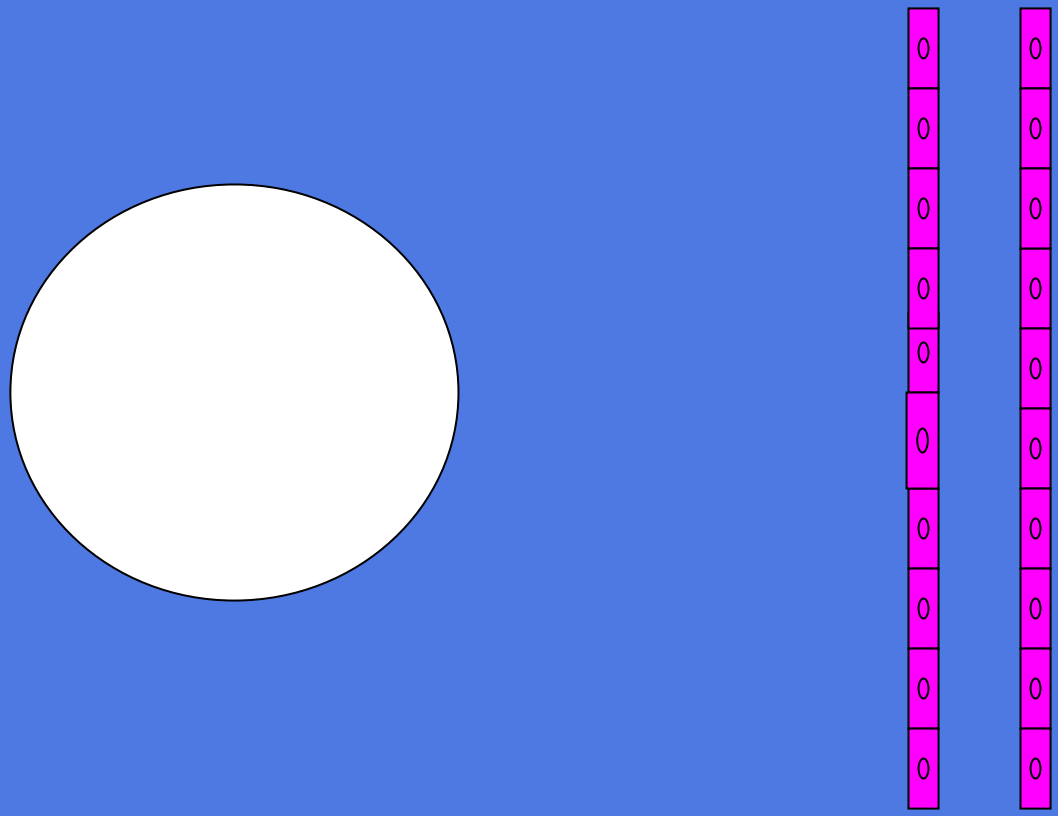
Tumor angiogenesis

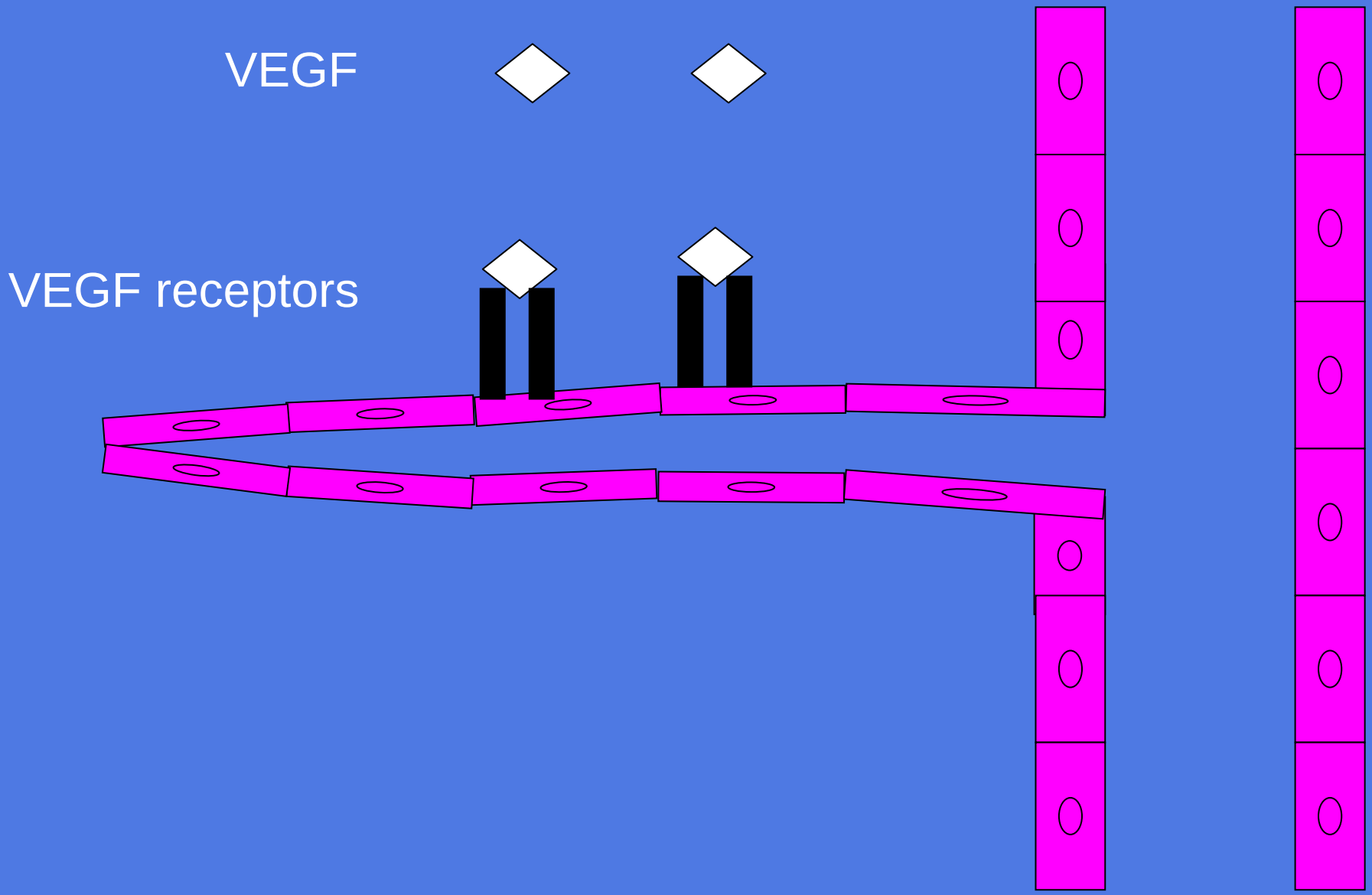


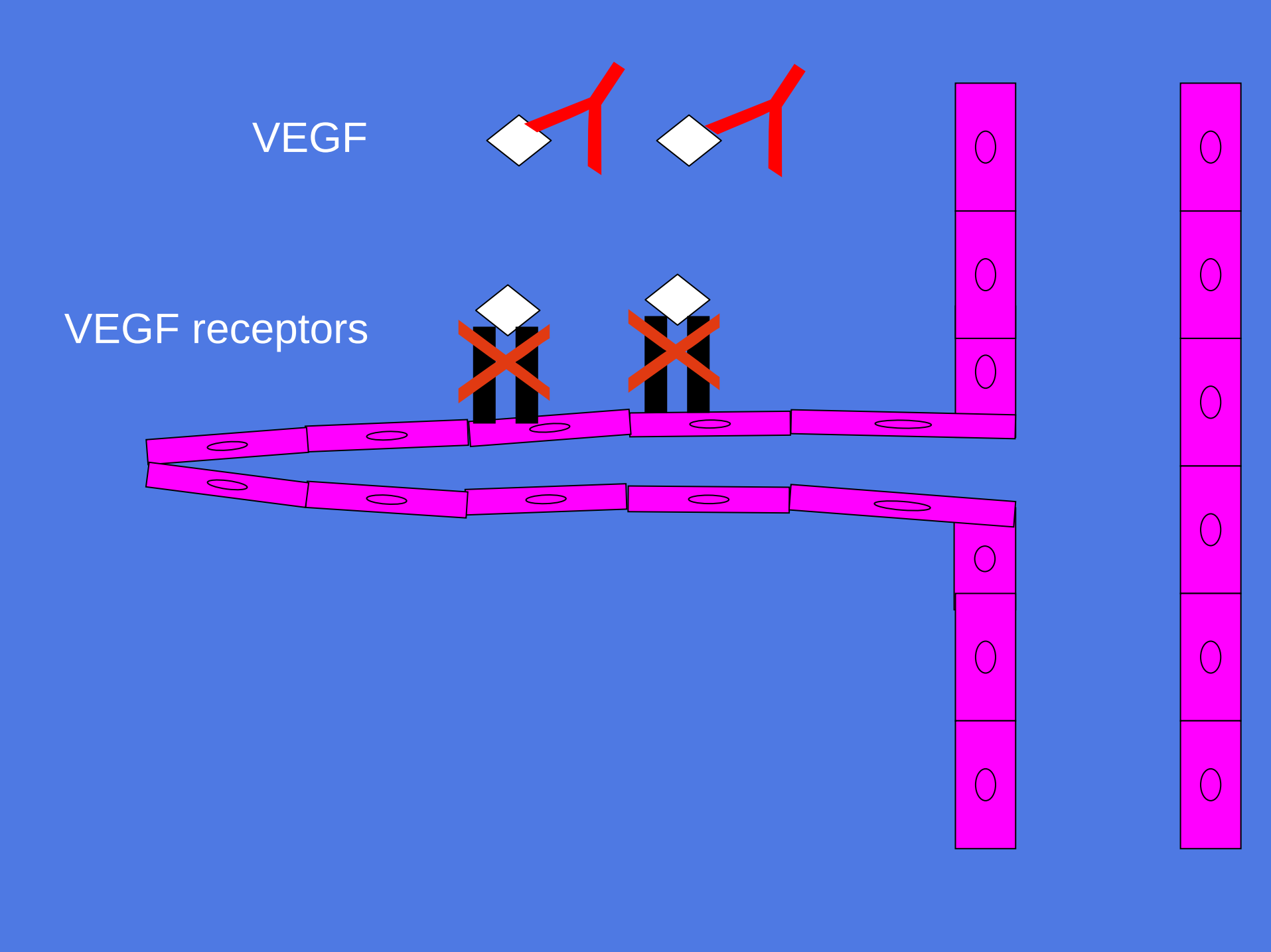
Anti-angiogenic therapy

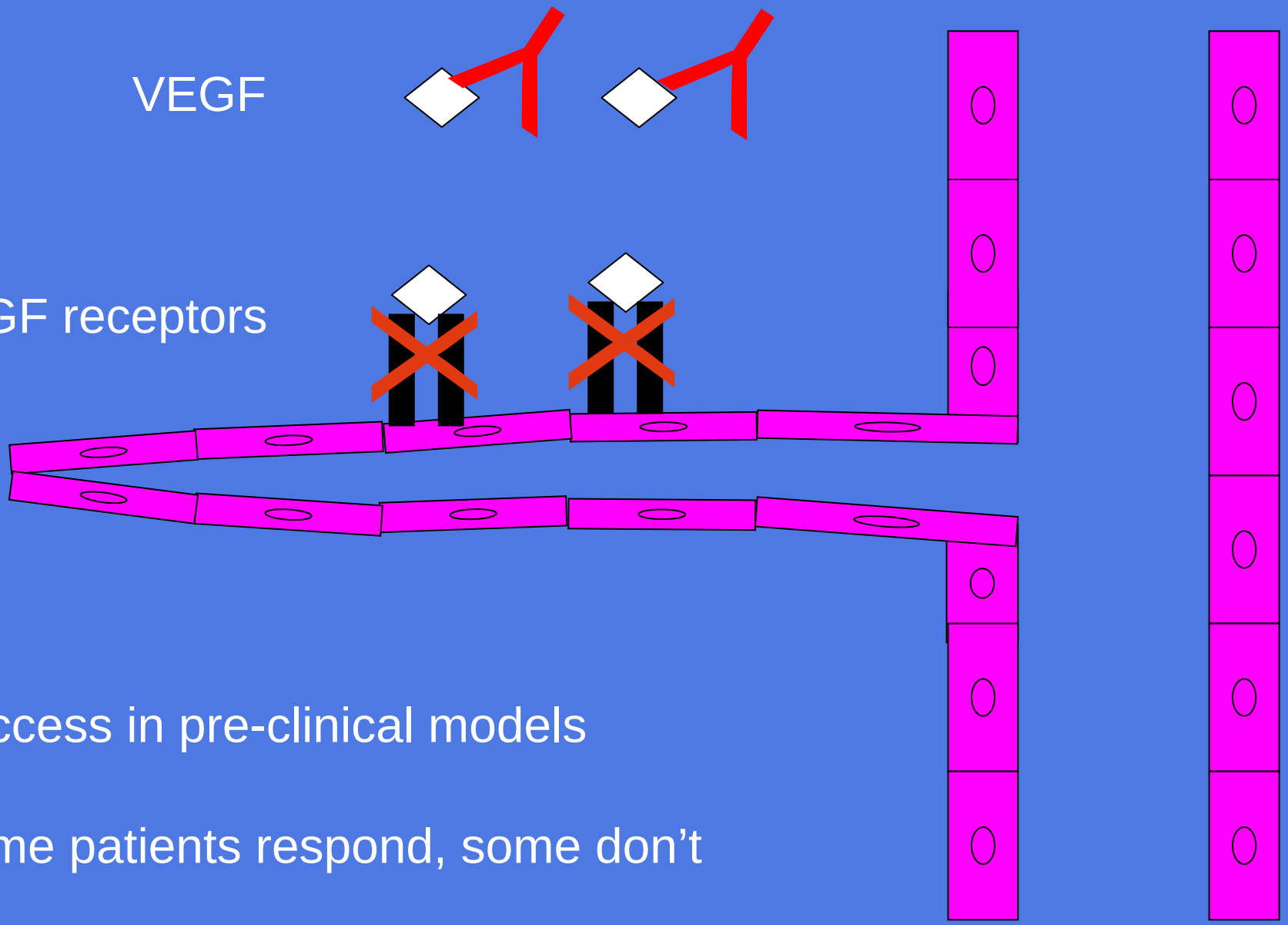


Anti-angiogenic therapy

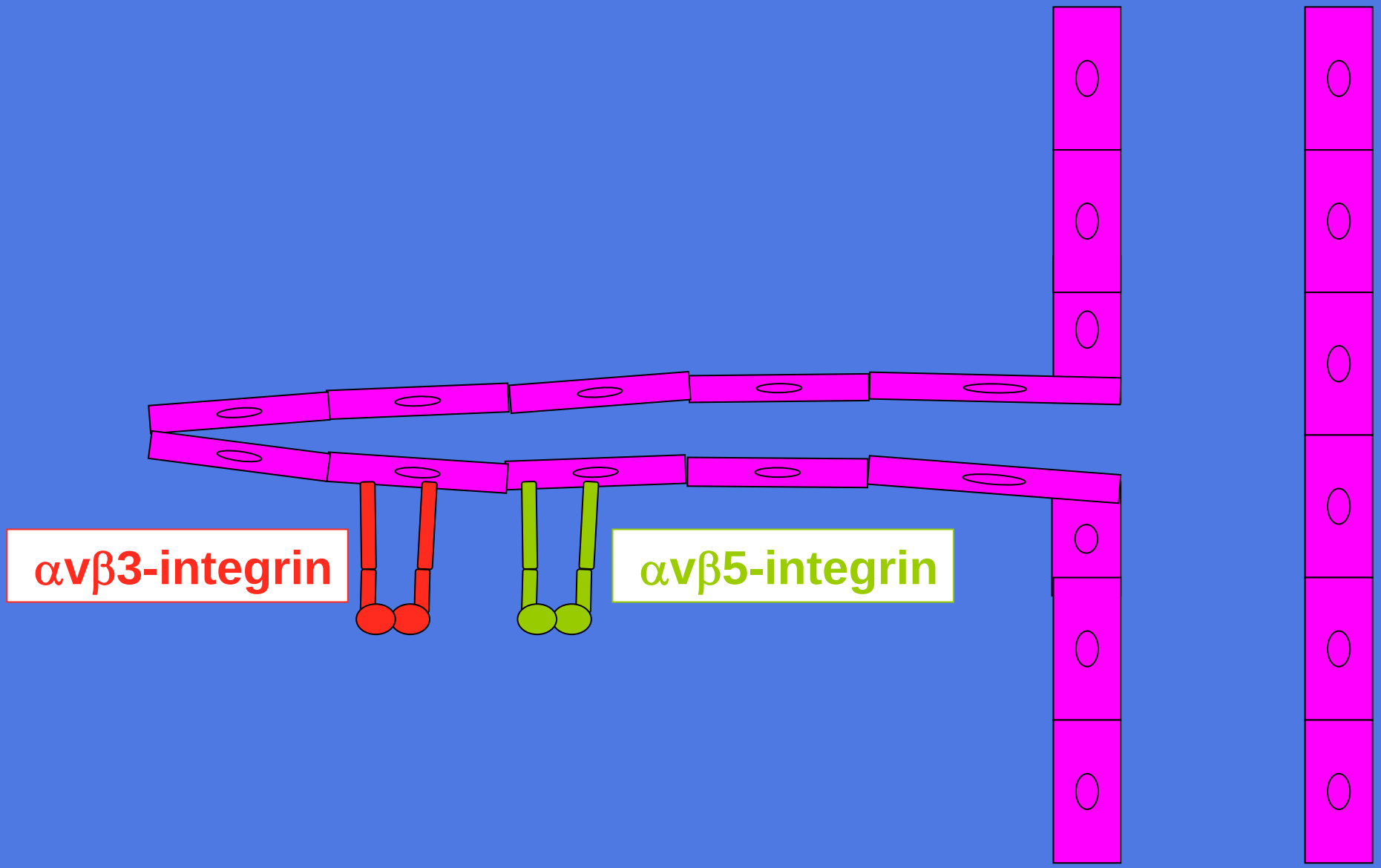



VEGF
VEGF receptors

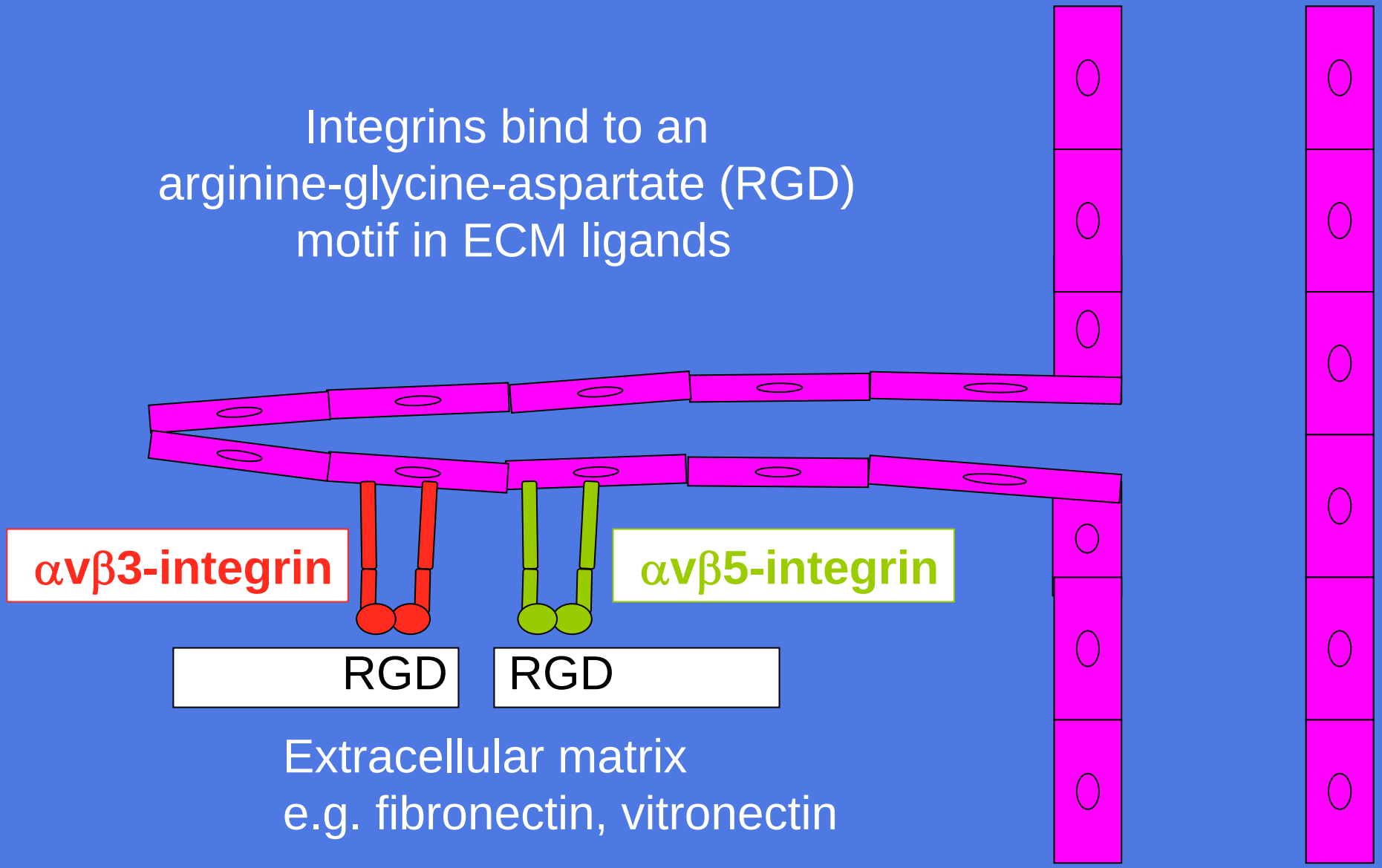

VEGF
VEGF receptors



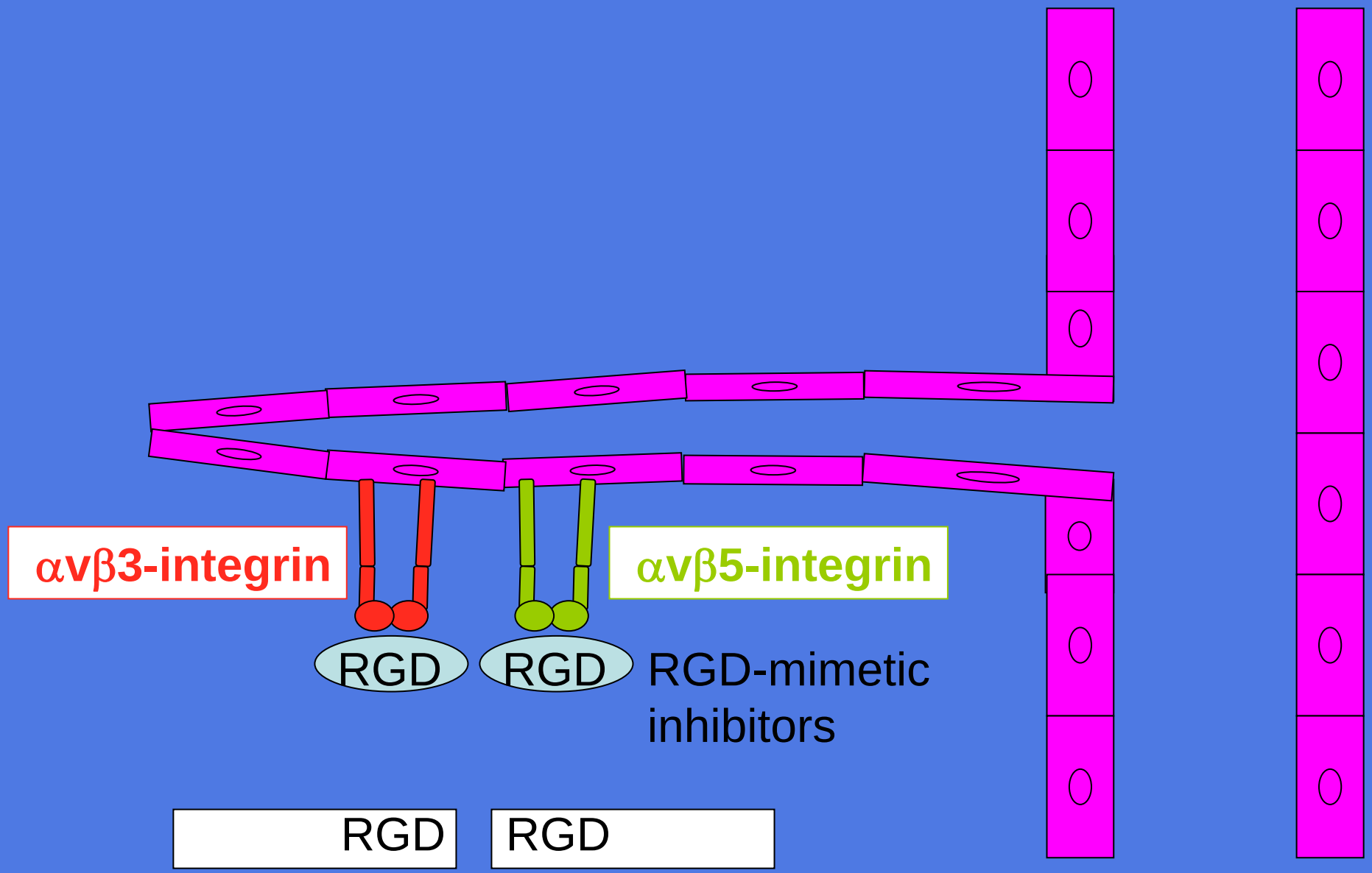
- Success in pre-clinical models
- Some patients respond, some don’t
- Delayed disease progression in some cancer types


αvβ3-integrin
αvβ5-integrin

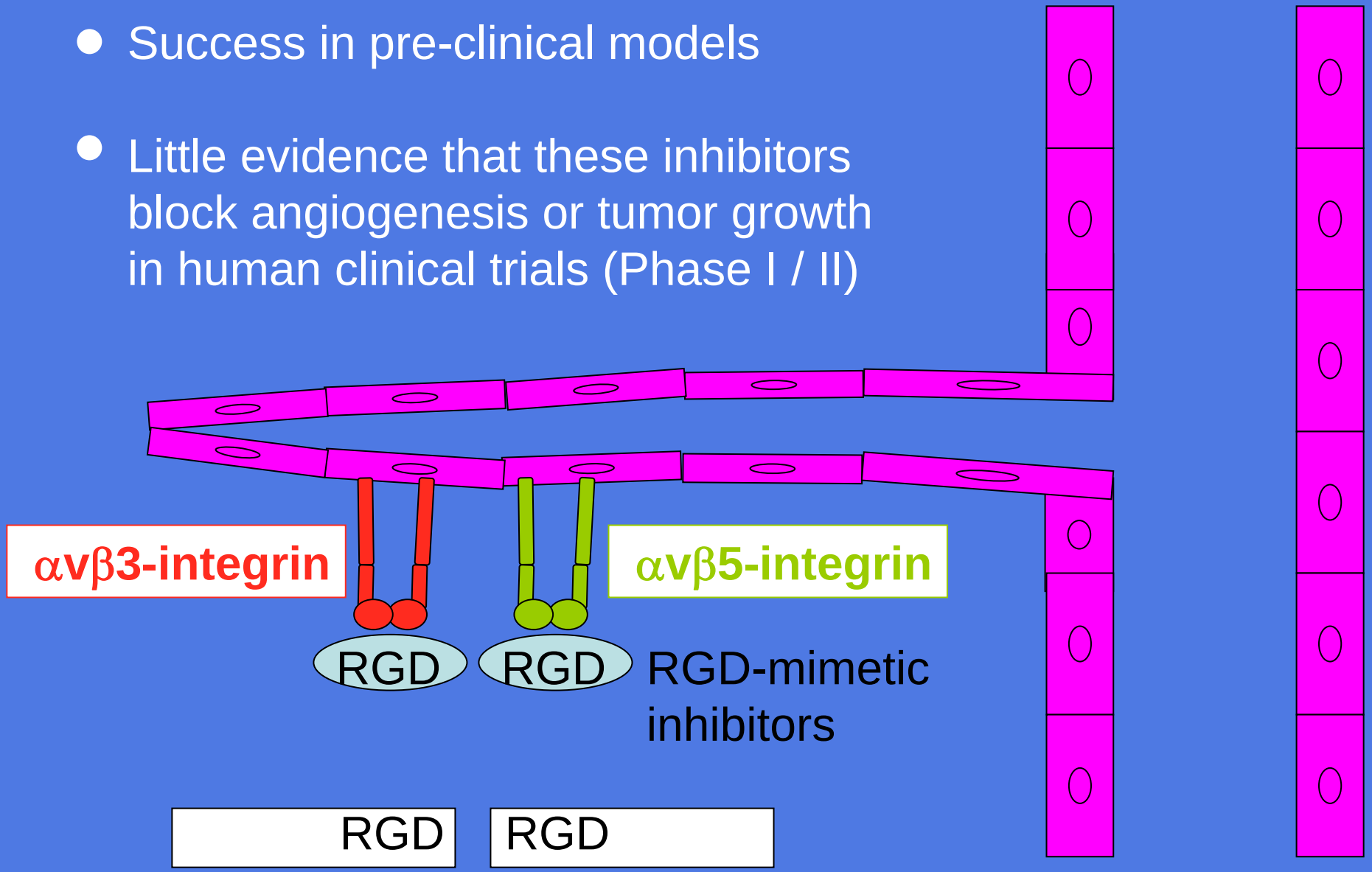
Integrins bind to an arginine-glycine-aspartate (RGD) motif in ECM ligands



Extracellular matrix
e.g. fibronectin, vitronectin

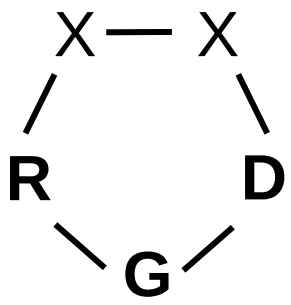

αvβ3-integrin
αvβ5-integrin
RGD
RGD
RGD-mimetic inhibitors
RGD
RGD

- Success in pre-clinical models
- Little evidence that these inhibitors block angiogenesis or tumor growth in human clinical trials (Phase I / II)



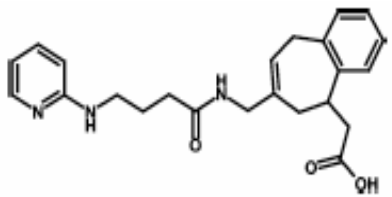
Inhibitors of αvβ3- and αvβ5-integrins

1. Cyclic RGD peptides



e.g. Cilengitide (Merck)

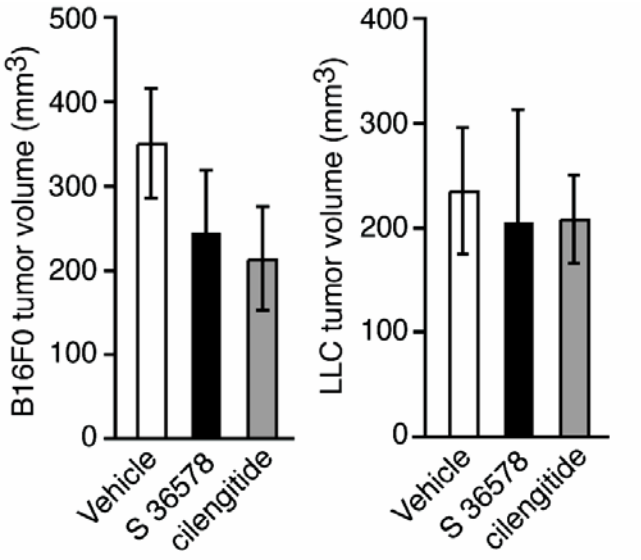
2. RGD-mimetic small molecule inhibitors



e.g. S 36578 (Servier)

Growth of B16 and LLC tumors in mice is not suppressed by 200 mg/kg/day integrin inhibitor

Tumor volume



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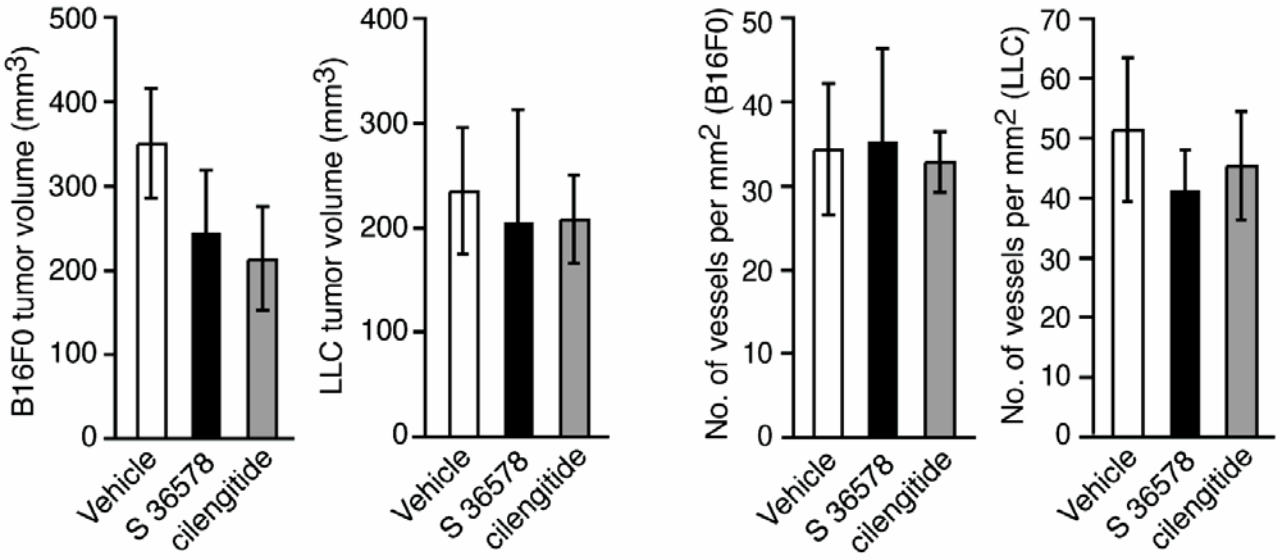
Tumor volume

Tumor angiogenesis



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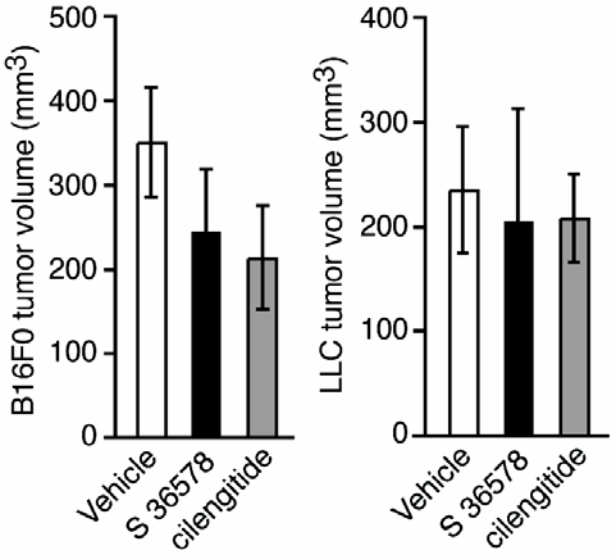
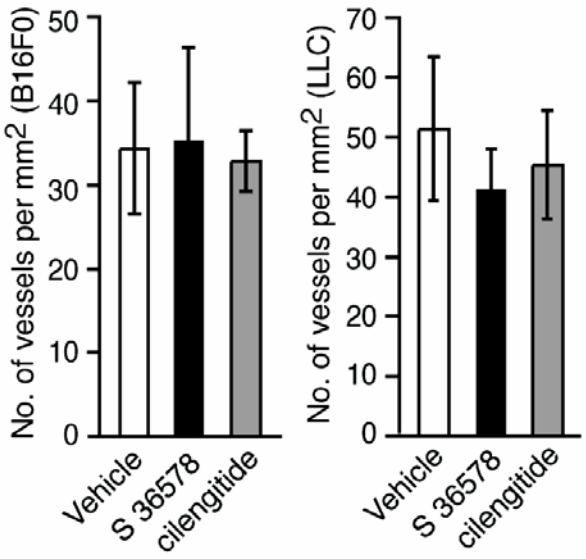
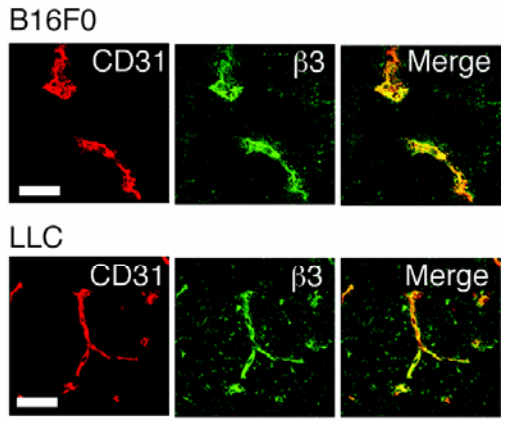
Tumor volume



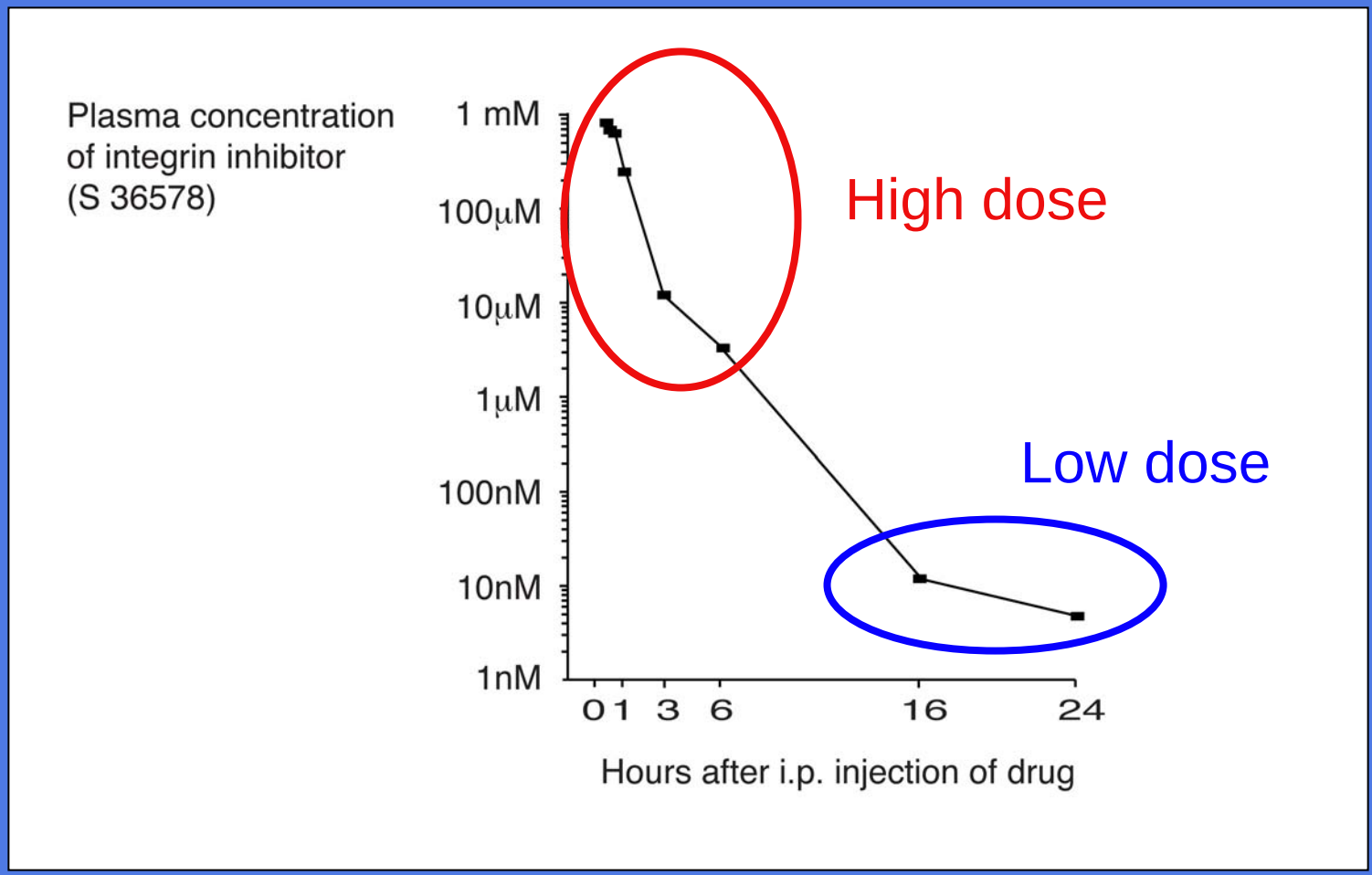
Tumor angiogenesis



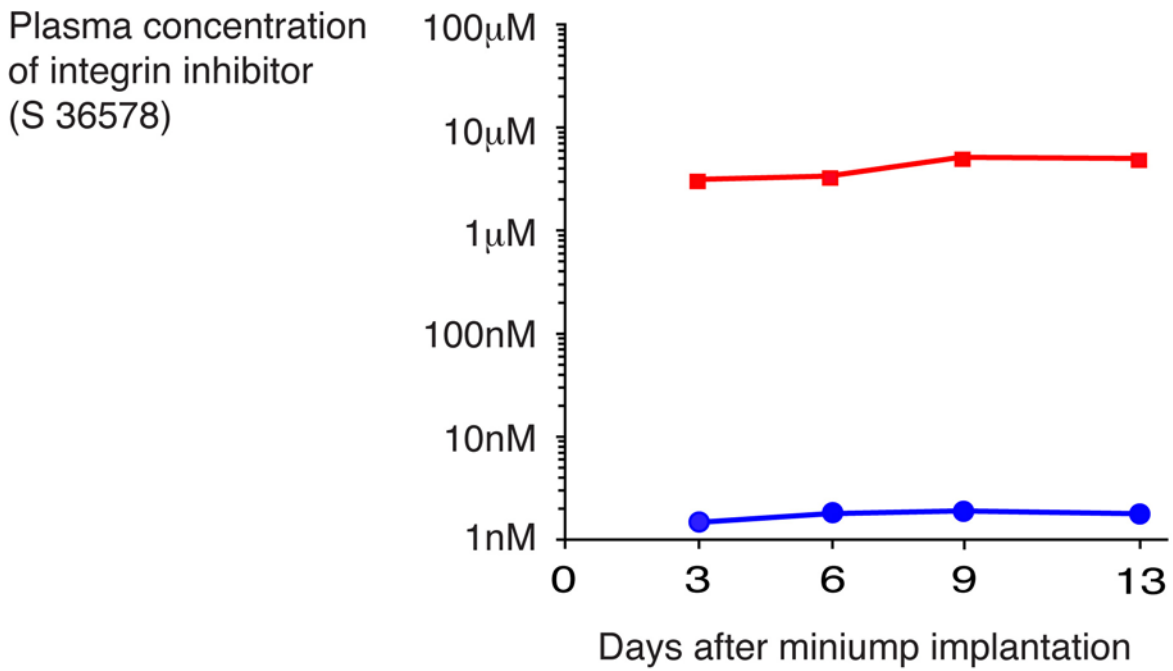
Integrin expression



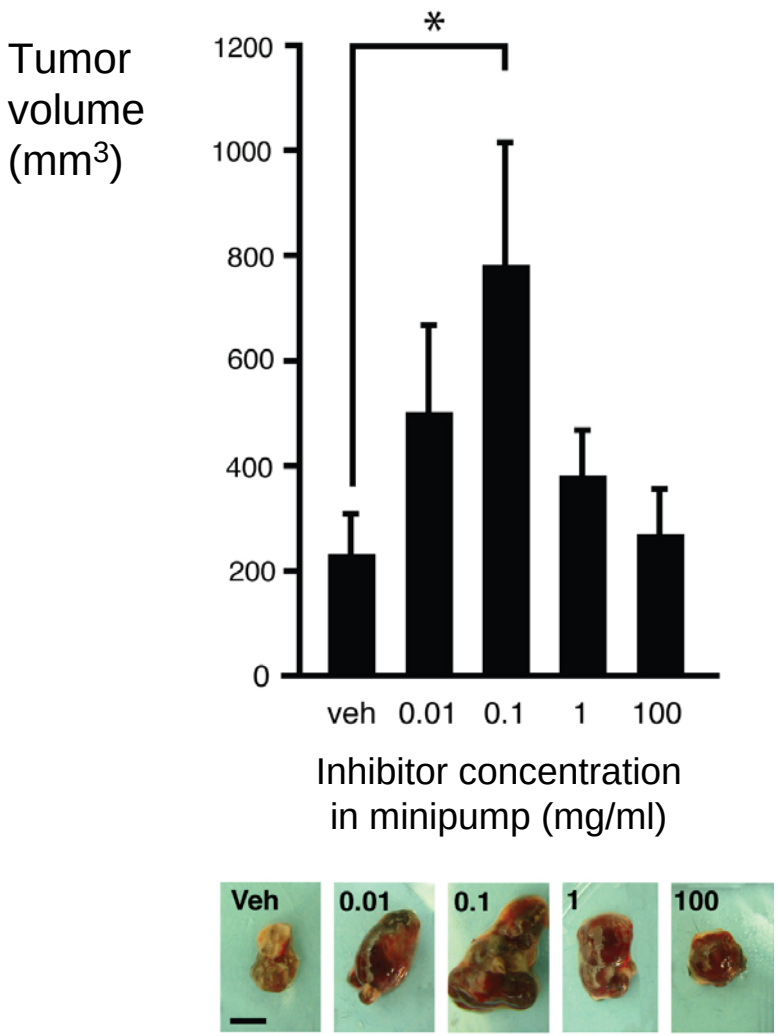
Pharmacokinetics of integrin inhibitors *in vivo*



Constant plasma concentrations of integrin inhibitors can be achieved using osmotic minipumps

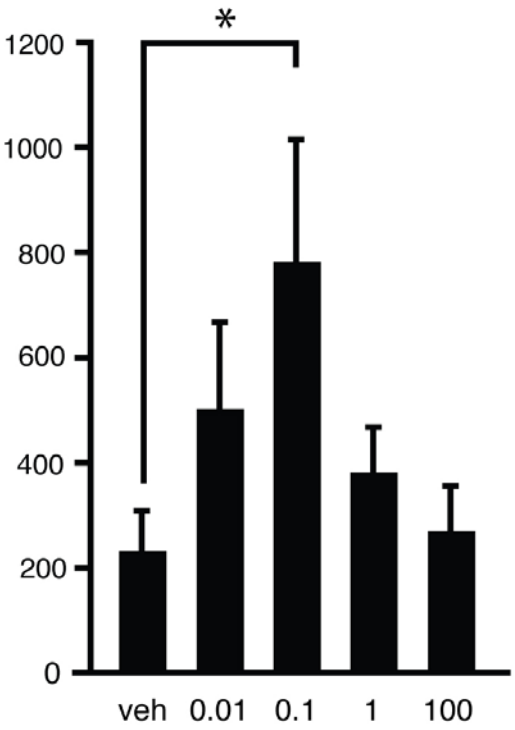


Low doses of integrin inhibitors can stimulate B16 tumor growth and angiogenesis *in vivo*

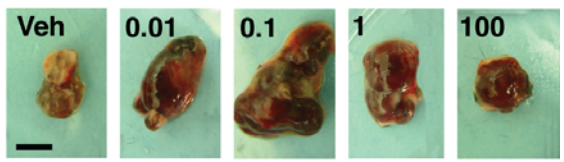


Low doses of integrin inhibitors can stimulate B16 tumor growth and angiogenesis *in vivo*

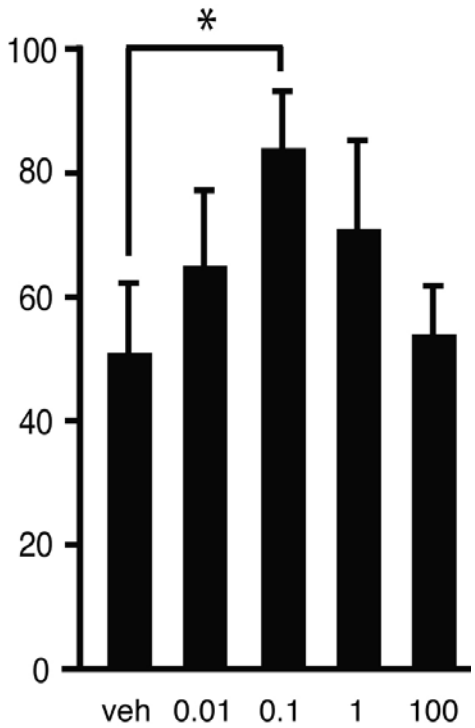
Tumor volume (mm^3)



Inhibitor concentration in minipump (mg/ml)



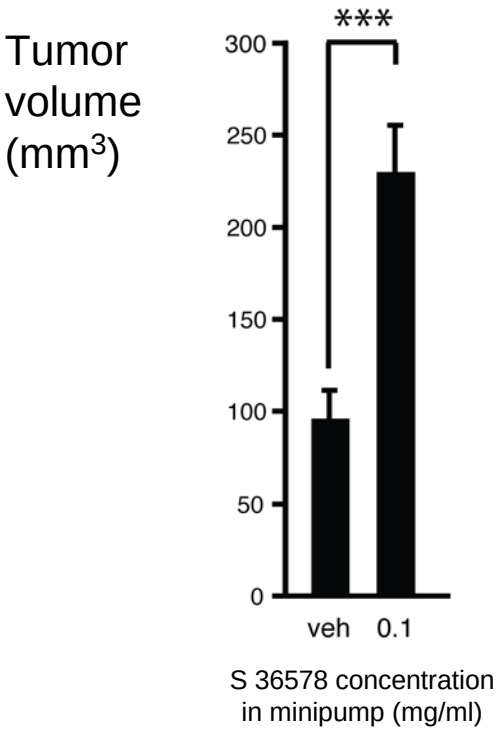
Tumor vessels per mm^2



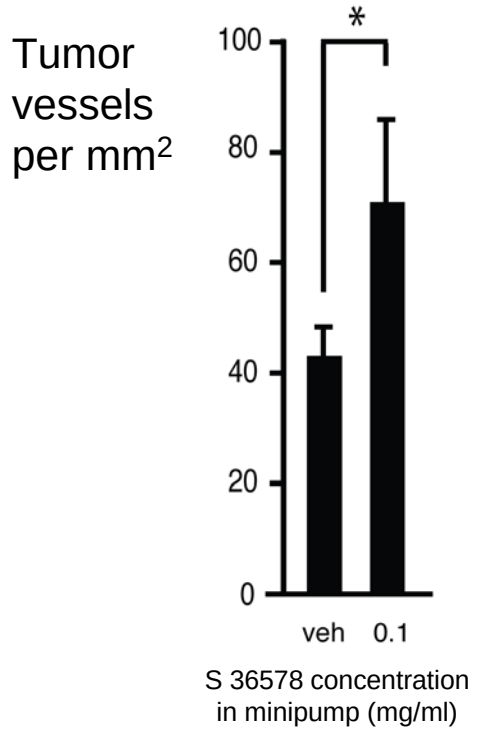
Inhibitor concentration in minipump (mg/ml)

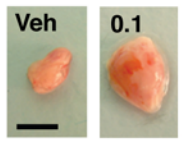
Low doses of integrin inhibitors can stimulate LLC tumor growth and angiogenesis *in vivo*

LLC tumor size

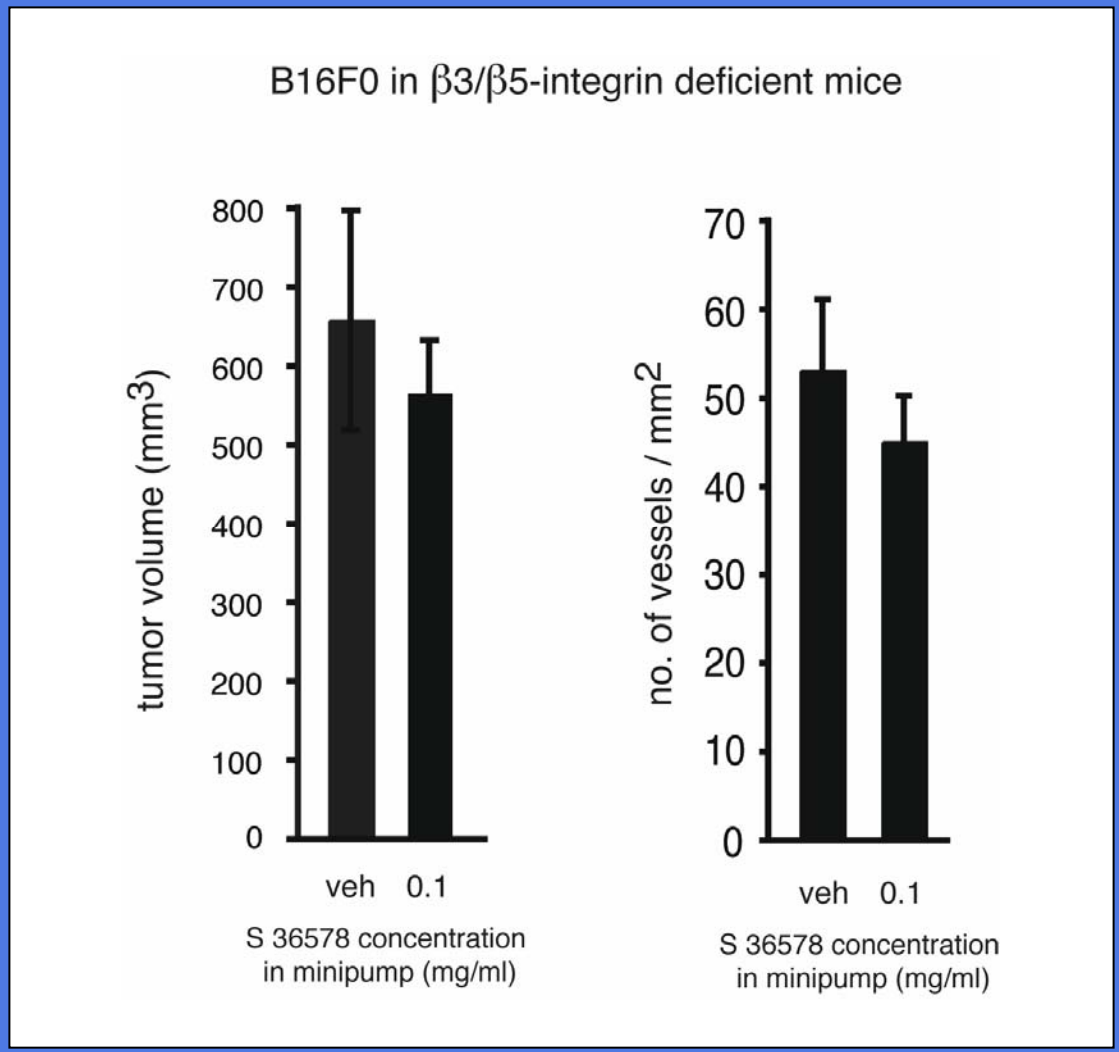


LLC vessels

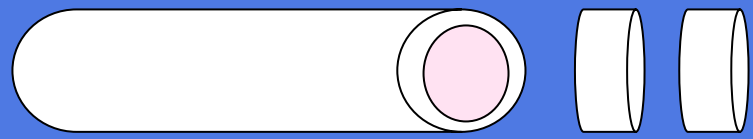




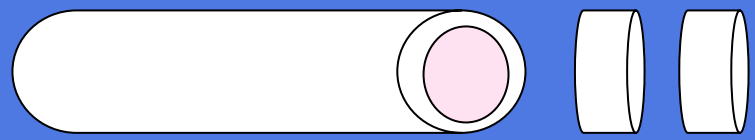
Expression of β3- and β5-integrin is required for integrin inhibitors to promote tumor growth and angiogenesis

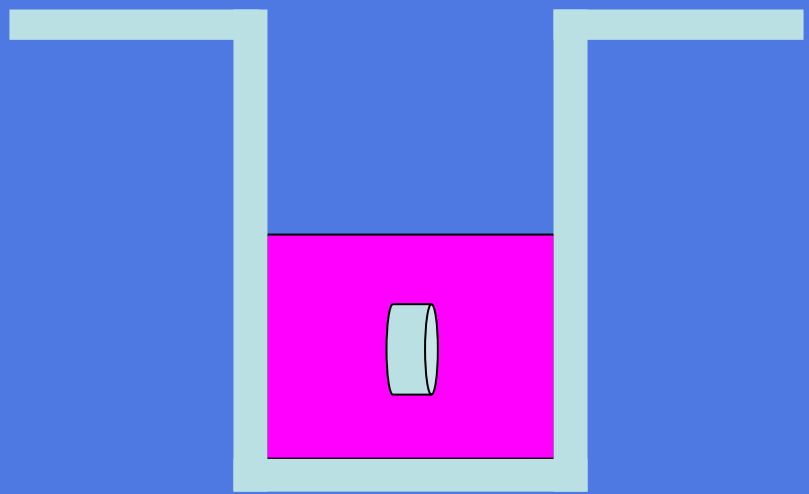


The mouse aortic ring assay



The mouse aortic ring assay





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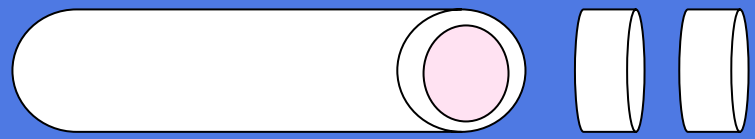
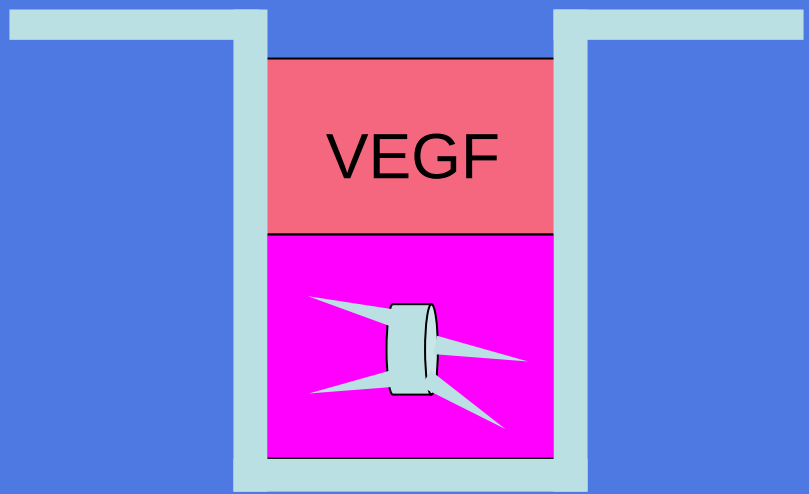
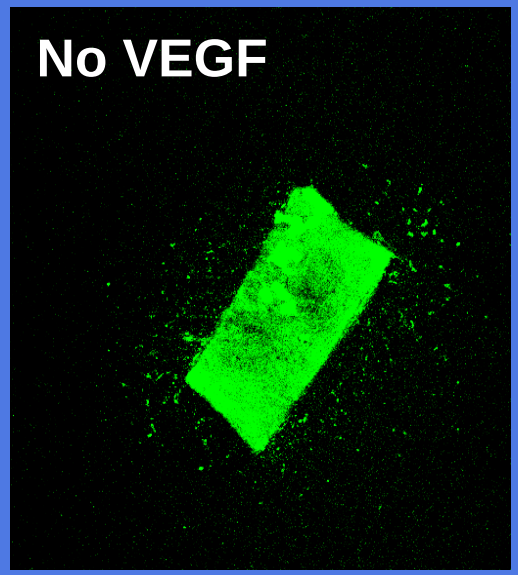
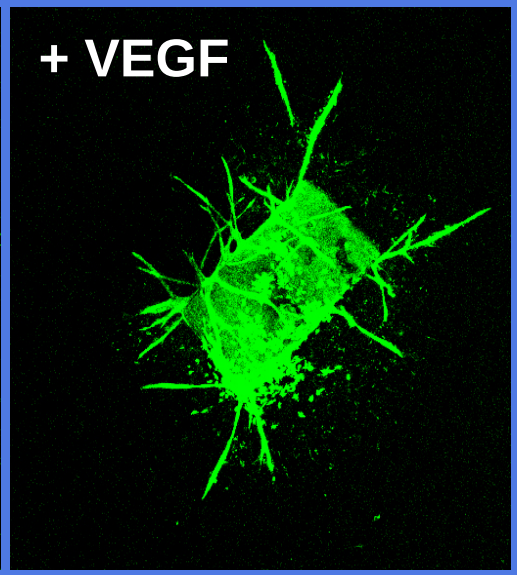
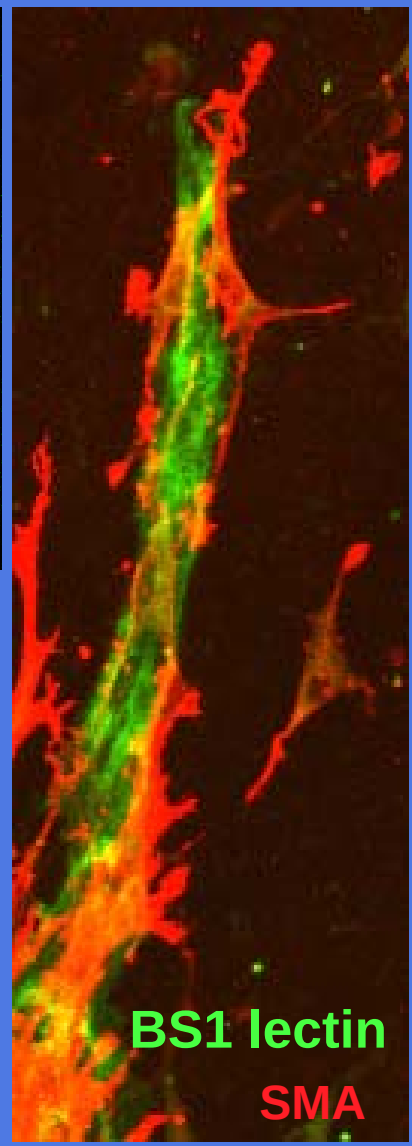




Low doses of integrin inhibitors stimulate angiogenesis *in vitro*







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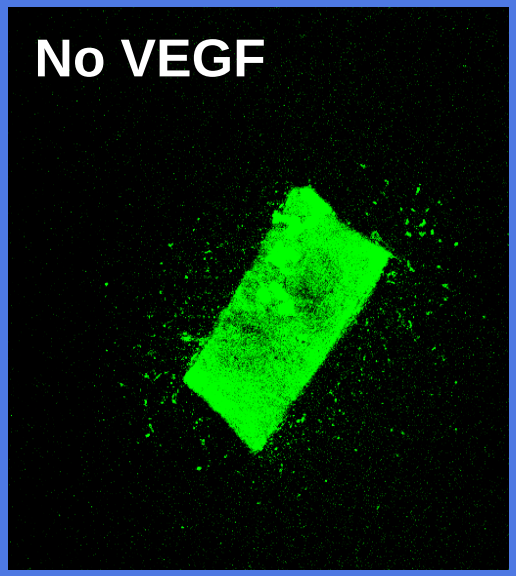
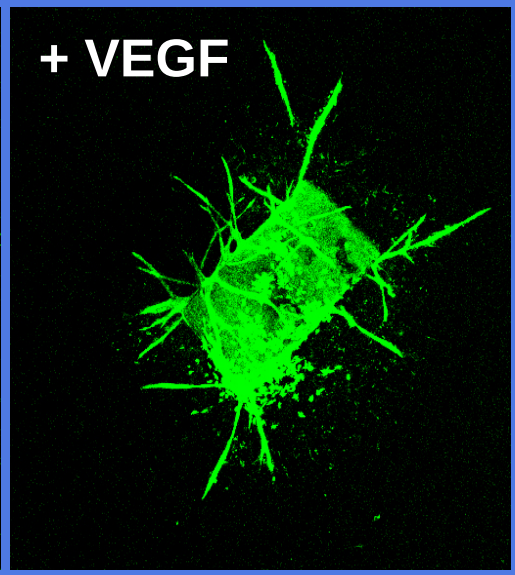
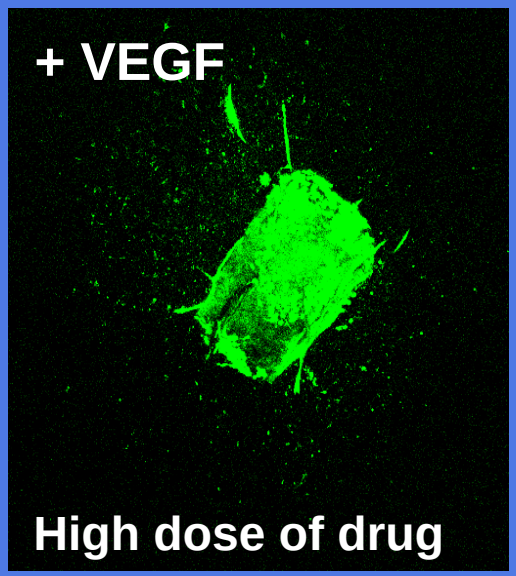
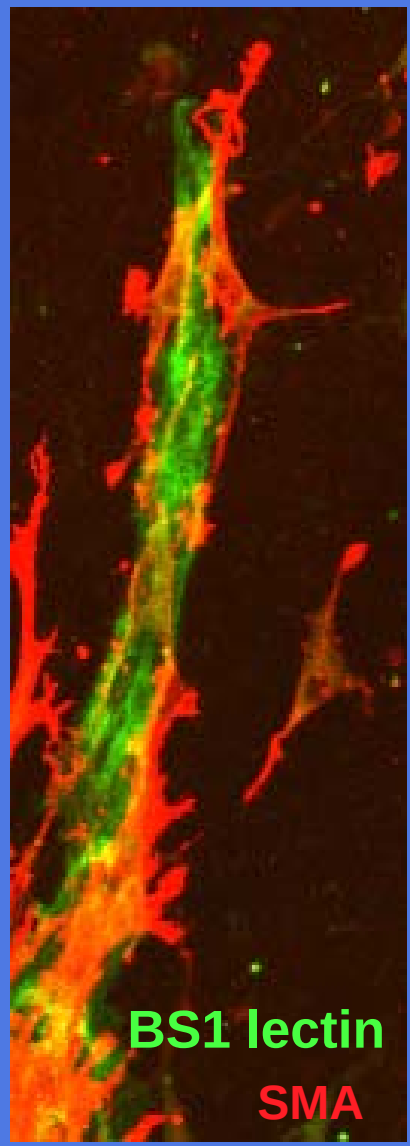
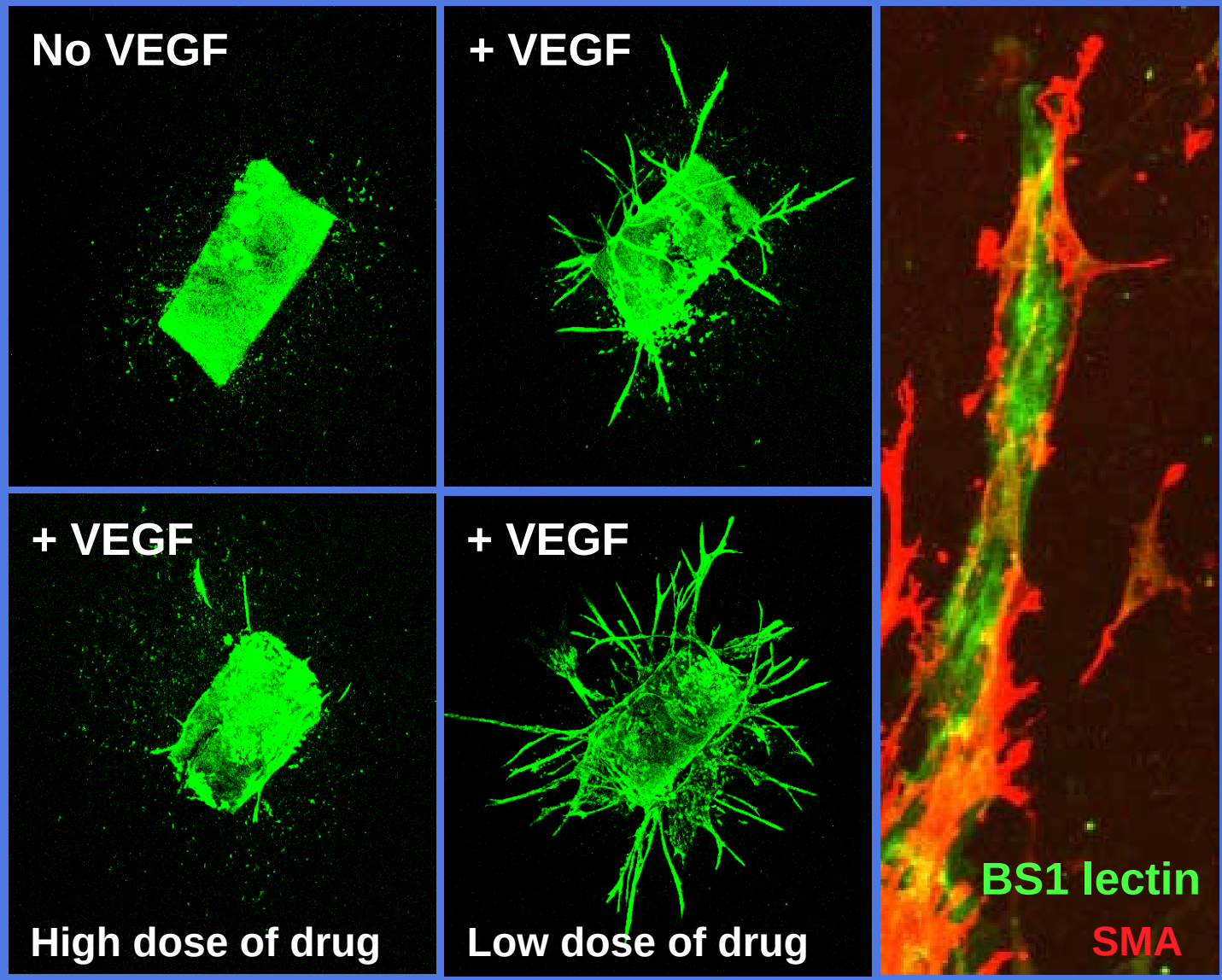






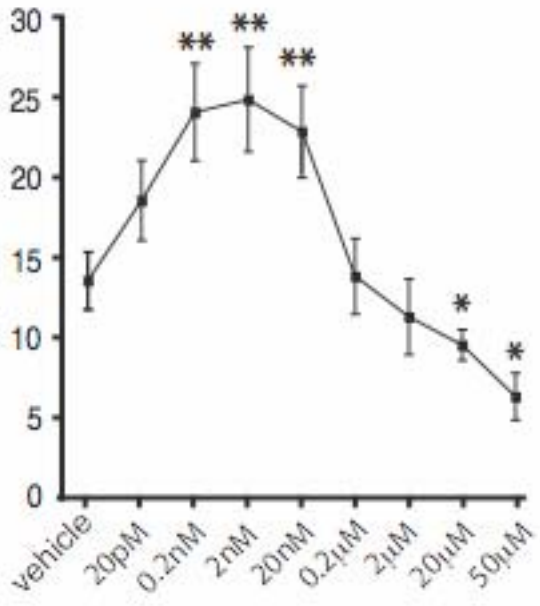


Low doses of integrin inhibitors stimulate angiogenesis *in vitro*



Low doses of integrin inhibitors stimulate VEGF-mediated angiogenesis *in vitro*

Number of sprouts per ring



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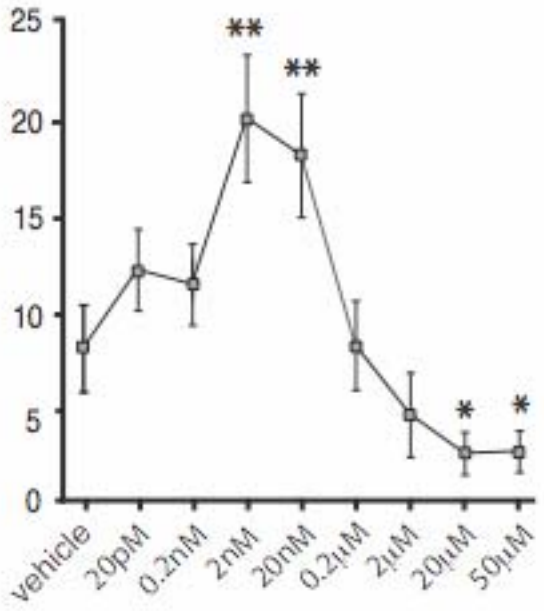
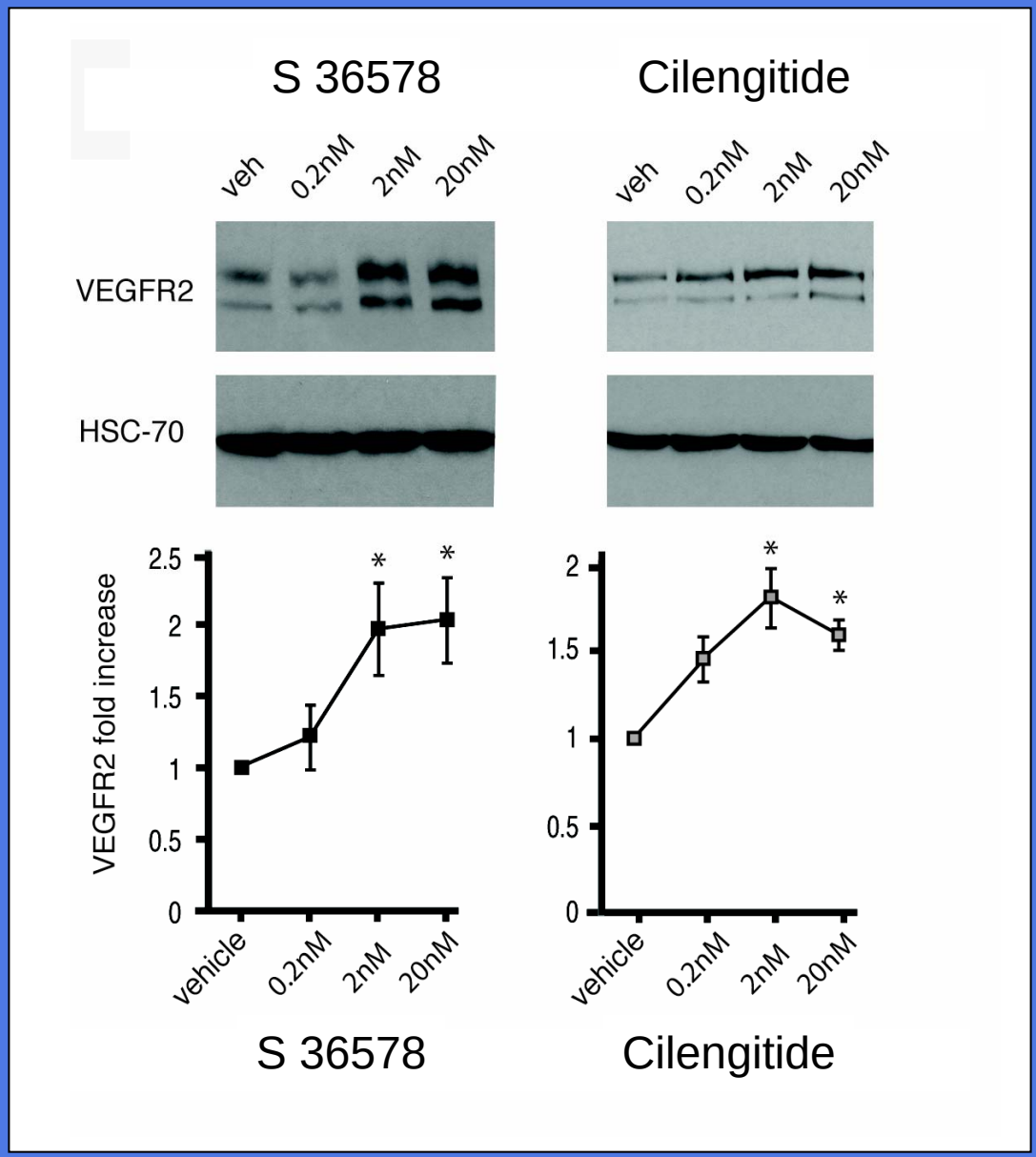
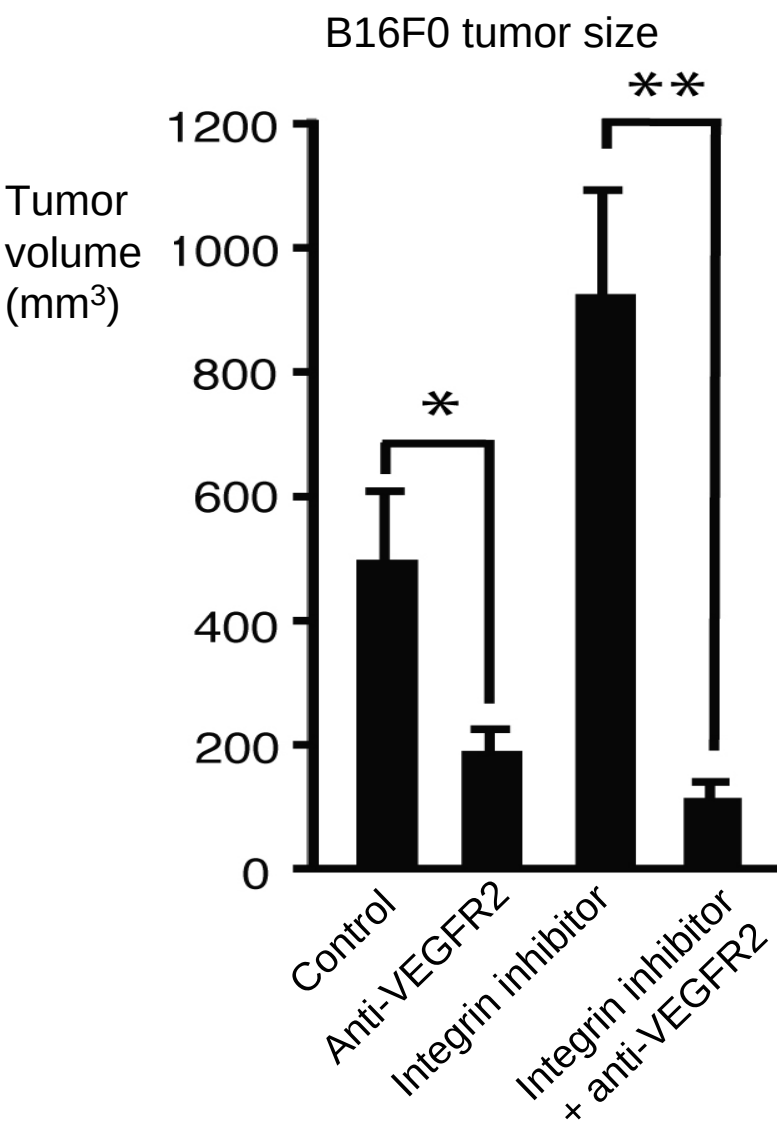


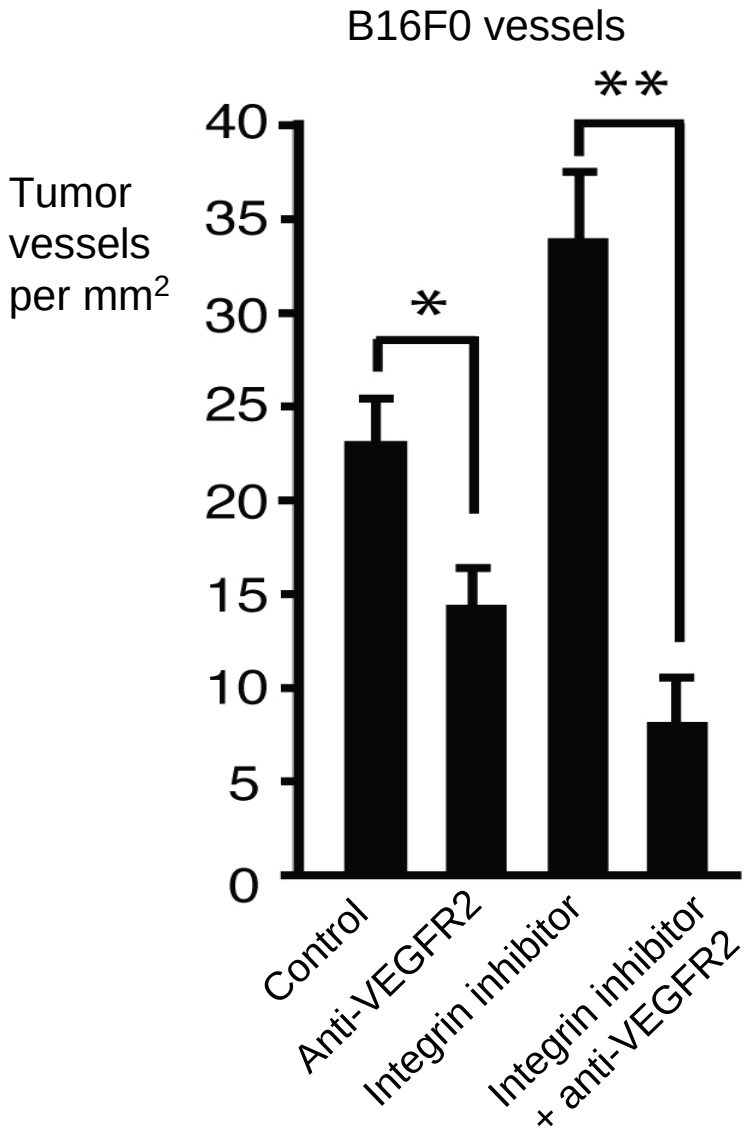
Cilengitide

Low doses of integrin inhibitors stimulate expression of VEGF receptor 2 protein

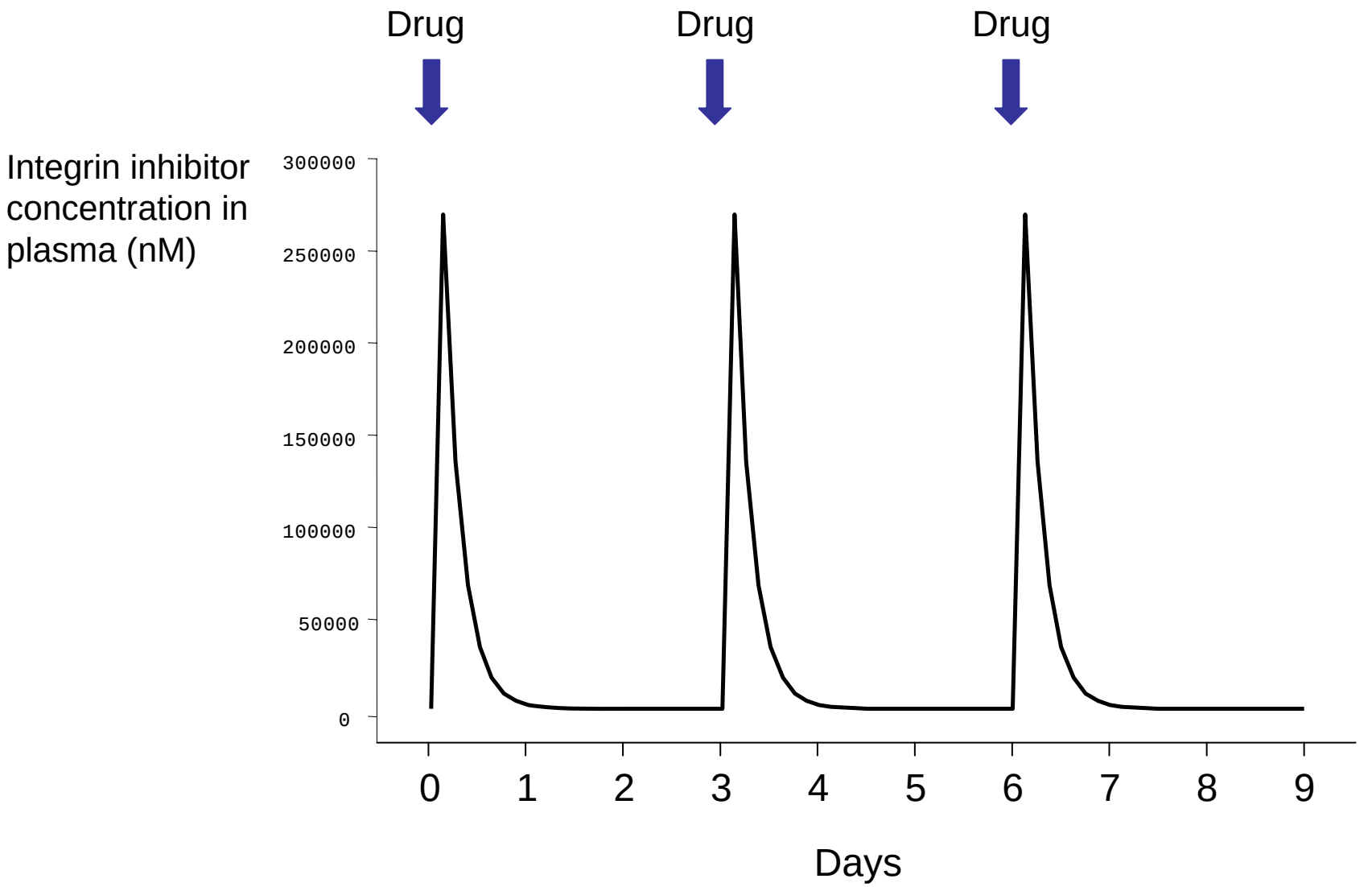


A VEGFR2 inhibitory antibody blocks the enhanced tumor growth and angiogenesis *in vivo*





Pharmacokinetics of an integrin inhibitor (cilengitide) in patients demonstrates high and low dose exposure



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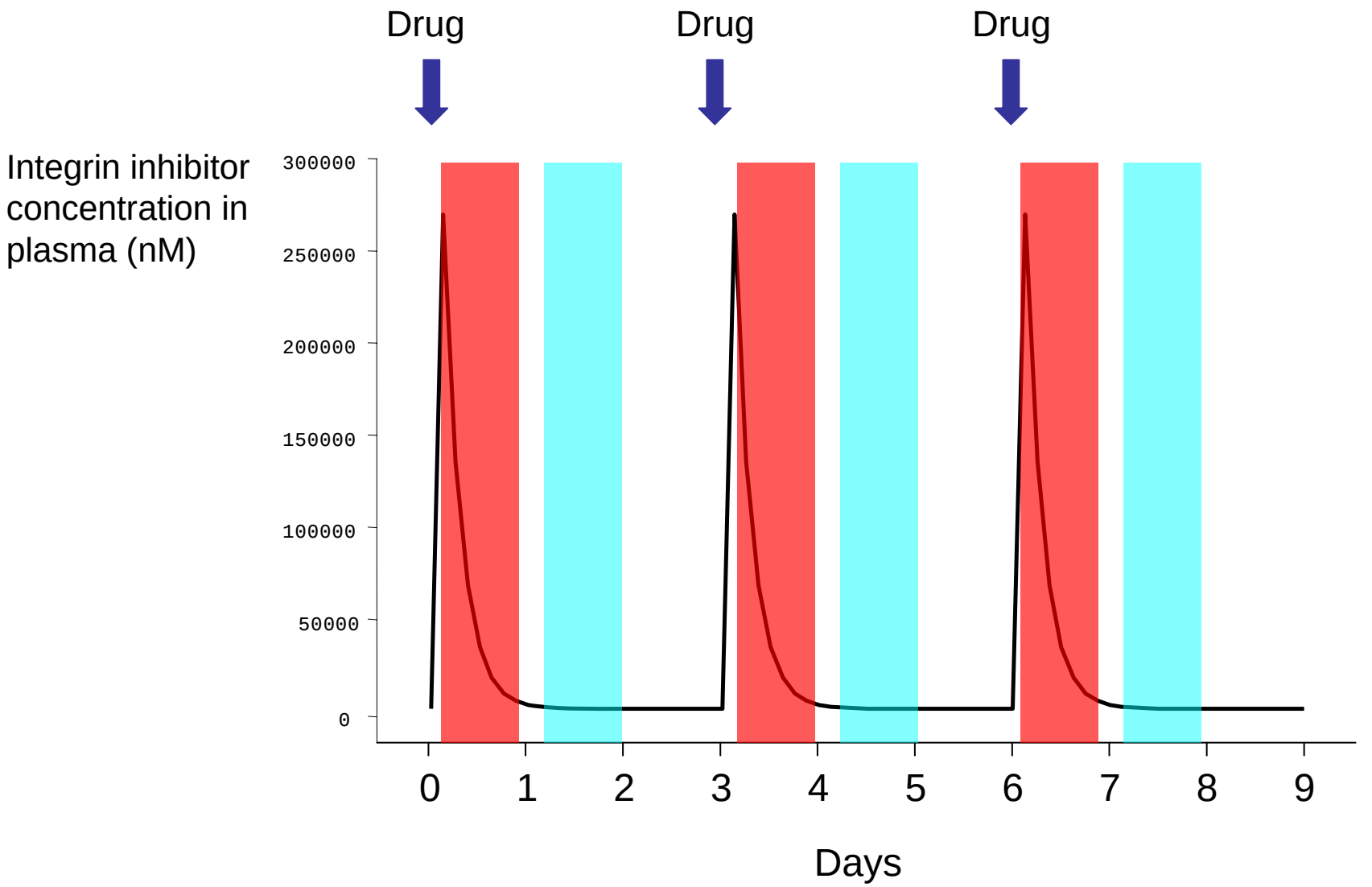


Conclusions

Low concentrations of RGD-mimetic integrin inhibitors can promote tumor growth and VEGF-mediated tumor angiogenesis in mice.

These effects are dependent on:

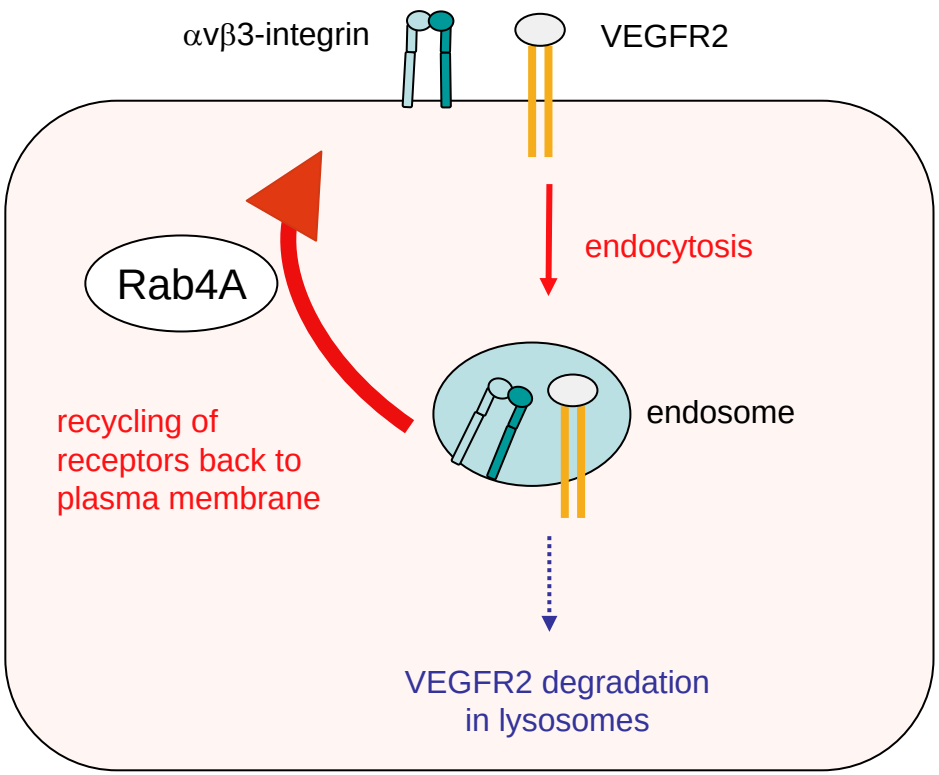
(1) Expression of the drug targets: αvβ3-integrin and αvβ5-integrin
(2) Signalling through the pro-angiogenic receptor VEGFR2

Intermittent exposure to low concentrations of RGD-mimetic integrin inhibitor could compromise the efficacy of these drugs in the clinic.

Mechanism

How do low doses of RGD-mimetic integrin inhibitors promote tumor growth and tumor angiogenesis?

Role of VEGFR2 and β3-integrin recycling



↑ β3-integrin recycling

↓

↑ VEGFR2 recycling

↓ VEGFR2 degradation

↓

↑ VEGF-mediated migration

↑ VEGF-mediated angiogenesis

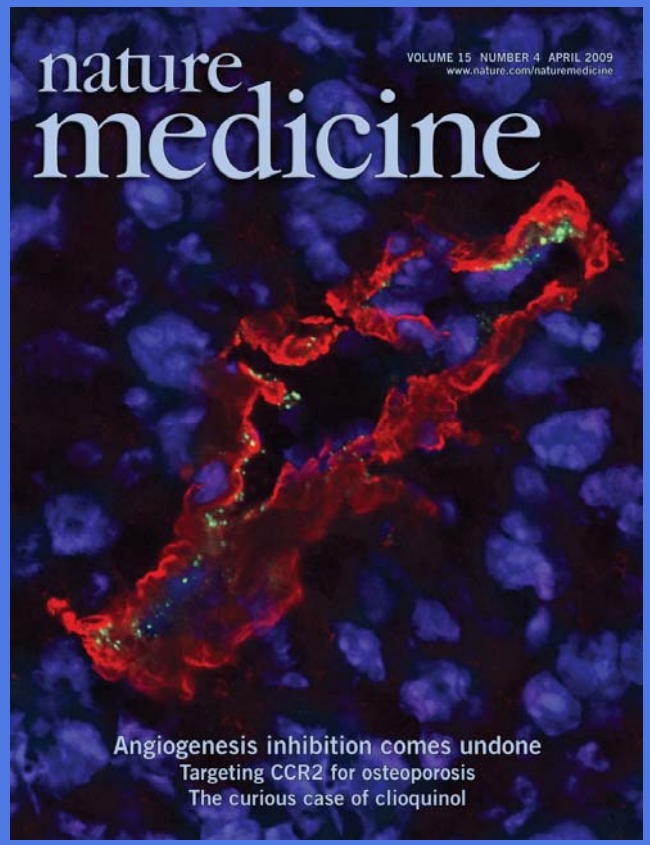
Reynolds *et al.*, Nature Medicine April 2009

nature medicine

Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors

Andrew R Reynolds[1,2], Ian R Hart[3], Alan R Watson[2], Jonathan C Welti[1], Rita G Silva[2], Stephen D Robinson[2], Georges Da Violante[4], Morgane Gourlaouen[1], Mishal Salih[2], Matt C Jones[5], Dylan T Jones[2], Garry Saunders[6], Vassiliki Kostourou[2], Françoise Perron-Sierra[7], Jim C Norman[5], Gordon C Tucker[8] & Kairbaan M Hodivala-Dilke[2]

Inhibitors of $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrin have entered clinical trials as antiangiogenic agents for cancer treatment but generally have been unsuccessful. Here we present *in vivo* evidence that low (nanomolar) concentrations of RGD-mimetic $\alpha_v\beta_3$ and $\alpha_v\beta_5$ inhibitors can paradoxically stimulate tumor growth and tumor angiogenesis. We show that low concentrations of these inhibitors promote VEGF-mediated angiogenesis by altering $\alpha_v\beta_3$ integrin and vascular endothelial growth factor receptor-2 trafficking, thereby promoting endothelial cell migration to VEGF. The proangiogenic effects of low concentrations of RGD-mimetic integrin inhibitors could compromise their efficacy as anticancer agents and have major implications for the use of RGD-mimetic compounds in humans.



What are the implications for the use of RGD-mimetic integrin inhibitors in patients?

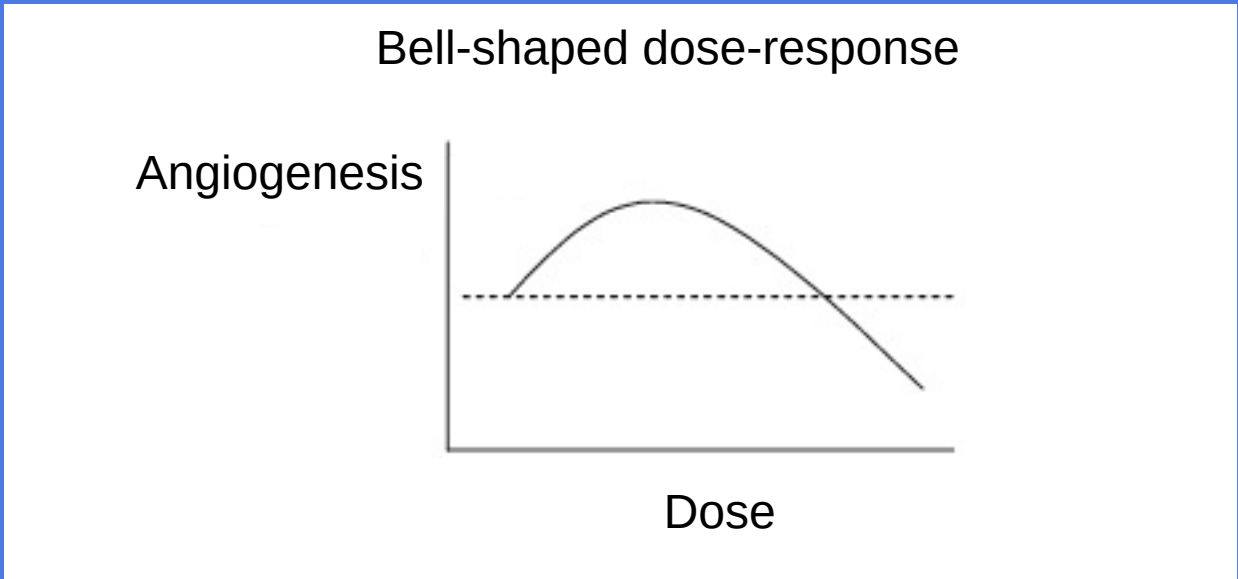
Low concentrations of RGD-mimetic integrin inhibitor could promote angiogenesis and compromise efficacy of therapy.

RGD-mimetic integrin inhibitors need to be delivered at continuously high doses in order to be effective.

Integrin inhibitors are likely to be more effective when used in combination with other agents e.g. VEGF signalling inhibitors.

Is it time for a ‘new generation’ of integrin inhibitors?
e.g. non-RGD based.

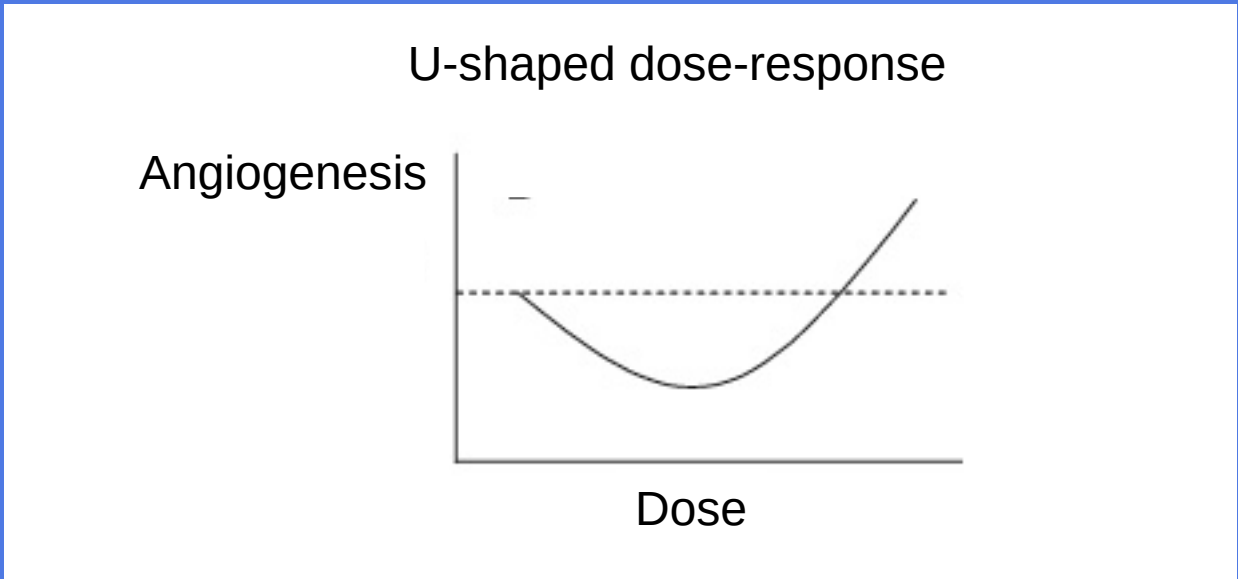
Is there other evidence for agents that induce low dose stimulation of angiogenesis and high dose inhibition of angiogenesis?



Adapted from Calabrese et al 2009

- Cilengitide, S 36578 (inhibitors of integrins) *Reynolds et al 2009*
- Statins (cholesterol lowering drugs) *Weis et al 2002, Urbich et al 2002*
- VEGF (major pro-angiogenic ligand) *A. Reynolds, unpublished data*

Is there evidence for agents that induce low dose inhibition of angiogenesis with loss of efficacy at higher doses?



Adapted from Calabrese et al 2009

- ATN-161 (integrin inhibitor) *Donate et al 2006*
- Endostatin (endogenous angiogenesis inhibitor) *Celik et al 2005, Sjin et al 2006*
- Angiostatin (endogenous angiogenesis inhibitor) *Benelli et al 2003*
- Thrombospondin-1 (endogenous angiogenesis inhibitor) *Motegi et al 2002*
- Rosiglitazone (PPARγ ligand) *Panigraphy et al 2002*

Endostatin

- Proteolytic fragment of collagen type XVIII
- Anti-angiogenic, multiple receptors on endothelial cells
- Currently in Phase I / II trials

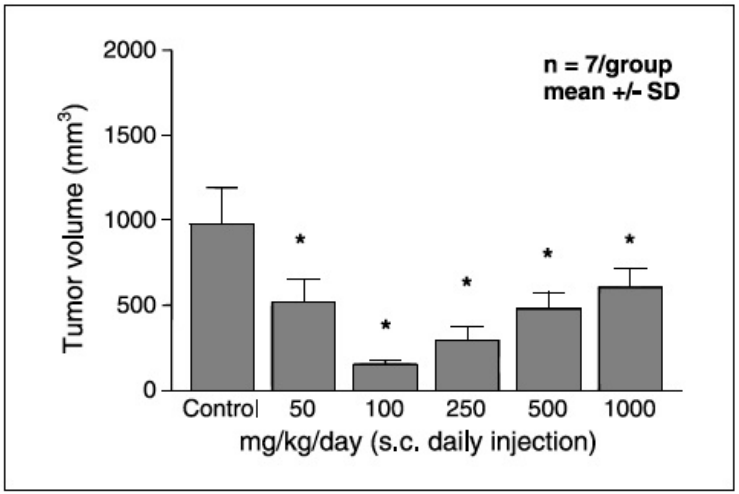


Figure 1. Treatment of human pancreatic carcinoma (BxPC-3) with human endostatin. Mean (± SD) tumor volume after a 20-day treatment with different dosages of rhEndostatin (50, 100, 250, 500, and 1,000 mg/kg/d) in BxPC-3 tumor–bearing mice (group sizes, $n = 7$). Endostatin was given s.c. once daily. Tumors were measured every 3 to 5 days. *, $P < 0.001$, tumor volume in all treatment groups were significantly different compared with the control group.

Plasma concentrations:

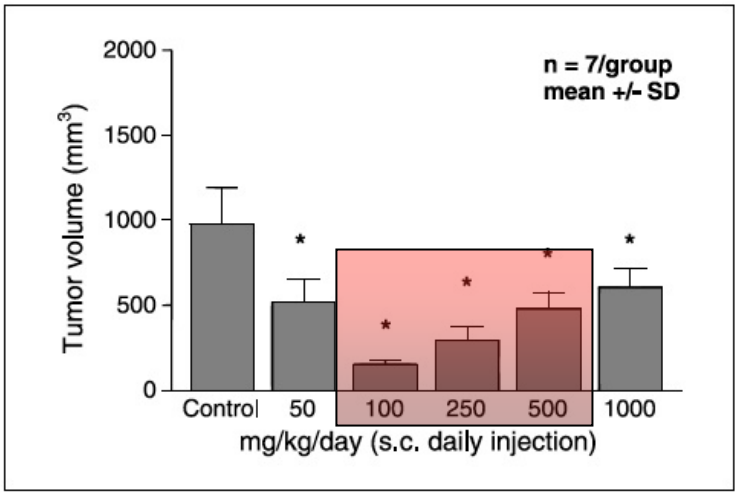
≤ 10 ng/ml - poor activity
10 - 200 ng/ml - anti-angiogenic
≥ 200 ng/ml - poor activity

i.e. narrow therapeutic window

Celik et al 2005, Sjin et al 2006

Endostatin

- Proteolytic fragment of collagen type XVIII
- Anti-angiogenic, multiple receptors on endothelial cells
- Currently in Phase I / II trials



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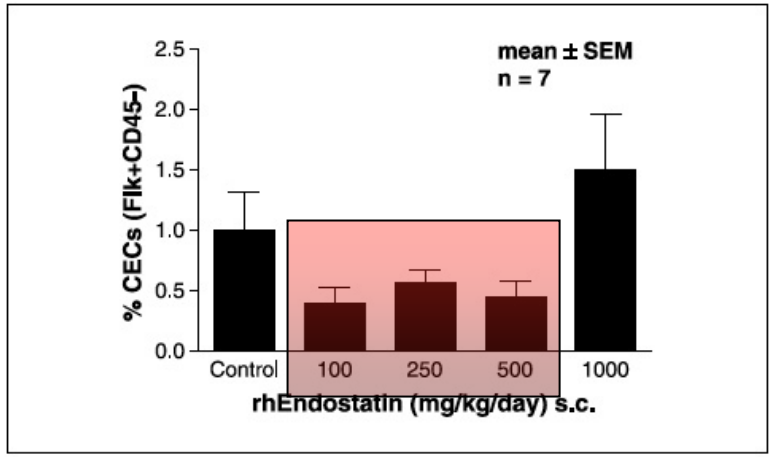

Figure 9. Inhibition of CEC by endostatin. CECs under endostatin therapy in tumor-bearing mice (BxPC-3). To investigate the correlation between changes in CECs and the antitumor efficacy of endostatin, blood was drawn from the retro-orbital plexus at the time of sacrifice (after 20 days) and CECs were measured as previously described (17). Flow cytometry was done using a FACSCalibur flow cytometer (Becton Dickinson Biosciences).

Celik et al 2005, Sjin et al 2006

Consequences for effective dosing of patients with anti-angiogenic drugs

- Dosing to the maximum tolerated dose (MTD) is inappropriate.
- Pharmacodynamic / biomarker-driven dosing.
- In the future, hormetic effects should be considered during the development of anti-angiogenic agents.

Recent papers suggest that angiogenesis inhibitors could sometimes promote tumour progression

nature medicine

Stimulation of tumor growth and angiogenesis by low concentrations of RGD-mimetic integrin inhibitors

Andrew R Reynolds[1,2], Ian R Hart[3], Alan R Watson[2], Jonathan C Welti[1], Rita G Silva[2], Stephen D Robinson[2], Georges Da Violante[4], Morgane Gourlaouen[1], Mishal Salih[2], Matt C Jones[5], Dylan T Jones[2], Garry Saunders[6], Vassiliki Kostourou[2], Françoise Perron-Sierra[7], Jim C Norman[5], Gordon C Tucker[8] & Kairbaan M Hodivala-Dilke[2]

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Cell PRESS

Cancer Cell Report

Accelerated Metastasis after Short-Term Treatment with a Potent Inhibitor of Tumor Angiogenesis

John M.L. Ebos,[1,2] Christina R. Lee,[1] William Cruz-Munoz,[1] Georg A. Bjarnason,[3] James G. Christensen,[4] and Robert S. Kerbel[1,2,*]
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*Correspondence: robert.kerbel@sunnybrook.ca
DOI 10.1016/j.ccr.2009.01.021

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Report

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Cancer Cell
Article

Antiangiogenic Therapy Elicits Malignant Progression of Tumors to Increased Local Invasion and Distant Metastasis

Marta Pàez-Ribes,[1,6] Elizabeth Allen,[2,6] James Hudock,[3] Takaaki Takeda,[4] Hiroaki Okuyama,[4] Francesc Viñals,[1,5] Masahiro Inoue,[4] Gabriele Bergers,[3] Douglas Hanahan,[2,*] and Oriol Casanovas[1,*]
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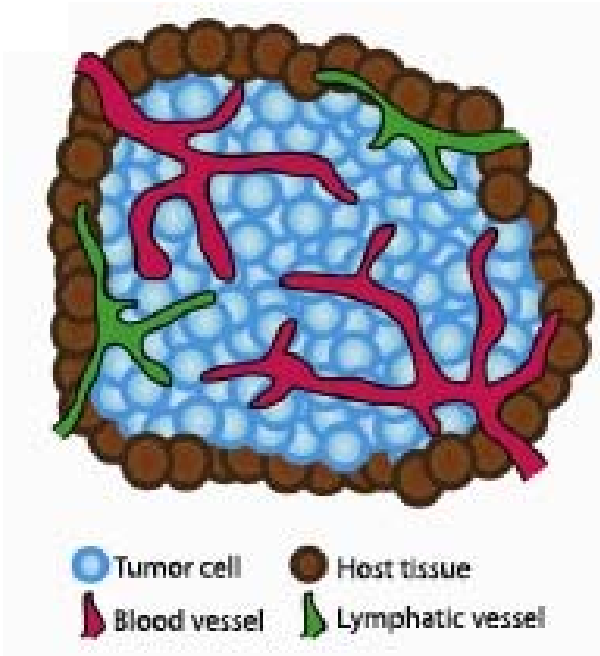
VEGF inhibitors can paradoxically promote tumour progression in mice

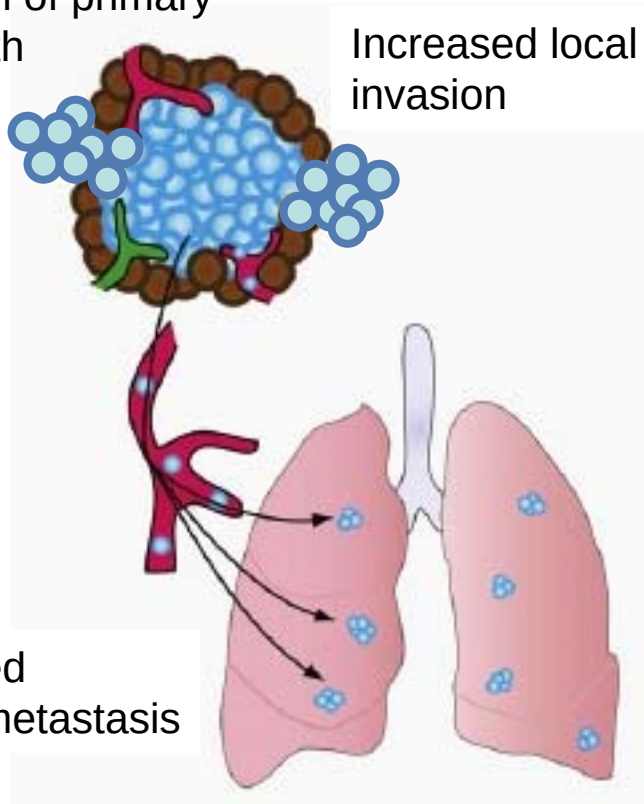


anti-VEGF therapy

Suppression of primary tumor growth

Increased local invasion

Increased distant metastasis



Ebos et al., 2008, Paez-Ribes et al., 2008
Figure adapted from Loges et al., 2008

True for other chemotherapeutics?

Int. J. Cancer: **15**, 588-595 (1975)

ENHANCEMENT BY DRUGS OF METASTATIC LUNG NODULE FORMATION AFTER INTRAVENOUS TUMOUR CELL INJECTION

by

L. M. VAN PUTTEN, L. K. J. KRAM, H. H. C. VAN DIERENDONCK, T. SMINK and M. FÜZY [1]
Radiobiological Institute TNO, Lange Kleiweg 151, Rijswijk (ZH), The Netherlands

In studies on a model of induced pulmonary metastasis in mice a tumour host system was analysed which was not affected by immunogenicity of the tumour for the host; neither intensive immunosuppression nor immunization caused a significant change in the quantity of pulmonary metastatic nodules. In contrast the application of cytostatic drugs and of Corynebacterium parvum *could modify the pulmonary resistance to the formation of tumour nodules by a factor greater than 100 in either direction. This finding confirms the observation of others that major modification of the resistance to metastatic tumour formation can occur independently of classical immunological mechanisms. Special attention is drawn to the fact that cyclophosphamide enhances the formation of metastatic nodules in this model by factors of 100 to more than 1,000, whereas other cytostatic drugs including the cyclophosphamide congeners iphosphamide and trophosphamide are active only by factors between 2 and 12. The possible practical significance of these findings is discussed.*

Van Putten et al., 1975

Key research questions and goals

What regulates tumor sensitivity and tumor resistance to angiogenesis inhibitors?

Can anti-cancer agents sometimes promote angiogenesis and tumor progression and how does this occur?

Goal: by understanding these unusual dose-response phenomena, develop better strategies for treating cancer

Cancer Research UK

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Imclone (USA)

Dan Hicklin

Beatson Institute (Scotland)

Jim Norman
Matt Jones

Breakthrough Breast Cancer

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