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An:
Prof. Dr. med. Heiko Koller
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Datum: 28.06.2017

Sehr geehrter Herr Prof. Koller!

Anbei sende ich Ihnen den Abschlussbericht zum Forschungsprojekt:

„Zirkulierende Tumorzellen (CTC) nach Zementaugmentation (Vertebroplastie) spinaler Tumore“

Antragsteller:

Priv.-Doz. Dr. med. Sven O. Eicker

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Projektname:

Zirkulierende Tumorzellen (CTC) nach Zementaugmentation (Vertebroplastie) spinaler Tumore

Projekt-Code der Verwaltung des Förderbetrag-Empfängers:

SAP-Projektkonto 1489/101

Datum des Zuwendungsbescheides:

12.12.2014

Einleitung:

The spinal column is the most frequent site of bone metastasis in the body.

Primarily intended for the apparent and rapid pain relief, cement augmentation due to percutaneous Vertebroplasty (VP) or Kyphoplasty (KP) for treatment of spinal metastasis is a well-established treatment with a less invasive nature compared to open spinal surger. However, there is a well-known potential risk of leakage of the liquid cement out of the vertebral body into the surrounding vessels with subsequent embolization. Furthermore, reports about tumor extravasation after VP are known. These tumor extravasations probably spread by one of the above-mentioned routes.

Zielsetzung:

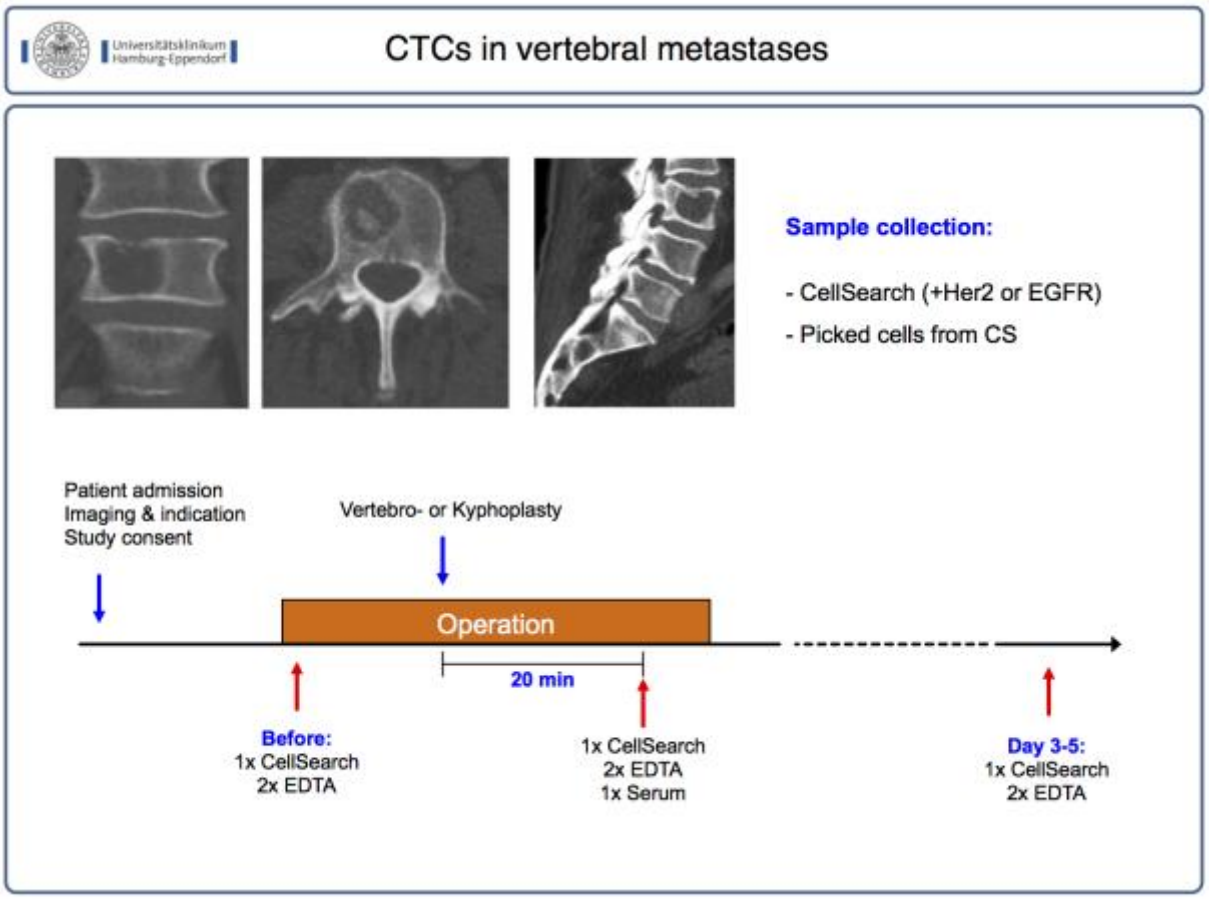
Based on these facts we assessed here whether elevated intrametastatic pressure during cement augmentation results in an increased dissemination of tumour cells into the vascular circulation.

Methodik:

Patients and Study design

This prospective, single-institution study was conducted at the University Medical Hamburg-Eppendorf (Hamburg, Germany). We enrolled 21 patients with metastatic involvement of the spinal column. Informed consent was obtained from all patients. The study was approved by the medical ethics committee (ID# PV4904) of the Chamber of Physicians of Hamburg. Peripheral blood samples for CTC analyses were obtained in all patients at three time points: preoperatively, 20 minutes post cement augmentation and 3-5 days postoperatively. In 5 patients a fourth samples in the follow up

could be analysed. For the vertebroplasty procedure, a 13-gauge needle was advanced to the central aspect of the lesion at the vertebral body. Cement (VertaPLex HV, Stryker, Duisburg, Germany) was prepared on the bench and infused under lateral and anterior-posterior (ap) fluoroscopy into the vertebral body. Infusion was stopped when the cement reached to the posterior aspect of the vertebral body or entered an extraosseous space, such as the intervertebral disk or an epidural or paravertebral vein. For kyphoplasty, ballon dilatation was performed before cement was applied.



Detection of circulating tumor cells

For CTC enumeration, 7.5 ml peripheral whole blood was collected CellSave tubes (Immunicon, Inc., Huntingdon Valley, PA). The semi-automated analysis was performed as described elsewhere. Blood samples were kept at room temperature for ≤72 hours before analysis using the CellSearch™ assay (CellSearch™ Epithelial Cell Kit/CellSpotter™ Analyzer, Veridex LLC, Raritan, NJ, USA). The assay uses a ferrofluid coated with antibodies to epithelial cell adhesion molecule (EpCAM) to immunomagnetically separate cells of epithelial origin from blood, and fluorescent staining to differentiate between debris, hematopoietic cells, and epithelial-derived circulating tumor cells. CTCs enumerated and characterized in this study were cells with a positive staining for keratins and nuclear DAPI staining but negative for the pan-leukocyte marker CD45. The accuracy and reproducibility of the CellSearch system have been described previous. As an additional tumor marker we used HER2.

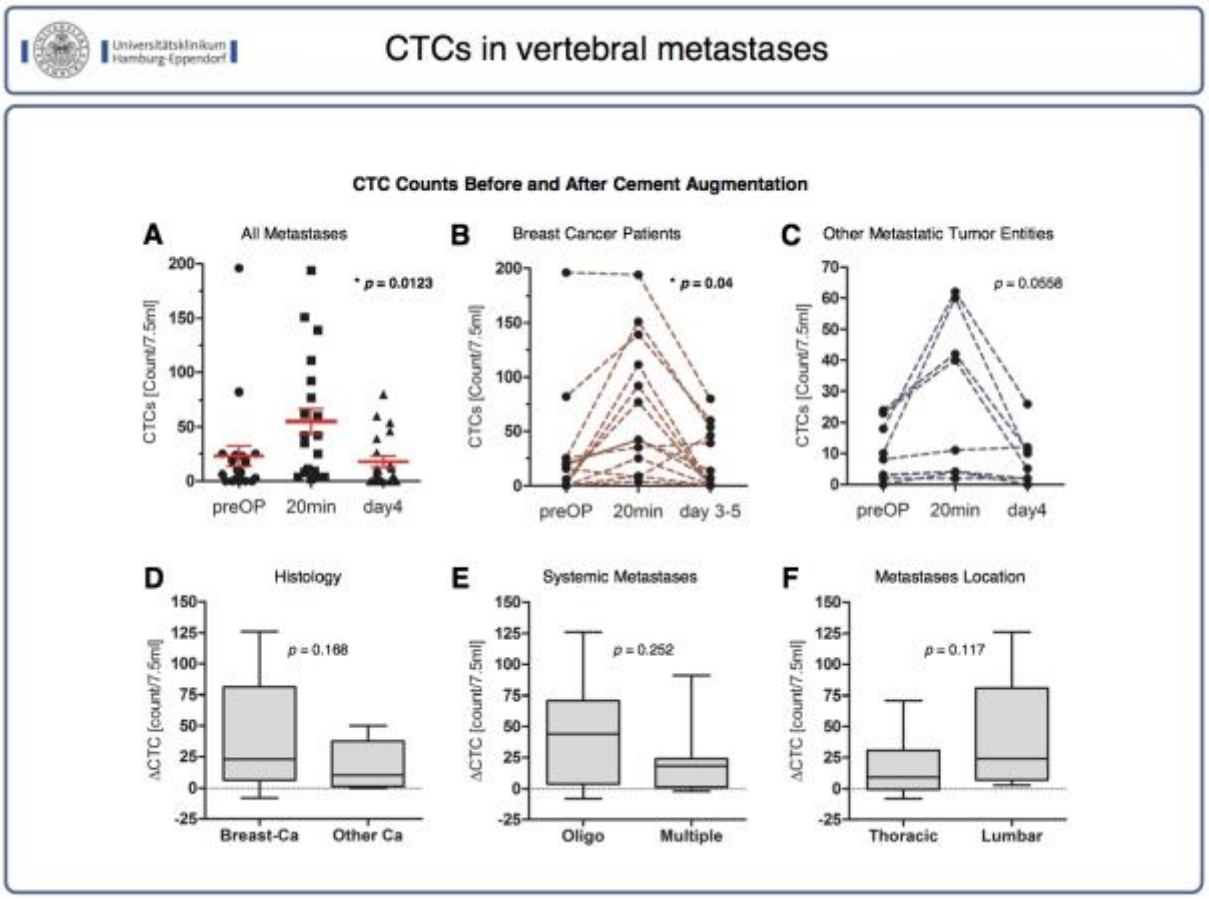
Whole genome amplification and array CGG analyses

Single keratin positive CTCs were picked in 0.5 ul PCR tubes using the micromanipulator as described previously (Babayan et al., 2016). CTCs were amplified using the Amply kit (Silicon Biosystem) Whole genome amplification was done at the University Düsseldorf.

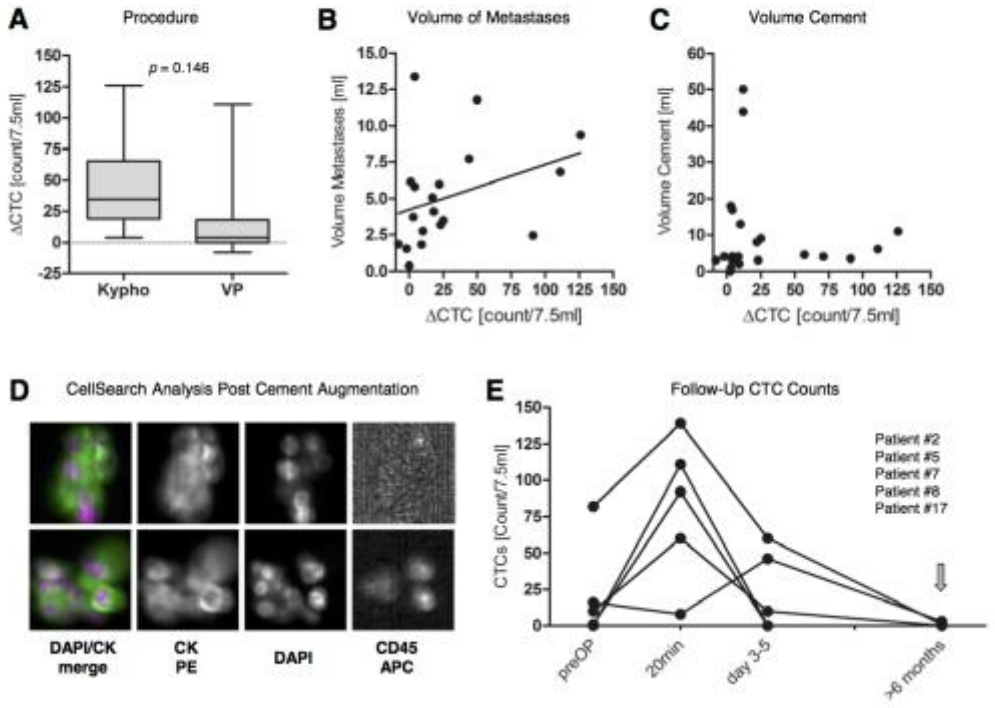
Data analysis and statistics

CTC counts were expressed as means with standard deviation. Comparison of CTC counts before and after VP and KP was made using one-way ANOVA test. Additional comparisons applied a student`s t-test. A p-value <0.05 was considered as statistically significant. All statistical analyses were performed using the GraphPad Prism 5.0 and SPSS version 18.0 software (SPSS, Chicago, IL, USA).

Ergebnisse:



CTCs in vertebral metastases



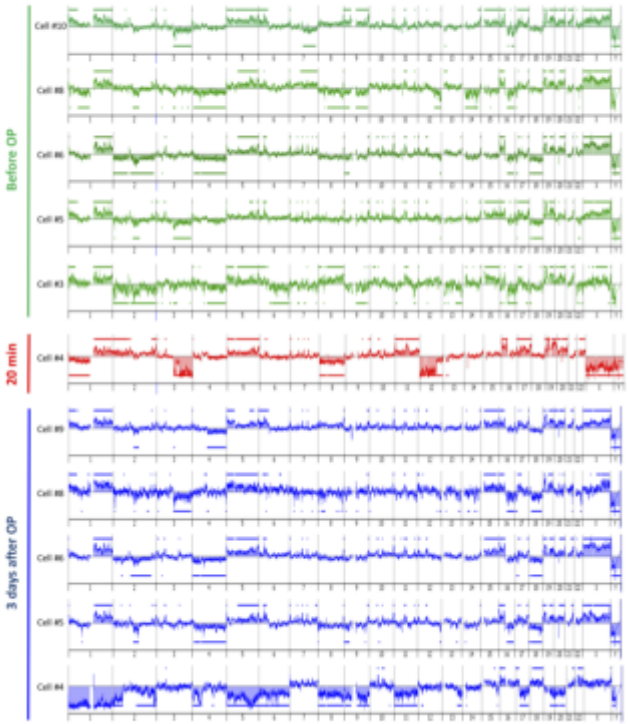
CTCs in vertebral metastases

Molecular Analysis of single CTCs

→ Array CGH for CNV

→ Example of picked CTCs from one patient before, 20min and on day 3 postoperatively

→ 20min CTC shows unique profile

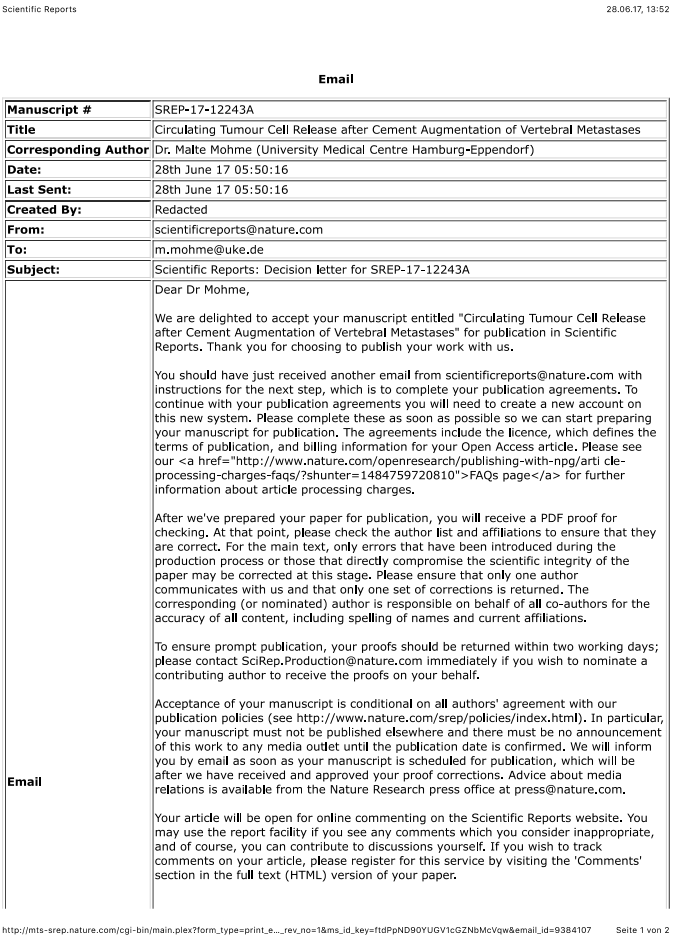


Diskussion:

This is the first study to report that peripheral circulating tumor cells (CTCs) are temporarily significantly increased due to the vertebral cement augmentation procedure. Furthermore the genetic profile of this detected Cells is unique. If this CTC dissemination leads to new metastatic seeding or affects the prognosis will be assessed in future studies. But taken together, our findings provide a rationale for the development of new strategies to reduce the increased dissemination of CTC after vertebroplasty.

Publikation:

Das Manuskript wurde am 28.06.2017 zur Publikation bei ***scientific reports (IF:4.3)*** angenommen.



Scientific Reports 28.06.17, 13:52

Email

Manuscript #	SREP-17-12243A
Title	Circulating Tumour Cell Release after Cement Augmentation of Vertebral Metastases
Corresponding Author	Dr. Malte Mohme (University Medical Centre Hamburg-Eppendorf)
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Last Sent:	28th June 17 05:50:16
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From:	scientificreports@nature.com
To:	m.mohme@uke.de
Subject:	Scientific Reports: Decision letter for SREP-17-12243A
Email	Dear Dr Mohme, We are delighted to accept your manuscript entitled "Circulating Tumour Cell Release after Cement Augmentation of Vertebral Metastases" for publication in Scientific Reports. Thank you for choosing to publish your work with us. You should have just received another email from scientificreports@nature.com with instructions for the next step, which is to complete your publication agreements. To continue with your publication agreements you will need to create a new account on this new system. Please complete these as soon as possible so we can start preparing your manuscript for publication. The agreements include the licence, which defines the terms of publication, and billing information for your Open Access article. Please see our FAQs page for further information about article processing charges. After we've prepared your paper for publication, you will receive a PDF proof for checking. At that point, please check the author list and affiliations to ensure that they are correct. For the main text, only errors that have been introduced during the production process or those that directly compromise the scientific integrity of the paper may be corrected at this stage. Please ensure that only one author communicates with us and that only one set of corrections is returned. The corresponding (or nominated) author is responsible on behalf of all co-authors for the accuracy of all content, including spelling of names and current affiliations. To ensure prompt publication, your proofs should be returned within two working days; please contact SciRep.Production@nature.com immediately if you wish to nominate a contributing author to receive the proofs on your behalf. Acceptance of your manuscript is conditional on all authors' agreement with our publication policies (see http://www.nature.com/srep/policies/index.html). In particular, your manuscript must not be published elsewhere and there must be no announcement of this work to any media outlet until the publication date is confirmed. We will inform you by email as soon as your manuscript is scheduled for publication, which will be after we have received and approved your proof corrections. Advice about media relations is available from the Nature Research press office at press@nature.com. Your article will be open for online commenting on the Scientific Reports website. You may use the report facility if you see any comments which you consider inappropriate, and of course, you can contribute to discussions yourself. If you wish to track comments on your article, please register for this service by visiting the 'Comments' section in the full text (HTML) version of your paper.

http://mts-srep.nature.com/cgi-bin/main.plex?form_type=print_e..._rev_no=1&ms_id_key=ftdPpND90YUGV1cGZNbMcVqw&email_id=9384107 Seite 1 von 2

Die Daten wurden auf folgenden Tagungen präsentiert:

DWG Jahrestagung Best of Session (2. Preis), DGNC Jahrestagung, Sektionstagung für Neuroonkologie der DGNC, Sektionstagung Wirbelsäule der DGNC, Frankfurt spine Symposium, ISMRC, Dresden spine Tumor day.

Bemerkung:

- Wie damals telefonisch besprochen wurde ein Teil des beantragten Geldes umgewidmet. Das Analysegerät benötigte einen neuen Rechner welcher angeschafft wurde. Auch wurden die Verbrauchmaterialien diskret an unsere Ergebnisse angepasst (Besonders viele Mamma CA Patienten). Aufgeführt in der beigefügten Auflistung der Mittelverwendung.
-
- Den noch vorhandenen Restbetrag würden wir gerne für weitere 1-2 Patienten nutzen und
- Die open access Gebühr des Journals bezahlen. Scientific report ist immer ein open Access Journal mit einer Gebühr von 1.305 Euro plus Steueren. Nach Begleichung der Rechnung würde ich die Endabrechnung schicken und den Restbetrag Rückerstatten.

Mit freundlichen Grüßen

S.O.E.

Priv.-Doz. Dr. med. S.O. Eicker
Ltd. Oberarzt
Neurochirurgische
Wirbelsäulenchirurgie

Anlage:

Mittelverwendungsnachweis des Drittmittelverwalters des UKE (**CAVE:** Publikationsgebühr und Untersuchung von 2 Patienten wird noch abgezogen und dann die Endabrechnung geschickt)

Mittelverwendungsnachweis zum 26.10.2016

	Objektbezeichnung	Bezeichnung des Gegenkontos	Belegdatum	Buchungsdatum	Bezeichnung	Wert/KWähr	K.Währung	Nr Referenzbeleg	Kostenart
2	Geldeingang	DP Drittmittel ZE/ZA	23.01.15	23.01.15	deutsche wirbelsäulen-stiftung	-30.000,00	EUR	90024001	473005
3	PSP-Element 1489/101/0-0 Geldeingang					-30.000,00	EUR		
4	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	24.04.15	24.04.15	NUCLEO SPIN XS KITS 1 PAK	251,97	EUR	5000038076	795011
5	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	29.04.15	29.04.15	RosetteSep Human CD45 Depletion Cocktai	248,71	EUR	5000039611	795011
6	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	30.04.15	30.04.15	MEGM BulletKit, 1Pck.	198,97	EUR	5000040047	795011
7	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	06.05.15	06.05.15	RT2 PreAMP cDNA Synthesis Kit (12)	548,23	EUR	5000041664	795011
8	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	06.05.15	06.05.15	RT2 SYBR Green qPCR Mastermix (12)	1.306,86	EUR	5000041664	795011
9	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	08.05.15	08.05.15	PAHS-131ZD-12 - RT² Profiler PCR Array	1.915,78	EUR	5000042611	795011
10	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	08.05.15	08.05.15	RT2 PreAMP Pathway Primer Mix	179,04	EUR	5000042611	795011
11	Verbrauchsmaterialien	Lonza Sales Ltd	28.04.15	12.05.15	MEGM BulletKit, 1Pck.	58,67	EUR	45319	795011
12	Verbrauchsmaterialien	STEMCELL Technologies Ltd.	28.04.15	13.05.15	RosetteSep Human CD45 Depletion Cocktai	6,95	EUR	46351	795011
13	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	23.07.15	23.07.15	Cell-Search-Kit, 1 Kit	7.804,38	EUR	5000068180	795011
14	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	11.08.15	11.08.15	EGF-R Tumor Phenotyping Reagent 3ml	1.302,81	EUR	5000074308	795011
15	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	08.09.16	08.09.16	Cell-Search-Kit, 1 Kit	2.601,46	EUR	5000092081	795011
16	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	09.09.16	09.09.16	Her2/neu Tumor Phenotyping Reagent 1 Kit	1.302,81	EUR	5000092703	795011
17	Verbrauchsmaterialien	WERE-Kto. Chemik./Di	09.09.16	09.09.16	Cell-Search-Kit, 1 Kit	2.601,46	EUR	5000092703	795011
18	PSP-Element 1489/101/2-1 Verbrauchsmaterialien					20.328,10	EUR		
19	Aufträge	WERE-Kto. Chemik./Di	07.07.16	07.07.16	SurePrint G3 Human CGH array	1.777,52	EUR	5000088185	795013
20	Aufträge	WERE-Kto. Chemik./Di	07.07.16	07.07.16	SureTag Complete DNA Labeling Kit	1.260,08	EUR	5000088185	795013
21	Aufträge	WERE-Kto. Chemik./Di	07.07.16	07.07.16	Oligo aCGH/ChIP-Chip Hybridization Kit	254,02	EUR	5000088185	795013
22	Aufträge	WERE-Kto. Chemik./Di	07.07.16	07.07.16	Cot-I Human DNA	137,59	EUR	5000088185	795013
23	Aufträge	WERE-Kto. Chemik./Di	07.07.16	07.07.16	OligoCHG Wash Buffers 1 and 2,4L	211,68	EUR	5000088185	795013
24	Aufträge	WERE-Kto. Chemik./Di	07.07.16	07.07.16	Pack 5 Backings 4 Arrays per Slide	96,43	EUR	5000088185	795013
25	Aufträge	Agilent Technologies	29.03.16	08.07.16	SurePrint G3 Human CGH array	380,90	EUR	73782	795013
26	Aufträge	Agilent Technologies	29.03.16	08.07.16	SureTag Complete DNA Labeling Kit	270,02	EUR	73782	795013
27	Aufträge	Agilent Technologies	29.03.16	08.07.16	Oligo aCGH/ChIP-Chip Hybridization Kit	54,43	EUR	73782	795013
28	Aufträge	Agilent Technologies	29.03.16	08.07.16	Cot-I Human DNA	29,49	EUR	73782	795013
29	Aufträge	Agilent Technologies	29.03.16	08.07.16	OligoCHG Wash Buffers 1 and 2,4L	45,36	EUR	73782	795013
30	Aufträge	Agilent Technologies	29.03.16	08.07.16	Pack 5 Backings 4 Arrays per Slide	20,66	EUR	73782	795013
31	PSP-Element 1489/101/2-4 Aufträge					4.538,18	EUR		
32	Geräte	Cancom GmbH	05.02.15	11.02.15	Apple MacBook Pro 2,8GHz 4656371	1.754,06	EUR	12958	099004MM
33	Geräte	Cancom GmbH	05.02.15	11.02.15	Cancom Care60 3432606	153,51	EUR	12958	099004MM
34	Geräte	Cancom GmbH	05.02.15	11.02.15	Apple MacSafe 2 Netzteil 4124807	67,83	EUR	12958	099004MM
35	PSP-Element 1489/101/2-6 Geräte					1.975,40	EUR		
36						-3.158,32	EUR		