Abstract # 3288

The Anti-tumour Efficacy of the Novel Peptide Inhibitor of Angiogenesis ALM201

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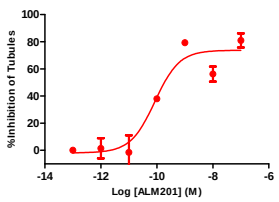
Overview

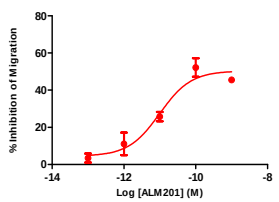
- Inhibition of angiogenesis is a valuable therapy in the treatment of cancer.
- Here we describe the characterisation of a novel peptide, ALM201; derived from the natural protein FKBP-like binding protein (FKBPL), which has potent anti-angiogenic activity ***(Clinical Cancer Research Highlights March 2011; Vol.7 Pp 947)***.
- ALM201 sequence: IRQQPRDPPTETLELEVSPDPAS
- ALM201 has been profiled in a range of *in vitro* human microvascular endothelial cell (HMEC-1) and *ex-vivo* models of angiogenesis.
- The peptide was efficacious and well tolerated with no signs of toxicity observed in mouse xenograft models up to 80 days of dosing.
- In cells that express CD44, ALM201 disrupts microtubule organisation and prevents migration.
- A significant difference between the PK and PD *in vivo* is a major advantage, allowing dosing every three days.
- ALM201 is in pre-clinical development with clinical development planned for Q4 2011.

In vitro Efficacy of ALM201

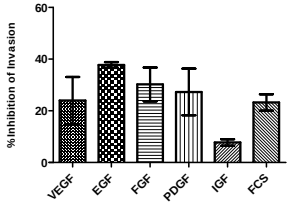
Tubule formation on Matrigel



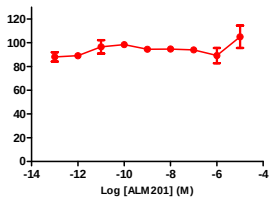
Wound scrape model of migration



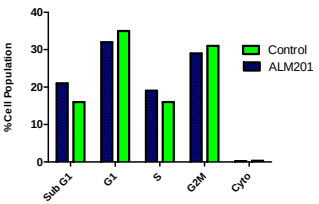
Invasion in response to growth factors



Effect on HMEC-1 cell viability at 72hrs



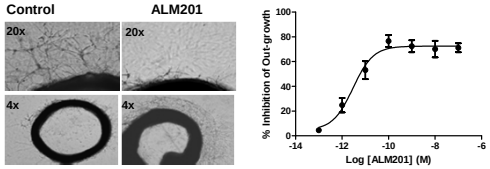
Effect on cell cycle at 72hrs



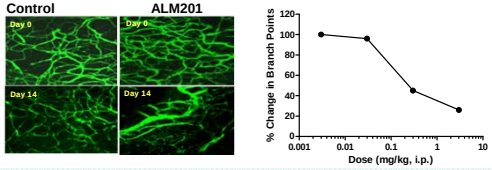
- ALM201 dose and time dependently inhibits HMEC-1 tubule formation and significantly reduces cell migration with picomolar potency
- ALM201 (1nM) significantly inhibits invasion *in vitro* stimulated by a range of growth factors in a modified Boyden chamber assay

- ALM201 has no effect on cell viability as measured by Cell Titre Glo (Promega) or cell cycle when compared to vehicle over a 72hr period

Ex-vivo Models of Angiogenesis



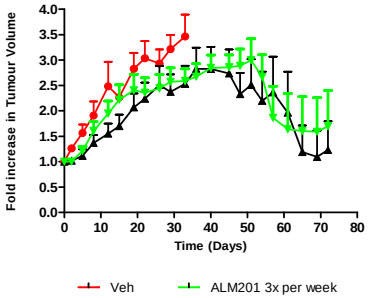
- ALM201 induces dose dependent inhibition of tubule out-growth in the *ex-vivo* Matrigel embedded aortic ring assay



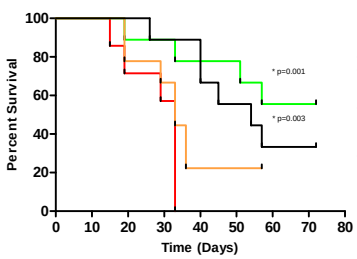
- ALM201 significantly inhibits branch formation in the window chamber model of angiogenesis, in a dose dependent fashion when administered i.p.

In vivo Efficacy Studies with ALM201

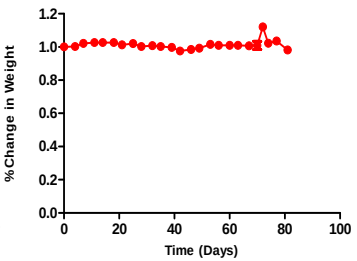
Du145 xenograft growth delay



Kaplan-Meier plot showing time to tumour quadrupling



Animal weight loss in the daily 0.3mgkg^{-1} group

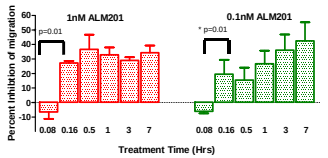


PK profile

Group	T½ (Mins)	Cl (l/h/kg)	Cmax (ng/ml)	AUC (0-INF) (ng.h/ml)	Tmax (Mins)
ALM201 3.0mg/kg i.v.	9	2.2	5927	1342	
ALM201 0.3mg/kg s.c.	9	1.4	267	208	15

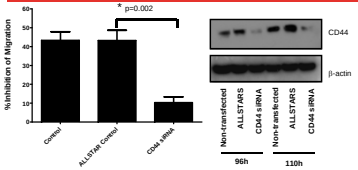
- ALM201 has a short half life and rapid clearance in contrast to the excellent prolonged efficacy *in vivo*

Long-lasting effects can be mimicked in the wound scrape assay *in vitro*

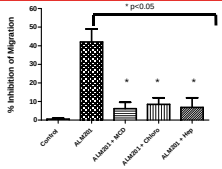


- Administration of ALM201 (0.3mgkg^{-1} s.c.) daily or three times per week significantly inhibited tumour size and time to quadrupling in the subcutaneous Du145 xenograft model
- No significant weight loss observed when ALM201 was administered for eighty days s.c.
- Short exposure to ALM201 followed by wash-out had a similar effect on cells as continuous 7hr exposure

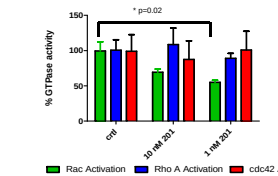
ALM201 Dependency on CD44 Expression and Internalisation Into the Cell



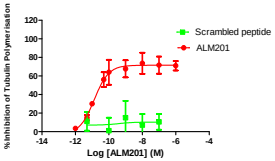
- Knock down of CD44 with siRNA significantly attenuated ALM201 activity in migration models



- Inhibition of known internalisation mechanisms attenuated ALM201 efficacy in migration models

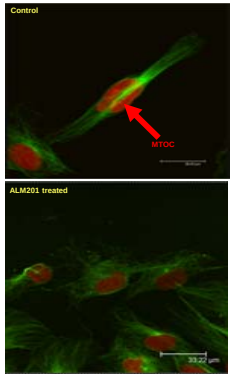


- ALM201 significantly inhibited Rac activation in migrating cells with no effect on other GTPase's

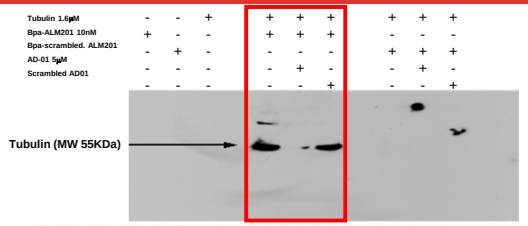


- ALM201 dose dependently inhibited tubulin polymerisation *in vitro*

ALM201 Inhibits Cytoskeletal Elements and Tubulin Assembly



- ALM201 disrupts microtubule organisation in migrating HMEC-1 cells



- ALM201 specifically binds to sites on tubulin, the interaction was competed off with excess active peptide AD-01 but not inactive scrambled peptide

Assay	IC50 (nM)				
	ALM201	Bevacizumzab	Sunitinib	Combretastatin	Vinblastine
Wound Scrape	0.009	59	8.9	Not determined	Not determined
Proliferation	No effect	158	1300	0.2	0.02
Tube Formation	0.082	2400	4.4	Not determined	Not determined
Microtubule Polymerisation	0.11	No effect	No effect	360	502
Cell Cycle	No effect	Not determined	Not determined	60% cells sub G1	60% cells sub G1

- ALM201 has a novel profile when compared to existing anti-angiogenics and vascular disrupting agents, targeting microtubules with no toxicity or effect on cell cycle

Conclusions

- ALM201 is a novel peptide which significantly inhibits angiogenesis with potency in the picomolar range *in vitro*.
- No effect on cell viability or cell cycle was observed with ALM201 *in vitro*.
- ALM201 has a short half life and high clearance but prolonged activity *in vivo*, which can be modelled *in vivo*
- The peptide significantly delays tumour growth in a Du145 xenograft model, with efficacy observed when administered daily or three times per week.
- No significant weight loss was observed in animals treated daily for 80 days.
- The mechanism of action depends on internalisation via CD44.
- ALM201 disrupts the cytoskeleton of HMEC-1 cells preventing migration by directly interacting with tubulin assembly.
- ALM201 exhibits a novel profile when compared to existing anti-vascular agents.
- ALM201 is in pre-clinical development with clinical development planned for Q4 2011.

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