6. Antrag Simona Vincenti vom 01.3.2023

Projekttitel:	Untersuchung autologer Gadolinium-markierter extrazellulärer Vesikel zur Evaluation der kompletten Resektion von kaninen Hirntumoren
Name der Organisation:	Universität Bern, Vetsuisse Bern, Department für Klinische Veterinärmedizin, Kleintierchirurgie + Universität Mailand, Abteilung Gesundheitwissenschaft
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Datum der Einreichung:	28/02/2023
Thema:	Veterinärmedizin
Beantragte Unterstützung (CHF):	13640
Bankverbindung (IBAN):	CH9009000000300066183
Vorgesehener Projektstart:	01/01/2022
Vorgesehener Projektabschluss:	31/12/2023
Kurzzusammenfassung des Projekts:	Hunde zeigen nach dem Menschen als zweithäufigste Spezies spontane Hirntumore1-3. Neben der chirurgischen Resektion spielen insbesondere Chemotherapie und Bestrahlung eine fundamentale Rolle in der Therapie dieser Erkrankung. Unglücklicherweise entwickeln Tumore häufig eine Resistenz gegen die beiden letztgenannten Therapien4-10. Ein entscheidendes Problem bei der chirurgischen Exzision von Hirntumoren ist die relativ hohe Inzidenz inkompletter Exzision, welche zu einem frühen Rezidiv und einer höheren post-operativen Morbidität führt11. Aus diesem Grund streben wir an die Lebensqualität und Prognose unserer kaninen Patienten durch Nutzung einer innovativen Therapie zu verbessern. Diese Therapie basiert auf der Verwendung von extrazellulären Vesikeln (EVs). EVs bezeichnet eine gemischte Population von Nanopartikeln, die von gesunden und neoplastischen Zellen produziert werden und zu verschiedenen physiologischen und pathologischen Prozessen, einschließlich des Tumorwachstums, beitragen12-14. EVs liegen im Blut vor und können aus dem Kreislauf extrahiert und mit verschiedenen Markern und Kontrastmitteln versetzt werden. Das Besondere an EVs ist ihre Fähigkeit, zu der Zelle zurückzukehren, die sie ursprünglich produziert hat, einschließlich Tumorzellen15-17. Diese Fähigkeit, zur Ursprungszelle zurückzukehren, eröffnet neue, spannende Möglichkeiten, um selektiv neoplastische Zellen anzugreifen.

Gadolinium und Iohexol sind die am häufigsten verwendeten Kontrastmittel in der Magnetresonanztomographie (MRT) bzw. Computertomographie (CT). Da MRT und CT grundlegende bildgebende Verfahren zur Lokalisierung eines Hirntumors und zur Planung seiner Behandlung sind, wollen wir untersuchen, ob die Extraktion von EVs aus Hunden mit Hirntumoren durchführbar ist. Darüber hinaus wollen wir evaluieren ob es möglich ist, diese EVs mit Iohexol und Gadolinium zu versetzen und sie anschließend wieder in den Patienten zu injizieren, aus dem sie entnommen wurden. Auf diese Weise wollen wir ihre Fähigkeit bestätigen, Kontrastmittel selektiv nur in Tumorzellen zu bringen.

Untersuchung autologer Gadolinium-markierter extrazellulärer Vesikel zur Evaluation der kompletten Resektion von kaninen Hirntumoren

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1 - Zusammenfassung mit Keywords

Hunde zeigen nach dem Menschen als zweithäufigste Spezies spontane Hirntumore[1-3]. Neben der chirurgischen Resektion spielen insbesondere Chemotherapie und Bestrahlung eine fundamentale Rolle in der Therapie dieser Erkrankung. Unglücklicherweise entwickeln Tumore häufig eine Resistenz gegen die beiden letztgenannten Therapien[4-10]. Ein entscheidendes Problem bei der chirurgischen Exzision von Hirntumoren ist die relativ hohe Inzidenz inkompletter Exzision, welche zu einem frühen Rezidiv und einer höheren post-operativen Morbidität führt[11]. Aus diesem Grund streben wir an die Lebensqualität und Prognose unserer kaninen Patienten durch Nutzung einer innovativen Therapie zu verbessern. Diese Therapie basiert auf der Verwendung von extrazellulären Vesikeln (EVs). EVs bezeichnet eine gemischte Population von Nanopartikeln, die von gesunden und neoplastischen Zellen produziert werden und zu verschiedenen physiologischen und pathologischen Prozessen, einschließlich des Tumorwachstums, beitragen[12-14]. EVs liegen im Blut vor und können aus dem Kreislauf extrahiert und mit verschiedenen Markern und Kontrastmitteln versetzt werden. Das Besondere an EVs ist ihre Fähigkeit, zu der Zelle zurückzukehren, die sie ursprünglich produziert hat, einschließlich Tumorzellen[15-17]. Diese Fähigkeit, zur Ursprungszelle zurückzukehren, eröffnet neue, spannende Möglichkeiten, um selektiv neoplastische Zellen anzugreifen. Gadolinium und Iohexol sind die am häufigsten verwendeten Kontrastmittel in der Magnetresonanztomographie (MRT) bzw. Computertomographie (CT). Da MRT und CT grundlegende bildgebende Verfahren zur Lokalisierung eines Hirntumors und zur Planung seiner Behandlung sind, wollen wir untersuchen, ob die Extraktion von EVs aus Hunden mit Hirntumoren durchführbar ist. Darüber hinaus wollen wir evaluieren ob es möglich ist, diese EVs mit Iohexol und Gadolinium zu versetzen und sie anschließend wieder in den Patienten zu injizieren, aus dem sie entnommen wurden. Auf diese Weise wollen wir ihre Fähigkeit bestätigen, Kontrastmittel selektiv nur in Tumorzellen zu bringen.

2 - Aktueller Stand der Forschung auf diesem Gebiet

Einige aktuelle Veröffentlichungen befassen sich zwar mit der Nutzung von EVs bei Tieren, jedoch wurde das große diagnostische und therapeutische Potenzial der EVs bei klinischen, kaninen Patienten noch nicht untersucht. Eine Studie zeigte, dass EVs, die von menschlichen Patienten mit Dickdarmtumoren extrahiert und in Mäuse mit subkutanen Tumoren injiziert wurden, die Tumorzellen in der Maus erreichen konnten[15]. In dieser Studie wurden von murinen und humanen

Tumorzellen stammende EVs mit Fluoreszenzfarbstoffen (Indocyaningrün) allein oder in Kombination mit einem onkolytischen Adenovirus (OV) beladen und den Mäusen intravenös (i.v.) verabreicht. Die vom Tumor stammenden EVs zeigten 24 Stunden nach der Injektion eine selektive Anreicherung der Fluoreszenz im Tumor (Abbildung 1). Darüber hinaus änderte die Beladung der EVs mit großen OVs den tumorspezifischen Tropismus der EVs in vivo nicht, was die Möglichkeit der Verwendung von EVs als Träger von niedermolekularen Medikamenten und Biopharmazeutika bestätigt. In einer weiteren Studie wurde die Möglichkeit der Versetzung von EVs mit dem MRT-Kontrastmittel Gadolinium und die Akkumulation von mit Gadolinium beladenen EVs in tumortragenden Mäusen nachgewiesen[16]. Eine andere Studie bestätigte, dass vom Tumor stammende EVs die natürliche Barriere zwischen dem Gehirn und dem peripheren Blutkreislauf (Blut-Hirn-Schranke - BHS) bei Mäusen passieren können[18]. Außerdem wurden EVs bei kaninen Patienten mit verschiedenen soliden Tumoren wie Adenokarzinomen der Brustdrüse, Gebärmutter-Sarkomen und Leber-Adenokarzinomen identifiziert und isoliert[19,20]. Bisher wurden jedoch keine EVs von Hunden mit bösartigen Hirntumoren isoliert. Die oben beschriebenen, außergewöhnlichen Erkenntnisse stellen einen großartigen Ausgangspunkt dar, um völlig neue und revolutionäre Szenarien für die Diagnose und Therapie von Hunden und Menschen mit Tumoren zu entwickeln. Insbesondere kann die Kombination dieser Technologien den onkologischen Veterinär-Chirurgen in die Lage versetzen, bei kaninen Hirntumoren die bestmögliche Operation zu planen und durchzuführen (die dem Konzept "so viel wie nötig, so wenig wie möglich" folgen sollte). Vor diesem Hintergrund sind mehrere Schritte zur Verbesserung der chirurgischen Therapie und der Prognose von Hirntumoren bei Hunden erforderlich. Die Identifizierung von EVs bei Hunden mit Hirntumoren und der Nachweis ihrer Fähigkeit, die BHS zu passieren, sind grundlegende Ausgangspunkte.

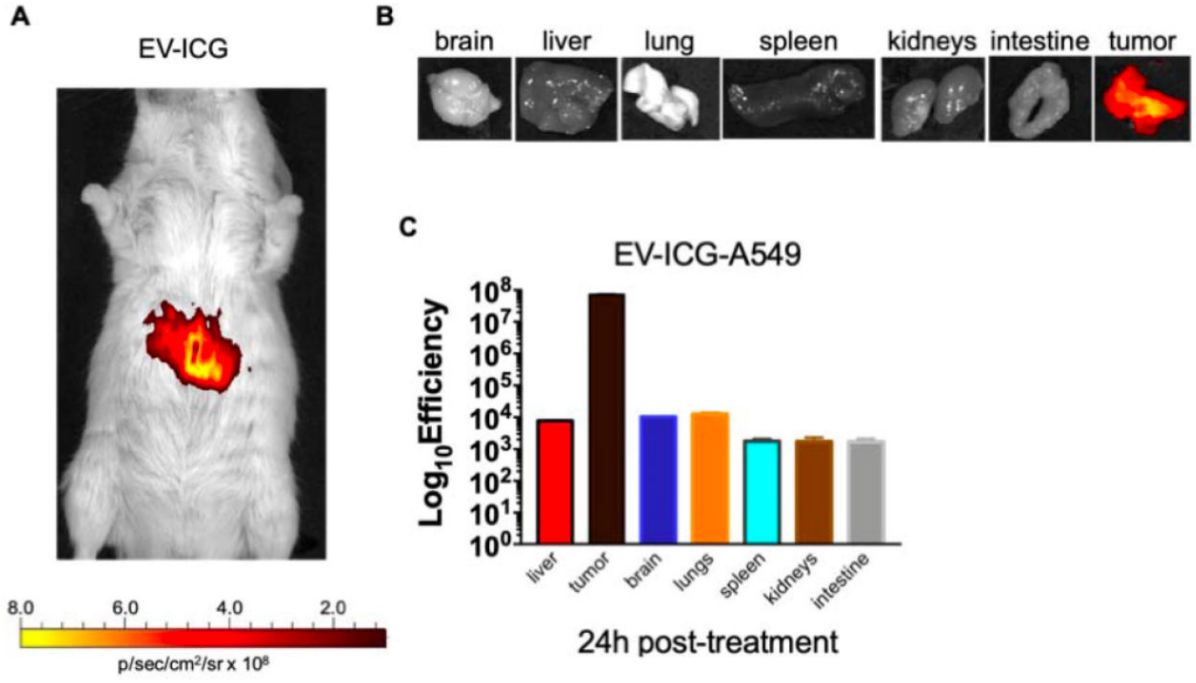


Abbildung 1: von Garofalo et al 2019. Bild einer Maus mit subkutanem Tumor nach Injektion von mit Indocyaningrün geladenen EVs. Zu beachten ist, dass die Fluoreszenz nur im Tumor und nicht in anderen Organen sichtbar ist.

Literaturverzeichnis:

1. Meuten DJ, Tumor in Domestic Animals. (Kohn Wiley & Sons, 2016).
2. Bentley RT, Ahmed AU, Yanke AB et al. Dogs are man's best friend: in sickness and in health. Neuro-Oncology 2017;19:312-322.

3. Snyder JM, Shofer FS, Van Winkle TJ et al. Canine intracranial primary neoplasia: 173 cases (1986-2003). J Vet Intern Med 2019;20:669-675.
4. Pang LY, Saunders L, Argyle DJ. Epidermal growth factor receptor activity is elevated in glioma cancer stem cells and is required to maintain chemotherapy and radiation resistance. Oncotarget 2017;8:72494-72512
5. Sordillo PP, Sordillo LA, Hlson L. The Kynurenine pathway: a primary resistance mechanism in patients with glioblastoma. Anticancer Res 2017;37:2159-2171.
6. Fianco G, Mongiardi MP, Levi A et al. Caspase-8 constributes to angiogenesis and chemotherapy resistance in glioblastoma. Elife 2017;6:979748.
7. Hombach-Klonisch S, Mehrpour M, Shojaei S et al. Glioblastoma and chemoresistance to alkylating agents: involvement of apoptosis, autophagy and unfolded protein response. Pharmacol.Ther 2018;184:13-41.
8. Wang J, Zhou F, Li Y et al. Cdc20 overexpression is involved in temozolomide- resistant glioma cells with epithelial-mesenchymal transition. Cell Cycle 2017;16:2355-2365.
9. Hatanpaa KJ, Burma SZ, Habib AA. Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging and radioresistance. Neoplasia 2010;12:675-684.
10. Kelley K, Knisely J, Symons M et al. Radioresistance of Brain Tumors. Cancers (Basel) 2016;8:42.
11. Shah JL, Gordon L, Shaffer JL et al. Stereotactic Radiosurgery and Hypofractionated Radiotherapy for Glioblastoma. Neurosurgery 2018;82:24-34.
12. Paolicelli RC, bergamini G, Rajendran L. Cell-to-cell communication by extracellular vesicles: focus on microglia. Neuroscience 2018;405:148-157.
13. Asfar SA, Bin Bao, FHS. Cancer Metastasis Rev. 2014;32:1-33.
14. An T, Qin S, Xu Y et al. Exosomes serve as tumour markers for personalized diagnostics owing to their important role in cancer metastasis. J Extracell Vesicles. 2015;4:1-5.
15. Garofalo M, Villa A, Crescenti D et al. Heterologous and cross-species tropism of cancer-derived extracellular vesicles. Theranostics. 2019;9:5681-5693.
16. Abello J, Nguyen TDT, Marasini R et al. Biodistribution of gadolinium- and near infrared-labeled human umbilical cord mesenchymal stromal cell-derived exosomes in tumor bearing mice. Theranostics. 2019;9:2325-2345.
17. Villa, A.; Garofalo, M.; Crescenti, D.; Rizzi, N.; Brunialti, E.; Vingiani, A.; Belotti, P.; Sposito, C.; Franze, S.; Cilurzo, F.; et al. Transplantation of autologous extracellular vesicles for cancer-specific targeting. Theranostics 2021, 11, 2034–2047.
18. Morad G, Carman CV, Hagedors EJ et al. Tumor-Derived extracellular vesicles brach the intact bloodbrain barrier via transcytosis. ASC Nano. 2019;13:13853- 13865.
19. Zmigrodzka M, Witkowska-Pilaszewicz O, Rzepecka A et al. Extracellular vesicles in the blood of dogs with cancer – a preliminary study. Animals. 2019;9:575.
20. Sammarco A, Finesso G, Cavicchioli L et al. Preliminary investigation of extracellular vesicles in mammary cancer of dogs and cats: identification and characterization. Vet Comp Oncol 2018;16:489-496.

3 - Aktueller Stand der eigenen Forschung

In den letzten 50 Jahren sind Hunde mehr und mehr zu Familienmitgliedern geworden. Dies hat zu einer erheblichen Verbesserung ihrer medizinischen Versorgung und ihrer allgemeinen Lebensqualität geführt, was sich in einer höheren Lebenserwartung niederschlägt. Wie beim Menschen steigt auch bei Hunden, die länger leben, die Wahrscheinlichkeit Tumore zu entwickeln. Aus diesem Grund sind Tumore bei unseren heutigen Hunden extrem häufig geworden, und es wurden mehrere, außerordentliche Verbesserungen zur Behandlung und Heilung vieler dieser Tumore erzielt. Es gibt jedoch weiterhin viel Raum für Forschung, um eine noch bessere medizinische Versorgung unserer Haustiere sicherzustellen. Um einen Beitrag zur Verbesserung der Lebensqualität und der Behandlungsmöglichkeiten von Hunden mit Tumoren zu leisten, liegt mein Forschungsschwerpunkt auf der chirurgischen Onkologie. Ein wichtiger Teil dieser Verbesserung erfolgt durch eine genauere Bestimmung der Tumorgrenzen. Mit einer genauen Kenntnis der Tumorausdehnung können wir die beste Behandlungsmodalität anbieten, die auf jeden einzelnen von einem Tumor betroffenen Hund zugeschnitten ist. Insbesondere möchte ich die

chirurgische Behandlung durch eine verbesserte intraoperative Erkennung der Tumorausdehnung optimieren und eine genauere, histologische Bewertung der Tumorentfernungsränder erreichen. Bereits seit Beginn meiner Forschungserfahrung, die mit meinem Dissertationsprojekt an der Universität Mailand in den Jahren 2008-2010 begann, konzentriere ich mich auf dieses Gebiet. Die Dissertation stellt eine prospektive Studie dar, welche das Vorhandensein einer minimalen Resterkrankung bei Hunden mit multizentrischem Lymphom nach Chemotherapie untersucht. Dabei konnten wir feststellen, dass 20 % der Hunde, bei denen das Lymphom klinisch in Remission war, eine minimale Resterkrankung aufwiesen und ein frühes Rezidiv des Tumors auftrat[16]. Im Anschluss an diese Studie führte ich eine retrospektive Studie über Hunde mit unvollständig exzidierten Mastzelltumoren und deren Ergebnis in Abhängigkeit von der nach der Operation durchgeführten weiteren Therapie durch[15]. Während dieser Studie wurde mir klar, dass es in der Veterinärmedizin keine klaren Richtlinien für die Definition tumorfreier Ränder nach einer chirurgischen Tumorexzision gibt und dass die Standardbewertung der Ränder in der Histopathologie einige Schwächen aufweist. Mein PhD befasst sich nun mit Hirntumoren bei Hunden und der Frage, wie man selektiv das neoplastische Gewebe hervorheben kann, indem man von jedem Patienten extrahierte („autologe“) EVs verwendet, die mit verschiedenen Kontrastmitteln und Markern beladen sind: Gadolinium, Iohexol und Indocyaningrün. Wir konnten bereits zeigen, dass EVs erfolgreich mit Iohexol[4] beladen werden können. Nun arbeiten wir an der Beladung von EVs, die von Hunden mit primären Hirntumoren (Gliomen) entnommen wurden. Parallel zu meinem PhD-Projekt laufen drei Studien in Zusammenarbeit mit der Abteilung für Infektionskrankheiten und Pathobiologie (DIP) der Vetsuisse-Fakultät Bern, die sich mit der Verbesserung der histologischen Beurteilung der Operationsränder nach der Tumorexzision befassen. Dafür sammeln wir bösartige Haut- und Brustdrüsentumore, sowie orale und hepatische Tumore. Zusammen mit den Pathologen des DIP haben wir einen neuen Ansatz für die Bewertung der Ränder erarbeitet, der wesentlich vollständiger und präziser ist. Wir haben begonnen, das erste Manuskript über Hauttumore zu schreiben, und wir machen Fortschritte bei der Sammlung von Fällen von oralen Tumoren und Lebertumoren.

Liste der eigenen Publikationen:

1. Zimmermann J, Brunner A, Underberg J, **Vincenti S**. Computed tomographic measurements of tracheal diameter and length in normocephalic cats. J of Feline Medicine and Surgery, JFMS-22-0165.R2 Feb 2023. 10.1177/1098612X231158578
2. Brandstetter V, Schmid J, Findji L, Selmic LE, Murgia D, de Mello Souza CH, Liehmann LM, L'Eplattenier H, Tichy A, **Vincenti S**. Feline primary nonhematopoietic malignant liver tumors: A multicenter retrospective study (2000-2021). *Veterinary and Comparative Oncology* January 2023 DOI:10.1111/vco.12874.
3. Szabo Z, Moser J, **Vincenti S**. Persistent Mullerian duct syndrome in a dog. SAT 2023; 165:625-633. DOI:10.17236/sat00275.
4. Vincenti S, Villa A, Crescenti D, Crippa E, Brunialti E, Shojaeighahrizjani F, Rizzi N, Rebecchi M, Dei Cas M, Del Sole A, Paroni R, Mazzaferro V, Ciana P. Increased sensitivity of computed tomography scan for neoplastic tissues using the extracellular vesicle formulation of the contrast agent ioexhol. *Pharmaceutics 2022*, 14, 2766. DOI: 10.3390/pharmaceutics14122766.
5. Metzger MD, Van der Vekens E, Rieger J, Forterre F, **Vincenti S**. Preliminary studies on the intrahepatic anatomy of the venous vasculature in cats. *Veterinary Science*, September 2022. DOI:10.3390/vetsci9110607.
6. Goffart L, Durand A, Dettwiler M, Vincenti S. Feline gastrointestinal eosinophilic sclerosing fibroplasia presenting as a rectal mass. *J Fel Int Med and Surg*, June 2022. DOI:10.1177/20551169221114330.
7. Zimmermann J, Vincenti S. Comparing aspects of permanent tracheostomy between cats and children – can we learn from pediatric medicine? *Kleintierpraxis* 2022;67:07/2022. DOI:10.2377/0023-2076-67-372.
8. Gamez E, Spadavecchia C, **Vincenti S**, Mirra A. Anesthetic management of a Labrador Retriever undergoing adrenalectomy for pheochromocytoma excision, a case report. *Frontiers in Veterinary Science*, Feb 2022. DOI: 10.3389/fvets.2022.789101.

9. Barge P, **Vincenti S**, Geissbühler U. “Subclavian steal phenomenon demonstrated by ultrasound in a dog with hypoplastic aberrant left subclavian artery”. *J of Small Anim Prac*, 2021. DOI: 10.1111/jsap.13383.
10. **Vincenti S**, Betting A, Durand A, Campos M, Scanziani E, Soto S. “Total laryngecomy in a cat with laryngeal peripheral nerve sheath tumor”. *Veterinary Surgery* 2021. DOI: 10.1111./vsu.13646.
11. “Type Ib (tubular) non-communicating colonic and rectal duplication in a cat” Hammer M, Schmidli M, Campos M, **Vincenti S**. *Vet Record*, Case Report, Nov 2020. DOI: 10.1111/jsap.13383.
12. “Characterization of canine epidermal organoid cultures by immunohistochemistry and qPCR” Wiener D, Studer I, Brunner M, Hermann A, **Vincenti S**, Zhang M Groch K, Welle M. *Veterinary Dermatology* 2020. DOI:10.1111/vde.12914.
13. “An anatomical study of plate-rod fixation in feline tibiae” Gutbrod A, **Vincenti S**, Knell, SC, Schmierer PA Pozzi A. *Veterinary Surgery* 2017 Oct;46(7)909-914. DOI: 10.1111/vsu.12678.
14. “Surgical treatment of a proximal tibial deformity associated with a partial caudal and cranial cruciate ligament deficiency and patella baja” **Vincenti S**, Knell S, Pozzi A *Schweiz Arch Tierheilkd*. 2017 Apr;159(4):237-242. DOI: 10.17236/sat00113.
15. “Influence of treatment on the outcome of dogs with incompletely excised grade-2 mast cell tumours” **Vincenti S**, Findji L. *Schweizer Arch Tierheilkd*. 2017 Mar;159(3):171-177. DOI: 10.17236/sat00109.
16. “Flow cytometric evaluation of minimal residual disease (MRD) in canine lymphoma treated with chop-based protocol: preliminary results”. **Vincenti S**, Gelain ME, Stefanello D. Published as monography in 2010 at University of Milano and presented as poster at the ESVONC Congress 2010, Turin.

4 - Detaillierter Forschungsplan

Die hier beschriebene Pilotstudie gehört zum größten Forschungsprojekt meines PhDs. Die 3 Fälle der Kontrollgruppe und die ersten 3 Fälle der Pilotstudie, aus denen wir erfolgreich EVs aus Patienten mit Hirntumoren extrahiert haben, konnte ich bereits sammeln. Nun werden wir die letzten 3 Fälle der Pilotstudie rekrutieren, welche eine Injektion von mit Gadolinium beladenen EVs erhalten werden. Für diese letzten 3 Fälle beantragen wir derzeit die Finanzierung. Die gesamte Studie wurde an der Vetsuisse-Fakultät Bern durchgeführt und am Labor für molekulare Pharmakologie und Krebsbiologie Abteilung Gesundheitwissenschaft Universität Mailand.

Hypothese

Autologe, vom Patienten stammende Gad-EVs können die Blut-Hirn-Schranke (BHS) passieren und sich selektiv mit Tumorzellen des Zentralnervensystems im Hund verbinden.

Ziele

1. Nachweis des Vorhandenseins von aus Hirntumoren stammenden EVs beim Hund.
2. Demonstration der Fähigkeit von Gad-EVs, die BHS zu passieren, und somit detaillierte Darstellung der Ausdehnung eines Hirntumors beim Hund.
3. Nachweis der sicheren Anwendung von Gad-EVs bei Hunden.
4. Nachweis der höheren Kapazität von Gad-EVs-MRT-Bildern im Vergleich zu Standard-MRT-Bildern zur Unterstützung des Tierarztes bei der Planung einer genauen chirurgischen Entfernung des Tumors. Dieser Teil der Studie würde die Grundlage für künftige Studien an Hunden und Menschen bilden.

Rekrutierung von kaninen Patienten, Erzeugung und Injektion von Gad-EVs von kaninen Patienten mit spontanen Hirntumoren.

Insgesamt werden 9 kanine Patienten mit spontanem Hirntumor und einem Körpergewicht von mehr als 15 kg in die Studie aufgenommen. Sechs Patienten werden der Pilotstudiengruppe und drei der Kontrollgruppe zugewiesen. Jeder der 9 Patienten erhält ein MRT des Gehirns mit i.v.-Injektion von Gadolinium (0,15mmol/kg Gadotersäure, Clariscan). Von sechs Patienten werden 15 ml Blut entnommen und zur Herstellung autologer Gad-EVs verwendet. Bei 3/6 Patienten der Pilotstudiengruppe haben wir nachgewiesen, dass es möglich ist, vom Tumor stammende EVs zu extrahieren und diese EVs mit Gadolinium zu beladen. Wir schließen derzeit die Sicherheitstests ab, um die Sterilität der Gad-EVs zu bestätigen und somit jegliches Risiko für unsere Patienten auszuschließen. Die verbleibenden 3/6 Patienten der Pilotstudiengruppe werden eine Injektion von autologen, aus dem Tumor stammenden, Gad-EVs erhalten.

Durchführung des Post-Injektions GAD-EVs MRTs

Nach der Herstellung werden die patientenspezifischen Gad-EVs, die in 5 ml physiologischer Lösung (NaCl 0,9 %) dispergiert sind, wieder in den Hund injiziert. Anschließend wird der Patient einer MRT-Untersuchung nach der Injektion unterzogen. Diese MRT-Untersuchung wird als "MRT 1" bezeichnet. Im Anschluss an diese MRT-Untersuchung wird ein chirurgischer Plan zur vollständigen chirurgischen Entfernung des Hirntumors von zwei Veterinärchirurgen (DECVS - FF und SV) durchgeführt.

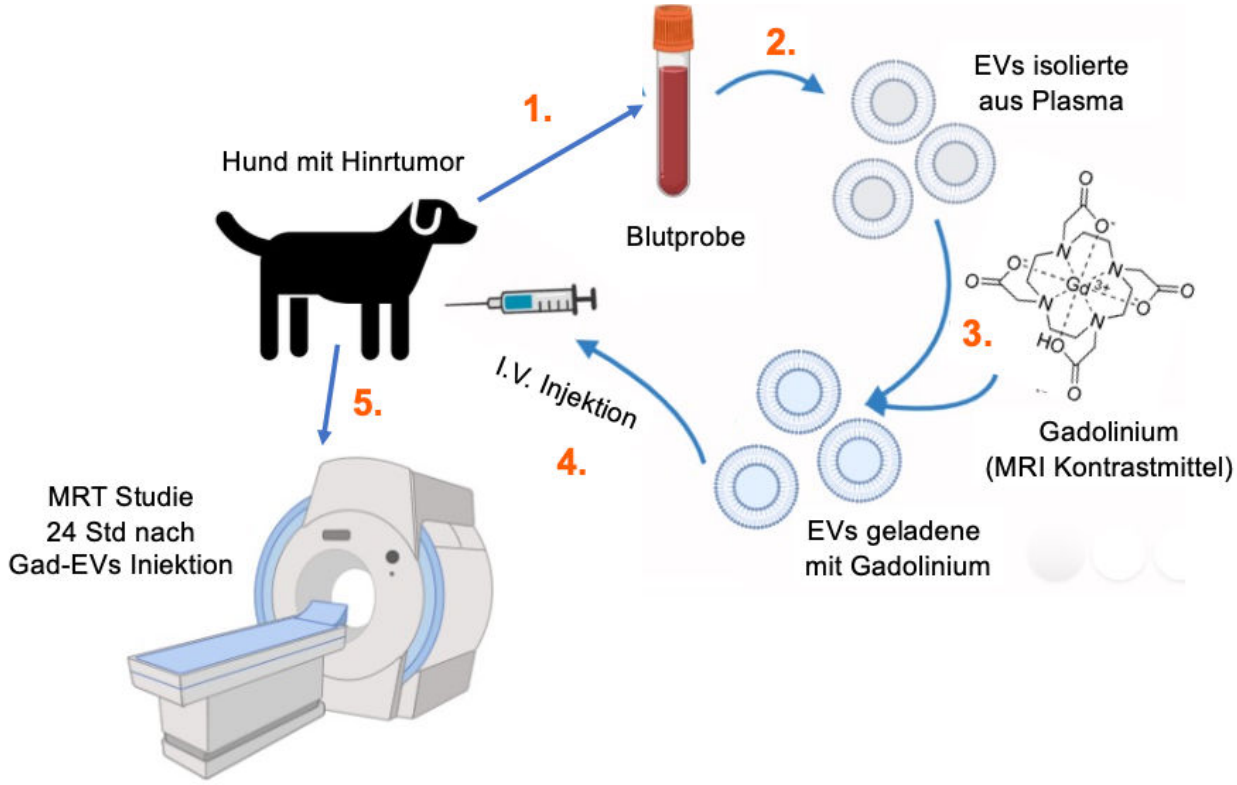


Abbildung 1: Darstellung der Pilotstudie. Ein Hund, bei dem bereits im MRT (MRT 0) ein Hirntumor diagnostiziert wurde, wird in die Studie aufgenommen. Punkt 1. und 2. Entnahme der EVs aus dem Hund; Punkt 3. Beladung der EVs mit Gadolinium (Gad-EVs); Punkt 4. Injektion der Gad-EVs zurück in den Hund; 5. Durchführung der zweiten MRT (MRT 1) 24 Stunden nach Injektion der Gad-EVs.

Verfügbare Infrastruktur und Ressourcen

- Kleintierklinik der Universität Bern, wo die Hunde aufgenommen, die Blutentnahme, die Injektion von Gad-EVs und die MRT-Untersuchungen durchgeführt werden.
- Labor des Instituts für Virologie und Immunologie, Abteilung für Infektionskrankheiten und Pathobiologie (DIP), der Vetsuisse-Fakultät Bern, wo EVs aus dem Plasma von Hunden mit Hirntumoren durch Ultrazentrifugation extrahiert und mit Gadolinium beladen werden.
- Labor für molekulare Pharmakologie und Krebsbiologie Abteilung Gesundheitwissenschaft Universität Mailand, wo die Technologie zur Extraktion und Herstellung der EVs entwickelt und verbessert wurde.

Zeitplan und Meilensteine

Zeitplan

- Dauer: 2 Jahre (Beginn: Januar 2022)
- Der erste Teil (Sammlung der 3 Fälle der Kontrollgruppe und der ersten 3 Fälle der Pilotstudie) ist bereits abgeschlossen, im Jahr 2023 werden wir die letzten 3 Fälle der Pilotstudie sammeln. Diesen 3 Hunden werden mit Gadolinium beladene EVs injiziert und 24 Stunden nach der Injektion wird ein MRT durchgeführt.

Meilensteine

2022:

- Erfolgreiche Extraktion von tumorbedingten EVs aus 3 Hunden mit Hirntumor. **Erreicht.** Siehe in der Tabelle "Erste 3 Hunde Pilotstudie".

2023:

- Extraktion, Beladung mit Gadolinium und Rückinjektion von aus Tumoren stammenden EVs von 3 Hunden mit Hirntumor und Durchführung einer MRT 1 Stunde nach Injektion der EVs. Siehe in der Tabelle "Letze 3 Hunde Pilotstudie".
- Abschluss der Studie und Manuskriptverfassung

Aktivitäten	2022		2023	
	I	II	I	II
Sammlung Kontrollfälle				
Erste 3 Hunde Pilostudie				
Letze 3 Hunde Pilostudie				
Manuskriptverfassung				

Meilensteine Tabelle: in Grün die bereits erreichten Meilensteine, in Gelb die für dieses Jahr geplanten.

Bedeutung des geplanten Forschungsprojekts

Dieses Forschungsprojekt, welches auf der Verwendung von autologen, mit Gadolinium beladenen Tumor-EVs basiert, stellt eine einzigartige Möglichkeit dar, die Diagnose, Therapie und Prognose von Hunden mit Hirntumoren zu verbessern. Zum ersten Mal in der Geschichte der Veterinärmedizin können wir die außergewöhnliche *theranostische* Methode anwenden, die diese Population von EVs

bietet, um das neoplastische Gewebe gezielt zu markieren und zu bekämpfen. Diese Studie ist ein erster Schritt zur Bewertung der intrazerebralen Bioverteilung der EVs und ihres Nutzens für eine gezieltere chirurgische Planung.

Budget

Vorhandene Mittel

Personal:
- Sammlung von klinischen Fällen bei Hunden: S. Vincenti and A. Maiolini
- Extraktion, Bearbeitung und Einspritzen EVs: S. Vincenti
- Betreuung der Bildanalyse: D. Schwarz (Radiologie, Dipl. ECVDI)
- Chirurgische Planung auf MRT 1-Bildern: S. Vincenti und F. Forterre

Kostenpunkt	**Betrag (CHF)**
Verbrauchsmaterial und Wartung	
. Intravenöse Katheter, Anästhesiematerial und Medikamente	. 900CHF
. Gadotersäure	. 750CHF
Wissenschaftliche Ausrüstung	
. Violen für die Ultrazentrifuge	. 1.000CHF
Weitere Kosten	
. Anästhesie für Hunde von 15-25kg (jedes MRT 260CHF)	. 2.340CHF
. MRT 0 für 6 Patienten (1000CHF pro Patient)	. 6.000CHF
. MRT 1 für 3 Patienten (250CHF pro Patient)	. 750CHF
. Klinische Untersuchungen, Krankenhausaufenthalt, Medikamente (falls erforderlich)	. 1.600CHF
. Blutuntersuchungen (100CHF pro Patient)	. 300CHF
TOTAL	**13.640CHF**

5 - Erwartete Resultate und Ziele des Projektes

- Nachweis der Fähigkeit von autologen, vom Patienten stammenden Gad-EVs, die BHS zu passieren und sich selektiv mit neoplastischen Zellen von Hirntumoren zu verbinden
- Drastische Verbesserung der Fähigkeit des Tierarztes, eine hochpräzise chirurgische Resektion Hirntumoren zu planen und durchzuführen
- Eröffnung neuer, aufregender Szenarien für die Diagnose, das Staging, die chirurgische Planung, die Nachsorge und möglicherweise die *Theragnostik* bei Hinrtumoren bei Hunden
- Großes Potenzial für die Anwendung der Ergebnisse dieses Promotionsprojekts in der translationalen Medizin.

CURRICULUM VITAE

Personal information

Name:	**Simona Vincenti**
Title:	DVM, MRCVS, Dipl ECVS, PhD Fellow RCVS Recognized Specialist in Small Animal Surgery
Address:	Kleintierklinik Vetsuisse Faculty, University of Bern Länggassstrasse 128, 3012, Bern, Switzerland Phone: +41 76 681 6348 Phone 2: +39 348 155 4058 e-mail: simona.vincenti@unibe.ch
Date of birth:	21/01/1986
Place of birth:	Milano, Italy

Education and Professional Training

2004-2010:	Veterinary Medicine Faculty, University of Milan, IT
1999-2004:	Scientific High School, Liceo Luigi Cremona Viale Marche 71/73, 20159, Milan, IT

Qualifications

Mar 2021:	**Royal College of Veterinary Surgeon (RCVS) Recognized Specialist in Small Animal Surgery**
Feb 2019:	**Diplomate European College of Veterinary Surgeons (Dipl ECVS)** **European Board Veterinary Specialization (EBVS) Recognized Specialist in Small Animal Surgery**
Nov 2010:	**State Exam**
Jul 2010:	**Doctor in Veterinary Medicine (DVM)** Graduation in Veterinary Medicine degree (Doctor Degree). Vote: 110/110 with honors. Title of Thesis: “Flow cytometric evaluation of minimal residual disease (MRD) in canine lymphoma treated with chop-based protocol: preliminary results”. Relator: Dr. Damiano Stefanello DVM, PhD

Working experience

Since Mar 2019:	Senior surgeon (Oberärztin), lecturer and researcher (PhD Fellow) in small animal surgery Kleintierklinik Vetsuisse Faculty, University of Bern Länggassstrasse 128, 3012, Bern, CH
Oct 2018-Feb 2019:	Part-time senior surgeon (soft tissue, oncologic, orthopedic and neurologic surgery) Kleintierklinik Vetsuisse Faculty, University of Bern

	Länggassstrasse 128, 3012, Bern, CH
Sep 2016-Sep 2018:	Small animal surgeon (orthopedic, soft tissue and oncologic surgery) Tierklinik Sonnenhof, Luzernstrasse 55a, 4552 Derendingen, (SO), Switzerland, CH
Jul 2013-Aug 2016:	ECVS Residency in Small Animal Surgery Tierspital Zurich, Vetsuisse Faculty, University of Zurich Winterthurerstrasse 260, 8050 Zurich, CH
Feb 2012-Feb 2013:	Rotating Internship VRCC Veterinary Referrals, No 1 Bramston Way, Southfield, Laindon, Essex SS15 6TP, UK
Sep 2011-Jan 2012:	Veterinary Surgeon – General practitioner Ambulatorio veterinario Dr Edmondo Vatta, Via Borgazzi 38, 20900 Monza (MB), IT
Jan 2011-Jul 2011:	Leonardo Fellowship in surgical oncology VRCC Veterinary Referrals, No 1 Bramston Way, Southfield, Laindon, Essex SS15 6TP, UK

Faculty participations

Since 2022:	Member of the Research Committee (Forschungskommission)
Since 2022:	Member of Appeal Committee (Berufungskommission) for an Assistant Professorship position
Since 2020:	Representative for the Intermediate Faculty Members (Mittelbau) of the Small Animal Clinic in the Strategiegremium
Since 2019:	Representative for the Intermediate Faculty Members (Mittelbau) of the Small Animal Clinic (DKV - KKH) since 2019

ECVS College participations

Since Sep 2020:	Member of the ECVS Credential Committee

Publications

1. Zimmermann J, Brunner A, Underberg J, **Vincenti S**. Computed tomographic measurements of tracheal diameter and length in normocephalic cats. J of Feline Medicine and Surgery, JFMS-22-0165.R2 Feb 2023. 10.1177/1098612X231158578
2. Brandstetter V, Schmid J, Findji L, Selmic LE, Murgia D, de Mello Souza CH, Liehmann LM, L'Eplattenier H, Tichy A, **Vincenti S**. Feline primary nonhematopoietic malignant liver tumors: A multicenter retrospective study (2000-2021). *Veterinary and Comparative Oncology* January 2023 DOI:10.1111/vco.12874.
3. Szabo Z, Moser J, **Vincenti S**. Persistent Mullerian duct syndrome in a dog. SAT 2023; 165:625-633. DOI:10.17236/sat00275.
4. **Vincenti S**, Villa A, Crescenti D, Crippa E, Brunialti E, Shojaeighahrizjani F, Rizzi N, Rebecchi M, Dei Cas M, Del Sole A, Paroni R, Mazzaferro V, Ciana P. Increased sensitivity

of computed tomography scan for neoplastic tissues using the extracellular vesicle formulation of the contrast agent ioexhol. *Pharmaceutics 2022*, 14, 2766. DOI: 10.3390/pharmaceutics14122766.

5. Metzger MD, Van der Vekens E, Rieger J, Forterre F, **Vincenti S**. Preliminary studies on the intrahepatic anatomy of the venous vasculature in cats. *Veterinary Science*, September 2022. DOI:10.3390/vetsci9110607.
6. Goffart L, Durand A, Dettwiler M, Vincenti S. Feline gastrointestinal eosinophilic sclerosing fibroplasia presenting as a rectal mass. *J Fel Int Med and Surg*, June 2022. DOI:10.1177/20551169221114330.
7. Zimmermann J, Vincenti S. Comparing aspects of permanent tracheostomy between cats and children – can we learn from pediatric medicine? *Kleintierpraxis* 2022;67:07/2022. DOI:10.2377/0023-2076-67-372.
8. Gamez E, Spadavecchia C, **Vincenti S**, Mirra A. Anesthetic management of a Labrador Retriever undergoing adrenalectomy for pheochromocytoma excision, a case report. *Frontiers in Veterinary Science*, Feb 2022. DOI: 10.3389/fvets.2022.789101.
9. Barge P, **Vincenti S**, Geissbühler U. "Subclavian steal phenomenon demonstrated by ultrasound in a dog with hypoplastic aberrant left subclavian artery". *J of Small Anim Prac*, 2021. DOI: 10.1111/jsap.13383.
10. **Vincenti S**, Betting A, Durand A, Campos M, Scanziani E, Soto S. "Total laryngecomy in a cat with laryngeal peripheral nerve sheath tumor". *Veterinary Surgery* 2021. DOI: 10.1111./vsu.13646.
11. "Type Ib (tubular) non-communicating colonic and rectal duplication in a cat" Hammer M, Schmidli M, Campos M, **Vincenti S**. *Vet Record*, Case Report, Nov 2020. DOI: 10.1111/jsap.13383.
12. "Characterization of canine epidermal organoid cultures by immunohistochemistry and qPCR" Wiener D, Studer I, Brunner M, Hermann A, **Vincenti S**, Zhang M Groch K, Welle M. *Veterinary Dermatology* 2020. DOI:10.1111/vde.12914.
13. "An anatomical study of plate-rod fixation in feline tibiae" Gutbrod A, **Vincenti S**, Knell, SC, Schmierer PA Pozzi A. *Veterinary Surgery* 2017 Oct;46(7)909-914. DOI: 10.1111/vsu.12678.
14. "Surgical treatment of a proximal tibial deformity associated with a partial caudal and cranial cruciate ligament deficiency and patella baja" **Vincenti S**, Knell S, Pozzi A *Schweiz Arch Tierheilkd*. 2017 Apr;159(4):237-242. DOI: 10.17236/sat00113.
15. "Influence of treatment on the outcome of dogs with incompletely excised grade-2 mast cell tumours" **Vincenti S**, Findji L. *Schweizer Arch Tierheilkd*. 2017 Mar;159(3):171-177. DOI: 10.17236/sat00109.
16. "Flow cytometric evaluation of minimal residual disease (MRD) in canine lymphoma treated with chop-based protocol: preliminary results". **Vincenti S**, Gelain ME, Stefanello D. Published as monography in 2010 at University of Milano and presented as poster at the ESVONC Congress 2010, Turin.

Posters

1. "Feasibility of loading extracellular vesicles with iohexol". **Vincenti S**, Villa A, Crescenti D, Rizzi N, Rebecchi M, Mazzaferro V, Ciana P. **GCB Symposium 2022**, On-line Congress.
2. "Total laryngectomy in a cat with a laryngeal peripheral nerve sheath tumor: first description of the surgical technique and long-term outcome". **Vincenti S**, Betting A, Durand A, Campos M, Scanziani E, Soto S. **ECVS Congress 2020**, On-line Congress.
3. "Caudal cruciate ligament rupture associated with negative tibial plateau angle: correction usign a CORA-based cranial tibial opening wedge". **Vincenti S**, Turner J, Pozzi A. **ECVS Congress 2016**, Lisbon.
4. "Flow cytometric evaluation of minimal residual disease (MRD) in canine lymphoma treated with chop-based protocol: preliminary results". Stefanello D, Gelain ME, Valenti P, Barbieri L, **Vincenti S**, Comazzi S. **ESVONC congress 2010**, Turin.

Oral presentations at Congresses

- **"**Yellow: Biliary Obstruction" presented at **KIS 2023 (Kleintier-Intensiv-Symposium) University of Bern**
- **"**Feasibility of loading EVs with Iohexol". **Flash presentation GCB Symposium 2022**
- **"**Masse surrenaliche nel cane: quando l'invasività non deve scoraggiare". **UNISVET National Congress 2022**.
- **"**PSS, one ring to rule them all" presented at **KIS 2022 (Kleintier-Intensiv-Symposium) University of Bern**
- **"**Prophylaxemäglichkeiten zur Vermeidung chirurgischer Erkrankungen des Magen-Darm-Traktes (Gastropexie, Plikation und Co.)" **66. DGK-DVG (Deutsche Gesellschaft für Kleintiermedizin) Berlin 2020**.
- **"**Komplikationen bei der Versorgung von Perinealhernien – Wie vermeiden?" **66. DGK-DVG (Deutsche Gesellschaft für Kleintiermedizin) Berlin 2020**
- **"**Oral tumors and therapy in the dog" presented at **KIS 2020 (Kleintier-Intensiv-Symposium) University of Bern**
- "Canine mammary tumors, future perspectives" **presented at KIS 2020 (Kleintier-Intensiv-Symposium) University of Bern**
- "Objective Assessment of goals achieved by decompressive surgery" **ECVS Congress, Budapest 2019**
- "Plate-rod constructs for tibial fractures in cats: an anatomical study". Vincenti S, Pozzi A, Gutbrod A. **Resident forum ECVS Congress, Lisbon 2016.**
- "Septic Abdomen" **In House Seminar, October 2015 University of Zurich**
- "Sacro-coccygeal luxation" **Small Animal Surgery Courses 2014 University of Zurich**
- "Influence of treatment on the outcome of dogs with incompletely excised grade-2 mast cell tumours" Vincenti S, Findji L" **AVSTS Spring Meeting 2nd April 2014 – Peter Holt Award**
- Influence of treatment on the outcome of dogs with incompletely excised grade-2 mast cell tumours" Vincenti S, Findji L. **Award as best presentation of the resident forum ECVS Congress, Rome 2013.**

Teaching Experience

Faculty – Bern University

Student lectures

- Akutes Abdomen – **Schwerpunkt Kleintiere 4.JK 2019, 2020, 2021, 2022**
- Leitsymptome – **4.JK 2019, 2020, 2021, 2022, 2023**
- Hauttumoren – **3.JK 2022, 2023**
- Prinzipien von Onkochirurgie – **3.JK 2022, 2023**

Student courses

- Probelaparotomie an Kadavern – **4.JK 2021, 2022, 2023**
- Chirurgie für Hauttumoren an Kadavern – **4.JK 2022, 2023**

Students, Interns, Residents clinical rounds – weekly and monthly **since 2019**

International

- Faculty Presenter at **ESAVS SOFT TISSUE SURGERY I Warsaw, 2023**
- Faculty Presenter at **ESAVS ECC I Bern, 2020, 2021, 2022, 2023**
- Faculty Presenter at **ESAVS ECC II Bern, 2019, 2020, 2021, 2022, 2023**
- Faculty Presenter at **UNISVET/University of Padova Master of Oncology 2022**
- Faculty Presenter at **UNISVET Focus on Hepato-biliary Surgery Milan, 2022**
- Faculty Presenter at **Vets4Ukraine, Online CPD 2022**
- Faculty Presenter at **UNISVET Surgery Focus on GDV, Online CPD 2021**
- Faculty Presenter at **UNISVET Surgery Focus on Diaphragmatic Hernia Online CPD 2021**

- Faculty Presenter at **UNISVET Surgical Oncology Seminary Online Course 2020**
- Faculty Presenter **ESAVS-ASIA Course, SOFT TISSUE SURGERY II Chengdu, 2018, 2019**
- Faculty Presenter **ESAVS-ASIA Course, SOFT TISSUE SURGERY II Shanghai, March 2018**

Awards

- **Peter Holt Award 2014 with the presentation** "Influence of treatment on the outcome of dogs with incompletely excised grade-2 mast cell tumors" **Vincenti S**, Findji L" **AVSTS Spring Meeting 2nd April 2014**
- **1st prize as best resident forum presentation ECVS Congress 2013 with the presentation** "Influence of treatment on the outcome of dogs with incompletely excised grade-2 mast cell tumours" **Vincenti S**, Findji L. **Rome 2013.**

Funding

- Burgergemeinde Bern Wissenschaft Funding 2021– 3.700CHF
- Leonardo Project Fellowship 2011 – 6.000Euros

Memberships

- February 2019: ECVS Diplomate
- January 2015: Member of VSSO, Veterinary Society of Surgical Oncology
- March 2012: Member of BSAVA, British Small Animals Veterinary Association
- February 2011: MRCVS, Member of the Royal College of Veterinary Surgeons
- January 2010: Member of SIONCOV, Italian Society of Veterinary Oncology
- January 2009: Member of SCIVAC, Cultural Italian Society of Companion Animals Veterinaries

Scientific Expert Activities

- Animals (ISSN: 2076-2615)
- Veterinary Record Case Reports (ISSN: 2052-6121)
- SAT (ISSN: 0036-7281)

Personal skills and competences

Mother tongue: Italian
Other languages: English, German, French
Computer skills and competences: good knowledge of the Microsoft office suite (Microsoft word, excel and power point), internet explorer, Firefox and safari; daily use of e-mail programs; knowledge of Macintosh operating system (apple).
Personal skills: very sociable, emphatic, and loyal person. High communicative (also in different languages) and cooperative ability inside the team. Strong team spirit thanks to a very outgoing behaviour cultivated by playing team sport such as volleyball. Great ability to take over the lead of a group, keeping a friendly, respectful, and horizontal communication among the group. This capacity to lead a group has been particularly cultivated during the last few years of work in the small animal clinic of the University of Bern. Important capacity to understand and empower students and young colleagues to reach their potentials during research and clinical supervision.

Form A

Application for licence to perform animal experiment

National no. **32996** Cantonal no. **BE134/2020**

art. 18 Animal Welfare Act (SR 455), art. 141 Animal Welfare Ordinance (SR 455.1), art. 30 Animal Experimentation Ordinance (SR 455.163)

Basics01-08

01 Address of the applicant

Institute

Name	Abteilung Chirurgie und Orthopädie
Street	Departement für klinische Veterinärmedizin Postfach
Postal code	3001
Town	Bern
Company	Uni-BE

Resource manager

Name	Franck Forterre
E-mail	franck.forterre@vetsuisse.unibe.ch
Tel. no	031 631 24 01

Study Director

Name	Franck Forterre
E-mail	franck.forterre@vetsuisse.unibe.ch
Tel. no	031 631 24 01

02 Address of the cantonal authority

Name	BE
Street	Münsterplatz 3a
Postal code	3000
Town	Bern 8
Delegated application input by the canton in animex-ch	No

03 Intercantonal experiment

Will the experiment be performed in more than one canton?	No
If yes: Secondary canton(s)	None

04 Title of application

Indication of application title	Investigation of autologous gadolinium-marked extracellular vesicles for estimation of complete resection of canine brain tumors

05 Title for the publication

Informative title to be used for the publication according to art. 20 let. a Animal Welfare Act (SR 455) after the end of the experiment.

Application title used for thepublication	Investigation of autologous gadolinium-marked extracellular vesicles for estimation of complete resection of canine brain tumors

06 Application type

Corresponding application type	New Application

07 Maximum prospective degree of severity

Indication of the maximum prospective degree of severity of this application	1

08 Duration of project and date of start

Duration of project	2 years / 11 months / 28 days
Date of proposed start	01.01.2021

Animals 9-10

09 Animal list

Animal List

Category info
Animal category: Dogs
Sex: Mixed
Use of genetically modified animals: No
Has this line been newly established in this facility especially for this experiment?: No
Constrained Line: No

Data sheet info and related forms

Documents
- No Records

Number info
Previously approved: 0
Requested number of animals: 18
Total number of animals requested: 18

Origin of the animals
- Origin type: Non-approved animal holding
    - Origin ID: Other / Description of animal origin: Private owned dogs, whole Switzerland

Place where the animals are kept
- Place type: Non-approved animal holding
    - Place ID: Tierspital Bern, Kleintierklinik

10 Location of the experiments

Address	Room number	Description
Tierspital Bern, Länggassstrasse 128, 3012 Bern		

Personnel 11-14

11 Personnel details

Name	Role	Qualification status	Deputy Study Director	Area of responsibility
Franck Forterre	Resource Manager (RM)			Resource manager
Franck Forterre	Study Director of Institute (SDI)	●		Principal study director
Helene Rohrbach	Involved Person of Institute (IPI)	●	✓	Anesthesia
Simona Vincenti	Involved Person of Institute (IPI)	●		Treatment of dogs

12 Resource Manager

Name	Franck Forterre
Statement of responsibility	The Resource Manager confirms that the persons named in the list of persons are familiar with the regulations of the Animal Welfare Act (SR 455) and Animal Welfare Ordinance (SR 455.1) applicable to animal experiments and that they satisfy the educational and further training requirements.

13 Principal Study Director

Name	Franck Forterre
Statement of responsibility	The Principal Study Director confirms his / her responsibility as stated in art. 131 Animal Welfare Ordinance (SR 455.1).
Name of Deputy Study Director	Helene Rohrbach

14 Animal Welfare Officer of Institute

Name	Isabelle Desbaillets
Statement of responsibility	By submitting to the cantonal veterinary office, the Animal Welfare Officer confirms that the application has been completed in full and contains the information required to assess its necessity (art. 129 let. a Animal Welfare Ordinance (SR 455.1)).
Timestamp of the submission	04.11.2020 12:54:40

Purpose of the experiment 15-22

In Sections 16-18, mark only one entry in each category and, where appropriate, enter a further mark in a sub-category.

15 Field of study

Indication of the scientific field	Oncology

16 Area of application

The project is associated with following area of application	Research, development and quality control (excluding safety testing) of products or devices for human and veterinary medicine

17 Association with diseases or disorders

The project is associated with following diseases or disorders	Animal diseases: Cancer, canine brain tumors

18 Associated procedures required by law

Indication of the regulatory requirements	No association with procedures required by law

19 External expertise

Has the project been appraised?	Yes
If yes: Expertise rendered by	Expertise rendered by: Prof. of Biothechnology and Farmacology Paolo Ciana, University of Milan

20 Objective of the experiment and background

Brief description of the objective of the experiment (maximum one page). To assess **compliance with the indispensability** (see section 38 to 40) of the experiment according to art. 17 Animal Welfare Act (SR 455): (1) Description of the **aim**, (2) **Current state of research**, (3) Anticipated **knowledge to be gained**.

1. Aim of the study:

The aim of the present study is to demonstrate the capacity of gadolinium-marked autologous, patient specific extracellular vesicles (Gad-Evs) to pass the brain-blood barrier (BBB) and underline in detail the extension of a central nervous system (CNS) tumor in the dog. The second aim is to confirm the safety of Gad-EVs in the dog and a further aim is to demonstrate a higher capacity of post-injection Gad-EVs magnetic resonance imaging (MRI) images to help the veterinary surgeon in planning an accurate surgical excision of the tumor. This part of the study would then constitute the basis for future studies in the dog and human being.

2. Current state of research:

Brain tumors cause severe neurological deficit and when malignant are associated with poor prognosis due to their aggressive growth pattern and high recurrence rates, despite initial adequate surgical treatment[1,2]. Canine brain tumours seem very similar to human primary brain tumours regarding incidence, clinical presentation, histopathological characteristics and survival times[1,2]. Over the last few decades, dramatic improvement was made concerning human and veterinary oncologic patients' therapy. However, intraaxial brain tumors remain very challenging and often frustrating to treat. Concerning surgical excision, a crucial aspect is the relatively high incidence of incomplete excision, which lead to early recurrence and higher post-surgical morbidity[3].

For this reason, many researches focused in developing alternative therapies.

Extracellular vesicles (EVs) are a group of cells-derived membrane vesicles present in body fluids and able to carry several molecules like proteins, lipids and coding or noncoding RNA molecules[4]. Concerning tumorigenesis, EVs contribute to the acquisition of specific tumor characteristics like inhibition of cell death, invasion, metastasis and immunosuppression[5,6]. A very recent publication[7] demonstrated *in vivo* and *in vitro* an extraordinary cross-species tumor-tropism for cancer-derived EVs. In this study EVs generated from murine and human cancer lines and human liver biopsy from healthy individuals were loaded with fluorescent dyes alone or in combination with a biopharmaceutical agent (the oncolytical adenovirus - OV) and tested for their activity in cancer cell lines. Among the several results, it was found that when administered intravenously (i.v.) to the mouse model of cancer, the tumor-derived EVs, but not the healthy-tissue EVs demonstrated a selective accumulation of the fluorescence at the tumor site 24h after injection. A further finding was that loading the EVs even with a large OVs did not change the tumor-specific tropism of the EVs also in vivo, confirming the possibility of using EVs as carrier of small molecules drugs and biopharmaceuticals.

Other two recent studies described other interesting characteristics of EVs. One study demonstrated the feasibility of loading EVs with an MRI contrast agent, gadolinium, and the accumulation of gadolinium-loaded EVs within ectopic osteosarcoma tumour-bearing mice[8]. The other study demonstrated that tumor-derived EVs can breach the intact BBB in vivo[9]. These extraordinary findings constitute a great substrate to launch completely new and revolutionary scenarios concerning diagnostic, multimodal therapy planning and follow-up plan. In particular, the combination of these technologies can enable the veterinary oncological surgeon to plan and perform the best *surgical doses* in face of canine brain tumors.

-

3. Anticipated knowledge to be gained:

Autologous, patient-derived Gad-EVs are able to pass the BBB and link selectively to central nervous tumor cells in the dog

The selective link of Gad-EVs with CNS tumoral cells will enable the veterinary surgeon to plan a more accurate surgical excision of the tumor.

All dogs will be client-owned dogs and all owners will sign a consent form prior to study enrolment. The results of the present study should provide the rationale for a new avenue of precise surgical planning and multimodal therapeutic plan for canine patient with spontaneous CNS tumors. The results of the canine study might provide some important knowledge also for human medicine (translational value).

-

Literature:

1. Meuten DJ, Tumor in Domestic Animals. (Kohn Wiley & Sons, 2016)

2. Bentley RT, Ahmed AU, Yanke AB et al. Dogs are man's best friend: in sickness and in health. Neuro-Oncology 2017;19:312-322.

3. Shah JL, Gordon L, Shaffer JL et al. Stereotactic Radiosurgery and Hypofractionated Radiotherapy for Glioblastoma. Neurosurgery 2018;82:24-34.

4. Paolicelli RC, bergamini G, Rajendran L. Cell-to-cell communication by extracellular vesicles: focus on microglia. Neuroscience 2018;405:148-

157.

5. Asfar SA, Bin Bao, FHS. Cancer Metastasis Rev. 2014;32:1-33.

6. An T, Qin S, Xu Y et al. Exosomes serve as tumour markers for personalized diagnostics owing to their important role in cancer metastasis. J

Extracell Vesicles. 2015;4:1-5.

7. Garofalo M, Villa A, Crescenti D et al. Heterologous and cross-species tropism of cancer-derived extracellular vesicles. Theranostics.

2019;9:5681-5693.

8. Abello J, Nguyen TDT, Marasini R et al. Biodistribution of gadolinium- and near infrared-labeled human umbilical cord mesenchymal stromal cell derived exosomes in tumor bearing mice. Theranostics. 2019;9:2325-2345.

9. Morad G, Carman CV, Hagedors EJ et al. Tumor-Derived extracellular vesicles breach the intact blood-brain barrier via transcytosis. ASC Nano. 2019;13:13853-13865.

Document(s): 0

21 Results of the previous application

Maximum one page must be completed in the case of renewal applications. Brief summary of the results of the previous licensing procedure including the number of animals used, the degree of severity, and the rationale for renewal of the application.

Document(s): 0

22 Hypotheses

Formulate the research question to be answered in the experiment or the hypothesis (hypotheses) to be tested(confirmatory study).

. Hypothesis 1:

Autologous, patient-derived Gad-EVs are able to pass the BBB and link selectively to central nervous tumor cells in the dog

. Hypothesis 2:

The best Gad-EVs post-injection MRI (MRI 1. #) will be obtained 24 hours after Gad-EVs injection.

Document(s): 0

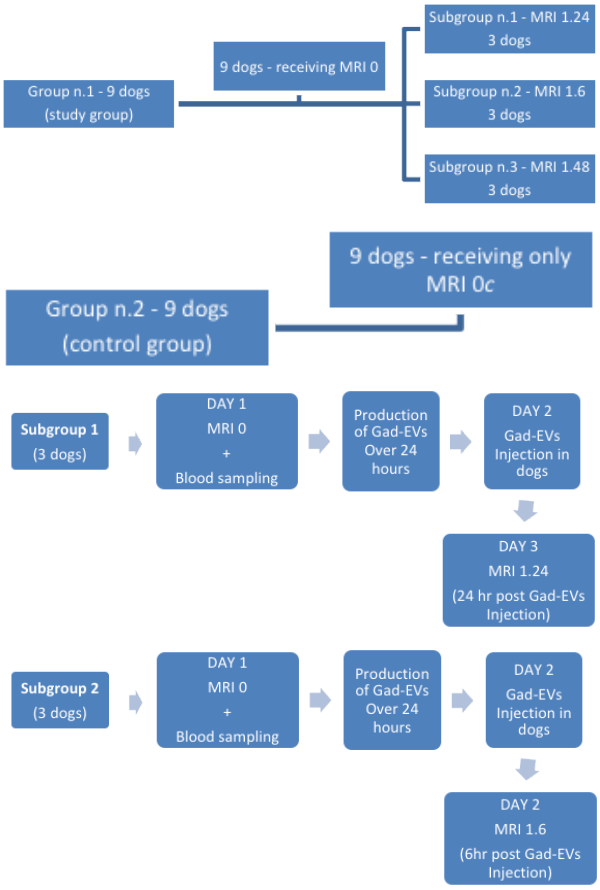
Course of the experiment (Method I) 23-27

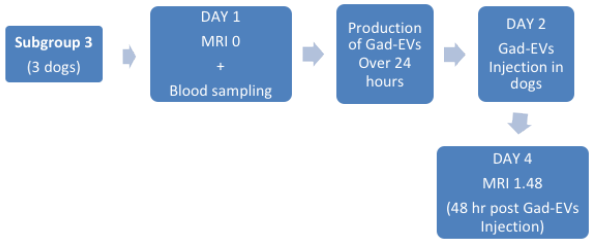
23 Course of the experiments: Schematic representation

Description of the course of the experiment from a **temporal perspective**. Representation of the course of the experiment or individual steps (e.g. flow chart, workflow diagram, table). To include: animal model, animal groups, the overall duration of the experiment for each group.

The whole project is planned in two stages. The first part of the project will be performed on lab animals of the Centre of excellence on neurodegenerative disease of the University of Milan. In this first part we will demonstrate the efficacy of Gad-EVs MRI images in providing excellent and detailed information about the extend and infiltration pattern of the mice CNS tumor. This precise information will be used to perform an adequate surgical excision of the tumor.

The second part of the project will be performed in 18 client-owned dogs coming at the small animal clinic of the veterinary hospital of the University of Bern. The aim is to demonstrate the capacity of Gad-EVs to pass the BBB and guide the veterinary surgeon in planning the surgical excision of a spontaneous CNS tumor.





Duration of the different phases of the experiment:

- MRIs (MRI 0, MRI 1. #): 60minutes each MRI
- Blood sampling: 5 minutes
- Extraction of autologous, patient-specific EVs and elaboration to obtain Gad-EVs: 24 hours
- Gad-EVs Intravenous injection: 15 minutes without sedation, 30 minutes if a sedation is necessary

Document(s): 0

24 Preparation of animals for the experiment

Assessment of the preparation of animals for the experiment (art. 119 Animal Welfare Ordinance (SR 455.1)): Description of screening examination,preparation to the experimental conditions, method of marking or identification and combination with the method of genotyping.

The patients will be routinely prepared for conventional anaesthesia. Depending on the development and the urgency of the clinical presentation, the patients will be treated on a routine basis. Patients with CNS tumors will be presented for the MRI 0 and, if the clinical conditions will permit it, will be sent home immediately after the MRI and the blood sampling.

Patients belonging to the Subgroup n.1 (MRI 1.24) will be re-presented at the small animal clinic of the University of Bern 24 hours after MRI 0 to receive the Gad-EVs i.v. injection. Twenty-four hours after the Gad-EVs injection the second MRI (MRI 1.24) will be performed, following the same anaesthetic protocol followed during MRI 0.

Patients belonging to the Subgroup n.2 (MRI 1.6) will be re-presented at the small animal clinic of the University of Bern 24 hours after MRI 0 to receive the Gad-EVs i.v. injection. Six hours after the Gad-EVs injection the second MRI (MRI 1.6) will be performed, following the same anaesthetic protocol followed during MRI 0.

Patients belonging to the Subgroup n. 3 (MRI 1.48) will be re-presented at the small animal clinic of the University of Bern 24 hours after MRI 0 to receive the Gad-EVs i.v. injection. Forty-eight hours after the Gad-EVs injection the second MRI (MRI 1.6) will be performed, following the same anaesthetic protocol followed during MRI 0.

Document(s): 0

25 Procedures / manipulations on the animal

Assessment of licence conditions (art. 140 Animal Welfare Ordinance (SR 455.1)) and suitability(section 38) of the method (art. 137 para. 3 Animal Welfare Ordinance (SR 455.1): Indicate the details of the various manipulations/interventions on the animal.

2. MRI of the head (MRI 0 and MRI 1. #): the patient will be placed under general anaesthesia as during MRI the patient is not allowed to move and because due to the loudness of the MRI exam the canine patient could get scared and stressed.
3. Harvesting of 15ml of blood from the left jugular vein: this procedure will be performed when the patient will be already under general anaesthesia and will not be of any hurt for the patient. The left jugular vein will be use for the blood sampling in a standard fashion, in order to be able to sample 15ml of blood in a short period of time and to keep the right jugular vein intact in the perspective of placing a central venous catheter.
4. Intravenous injection of autologous, patient-derived Gad-EVs: the suspension containing the Gad-EVs will be injected through the right jugular vein using a standard intravenous catheter (20G, Jelco 2 IV Catheter Radiopaque, Smiths Medicals).

Document(s): 0

26 Anaesthesia and / or analgesia

Assessment of the application in accordance with the implementation provisions (art. 135 Animal Welfare Ordinance (SR 455.1): Indicate the medications to be used, the dose and volume, the intended routes of administrationand consider the need of continuing administration.

Anaesthesia protocol for brain MRI performed at the Tierspital Bern.

Anaesthesia protocol for CNS tumors performed at the Tierspital:

- Buthorphanol 0.2-0.4mg/kg IM or IV, Medetomidine 0.002-0.02mg/kg IM/IV if necessary
- Induction: Propofol to effect 2mg/kg IV, Midazolam 0.2-0.4mg/kg IM/IV if necessary
- Following endotracheal intubation, anaesthesia will be maintained with inhalant agents (Sevofluorane/Isofluorane)
- Lung ventilation will be kept under control aiming at an EtCO2 between 28 and 35mm Hg

Rationale of anaesthesia / analgesia

Reasons for selecting or for not using analgesics and anaesthesia should be stated.

Anesthesia is necessary for canine patients undergoing an MRI study in order to guarantee absolutely no stress for the patient and high imaging quality. In fact, in order to acquire high MRI quality, the patient is not allowed to move. Due to the loudness of the MRI exam, the canine patient could be scared and stressed. Anaesthesia protocols are adapted to the patients and have been used routinely with very good results. During the whole procedure, the patient will not feel any pain or distress.

Document(s): 0

27 Methods of euthanasia

Assessment of the application concerning the proper conduct of euthanasia (art. 135 Animal Welfare Ordinance (SR 455.1)): Indicate the method of euthanasia, stating the substance used, the dose and route of administration, and the procedure to ensure the death of the animal.

The study does not include a euthanasia. Owner dogs that will return to their owners immediately after MRI. For dogs deceased or euthanized for reasons unrelated to our study, the owners will decide on the further procedure.

Document(s): 0

Evaluation of the experiment (Method II) (28-31)

28 Recorded parameters

Brief description of the parameter, importance, relation to research question.

Recorded parameters	Comments
1. ability of the autologous, patient-specific Gad-EVs to breach the BBB and selectively link to CNS tumor cells 2. increased capacity for the veterinary surgeon to accurately plan a possible surgical excision of the CNS tumor and further therapy like stereotactic radiotherapy	1. Due to their neoplastic cell-specific origin, Gad-EVs are able to link selectively only to neoplastic cells. This will be shown and confirmed by the MRI contrast enhancement induced by the presence of Gadoteric acid inside the Gad-EVs. In comparison to a standard MRI with contrast medium (Gadoteric acid) injected IV, the capacity of isolation only neoplastic tissue will be greatly improved. 2. The higher and more specific imaging quality in isolating only neoplastic tissue will enable the veterinary surgeon to plan a much more targeted therapeutic approach. All these data will be then be translated to human oncology to improve the therapeutic strategies in face of malignant CNS tumors.

29 Experimental set-up and study design

Description of the study design and planning: Number of animals per experiment/series of experiments, number of groups and number of animals per group. To indicate a description of each group. Specific considerations to include: allocation concealment / randomization, blinding, sample size calculation (rationale to be entered in section 30), in- and exclusion criteria, definition of primary outcome variables, statistical analysis plan.

Number of animals, groups and subgroups:

. Number of animals (patients) enrolled in the study: 9 dogs in the study group

. Number of subgroups: 3 subgroups, each subgroup will have 3 patients

. Number of patients in the control group: 9 dogs which will not undergo any experiment

Randomization, blinding, sample size calculation:

. Randomization: the first 3 patients included in the study will undergo the MRI1.# 24 hours after the Gad-EVs injection (MRI 1.24). The following six patients will receive an MRI 1.# either 6 hours or 48 hours after the Gad-EVs injection. The allocation of those 6 patients to one or the other group (MRI 1.6 or MRI 1.48) will be randomized using the Excel function for random number calculation.

. Blinding: the clinician responsible to read the MRI study (Prof Forterre and Prof Schweizer) will not know the timing at which the MRI 1.# was performed. In this way a double-blinded evaluation of the post Gad-EVs injection MRI will be performed.

Sample size calculation:

See details at section 30.

Study design and planning:

1. Canine patient enrolment, generation and injection of Gad-EVs from canine patient presenting with spontaneous CNS.

Client owned dogs presenting with a confirmed diagnosis of intra-axial brain tumor and with a body weight >15kg will be included in the study. To be included in the study owners have to sign a consent form (see attached documents: *Information about animal experiment for dog owners.docx, Informationen über Tierversuche für Hundebesitzer.docx, Informations sur l'expérimentation animale pour les propriétaires de chiens.docx*).

Each patient (n=9) will receive an MRI examination of the brain including post contrast sequences after i.v. injection of Gadolinium (0.15mmol/kg gadoteric acid, Clariscan) This MRI study will be defined as "MRI 0". From each of the 9 patients undergoing the experiment 15ml of blood will be collected and used to generate autologous Gad-EVs at the University of Bern. All blood samples will be refrigerated and elaborated for the extraction and production of autologous patient-derived EVs that will be marked with Gadolinium within 24 hours by the blood sampling. Briefly, EVs will be isolated from canine plasma, centrifuged and then loaded with Gadolinium (gadoteric acid).

After production, the patient-specific Gad-EVs will be injected back into the canine patient through a jugular injection. Patient-specific Gad-EVs will be injected in the patients within 24 hours after their extraction and mark with Gadolinium.

The control group (n=9) will consist of canine patients with body weight >15kg which have a diagnosis of CNS tumor and received a standard brain-profile MRI (MRI 0*c*, where *c* stays for *control*) with peripheral i.v. injection of Gadolinium (0.15mmol/kg gadoteric acid, Clariscan). These 9 patients will not undergo any additional procedure and therefore are not included in the experiment itself. They will undergo an MRI study as patients presenting with intracranial disease signs.

2. Perform post-injection Gad-EVs MRI

Each of the 9 canine patient enrolled in the experiment will receive the patient-specific Gad-EVs injection via a jugular injection. Then the patient will undergo the post-injection MRI study. This MRI study will be defined as "MRI 1. #" where the # will depend on after how many hours will the post-injection MRI be performed.

. Subgroup 1: to this group will belong the first 3 patients enrolled in the study will receive an MRI 24-hours (MRI 1.24) after injection of patient-specific Gad-EVs.

If the Gad-EVs will reach the CNS tumor and will selectively mark the neoplastic cells at 24h post-injection, the following cases will undergo the Gad-EVs post-injection MRI at different times.

In particular:

. Subgroup 2: 3 patients will receive an MRI 6-hours (MRI 1.6) after injection of patient-specific Gad-EVs

. Subgroup 3: 3 patients will receive an MRI 48-hour (MRI 1.48) after injection of patient-specific Gad-EVs.

3. Define the best timing for Gad-Evs post-injection MRI plan the surgical excision of the spontaneous CNS tumor in the canine patient

Depending on the MRI images obtained with the post-injection Gad-EVs MRI the best images quality and therefore the best timing for the MRI 1. # will be defined.

Performing of a surgical plan by two DECVS depending on the MRI images obtained before and after Gad EVs injection in a blinded system. Compare the surgical planning between the standard Gadolinium-MRI and the Gad-EVs MRI.

Document(s): 3

30 Rationale for the numbers of animals

Indicate the **reasons** for the planned numbers of animals, including the method used for statistical analysis(e.g. t-test, ANOVA, mixed-effects model, etc.). If possible, indicate the test parameters (statistical coefficients, level of significance, power).

Power calculation:

Power calculation were performed with the online tool AusVet Epitools (https://epitools.ausvet.com.au/)

. A sample size of 3 animals is sufficient to detect the breaching capacity of the Gad-EVs through the canine BBB with a power of 95% if the breaching is present at 24hrs, 6hrs and 48hrs after Gad-EVs injection respectively in at least 65% of all animals.

Descriptive statistic will be used to analyse the study results.

Document(s): 0

31 Expertise for statistical analysis

Indicate whether the experimental design and the planned statistical analysis have been verified by a person with expertise in biostatistics.

Yes

Handling of animals 32-37

Indicate the effects of the experimental methods on the animals, stress-reducing measures and use of the animals after the end of the experiment.

32 Downgraded husbandry conditions

Assessment of the necessity of restricted housing (see art. 117 Animal Welfare Ordinance (SR 455.1)): Details and reasons for any deviations from conditions in which animals are kept as defined in the Animal Welfare Ordinance (SR 455.1).

Not applicable. Dogs are owner dogs that will be treated at the small animal clinic of the Vetsuisse Faculty of Bern.

Document(s): 0

33 Effects on the animals

Assessment of all expected adverse effects on the animals (art. 19 para. 4 Animal Welfare Act (SR 455)) and of further strain imposed on the animals through humiliation, through major interference in their appearance or their capabilities or through excessive instrumentalisation (art. 25 and 26 of the Animal Experimentation Ordinance (SR 455.163)).

None. The two anaesthetic procedures follow extremely secure and standardised protocols. The blood sampling will be conducted under general anaesthesia and in a very standardised fashion. The Gad-EVs i.v. injection involve the injection of autologous, patient-derived EVs charged with a molecule, gadoteric acid, which is normally used and well established to be a secure and harmless molecule for dogs and humans.

Document(s): 0

34 Monitoring of well-being

Assessment of the monitoring and documentation (art. 135 and 144 Animal Welfare Ordinance (SR 455.1)): To indicate are the criteria for **intervention** and **termination** (**humane endpoints**), and the frequency of checks (who carries out checks, documentation and how often during which study phase?). Add a **score sheet** if appropriate.

Since the study participants are also patients in the small animal clinic, the management and care of the animals is carried out by the veterinarian who will be responsible for the patient enrolled in the study. The veterinarian responsible for the study patients is Dr. Med. Vet. Simona Vincenti, a senior surgeon of the Kleintierklinik, which has high clinical experience with small animals.

Adequate analgesia (the analgesic protocol will be adapted depending on the patient necessity and safety) and patient care will be provided and the natural needs of the animals will be covered at any time (food twice daily, water always, regular -up to 6 times daily- possibility for urination and defecation).

Each patient should return to the owner the same day after the MRI 0, after the Gad-EVs and after the MRI 1. # unless the clinical condition related to the CNS tumor require a hospitalization.

For the pre-experiment clinical exam and the post-experiment monitoring, please refer to the following attached documents:

- Initial clinical exam patients.pdf
- Patient hospitalization sheet.pdf
- Well-being score (WBS) sheet during hospitalization.pptx
- Well-being score (WBS) sheet for owners – EN DE FR.docx

In case a hospitalization will be needed, the frequency of the re-check will be every 8 hours. If necessary the frequency of those re-check will be increased accordingly to the *WBS sheet during hospitalization* document.

If the patient will be discharged the same day after the MRI 0 and MRI 1.#, the owner will be provided with the *Well-being score (WBS) sheet for owners* and the contact of the responsible Dr.Med.Vet. (Simona Vincenti) will be given to the owner. In this way, in case of necessity, the owner will be able to contact Dr.med.vet. Simona Vincenti at any time after the discharge of his/her dog.

Document(s): 2

35 Refinement

Assessment concerning implementing rules**under the specific experimental conditions** according to art. 135, 137 para. 4 and 144 Animal Welfare Ordinance (SR 455.1). To indicate the details of mitigating measures or reasons for not using such measures. Which mitigating measures are taken to reduce stress or minimize anyharm imposed on the animalsunder the specific experimental conditions?

The experimental conditions do not include any particular procedure, which can induce stress or pain for the animal. In fact, the procedures, which will be performed on animals, belong to standard procedure done on patient, which undergo a general and neurological exam, a blood sampling and an MRI of the brain.

In particular:

. General and neurological exam: they do not include any painful procedure; therefore, any mitigation measure is to be taken. Furthermore, those exams will be done in the presence of the animal owner, which will reduce the potential stress to the animal.

. Blood sampling for extraction of the EVs: a standard jugular blood sampling will be performed. In order to reduce at minimum the stress for the patient a local anesthetic cream (EMLA Cream) will be applied on the shaved skin of the patient 10 minutes before the blood sampling. In this way, the blood sampling will be pain-free for the patient. Furthermore, in case the animal temperament (anxious, distrustful or aggressive) will prevent a stress-free blood sampling, the animal will undergo a short and safe sedation which will allow to perform the blood sampling without inducing any pain or stress to the animal.

The sedation protocol is:

. Buthorphanol 0.2-0.4mg/kg IM or IV, Medetomidine 0.002-0.02mg/kg IM/IV.

This protocol is a very safe and commonly used sedation protocol used for dogs. In case of necessity, the protocol will be adjusted to suits at best to the animal-specific clinical condition and requirement.

. Intravenous injection of autologous Gad-EVs: a standard jugular venous catheter will be placed to perform the Gad-EVs injection. In order to reduce at minimum the stress for the patient a local anaesthetic cream (EMLA Cream) will be applied on the shaved skin of the patient 10 minutes before the placement of the jugular catheter and the injection. In this way, the jugular catheter placement will be pain-free for the patient. Furthermore, in case the animal temperament (anxious, distrustful or aggressive) will prevent a stress-free jugular catheter placement, the animal will undergo a short and safe sedation which will allow to perform the blood sampling without inducing any pain or stress to the animal.

The sedation protocol is:

. Buthorphanol 0.2-0.4mg/kg IM or IV, Medetomidine 0.002-0.02mg/kg IM/IV.

This protocol is a very safe and commonly used sedation protocol used for dogs. In case of necessity, the protocol will be adjusted to suits at best to the animal-specific clinical condition and requirement.

. Performing of MRI 0 and MRI 1. #: to perform an MRI study in animals a general anaesthesia is required. In this way the patient will not suffer any stress from the loudness of the machine and from the prolonged, still positioning required achieving high quality MRI images. The anaesthetic protocol is specified at section n.26.

Document(s): 0

36 Distribution by degree of severity

Summary evaluation of the expected maximum stress on the animals (art. 26 Animal Experimentation Ordinance (SR 455.163)):Indicate the maximum expected degree of severity for each animal category and group. The expected number of animals for each anticipated degree of severity should be given as a percentage. The allocation is based on FSVO technical information no 1.04.

The canine patients enrolled in the study are private owned dogs. All dogs will undergo standard diagnostic imaging. Intravenous application of contrast medium, a repeated standard anesthesia and MRI investigation will be the supplementary stress applied to dogs enrolled in the study. Degree of severity: 1

Document(s): 0

37 Use of the animals at the end of the experiment

Assessment of the application concerning the implementing rules with regard to the further use of animals after an experiment (see art. 20 Animal Welfare Act (SR 455); art. 141 para 4 Animal Welfare Ordinance (SR 455.1).

As each canine patient included in the study is a private owned dog, the patients will immediately returned to his or her owners

Document(s): 0

Rationale and weighing of interests[38-40]

Details of the reasons and justifications for selecting the experimental method and animal category.

38 Suitability

Reasons for selecting the animal model with regard to the experimental objective and depiction of scientific validity (i.e. construct validity, internal validity, and external validity) and reproducibility of the expected findings, if appropriate. Show the extent to which it is possible to generalise or extrapolate to other study conditions, populations of animals or species, incl. humans.For regulatory experiments, indicate if an experiment is required by the authorities.

Dogs affected by spontaneous CNS malignant tumor are an excellent model for human patients affected by CNS malignant tumors. Because both species shear a very bad prognosis, there is a strong need for new diagnostic and therapeutic options.[1,2] Dogs with a spontaneous CNS malignant tumor can therefore represent a mid-way between laboratory mice models and human being. Such a study has not been done so far and represents the very first project evaluating the possibility for autologous, patient-specific Gad-EVs to breach the BBB and selectively mark only and all neoplastic cells (belonging to both primary and metastatic disease). Should the results from our study fulfill our expectation and confirm our hypothesis, these results would have a great impact in the diagnostic and therapeutic options for canine and human patients affected by malignant CNS tumors.

Document(s): 0

39 Necessity (3R)

Reasoning why the intended aim of the experiment **cannot be achieved by methods that comply better with the 3R criteria**. Explain also why a method that does not require animals does not exist (**Replace**), why the experiment cannot be carried out with fewer animals (**Reduce**), and how all possibilities to reduce the strain on the animals are exploited (**Refine**).

There is no other methodology, which could replace our methods. A 3D, *in vitro* reproduction of the BBB was used to test the capacity of the EVs to breach the BBB[9]. This interesting model however, cannot replace a natural BBB. As the dog is an excellent model for human CNS malignant tumors, another animal model or an in vitro methodology cannot replace the canine model.

Document(s): 0

40 Weighing of interests

Assessment of the application with regard to the balance between expected gains in knowledge or other results (interests) and the pain, suffering,harm, injury or anxiety inflicted (strain on the animals) in accordance with ethical considerations. For more details on the weighing of interests, see the “Explanatory notes to Form A” and the document “Weighing of interests in animal experimentation” [LINK www.blv.admin.ch].

The results of this preliminary study will indicate if a study at a larger scale is justified. In this case, a new study design based on a power analysis determining the number of participants to be included will be set-up. Because dogs are often used as models in human medicine, the present and similar studies could be regarded with great interest to gain further insight into the processes involved. A better understanding of the behaviour of CNS tumors in the dog would be advantageous in order to facilitate the development of more effective diagnostic and therapeutic strategies. Furthermore, EVs can have extraordinary applications in terms of early diagnosis,

staging, imaging, therapy and follow-up strategies of malignant tumors in general. Therefore evaluating these molecules on clinical veterinary patients, is essential to provide fundamental information to improve patient quality of life of veterinary but long term potentially also human patients.

Document(s): 0

List of documents

Type	Title	Description	Filename	Date
Application	Well-being score owners .pdf		Well-being score owners .pdf	
Application	Well-being score hospitalization.pdf		Well-being score hospitalization.pdf	
Field	Patient hospitalization sheet.pdf		Patient hospitalization sheet.pdf	
Field	Information for dog owners.pdf		Information about animal experiment for dog owners.pdf	
Field	Informationen für Hundebesitzer.pdf		Informationen über Tierversuche für Hundebesitzer.pdf	
Field	Informations pour les propriétaires de chiens.pdf		Informations sur l'expérimentation animale pour les propriétaires de chiens.pdf	
Field	Initial clinical exam patients.pdf		Initial clinical exam patients.pdf	