# PATENT ASSIGNMENT COVER SHEET

Electronic Version v1.1
Stylesheet Version v1.2

EPAS ID: PAT3520587

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| **SUBMISSION TYPE:** | NEW ASSIGNMENT |
| **NATURE OF CONVEYANCE:** | ASSIGNMENT |

**CONVEYING PARTY DATA**

| Name | Execution Date |
|---|---|
| KING'S COLLEGE LONDON | 01/28/2015 |

**RECEIVING PARTY DATA**

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|---|---|
| **Name:** | CANCER RESEARCH TECHNOLOGY LIMITED |
| **Street Address:** | 407 ST. JOHN STREET |
| **Internal Address:** | ANGEL BUILDING |
| **City:** | LONDON |
| **State/Country:** | UNITED KINGDOM |
| **Postal Code:** | EC1V 4AD |

**PROPERTY NUMBERS Total: 1**

| Property Type | Number |
|---|---|
| **Application Number:** | 14798825 |

**CORRESPONDENCE DATA**

**Fax Number:** (312)474-0448

***Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.***

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| **ATTORNEY DOCKET NUMBER:** | 30699/45341A |
| **NAME OF SUBMITTER:** | LYNN L. JANULIS |
| **SIGNATURE:** | /Lynn L. Janulis, Reg. No. 53,066/ |
| **DATE SIGNED:** | 09/10/2015 |

**Total Attachments: 15**
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**THIS ASSIGNMENT is made the 23rd day of November 2014** (the "**Assignment Date**")

**BETWEEN**

(1) **KING'S COLLEGE LONDON,** a corporation created by Royal Charter and registered under number RC000297, of Strand, London WC2R 2LS (the "**University**"); and

(2) **CANCER RESEARCH TECHNOLOGY LIMITED** a company registered in England under number 1626049 whose registered office is situated at Angel Building, 407 St. John Street, London, EC1V 4AD ("**CRT**").

**WHEREAS:**

(A) The Inventors (as defined below), conducted a programme of research at the University resulting in the identification of cyclic peptide inhibitors of ADAM8 (the "**Invention**"). The Invention is the subject of the Assigned Patent Rights (as defined below) filed by the University.

(B) Further validation studies of the Invention have been undertaken by Professor Joerg Bartsch with the assistance of CR-UK and third party funding.

(C) Under an agreement dated 19 September 2010 CRT granted the University the right to utilise certain data generated through one such CR-UK funded validation study referred to in Recital B for the purpose of marketing the Invention and Assigned Patent Rights (the "**Side Letter**").

(D) Subsequently, under an agreement between CRT, the University and King's College London Business Limited (now part of the University) dated 23 November 2012 (the "**Commercialisation Agreement**"), CRT acquired the exclusive right to commercialise the Invention and Assigned Patent Rights.

(B) The University and CRT have now agreed that the Assigned Rights (as defined below) would be assigned to CRT on the terms of an agreement dated 10 May 2010 made between the University, King's College London Business Limited and CRT (the "**Technology Transfer Agreement**").

(C) The University owns the Assigned Rights and assigns such rights to CRT subject to the terms of this Assignment.

**NOW IT IS HEREBY AGREED** as follows:

## 1. DEFINITIONS AND INTERPRETATION

1.1 In this Assignment and in the schedule to this Assignment the following words and phrases shall have the following meanings unless the context requires otherwise:

| | |
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| **"Assigned Know-How"** | means Know How generated by the Inventors and related to the Invention particulars of which are set out in the schedule. |
| **"Assigned Materials"** | means the Materials, particulars of which are set out in the schedule. |
| **"Assigned Patent Rights"** | means the Patent Rights, particulars of which are set out in the schedule, together with any Patent Rights derived or claiming priority therefrom. |
| **"Assigned Rights"** | means Assigned Patent Rights, Assigned Material and Assigned Know How. |
| **"Assignment"** | means this assignment and its schedule, as may be varied from time to time in accordance with the provisions of this assignment. |
| **"CR-UK"** | means Cancer Research UK, a charity and company registered in England and Wales with registered charity number 1089464 and registered company number 4325234. |
| **"Direct Costs"** | means the following costs incurred by the relevant Commercialising Party in prosecuting, maintaining, enforcing, defending and commercialising Transferred Intellectual Property (in the case of CRT) or Funded Intellectual Property (in the case of King's Business and/or the University):<br>(1) official filing fees;<br>(2) any amounts payable to the University in order to fully reimburse them for the King's Historic Patent Costs;<br>(3) all reasonable external patent agents' costs, legal fees and expenses and other third party advisory and consultancy fees and expenses incurred in prosecuting, maintaining, enforcing, defending and commercialising Transferred |

Intellectual Property or Funded Intellectual Property (as the case may be);

(4) reasonable in-house legal costs of qualified legal staff, calculated on a time basis at an index-linked daily rate which at the Effective Date is one hundred and fifty pounds (£150);

(5) reasonable travel expenses (using the cheapest tickets reasonably available for the purpose) and reasonable out-of-pocket expenses;

(6) courier charges and third party printing costs;

(7) any non-recoverable taxes or charges including Value Added Tax which may be imposed; and

(8) any other similar costs and expenses,

provided that where such costs are recovered from a Third Party they shall not be treated as Direct Costs for the purposes of this Agreement.

**"Gross Revenue"** means all sums received by CRT from its commercial exploitation of the Assigned Rights.

**"Inventors"** means Professor Joerg Bartsch and Dr Garrit Koller.

**"King's Historic Patent Costs"** means the sum of twenty four thousand eight hundred seventy two pounds and ninety five pence (£24,872.95);

**"Know How"** means technical and other information which is not in the public domain including, ideas, concepts, inventions, discoveries, data, formulae, algorithms, specifications, clinical data, information relating to Assigned Materials (including biological and chemical structures and functions as well as methods for synthesising chemical compounds), procedures for experiments and tests, results of experimentation and testing, results of research and development including laboratory records and data analyses. Information in a compilation or a compilation of information may be Know How



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notwithstanding that some or all of its individual elements are in the public domain.

**"Materials"** means reports, research notes, charts, graphs, comments, computations, analyses, recordings, photographs, paper, notebooks, books, files, ledgers, records, tapes, discs, diskettes, CD-ROMs, computer programs and documents thereof, computer information storage means, samples of material, other graphic or written data and any other media in or on which Know-How (as defined in the Technology Transfer Agreement) can be permanently stored and any chemical or biological substances including any:

(1) organic or inorganic element or compound;

(2) nucleotide or nucleotide sequence including DNA and RNA sequences;

(3) gene;

(4) vector or construct including plasmids, phages, bacterial vectors, bacteriophages and viruses;

(5) host organism including bacteria, fungi, algae, protozoa and hybridomas;

(6) eukaryotic or prokaryotic cell line or expression system or any development strain or product of that cell line or expression systems;

(7) protein including any peptide or amino acid sequence, enzyme, antibody or protein conferring targeting properties and any fragment of a protein or a peptide enzyme or antibody;

(8) drug or pro-drug;

(9) assay or reagent; or

(10) any other genetic or biological material or micro-organism;

(11) transgenic animals;

(12) multi-cellular plants;

(13) data for the derivation of molecular structures including NMR spectra, X Ray diffraction patterns, and other primary experimental information, assignments and other calculations, required for determination of the structure, and co-ordinates of the derived molecular structure; or

(14) computer programmes or algorithms.

**"Milestone"** means any defined stage up to and including the market launch of a product developed using the Invention and/or Assigned Rights, agreed with a Third Party under a commercialisation agreement, including, but not limited to signature fee, annual fee, and fees at candidate selection, IND filing and grant, EMA filing and grant and clinical development stages (Phase 1, 2, 3).

**"Net Revenue"** means CRT's Gross Revenue less its Direct Costs.

**"Parties"** means the University and CRT and "Party" shall be construed accordingly.

**"Patent Rights"** means any patent applications, patents, author certificates, inventor certificates, utility certificates and models and certificates of addition and all foreign counterparts of them including any divisional applications and patents, refilings, renewals, continuations, continuations-in-part, patents of addition, extensions, reissues, substitutions, confirmations, registrations, revalidations and additions of or to them, as well as any supplemental protection certificate, and equivalent protection rights in respect of any of them.

**"Medivir Field"** means commercialisation of the Assigned Rights for development of a small molecule or peptide inhibitor as a diagnostic or therapeutic agent which will be developed under a licence (which is currently in negotiations as at the Assignment Date) with Medivir AB.

**"Phase 1 Clinical Trial"** means a small scale human clinical trial with the aim of establishing preliminary pharmacokinetic and/or pharmacodynamic data on a pre-clinical target and/or early stage toxicology data and/or the maximum tolerated dose of a pre-clinical target.

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| **"Product"** | means any diagnostic or therapeutic agent whether of a biological or chemical nature, at any stage in its development, developed using the Assigned Rights. |
| **"Third Party"** | means any entity or person other than the Parties and their respective affiliates. |

1.2 The headings used in this Assignment shall not affect its interpretation.

## 2. ASSIGNMENT

2.1 In consideration of the payment of the sum of one pound sterling (UK £1) by CRT to the University, receipt of which is hereby acknowledged by the University, the University hereby assigns to CRT absolutely all of its right, title and interest throughout the world in and to the following:

2.1.1 the Assigned Rights;

2.1.2 all rights and powers arising or accrued in respect of the Assigned Patent Rights including the right to recover and take all such proceedings as may be necessary for the recovery of damages and/or other remedies in respect of all infringements whether committed before, on, or after the Assignment Date and to have the benefit of any remedy (other than a monetary remedy) obtained on any infringement or alleged infringement before the Assignment Date;

2.1.3 the right to apply for, prosecute and to obtain Patent Rights in any part of the world (including but not limited to Canada and the United States of America) in respect of the Assigned Patent Rights and to claim priority from the date of any application within the Assigned Patent Rights, with the intent that the grant of any patents or similar protection shall be in the name of and vest in CRT.

2.2 The University, having made due and careful enquiry, confirms that:

2.2.1 neither the University nor any of the employees of the University have done anything which may cause the whole or any part of the Assigned Patent Rights to be invalidated or registration of them refused;

2.2.2 no person other than the Inventors are entitled to be named as an inventor in relation to the Assigned Patent Rights;

2.2.3 notwithstanding that Professor Joerg Bartsch is no longer an employee of the University and may have taken physical possession of the Assigned Materials to his new employer, all right, title and interest in the Assigned Materials remain entirely in the possession or control of the University; no employee of the University or Professor Bartsch,

either during his term of employment or subsequently, has supplied or permitted the supply of any Materials within the Assigned Materials to any person other than as permitted by the terms of the Technology Transfer Agreement or otherwise as permitted by CRT; all of such transfers have been declared to CRT; and no person is entitled to call for the possession or control of any Materials within the Assigned Materials.

2.3 The University warrants that:

2.3.1 it is the sole beneficial owner of the Assigned Rights and has done or procured to be done all necessary acts and executed or procured to be executed all necessary documents to effect the transfer to it of full legal and beneficial ownership in the Assigned Rights, and to vest title to the Assigned Rights in the University;

2.3.2 there are no third parties with any right, title or interest in or to the Assigned Rights;

2.3.3 so far as permitted by law, the Inventors have waived all moral rights to which they are entitled throughout the world which exist in or in respect of the Materials;

2.3.5 the Inventors were employees of the University at the time or times of making the Invention and any other inventions which are the subject of the Assigned Patent Rights;

2.3.6 it has full right, power and authority to assign to CRT all of its right, title and interest in the Assigned Rights under the terms of this Assignment.

2.4 Each of CRT and the University acknowledge that the other and CR-UK shall be entitled to use the Assigned Patent Rights and Assigned Materials in accordance with Clause 2.13 of the Technology Transfer Agreement and subject to the provisions of Clause 7 of the Technology Transfer Agreement despite the fact the Assigned Patent Rights and Assigned Materials may not fall within the definitions of Solely Funded Intellectual Property or Jointly Funded Intellectual Property (as such terms are defined in the Technology Transfer Agreement), or as otherwise agreed between CRT and the University.

2.5 The University hereby requests the relevant authorities in all countries of the world to issue any patents granted for and/or in respect of the Assigned Patent Rights in the name of CRT or its successor or assignee in accordance with this Assignment.

2.6 The University agrees to procure that on request of CRT and at the University's cost, the Inventors acknowledge that, subject to the terms of this Assignment, all the Assigned Rights shall belong exclusively to CRT, that they have and will make no claim against CRT as to the ownership of, or for any compensation in respect of, the Assigned Rights and that any claim for

compensation in respect of the commercialization of the Assigned Rights shall be the sole responsibility of the University.

## 3. FURTHER ASSURANCE

3.1 At the request and cost of CRT and without unreasonable delay, the University shall and shall procure that, so far as they are reasonably able, Dr Garrit Koller

3.1.1 provides reasonable assistance to CRT in the prosecution of the Assigned Patent Rights and in defending and/or enforcing the Assigned Patent Rights, and (subject to CRT or its licensee or sub-licensee providing an appropriate indemnity to the University) participate in legal action for such defence or enforcement; and

3.1.2 sign, execute, make and do all such further deeds, documents, acts and things as CRT may reasonably require to give effect to this Assignment and to any necessary confirmatory assignments or other documents necessary to give effect to the assignment or registration of the assignment of the Assigned Rights or any of them and to allow CRT to enjoy the full benefit of the rights hereby assigned.

3.2 The University shall, at its own cost, make the Assigned Materials available to CRT and to any persons nominated by CRT and at CRT's cost provide and procure that the Inventors or other relevant employees of the University provide CRT and such nominees with such assistance in relation to the use and understanding of the Assigned Materials as CRT may reasonably request in order to facilitate the commercialization of the Assigned Rights in accordance with the terms of the Technology Transfer Agreement.

3.3 The University shall within thirty (30) days of the Assignment Date transfer, or procure the transfer, to CRT of all files and documents not already provided to CRT relating to the prosecution and maintenance of the Assigned Patent Rights in the power, possession or control of the University and at the cost of the University. For avoidance of doubt the University shall be entitled to retain a copy of any such files or documents for its records.

3.4 The University undertakes to use its reasonable endeavours to notify CRT promptly in writing should it become aware of any of the matters referred to in Clauses 2.2 and 2.3.

## 4. MATERIALS

4.1 The University undertakes to keep confidential and not disclose to any third party any confidential subject matter of the Assigned Patent Rights nor to use or provide to any third party the Assigned Materials for any purpose

whatsoever except in accordance with the terms of the Technology Transfer Agreement or in accordance with Clause 3.2.

4.2 CRT acknowledges that the Assigned Materials are experimental in nature, Save as provided in this Assignment and the Technology Transfer Agreement the University makes no representation and gives no warranty or undertaking in relation to the Assigned Materials. The University has notified CRT of any matters of which it is aware relating to the characteristics of the Assigned Materials or which may affect their commercialization but gives no warranty: (a) that use of the Assigned Materials will not infringe any intellectual property rights of any third party; or (b) that the Assigned Materials are of merchantable or satisfactory quality or fit for any particular purpose, have been developed with reasonable care and skill or tested, for the presence of pathogens or otherwise, or are viable, safe, or non-toxic.

4.3 The University accepts no responsibility for any use which CRT may make of any Assigned Materials or any product or process that may be developed, manufactured or used arising from them or their commercialization. Save as provided in this Assignment or the Technology Transfer Agreement, the University shall have no liability to CRT, whether in contract, tort, including but not limited to negligence, or otherwise, in relation to the supply of the Assigned Materials to CRT or their use or keeping by CRT or by any other person, or the consequences of their use, to the maximum extent permitted under applicable law.

## 5. PAYMENT

5.1 In place of the revenue sharing provisions expressed in Clause 4.1 of the Technology Transfer Agreement CRT and University have agreed that the University shall receive the following proportions of Net Revenues:

5.1.1 Where a Product falls within the Medivir Field

5.1.1.1 Forty percent (40%) where Gross Revenues are received by CRT from a Third Party in respect of (a) Milestones up to but excluding commencement of a Phase 1 Clinical Trial; and (b) Milestones including commencement of a Phase 1 Clinical Trial where a Product falls within the scope of the Assigned Patent Rights; and (c) royalty payments on a Product which falls within the scope of the Assigned Patent Rights; and

5.1.1.2 Twenty three percent (23%) where Gross Revenues are received by CRT from a Third Party in respect of (a) Milestones including commencement of a Phase 1 Clinical Trial where a Product falls outside the scope of the Assigned Patent Rights; and (b) royalty payments on a Product which falls outside the scope of the Assigned Patent Rights, but within the scope of the Assigned Know How.

5.1.2 Where a Product falls outside the Medivir Field CRT and the University shall agree an appropriate proportion of Net Revenues prior to the final execution of any commercialisation agreement.

5.2 In all other respects to the sharing of revenues, other than the proportions as amended by Clause 5.1 of this Agreement, the terms of the Technology Transfer Agreement shall apply.

## 6. GENERAL

6.1 Failure by either of the Parties to enforce at any time or for any period any one or more of the terms or conditions of this Assignment shall not be a waiver of them or the rights at any time subsequently to enforce all terms and conditions of this Assignment.

6.2 This Assignment shall be binding on and for the benefit of each Party's successors and personal representatives.

6.3 The obligations contained in this Assignment which have not been performed at completion of this Assignment shall survive completion.

6.4 To the extent that they have not already expired or been terminated, the Side Letter and Commercialisation Agreement referred to in Recitals C and D respectively, shall terminate with immediate effect from the date of this Assignment.

## 7. GOVERNING LAW AND JURISDICTION

7.1 All matters relating to this Assignment shall be governed by English law and the Parties submit to the exclusive jurisdiction of the courts of England and Wales.

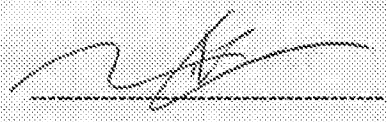
**SIGNED on behalf of CRT**

Print name of signatory: P J L'Huillier
Director, Business Management

Position: .....

Date: 24/2/15

**SIGNED on behalf of the University**

Print name of signatory: 

Position: Mike Shaw
Director, IP & Licensing
For and on behalf of King's College London

Date: 28-01-2015



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## SCHEDULE

1. Details of the Assigned Patent Rights:

| Country | Application Number | Title | Filing Date | Inventor(s) | Status |
|---|---|---|---|---|---|
| UK | GB 0719997 D0 | Protease Inhibition | Nov 21, 2007 | Joerg Bartsch, Garrit Koller | Application |
| UK | GB 2453589 A | Protease Inhibition | Apr 15, 2009 | Joerg Bartsch, Garrit Koller | Application |
| | WO 2009/047523 A1 | Protease Inhibition | Apr 16, 2009 | Joerg Bartsch, Garrit Koller | Application |
| EP | EP 2197898 A1 | Protease Inhibition | Jun 23, 2010 | Joerg Bartsch, Garrit Koller | Application |
| US | US 2010/0291063 A1 | Protease Inhibition | Nov 18, 2010 | Joerg Bartsch, Garrit Koller | Application |

2. Details of the Assigned Materials and Assigned Know How:

*Know How generated in the lead up to the following publications and associated with them (but not in the public domain)*:

Schlomann U, Wildeboer D, Webster A, Antropova O, Zeuschner D, Knight CG, Docherty AJ, Lambert M, Skelton L, Jockusch H, Bartsch JW. The metalloprotease disintegrin ADAM8. Processing by autocatalysis is required for proteolytic activity and cell adhesion. J Biol Chem. 2002 Dec 13;277(50):48210-9.

Naus S, Reipschläger S, Wildeboer D, Lichtenthaler SF, Mitterreiter S, Guan Z, Moss ML, Bartsch JW. Identification of candidate substrates for ectodomain shedding by the metalloprotease-disintegrin ADAM8. Biol Chem. 2006 Mar;387(3):337-46.

Kelly K, Hutchinson G, Nebenius-Oosthuizen D, Smith AJ, Bartsch JW, Horiuchi K, Rittger A, Manova K, Docherty AJ, Blobel CP. Metalloprotease-disintegrin ADAM8: expression analysis and targeted deletion in mice. Dev Dyn. 2005 Jan;232(1):221-31. PubMed PMID: 15580619.

Wildeboer D, Naus S, Amy Sang QX, Bartsch JW, Pagenstecher A. Metalloproteinase disintegrins ADAM8 and ADAM19 are highly regulated in human primary brain tumors and their expression levels and activities are associated with invasiveness. J Neuropathol Exp Neurol. 2006 May;65(5):516-27.

*: Bartsch JW, Wildeboer D, Koller G, Naus S, Rittger A, Moss ML, Minai Y, Jockusch H. Tumor necrosis factor-alpha (TNF-alpha) regulates shedding of TNF-alpha receptor 1 by the metalloprotease-disintegrin ADAM8: evidence for a protease-regulated feedback loop in neuroprotection. J Neurosci. 2010 Sep 8;30(36):12210-8.

**: Moss ML, Powell G, Miller MA, Edwards L, Qi B, Sang QX, De Strooper B, Tesseur I, Lichtenthaler SF, Taverna M, Zhong JL, Dingwall C, Ferdous T, Schlomann U, Zhou P, Griffith LG, Lauffenburger DA, Petrovich R, Bartsch JW. ADAM9 inhibition increases membrane activity of ADAM10 and controls α-secretase processing of amyloid precursor protein. J Biol Chem. 2011 Nov 25;286(47):40443-51.

Romagnoli M, Mineva ND, Polmear M, Conrad C, Srinivasan S, Loussouarn D, Barillé-Nion S, Georgakoudi I, Dagg Á, McDermott EW, Duffy MJ, McGowan PM, Schlomann U, Parsons M, Bartsch JW, Sonenshein GE. ADAM8 expression in invasive breast cancer promotes tumor dissemination and metastasis. EMBO Mol Med. 2014 Feb;6(2):278-94.

Schlomann U, Koller G, Conrad C, Ferdous T, Golfi P, Molejon Garcia A, Höfling S, Parsons M, Costa P, Soper R, Bossard M, Hagemann T, Roshani R, Sewald N, Ketchem RR, Moss ML, Rasmussen FH, Miller MA, Lauffenburger DA, Tuveson DA, Nimsky C, Bartsch JW ADAM8 as a novel drug target in Pancreatic Cancer. Nature Comm, in press.

*Know How relating to the following assays:*
Cell binding assay, CD23 shedding assay, TNFR1 shedding assay, CD23 peptide fluorescence assay, tumor sphere assays, angiogenesis assay, kinase assays, FRET microscopy, Immunoprecipitation, Immunofluorescence, Immunohistochemistry, ADAM8 ELISA, PrAMA assays (detection of MMP activities), production and purification of recombinant ADAM8.
Biomarker know how: TFNR1-shedding*, CD23 shedding, PrAMA assays**.
Tumour models: Heterotopic injection of tumor cells, orthotopic injection (pancreas, fat pad, stereotactic brain injection), Mouse breeding and generation of genetic mouse tumor models, Peptide administration, Toxicity assays, PK study.
(* and ** refer back to the publications listed above)

*Specifically the following assays:*
1. Cell death assays in the presence of BK-1361 using PDAC and other tumor cell lines
2. Knowhow about endogenous ADAM8 expression levels in different tumor cell lines
   - PDAC cell lines (n=12), either established cell lines or genetically modified as follows: Panc1, AsPC-1, Capan-1, Capan-3, BxPC-3, MiaPaCa-2, Colo-357, Su8686 T3M4; DT8066

     Cell lines generated to express human ADAM8: Panc1_ctrl, Panc1_A8
   - NSCLC cell lines (n=10), SK-LU-1, HCC2935, HCC4006, HCC827, NCI-H1581, A549, A-427, NCI-H596, SW 1573, NCI-H1688

- BC cell lines (n= 6), MB-231, T47D, MCF-7, BT474, SK-H, SKBR3

3. Know-how about other ADAM proteases expressed in tumor cell lines.
PDAC cell lines (n=12), see above
NSCLC cell lines (n=10), see above
BC cell lines (n=6), see above
4. Know How about experimental use of antibodies against mouse and human ADAM8.
Know How around generation of antibodies by immunisation of rabbits with peptides covering the cytoplasmic domain of mouse ADAM8, which cross-reacts with human ADAM8; anti-rabbit polyclonal antibody against mouse ADAM8, which can be used for neutralization.
Also Know How around anti-goat antibody against human ADAM8 ectodomain and the cytoplasmic domain.
5. Know-how about ADAM8 constructs (i.e. domains deletions, constructs for secreted ADAM8, functional analysis of ADAM8)
Relevant constructs are listed under Materials
6. Assay development (experimental conditions) and Experimental details on newly developed assays
Development and Optimisation of fluorescence activity assay specific for ADAM8 activity
Peptide test assay utilizing cell binding to ADAM8 coated surfaces
7. Know How around development of cell lines with ADAM8 modification
Panc1
AsPC-1
MB-231
U87MG
U251

*Relevant Materials are listed below:*

A) Constructs:

All mouse constructs for expression of ADAM8 protein variants in eukaryotic cells
WT-ADAM8 (full length mouse); EQ-ADAM8, ADAM8-□DC; ADAM8-MP, ADAM8-EC, ADAM8-deletion mutants of Integrin Binding Loop
Constructs for ADAM8 expression in fusion with fluorescent proteins (GFP, dsRed, mCherry)
Human constructs for expression of ADAM8 protein variants in eukaryotic cells:
WT-ADAM8 (full-length human); EQ-ADAM8
Human ADAM8 constructs for the production of recombinant ADAM8 in eukaryotic cells (KCL [Bicycle])
Several recombinant ADAM8 proteins were generated and purified in large quantities
ADAM8ecto-Fc: few micrograms (~50ug)
ADAM8ecto-His: 1 mg
ADAM8DCEdomain-Fc: 250 micrograms
ADAM8DCEdomain-His: 1 mg
ADAM8ecto-Fc(TEV cleavable): few micrograms
ADAM8DCE-Fc(TEV cleavable): ~ 10 micrograms

B) Cell lines with ADAM8 modifications in Panc1:

Panc1_ctrl cells (empty control vector)

Panc1_A8 cells (expressing human ADAM8 under control of an CMV promoter)

C) Antibodies

- Custom-made antibody: Rabbit-anti-A8 generated by immunization of rabbits with peptides covering the cytoplasmic domain of mouse ADAM8; cross-reacts with human ADAM8 (2 mg total IgG)
- Anti-rabbit polyclonal antibody against mouse ADAM8; can be used for neutralization (100 micrograms).
- Anti-goat antibody against human ADAM8 (ectodomain) (100 micrograms)
- Anti-goat antibody against ADAM8 cytoplasmic domain (100 micrograms)

*Know How covering the Mode of Action*
Functional analysis of ADAM8 in PDAC - understanding the underlying signalling cascade that ADAM8 activates in tumour cells (knockdown cells and BK-1361 peptide to analyse specific ADAM8 effects in these tumours) with focus is on the mechanism by which ADAM8 confers tumor progression.
Development of further assays to detect ADAM8 activities in body fluids. PrAMA assay to detect other MMP activities in tumor models. Screening for biomarkers.
Cell adhesion: Adhesion to endothelia and different ECM matrices
Migration: enhanced migration, transmigration through endothelia, transmigration through BBB, invasion into different ECM matrices including Collagen I, Fibronectin, and Collagen IV.
Binding/activation of β1 integrin: activation of integrin β1 pathway, enhances phosphorylation of ERK, Akt, and CREB (depending on the cell type).
Regulation of MMP activities: Increased ADAM8 leads to increased extracellular activities of MMPs (MMP-2, MMP-9 and MMP-14, depending on the cell type), causing a net increase in extracellular proteolytic activities.
Angiogenesis: ADAM8 leads to increased secretion of VEGF, Angiogenin, PIGS in breast cancer and GBM cells

*Indications tested & described by Joerg Bartsch*
Glioblastoma (GBM): ADAM8 in GBM, Wildeboer et al., 2006; Effect of ADAM8 on GBM growth, Schlomann et al., manuscript in preparation. ADAM8 causes chemoresistance of GBM cells, Dong et al., Neuro-Oncology, under revision.
Breast cancer (TNBC/DCIS): Sonenshein; see ms by Romagnoli et al., 2014
Pancreatic cancer (PDAC); Bartsch; see Schlomann et al., Nature Comm, in press.
Lung cancer (NSCLC): as published by Ishikawa et al., 2004; we have looked for stratification in samples from lung cancer patients (unpublished results)
Colon cancer: we have ongoing and promising collaborations with Prof. Dylan Edwards, Norwich, UK, and Prof. Irit Sagi, Weizmann Institute (unpublished results)
Asthma: asthma response is suppressed in ADAM8 deficient mice; BK-1361 was initially tested in OVA-challenged mice and found to be effective (Chen et al., submitted).

*Know How applied in manuscripts, which are not yet published:*
<u>Papers submitted (stage)</u>
PDAC paper: Nature Comm., in press
<u>Papers in preparation (> 80 % of data generated)</u>
Role of ADAM8 in colon cancer; Afik et al.,