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A phase I study of IMGN388, an antibody drug conjugate targeting α_v integrin, in patients with solid tumors

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Background: IMGN388 is a novel antibody–drug conjugate (ADC) composed of an α_v integrin-targeting fully human antibody and the maytansinoid, DM4, attached via a covalent bond. Its target is expressed in a wide variety of solid tumors and also on endothelial cells in the process of forming new blood vessels. In preclinical testing, IMGN388 has both anti-angiogenic and direct cytotoxic effects with strong activity against human xenograft lung, colon, pancreatic, ovarian and breast tumors in nude rat models.
Methods: In this first-in-human, phase I dose-escalating study, IMGN388 is administered to patients with advanced solid tumors using a standard 3 + 3 design. The primary study objectives are to establish the maximum tolerated dose and evaluate the safety and pharmacokinetics (PK) of IMGN388 when given intravenously every 3 weeks. Secondary objectives include evaluation of pharmacodynamics, immunogenicity and preliminary activity.
Results: A total of 35 patients (14M, 21F, median age = 63) have received the study drug at doses ranging from 5–160 mg/m^2. Most adverse events (AEs) have been Grade 1 or 2; the most common related events include nausea (26%), vomiting (23%), headache (13%), anorexia (13%), diarrhea (6%), fatigue (6%) and peripheral neuropathy (6%). There have been no related grade 4 AEs. One patient had grade 3 nausea/vomiting. One dose-limiting toxicity has been observed; grade 3 headache with confusion 24 hours after the first infusion of IMGN 388 at a dose of 45 mg/m^2. Subsequent patients have received steroid prophylaxis and no further grade 3/4 headache has been noted. There has been no evidence of human anti-human or anti-maytansinoid antibody formation (data available for doses up to 105 mg/m^2). Preliminary PK reveals an elimination phase $t_{1/2}$ of approximately 28 hrs; maximal plasma concentration increases in a generally dose-proportional manner. Five patients (breast, prostate, neuroendocrine, and 2 NSCLC) treated at doses $\geqslant$ 45 mg/m^2 have acheived stable disease for $\geqslant$4 cycles; two of these patients remain on therapy in cycles 6 and 8, respectively.
Conclusions: IMGN388 has been well tolerated at the doses tested. Dose escalation is ongoing. Updated results including α_v integrin expression data by immunohistochemistry will be presented.

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Mesenchymal stromal cells enhance the malignant potential of human colorectal cancer cells by inducing epithelial–mesenchymal transition (EMT)-related phenomena

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Background: Mesenchymal stromal cells (MSCs) are recruited to primary and metastatic sites of several tumour types, including colorectal cancer (CRC), and might contribute to tumour progression. The actual role played by MSCs and the mechanisms underlying MSC-tumour interactions remain to be clarified. We investigated the effects of human bone-marrow-derived MSCs (BM-MSCs) on CRC, *in vitro* and *in vivo*.
Material and Methods: Human established CRC cell lines were cultured in the presence or absence of BM-MSCs, in direct contact or in transwell plates. After a five day culture, tumour cell proliferation was assessed by differential cell counts, surface molecule expression was analyzed by flow cytometry, and production of soluble factors in culture supernatants was measured by Raybio antibody array® and ELISA. Tumour cells, sorted upon co-culture by flow cytometry, were evaluated for the expression of EMT-related genes by quantitative PCR and for *in vitro* invasiveness, by chemoinvasion assay. Furthermore, their tumorigenicity was assessed upon injection in NOD/SCID mice and developing tumours were analyzed by immunofluorescence.
Results: MSCs significantly increased tumour cell proliferation and decreased CD44 expression, independently of cell-to-cell contact. Analysis of co-culture supernatants revealed higher amounts of IL-6, MCP-1, RANTES and Angiogenin, in comparison to supernatants derived from single cultures. Moreover increased expression of several EMT-related genes, including SNAI2, TWIST, N-Cadherin, was detected on CRC cells sorted upon co-culture as compared with controls. Importantly, CRC cells co-cultured with MSCs showed higher invasive behaviour *in vitro*, than CRC cells cultured alone. No significant changes were observed in tumorigenicity. However, tumours originated from tumour cells co-cultured with MSCs showed a significantly higher vessel density as compared to controls.
Conclusions: MSCs reduce adhesiveness, induce expression of EMT-related genes and increase proliferation, invasiveness and angiogenic potential of CRC cells. These effects might contribute to CRC progression and spreading.

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The role of epithelial to mesenchymal transition (EMT) in the establishment of colorectal liver metastases. A potential source of prognostic biomarkers

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Background: Understanding of the biological processes involved in the development of metastatic cancer continues to develop, and the importance epithelial to mesenchymal transition (EMT) is well established. Several markers of EMT are recognised including various cytokines, transcription factors and markers of cell adhesion. Colorectal cancer will commonly metastasize to the liver but why some primary and secondary cancers behave in a more aggressive way is not fully understood.
Materials and Methods: A series of 20 colorectal liver metastases (LM) were obtained from surgical resection specimens, as well as 13 unmatched colorectal primary cancers (CPCs). Normal tissue was also retrieved from colon and liver. The expression of 13 key EMT genes were quantified by RT-PCR.
Results: LM samples were initially grouped according to the size and by number of synchronous metastases, being known predictive factors of recurrence and survival in LM. However, EMT profiles were similar across these groups. We then grouped LM by the Dukes stage of the primary cancer from which it arose, with Dukes A and B (LMAB) together (localised) and C and D (LMCD) together (metastatic biology). We saw that LMAB had up-regulation of EMT markers compared to LMCD with a significant increase in vimentin, s100a4 and TGFβ1 ($p < 0.05$).
When LMAB were compared to normal colon a strong EMT profile was seen, with significant down-regulation of E-cadherin and up-regulation of MACC1, HGF2, c-Met, Snail, vimentin, s100a4 and TGFβ1.
LMCD demonstrated a less robust profile with significantly reduced E-cadherin and raised cMET and MACC1, but reduced Slug and MMP-2.
In CPCs we found that EMT profiles were similar in Dukes AB (CPC AB) and Dukes CD (CPC CD) cancers, with both up-regulating MACC1 and c-Met. E-cadherin reached significant down-regulation in CPC AB and MMP2 was down-regulated in CPC CD.
When LMAB was compared to CPC AB we saw up-regulation of EMT markers in LMAB reaching significance for TGFβ1 and approaching significance in vimentin ($p = 0.056$) and s100a4 ($p = 0.073$). LMCD and CPC CD had very similar profiles.
Conclusions: LM are likely to arise from an aggressive sub-population of low stage CPC and this aggressive phenotype can be detected by EMT profiling. As expected, low stage CPCs selected from the general population do not express such an aggressive phenotype.
EMT markers may have a role in detecting aggressive primary cancers in low stage disease that may benefit from adjuvant treatment.

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Protease nexin 1 cleavage by MMP-9 modulates prostate cancer cell proliferation and tumorigenesis via regulation of the hedgehog pathway

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Background: The Hedgehog (HH) pathway is implicated in the growth and metastasis of prostate cancer cells. We report that increased expression of protease nexin 1 (PN1), an extracellular matrix (ECM) protein and serine protease inhibitor, reduces HH mediated signalling by reduction of the HH ligand, Sonic. We also show that PN1 levels are regulated by MMP-9 mediated cleavage.
Methods: Alterations in PN1 and MMP-9 in metastatic prostate cell lines PC3 were used to determine their effect on Hedgehog signalling. We used intraprostate injection followed by magnetic resonance imaging and

histology to determine the effect of MMP-9 and SHH signalling in the growth of prostate tumour cells in mice.
Results: PN-1 inhibits signalling in the Hedgehog (HH) pathway in PC3 cells and acts to decrease cell proliferation and viability. These read-outs correspond to decreases in a trio of important HH proteins (GLI1, PTCH1, and Cyclin D1) as well as one ligand (Sonic) following PN-1 over-expression. The results were verified by the use of mutations devised to interrupt functionality of PN1. MMP-9 by affecting PN-1 is thus able to alter cellular hedgehog signalling. MMP-9 deficient mice have increased levels of PN1 in the prostate. Furthermore, ablation of MMP-9 correlated with decreased formation and growth of prostate tumours in an orthotopic *in vivo* prostate tumour model. Future research will identify the compartments responsible for regulating PN1 and MMP-9 during prostate tumourigenesis and progression.
Conclusion: PN1 levels may influence prostate tumour cell proliferation, viability, and cell invasion *in vitro* and *in vivo* by regulating sonic hedgehog and its downstream targets. MMP-9 is now shown to regulate hedgehog signalling by controlling the levels of PN1. Regulation of this pathway could constitute a significant therapeutic advance in treating cancers that exhibit high expression of HH markers.

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The selective alpha5beta1 integrin antagonist, CLT-28643, inhibits tumors angiogenesis and growth

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Background: Extracellular matrix is crucial for endothelial cells survival, proliferation and motility during new vessels formation. Integrins are cell adhesion receptors involved in pathological angiogenesis. Particularly, abnormal expression of a5b1-integrin and its ligand, fibronectin, is a hallmark of cancer and angiogenic endothelial cells. Clanotech's proprietary compound, CLT-28643, is a selective $\alpha 5\beta 1$ antagonist as it was confirmed in several in vitro assays competing for the RGD fibronectin binding site. We have evaluated the in vivo effect of CLT-28643 by oral once daily administration in xenografted human tumor mouse models (lung, glioma, VEGF-resistant, and renal) and obtained significant inhibition of both tumor growth and tumor angiogenesis. CLT-28643 was well tolerated with no signs of toxicity.
Material and Methods: The test compound was evaluated in vitro for the capacity of inhibiting cell migration (chemotaxis), cell adhesion and binding of recombinant a5b1-integrin to fibronectin. In vivo efficacy studies were performed in xenograft and synograft mice models. Tumor cells were implanted subcutaneously in the hind leg of SCID mice. Oral administration of the test compound started at tumor volume of 250 mm^3. Changes in tumor volume were monitored every two days and calculated according to $0.52 \times \text{length} \times \text{width}^2$. Tumor angiogenesis was determined by 3 D technique.
Results: CLT-28643 inhibited cell migration by 53 % at 10 uM, adhesion to fibronectin by 50 % at 100 uM and binding to fibronectin by 90 % at 10 uM.

Xenograft models	Oral dose (mg/kg/day)	Inhibition of tumor growth (%)	Inhibition of angiogenesis (%)
Lung tumor	25–50	52–72	30–50
Glioma	50	30	14
VEGF-resistant tumor‡	50	43	24
Renal Cell Carcinoma	50	18–28	11

‡Synograft.

Conclusion: We demonstrated that the a5b1-integrin antagonist, CLT-28643 inhibits tumor angiogenesis, thereby resulting in regression of several human tumors in animal models when given orally. CLT-28643 has also shown to reduce both tumor growth and metastasis in the metastatic VEGF resistant tumor. These results suggest CLT-28643 as a good candidate for cancer therapy.

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The high invasive activity of S-adenosylmethionine decarboxylase-transformed fibrosarcoma cells is regulated by hepatocyte growth factor–Met signaling

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Background: Tumor cell invasion and metastasis are the main cause of death of cancer patients. Understanding of the molecular mechanisms of these complex processes would therefore be of utmost importance. The signaling driven by the interaction of hepatocyte growth factor (HGF) with its tyrosine kinase receptor, Met, is thought to play an important role in the regulation of proliferation, survival and migration of various tumor cells, particularly the epithelial cancer cells.
Material and Methods: Here, we performed comparative DNA microarray (Affymetrix MOE430 Set), RT-PCR, Western blot, and ELISA analyses of normal and S-adenosylmethionine decarboxylase (AdoMetDC)-transformed fibroblasts, which are highly invasive in nude mice [1,2], to identify the invasion-related genes and proteins in fibrosarcoma cells. The candidate genes/proteins identified were further functionally tested by three-dimensional Matrigel assays.
Results: We found the AdoMetDC-transformants to show highly increased expression of HGF both at the mRNA and protein level relative to the normal fibroblasts. Most importantly, knock-down of HGF by short hairpin RNA expression or addition of neutralizing antibodies to the Met receptor was found to effectively inhibit the invasion of AdoMetDC-transformants in Matrigel. Further, we found the downregulation of HGF to block the attachment and spreading of the cells on laminin.
Conclusions: The results suggest an important role for the HGF/Met signaling axis in the regulation of the invasiveness of AdoMetDC-transformed fibrosarcoma cells. HGF and its receptor Met may thus offer good therapeutic targets for the prevention of fibrosarcoma cell invasion and metastasis.

References

[1] Paasinen-Sohns et al. J Cell Biol.151:801–10, 2000.
[2] Nummela et al. Cancer Res 66:701–12, 2006.

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Bcl-2 regulates HIF-1a protein stabilization in hypoxic melanoma cells via the molecular chaperone HSP90β

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Background: Hypoxia-Inducible Factor 1 (HIF-1) is a transcription factor that is a critical mediator of the cellular response to hypoxia. Enhanced levels of HIF-1α, the oxygen-regulated subunit of HIF-1, are often associated with increased tumour metastasis, therapeutic resistance and poor prognosis. In this context we previously demonstrated that the anti-apoptotic protein bcl-2 cooperates with hypoxia to promote HIF-1/Vascular Endothelial Growth Factor (VEGF)-mediated tumour angiogenesis.
Material and Methods: Expression vectors encoding human bcl-2, *wild type* or hydroxylation resistant HIF-1α were used for stable and transient transfections of M14 human melanoma line. The effect of bcl-2 stable transfection will be evaluated in cells under hypoxic conditions in terms of bcl-2 and HIF-1α protein expression and localization (*Pulse-chase, western blot and confocal microscopy analyses*) HIF-1α protein stability and ubiquitination (*Western blot and immunoprecipitation analyses*) and HIF-1 transcriptional activity (*reporter assay*). The role of Heat Shock Proteins (HSPs) in the bcl-2-mediated regulation of HIF-1α expression and transcriptional activity (*Western blot analysis and reporter assay*) was evaluated by using chemical or genetical inhibition. Immunoprecipitation experiments were also performed to investigate the possible effect of bcl-2 protein on the interaction of HIF-1α and HSPs.
Results: By using M14 human melanoma cell line and its derivative bcl-2 overexpressing clones, we demonstrated that bcl-2-induced accumulation of HIF-1α protein during hypoxia was not due to an increased gene transcription or protein synthesis. In fact, it was related to a modulation of HIF-1α protein expression at a post-translational level, indeed its degradation rate was faster in the control lines than in bcl-2 transfectants. The bcl-2-induced HIF-1α stabilization in response to low oxygen tension conditions was achieved through the impairment of ubiquitin-dependent HIF-1α degradation involving the molecular chaperone HSP90 but it was not dependent on the prolyl hydroxylation of HIF-1α protein. We also showed that bcl-2, HIF-1α and HSP90 proteins form a tri-complex that may contribute to enhancing the stability of the HIF-1α protein in bcl-2 overexpressing clones under hypoxic conditions. Finally, by using genetic and pharmacological approaches we proved that HSP90 is involved in bcl-2-dependent stabilization of HIF-1α during hypoxia, and in particular the isoform HSP90β is the main player in this phenomenon.
Conclusions: We identified the stabilization of HIF-1α protein as a mechanism through which bcl-2 induces the activation of HIF-1 in hypoxic tumour cells involving the β isoform of molecular chaperone HSP90.