Safety and efficacy of Blood Brain Barrier opening with implantable device Sonocloud® combined with Nivolumab used alone or an association with Ipilimumab in brain metastases from patients with malignant melanoma

SONIMEL 01

INTERVENTIONAL STUDY OF A MEDICAL DEVICE OR AN IN VITRO DIAGNOSTIC MEDICAL DEVICE

Version N° 6.1 of 12/12/2022
Project code number: P160950J / N° EUD2018-002568-26 / IDRCB 2018-A02646-49

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Signature Page for an Interventional Study of a Medical Device or an in Vitro Diagnostic Medical Device

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The study was approved by the CPP of **Nord-Ouest III** on **06 April 2019** and authorized by the ANSM on **15 January 2019**.

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1. SUMMARY

Full title	Safety and efficacy of Blood Brain Barrier (BBB) opening with implantable device Sonocloud® combined with Nivolumab used alone or an association with Ipilimumab in brain metastases from patients with malignant melanoma
Acronym	SONIMEL 01
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Sponsor	Assistance Publique-Hôpitaux de Paris
Scientific justification	Anti PD-1 monoclonal antibodies (nivolumab and pembrolizumab) alone or in association with antiCTLA4 (Ipilimumab) are established as indisputable treatment of metastatic melanoma, with unprecedented overall survival, and are indicated for first-line treatment including patients with BRAF mutation. Given their high molecular weight, their penetration in the brain sanctuary is uncertain and relies on disruption of the BBB which occurs occasionally. SonoCloud® is an implantable device delivering low intensity pulsed UltraSound (US). Along with systemic injection of an US resonator, SonoCloud® demonstrated safe and efficient at repetitively opening the BBB. We anticipate that BBB opening could help at increasing brain penetration of monoclonal antibodies and potentially boosting immunity in the brain. This could translate in controlling brain disease with the same magnitude as for extracranial disease. This would also open avenues for optimizing the treatment of brain metastases in combination with checkpoint inhibitors in many other cancers.
Main objective and primary endpoint	*One single objective, one single endpoint* The main objective will be the determination of the most successful dose (MSD), defined as the dose with the highest probability of BBB opening efficacy without toxicity directly related to the ultrasound emission. Primary endpoint: Success defined as grade 2 or 3 BBB opening/disruption with pulsed US using the SonoCloud® system without toxicity related to the device assessed clinically and using brain MRI (DLT evaluated during the first 4 weeks of treatment for Nivolumab alone and 6 week of treatment for Nivolumab + Ipilimumab) Thus, the primary endpoint will be the success defined as efficacy without toxicity during the first 4 weeks of treatment for Nivolumab alone and 6 week of treatment for Nivolumab + Ipilimumab.
Secondary objectives and endpoints	Secondary objectives: - clinical efficacy of Nivolumab +/- Ipilimumab in the context of BBB opening in the study population -Safety of Nivolumab alone or combined to Ipilimumab in the context of BBB opening

	Secondary endpoints: - Best overall response rate and ORR at 3 months - Best intracranial overall response rate (BICORR) and ICORR at 3 months - Best extracranial overall response rate (BECORR) and ECORR at 3 months - Progression free survival (PFS) at 3 months 6 months, 12 – 18 and 24 months - Intracranial progression free survival (intracranial iPFS) at 3 months 6 months, 12 – 18 and 24 months - Extracranial progression free survival (extracranial ePFS) at 3 months 6 months, 12 – 18 and 24 months - Overall survival (OS) at 4 months 6 months, 12 – 18 and 24 months - Safety using CTCAE version 5.0 Clinical activity will be assessed using TEP/TDM Scans (extracranial lesions) and MRI (intracranial metastases). Clinical objective response rate (ORR) (defined with the RANO and immunotherapy RANO and RECIST version 1.1 and the Immune-Related Response Criteria) and PFS defined as time from day 1 of immunotherapy perfusion and sonication to disease progression (according to the RECIST version 1.1and RANO) and the immune related Response Criteria- or death, whichever is first.
Design of the study	21 patients treated at maximum Considering that a maximum of 10% would withdraw from participating in the research a budget for 21+2 = 23 patients will be considered
Population of study participants	Advanced brain metastases melanoma patients, Age > 18 years
Inclusion criteria	- Patients with histologically confirmed metastatic melanoma - Patients must have recovered from all side effects of their most recent systemic or local treatment for metastatic melanoma (grade ≤ 1). - At least one measurable brain metastasis between 5mm and 35mm in diameter, not previously treated with surgery and/or radiosurgery and located less than 5 cm from the skull - Patients may have received -or not- prior radiosurgery and/or surgery for brain metastases; if they have received prior local treatment, they must have at least 1new RANO and RECIST assessable brain metastases. - BRAF status wild type or mutated (and in that case previous treatment with BRAF inhibitor and MEK inhibitor allowed) - Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 1. - Age > 18 years - Hemoglobin ≥10g/dL - Platelets ≥ 100000mm^3

	- Neutrophils ≥ 1500/mm^3 - Creatinine Clearance ≥ 50mL/mn - AST < 3N - ALT < 3N - Total bilirubin < 1.5N - Alkaline phosphatase < 3N - INR < 1.5 - Prothrombin ≥ 70% - TCA < 1.2 - No Hepatocellular insufficiency - No unhealed wound on the head - No allergy to poly isoprene - Signed informed consent - Patient with health insurance coverage - Life expectancy > 3 months
Non-inclusion criteria	- Ocular melanoma - Symptomatic lepto-meningeal involvement. - Symptomatic hemorrhagic brain metastases. - Symptoms of incoercible intracranial pressure; patients receiving corticosteroids and patients presenting intermittent seizures can be enrolled if they have a stable dose of corticosteroids (≤ 30mg/day corticotherapy) and anti-epileptic treatment since at least 2 weeks before enrolment. - Indication for urgent neurosurgery or radiotherapy - Prior malignancy active within the previous 2 years except for locally curable cancers that have been apparently cured or stage I untreated Chronic Lymphoid Leukemia. - Known HIV infection and any ongoing infectious disease or significant background. - Concurrent administration of any anticancer therapies other than those administered in this study. - Treatment with any cytotoxic and/or investigational drug, antiCTLA4 or targeted therapy ≤ 4 weeks or < 5 half-lives for targeted therapies or chemotherapy, prior to day 1 of study. - Prior whole brain radiotherapy - Pregnant or lactating women - Patient with auto immune disease - Contraindications to nivolumab alone or an association with Ipilimumab as defined in SPC (https://www.vidal.fr/substances/24410/nivolumab/) - Serious or uncontrolled medical disorders that, in the opinion of the investigator, may increase the risk associated with study participation or study drug administration impair the ability of the patient to receive protocol therapy, or interfere with the interpretation of study results. - Allergy to iodine, gadolinium, lidocaine - Contra-indications to SonoVue® : hypersensibility to sulfur hexafluoride, recent acute coronary syndrome or unstable ischemic heart disease

	congestive heart failure ≥ Class III or IV as defined by New York Heart Association concurrent treatment with Dobutamine severe pulmonary arterial hypertension uncontrolled systemic hypertension respiratory distress syndrome - Concurrent treatment for the CNS : such as - Benzodiazepin (or any other sedative/hypnotic) - Antihistaminic - Pro convulsing drug - Butyrophenons, phenothiazin or any other "conventional" antipsychotic - Barbituric - MAO inhibitor - Anticholinergic - Any other drug according investigator to cause cerebral toxicity due to BBB opening - Concurrent anticoagulant or antiplatelet therapy - Uncontrolled epilepsy - MRI contra indication (claustrophobia, intracorporal metallic material…) - Phlebitis, active pulmonary embolism - Prisoners or subjects who are involuntarily incarcerated - Psychological, familial, sociological, or geographical conditions that potentially hamper compliance with the study protocol and follow-up schedule; those conditions should be discussed with the subject before registration in the trial - Treatment with any drugs authorized under special conditions of stable dosage ≥ 1 month prior inclusion: • Inhibitors of acetylcholine esterase (i.e., donepezil Aricept, galantamine, rivastigmine) Memantine • Atypical antipsychotics (eg Quetiapine) • Antidepressants (other than MAO inhibitors and anticholinergics) Dose <20 mg / kg • Thyroid hormones
Exclusion criteria :	- Implantation of the SONOCLOUD® not possible according to neurosurgeon (Any patient morphological characteristics (e.g. skin characteristics, bone thickness, other), which, from neurosurgeons' opinion, prevent implantation of the device or may impair the ability of the patient to receive treatment with SonoCloud®, would be excluded)
Device(s) under investigation	SONOCLOUD®1 (SC1) SonoCloud® is manufactured by CarThera, France SonoCloud® 1 is composed of a cranial implant that uses ultrasound energy to yield BBB opening. SonoCloud® is an active implantable device (implantation duration until 16 weeks at maximum after inclusion), to be regulated as a Class III medical device. SonoCloud® is not CE marked yet.

Comparator arm	Non applicable
Interventions added for the trial	Sonocloud® implantation, US session, Sonocloud® explantation
Expected benefits for the participants and for society	Patients with advanced melanoma and brain metastasis are most often excluded from clinical trials. Therefore, clinical trials in this high unmet medical need population are awaited. Safety and feasibility of opening the BBB in patients with advanced melanoma and brain metastases treated with Nivolumab alone or an association with Ipilimumab would pave the way to (1) change SOC and prognostic of patients with advanced melanoma when brain metastases occur, (2) test other checkpoints or combination of checkpoint inhibitors in brain metastases of melanoma, (3) provide rationale for evaluation of antiPD-1 agents in other cancers where immunotherapy has been demonstrated efficient such as lung cancer.
Risks added by the trial	*Risks associated with the SonoCloud® device duringdevice implantation /explantation* *Risks associated with the US cures* *Risks associated with the a* 18F-FDOPA PET/CT
Scope of the study	1/ Study proposal to a patient during a consultation. Patient will have a delay of 2 days of reflection before signature of consent. 2/ Screening and inclusion visit to St Louis Hospital. • Verification of the inclusion / non-inclusion criteria of the study • Agreement of the patient to participate to the study and signature of the information note, 3/ Radiological assessment : scanner TAP + Brain IRM (15 to 21 days maximum before first dose), 4/ Consultation with the neurosurgeon and the anesthetist, (48h maximum after the radiological assessment). 5/ Sonocloud implantation (48h to 7 days after the consultation). 6/ Treatment (first treatment will be performed at least 7 days of implantation – a period of 15 days is possible if the patient's clinical condition allows it and if the investigator considers it necessary). 7 sonications will be performed for the patient with Nivolumab alone and 5 Sonications will be performed for patients with Nivolumab an association with Ipilimumab. 7/ A 18F-FDOPA PET/CT will be performed +/-8 days before the first sonication and 12 weeks after this first treatment course (±15 days). 8/ Explantation before the end study (16 weeks at maximum after inclusion).
Number of participants included	21 patients treated at maximum Considering that a maximum of 10% would withdraw from participating in the research a budget for 21+2 = 23 patients will be considered
Proposed number of sites	**2 AP-HP centers:**

	- St Louis Hospital for the patient's treatment (Nivolumab +/- Ipilimumab) and the sonication - La Pitié Salpétrière (for the implantation, and explantation of the device : Sonocloud®)
Duration of the trial	State: - inclusion period: 39 months - participation period (treatment + follow-up): 24 months (3 weeks maximum of selection, 12 weeks treatment + 81 weeks follow up) - total duration of the study : 63 months
Number of enrolments expected per site and per month	This is a single-center prospective interventional study
Statistical analysis	An adaptive Bayesian dose-escalation model (Continual Reassessment Method, CRM) will be used. The selected version of the CRM is a model-based design, where the dose–DLT relationship is modelled through a cumulative function corresponding to a one-parameter power model, the parameter of which is sequentially estimated from the data via a bayesian estimation. In this setting, the MTD is a population-parameter, and refers to the π=10th percentile of the dose-toxicity relationship. A total of 3 US dose levels will be evaluated (0.78, 0.9 and 1.03 MPa). A minimum of three patients will be included at each dose level. As of October 3, 2022, 3 patients were treated at level 1 (0.78MPa) and 1 patient was at level 2 (0.9MPa). No side effects or toxicity were observed (very good tolerance). Since the CRM design recommends that a new patient could be treated at the higher level (1.03MPa), the patient n°5 will receive the 1.03MPa dose.
Sources of monetary support	PHRC 2016
Trial will have a Data Safety Monitoring Board	Yes

2. SCIENTIFIC JUSTIFICATION FOR THE RESEARCH

2.1 Hypotheses for the research

Based on the experience in glioblastoma (GBM) where the Blood Brain Barrier (BBB) opening has been successfully achieved, opening of the BBB with the SonoCloud® pulsed ultrasound system is worth exploring during the sessions of anti PD-1 monoclonal antibody alone or an association with antiCTLA-4 treatment of patients with brain metastases of melanoma. BBB opening could help at boosting immunity in the brain, at increasing brain penetration of the systemically delivered monoclonal antibodies, translating in controlling brain disease with the same magnitude as for peripheral disease. In the SoniMel study we want to demonstrate the good safety of the procedure for patients with brain metastases from melanoma and determine the optimal dose for further developments in this indication.

2.2 Existing knowledge related to the condition under investigation

Melanoma, responsible for 90% of skin cancer-related cell deaths, is a relevant model for the implementation of precision medicine. A major progress has consisted in the development of immunotherapies inhibiting specific immune checkpoints. Ipilimumab (IPI), an anti CTLA-4 antibody (Ab), triggers long duration responses in about 20% of patients (Lebbe et al., 2014b; Schadendorf et al., 2015). More recently, two anti-PD-1 agents have proven benefit in phase III: Nivolumab with 41% overall response rate (ORR), median OS not reached, 73% and 58.2% survival at 1 year and 2 years, respectively (Robert et al., 2015; Topalian et al., 2012) and Pembrolizumab with up to 36.9% ORR and median OS not yet reached 68%, 55% and 50% survival at 1, 2 and 3 years respectively (Schachter J et al., 2017, Robert ASCO 2017). Anti-PD1 Ab are now approved as first line for advanced melanoma. The combination of Ipilimumab and Nivolumab, shows 57% ORR , 73%,64% and 58% survival at 1 ,2 and 3 years (Postow NEJM 2015 Larkin et al., 2015; Larkin 2017 Wolchok et al., 2017) and has been recently approved first line in advanced melanoma in Europe.
Apart from immunotherapy, targeted therapies combining BRAF and MEK inhibitors have been approved for BRAF mutated melanoma with up to 70 %ORR median PFS around 12 months and median overall survival up to 25.6 months (Ascierto P. et al. 2016; Long GV et al., 2017).

Melanoma is the most frequent cause of brain metastases after lung and breast cancer. Brain metastases are present in around 20% of patients when entering stage IV disease and up to 40% in the course of the disease (Barnboltz-Sloan 2004, Peruzzi 2011*, Sampson 1998, Jakob 2012, Zakrzewski et al Cancer 2011, Fife 2004* Forschner 2016).

Table 1 and 2 summarizes data from 6 phases II clinical trials recently published dedicated to melanoma patients with brain metastasis

Table 1 targeted therapies

Reference	Study type number of patients	Study population	intervention	Intracranial response	Median overall survival (months)
Falchhok Lancet. 2012 May 19;379(9829):1893-90	Phase I/n=10	Asymptomatic brain metastasis	Dabrafenib	1(40%) CR 4 (40%) PR	NR
Long	Phase 2/n=172 Break MB	BRAF mutant active brain metastasis	Dabrafenib	Cohort A	Cohort A 8.2 months

The Lancet Oncol. 2012; Vol. 13 (11) : 1087/95).		Cohort A no previous local treatment =n=89 Cohort B previous local treatment n=83		- 39.2% (V600E) CR+PR - 6.7 (V600k) CR+PR Cohort B -30.8%(V600E) CR+PR -22.2% (V600K) CR+PR	(PFS 4 months) Cohort B 7.8 months (PFS 4.1 months)
Dummer Eur J Cancer. 2014	Pilot/n=24	Non resectable, symptomatic on stable doses of steroids	Vemurafenib	16%	NR
McArthur GA, Ann Oncol. 2017 Mar 1;28(3):634-641.	Phase 2/n=146	Active brain metastasis Cohort A no previous local treatment =n=90 Cohort B previous local treatment n=56	Vemurafenib	Cohort A 18% Cohort B 20%	Cohort A 6.5 months (PFS 3.7 months) Cohort B 6.4 months (Median PFS 4.0 months)
Davies Lancet Oncol 2017	Phase 2/ Combi MB	Cohort A : n = 76 BRAF V600E asympto. pts, no prior RT, PS 0-1 Cohort B : n = 16 BRAF V600E asympto. pts, prior RT, PS 0-1 Cohort C : n = 16 BRAFV600D/K/R asympto. pts with or without prior RT, PS 0-1 Cohort D : n = 17 BRAF V600 D/E/K/R symptom. pts with or without prior RT, PS 0- 2	Combination of Dabrafenib and Trametinib	A : 58% B 56% C 44% D 59%	Median OS A :10.8 months Median PFS A :5.6 months B :7.2 months C :4.2 months D: 5.5 months

In summary, targeted therapies allow for a significant intracranial ORR which remains inferior and less durable than achieved in extracranial metastasis.

Table 2 immunotherapies

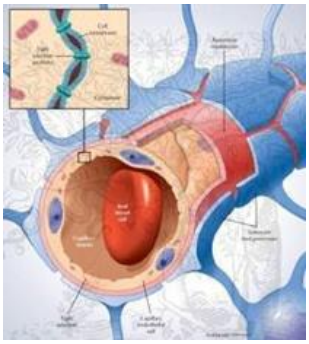
Reference	Study type number of patients	Study population	intervention	Intracranial response	Median overall survival (months)
Goldberg et Kluger the lancet.com/oncology Vol 17 July 2016	Phase I-II	18 melanoma patients with brain metastasis	pembrolizumab	IC ORR 22% (4/18)	NE
Long – Lancet March 27, 2018 http://dx.doi.org/10.1016/S1470-2045(18)30139-6 March 27, 2018	Phase II/ABC	Cohort A : n =35 asymptomatic pts, No prior WBRT	Ipilimumab+ nivolumab	IC and EC ORR 46% and 57%	6-mo IC and EC PFS 53% and 51% Med PFS IC NR EC 13.8 m
		Cohort B : n = 25 asymptomatic pts No prior WBRT	Nivolumab	IC and EC ORR 20%and 29% ORR30%	6-mo IC and ECPFS 20% and 35%
		Cohort C : n = 16 pts with previous WBRT or leptomenigeal melanoma	Nivolumab	IC and EC ORR 6% and 25% EC ORR 25%	6-mo IC and EC PFS 13%and 19%
Tawbi – ASCO 2017 – Abstract # 9507 CheckMate 204	Phase II Checkmate 204	N = 75 asymptomatic pts IC lesion : 0.5 – 3.0 cm No prior WBRT, CPI, Steroids ≥ 10 days, BRAFi or MEKi SRS allowed if still assessable lesion	Ipilimumab+ nivolumab	•1/3 received 4 doses Ipi + Nivo • IC ORR 55% (CR : 21%) • IC DCR 60% • EC ORR 49%	NR 6-mo PFS > 65%
IC intracranial EC extracranial					

Finally in a French retrospective review of 39/697 patients presenting with brain metastases of metastatic melanoma and receiving antiPD-1 agents in the MelBase cohort, survival was much lower than what was reported without brain metastases, around 11.3 months in median as compared to more than 24 months in patients without brain metastases (Allayous ASCO 2016).
In summary, ORR and survival data obtained with anti PD1 agents seems inferior to results achieved in patients without brain metastasis. Combining anti PD1 to anti CTLA4 improves the response rate and PFS, while data are too preliminary to extrapolate them to data observed in patients without active brain metastases. Noteworthy in the ABC trial the importance of progression in terms of sum of target lesions was higher in SNC targets as compared to extracranial targets (Long ASCO 2017). Altogether, such data support the concept of a decreased benefit of immunotherapy (as for targeted therapies) in brain metastasis as opposed to patients without brain metastases.
Decreased efficacy of immunotherapy such as anti PD1 agents in patients with brain metastasis could be related to the specificity of the microenvironment (Zhang et al., 2015). Another explanation could be a decreased penetration of immunotherapies because of the blood brain barrier. Indeed given their high molecular weight, penetration of monoclonal antibodies in the brain is uncertain and relies on disruption of the BBB which occurs occasionally. Until very recently, the dogma was that the CNS was immunologically privileged (Sampson et al., 2017). This has been challenged and it has clearly been shown that activated T cells can disrupt the BBB and penetrate the CNS (Hickey WF et al., 1991; Chen et al., 2015; Alkins et al., 2013). We hypothesize that we could improve the benefit of immunotherapy in melanoma brain metastasis using a device increasing the opening of the BBB. Such device could improve the penetration of therapeutic antibodies and facilitate the migration of activated T cells could be achieved.

Therefore opening the BBB concomitantly with use of immunotherapy could increase the control of brain disease.

2.3 Summary of relevant pre-clinical and clinical trials

BBB opening and immunotherapies
The low penetration of drugs into brain tissue is due to the presence of the blood-brain barrier (BBB) which separates the brain from the systemic blood flow (D. Fortin; 2004). This physiologic barrier is composed of a monolayer of endothelial cells, astrocytes and pericytes that line the cerebral microvessels and connect to each other by means of tight junctions. The BBB operates using active and passive transport mechanisms to limit the passage of potentially toxic molecules from the blood to the brain *[N. J. Abbott et I. A. Romero, Transporting therapeutics across the blood-brain barrier, Mol Med Today, vol. 2, n°. 3, p. 106–113, 1996], [W. M. Pardridge, Drug and gene delivery to the brain: the vascular route, Neuron, vol. 36, n°. 4, p. 555–558, 2002]*. As a consequence, approximately 98% of small-molecule drugs and 100% of large-molecule drugs do not cross the intact BBB *[**W.Pardridge,** Blood-brain barrier delivery, Drug Discov. Today, vol. 12, n°. 1–2, p. 54–61, 2007]*.



On the other hand, immunity is deficient in the brain with low or no presence of cytotoxic T lymphocytes (CTLs) and with immunosuppressive adaptation of tumors. A transient opening

of the BBB to allow for brain immunological changes and monoclonal AB and CTL entry into the brain might help at controlling the disease in the brain. In a mouse model of brain metastasis, specialized natural killer cells were systemically delivered and successfully targeted the brain tumor with simultaneous BBB disruption using US. In a murine model of brain amyloidosis, the opening of the BBB by US allowed brain penetration of anti-amyloid antibodies.

Recently, Liu et al published that BBB opening increased bevacizumab (anti HER2) concentration in the brain and retarded glioma progression with an increased median survival of treated mice (U87 glioma model).

In another murine model with concomitant BBB disruption using US and systemic infusion of trastuzumab, a high correlation was seen between brain concentrations of trastuzumab and grade of BBB opening. In their work, Kobus et al (2016) investigated whether the response of breast brain metastases to anti HER2 trastuzumab could be improved by temporary disruption of the BBB using focused ultrasound. In 30 nude rats inoculated with HER2-positive cells derived from a brain metastasis of a breast cancer patient (MDA-MB-361), they showed that six weekly treatments by trastuzumab and pertuzumab in combination with a six weekly sessions of BBB disruption using focused ultrasound led to response in four of the ten animals in the ultrasound + antibody-group (vs no responders in the antibody-only group). They concluded that disruption of the BBB using focused ultrasound is necessary to allow antibodies to inhibit growth of breast cancer brain metastasis.

Taken collectively, these data showed that BBB opening by ultrasound can effectively increase the concentrations of systemically administered monoclonal antibodies.

Sonocloud and BBB opening: preclinical and clinical data
SonoCloud® is an ultrasound transducer, to be implanted within the skull bone in the extradural space, delivering pulsed US in the brain with concomitant systemic injection of an US contrast agent and aiming at opening the BBB in the sonication field. It allowed repeated opening of the BBB in a primate experiment, with a good safety observed in 7 consecutive US procedures every 2 weeks (Jl Neurosurg 2016).

Summary of Pre-Clinical Animal Studies Performed by CarThera during the Development of the SonoCloud System.

Animal Study	Animal Model	Test Article(s)	Objective	Results
Acute safety of single BBB opening, irinotecan and temozolomide delivery	Rabbit	Non-implantable SonoCloud-1, SonoVue	To evaluate the effects of ultrasound-induced BBB opening in a rabbit model using histology and MR imaging.	At <0.8 MPa, minimal effects were observed in histological analysis and MR imaging of rabbit brain. Temozolomide and irinotecan delivery were significantly enhanced. (Beccaria et al. 2013 and Beccaria et al. 2016
Acute, 7-day safety of single BBB opening	Canine	Non-implantable SonoCloud-1, SonoVue	To evaluate the effects of ultrasound-induced BBB opening in a rabbit model	At <0.8 MPa, minimal effects were observed in canine brain after BBB opening and

			using histology and MR imaging.	no adverse effects were present at 7 days.
Long-term safety of repeated BBB opening	Baboon, Macaque	SonoCloud-1 Implant, Needle, SonoVue	To evaluate the long-term safety of repeated BBB opening with the SonoCloud device and the performance of the needle-implant connection system.	No long-term effects were observed after repeated (7 sonications) BBB opening in a primate model after 4 months. (Horodyckid et al. 2016)
Long-term safety of repeated BBB opening, carboplatin delivery	Baboon	SonoCloud-1 Implant, Needle, Generator, SonoVue	To evaluate the long-term safety of repeated BBB opening with the SonoCloud device and to evaluate the performance of the generator and needle-implant connection system. To measure the concentration of carboplatin chemotherapy after BBB opening.	The SC1 system performed without incident and safe BBB opening was repeatedly achieved. Carboplatin chemotherapy delivery was enhanced by more than 500% in sonicated regions of the brain. (Goldwirt et al. 2016)

The significant pre-clinical work summarized above demonstrates that pulsed-ultrasound can effectively and temporarily open the BBB at acoustic pressures without acute or subacute complications. This observation has been published in a variety of models and using a variety of drug agents.

A first-in-human phase I/IIa study of SonoCloud® in patients with recurrent glioblastoma (GBM) started in July 2014 has now completed its recruitment (EudraCT 2014-000393-19, NCT02253212). The main objective was to evaluate the safety of a transient and repeated opening of the BBB with pulsed ultrasounds delivered through SonoCloud® immediately before infusion of carboplatin, to improve penetration and efficacy of chemotherapy in the brain tumor. As of June 2018, 27 patients have been implanted and 25 patients treated representing more than 89 US treatments with concomitant systemic SonoVue® and followed with carboplatin have been delivered in 7 successive escalating levels of acoustic pressures (from 0.78 to 1.03MPa).The safety has been excellent so far; no adverse event related to the implantation of the device has been reported; no DLTs have been observed during US treatments. Two serious adverse events (SAE) with potential relationship to the treatment have been reported, one being a delayed (14 days after 0.78MPa US) brain oedema related to the progression of the disease possibly worsened by US; the second one was an oedema in the sonication field at 1.03 MPa, immediately post-sonication and reversible within 48 hours under corticosteroids. BBB opening was achieved from the dose of 0.78MPa, as shown in the immediate post sonication MRIs. Quality and depth of BBB opening increased with higher acoustic pressures, as shown in the immediate post sonication MRIs. When BBB opening was of good quality, stabilization of the tumor in the sonication field has been observed in some patients, contrasting with progression outside of the field.
The first results of the study have been published in June 2016 (Carpentier A. “Clinical trial of blood-brain barrier disruption by pulsed ultrasound”. Sci Transl Med. 2016 Jun 15;8(343) 1-8).

Sonocloud and immunotherapies
Our hypothesis is that BBB opening with SonoCloud will increase brain penetration of immunotherapies and will help at boosting immunity in the brain, with the goal to control brain disease with the same magnitude as for systemic disease.

It is not anticipated that any exacerbated safety issue might emerge from use of checkpoint inhibitor in the context of BBB opening with SonoCloud. Although checkpoint inhibitors do not target a specific organ, only very rare events concerning central nervous system have been reported so far. Indeed, in patients receiving nivolumab as a single agent, encephalitis occurred in 3 out of 1994 patients treated including one fatal limbic encephalitis which occurred in one patient after 7.2 months of exposure despite discontinuation of nivolumab. Encephalitis have also been reported in one patient receiving nivolumab with ipilimumab (0.2%) after 1.7 months of exposure (FDA).
In previously treated metastatic melanoma trial specifically, the most frequent Grade 3 and 4 adverse reactions reported in 2% to less than 5% of patients receiving nivolumab were abdominal pain, hyponatremia, increased aspartate aminotransferase, and increased lipase.

Adverse events in more than 10% of patients treated with nivolumab in trial 1

34]) (Trial 1) Adverse Reaction	OPDIVO (n=268)		Chemotherapy (n=102)	
	All Grades	Grades 3-4	All Grades	Grades 3-4
	Percentage (%) of Patients			
Skin and Subcutaneous Tissue Disorders				
Rasha	21	0.4	7	0
Pruritus	19	0	3.9	0
Respiratory, Thoracic, and Mediastinal Disorders				
Cough	17	0	6	0
Infections				
Upper respiratory tract infectionb	11	0	2.0	0
General Disorders and Administration Site Conditions				
Peripheral edema	10	0	5	0

Other clinically important adverse reactions in less than 10% of patients treated with nivolumab in Trial 1 were:
- Cardiac Disorders: ventricular arrhythmia
- Eye Disorders: iridocyclitis
- General Disorders and Administration Site Conditions: infusion-related reactions
- Investigations: increased amylase, increased lipase
- Nervous System Disorders: dizziness, peripheral and sensory neuropathy
- Skin and Subcutaneous Tissue Disorders: exfoliative dermatitis, erythema

Due to absence of clinical data regarding high dose effect of checkpoint inhibitors in brain, our trial will be designed however as a dose escalation study. The first US pressure cohort dose will be 0.78MPa (where mainly sub arachnoid and grey matter are concerned by BBB opening), subsequently increased at 0,9 (about half sub arachnoid grey matter/white matter BBB opening), 1,03 MPa (majority of grey and white matter BBB opening) with a maximum of 3 dose levels. In the GBM phase 1 study, where successive cohorts of patients were evaluated at increasing US pressure doses, the decision was made to not pursue evaluation of doses beyond 1,03MPa, even if the DLT was not reached at that dose; the main reason was the

observation of the excellent quality of the BBB opening at that dose, that was reproducible from one cycle to another and observed in all patients, without any significant toxicity.

Therefore, for the Sonimel study, we will evaluate conservative acoustic pressure doses at standard concentration of Nivolumab and Ipilimumab under strict surveillance.

2.4 Description of the population of research participants and justification for the choice of participants

Patients suffering from melanoma with brain metastasis are currently discussed in specific tumor boards. Treatment generally relies on systemic therapies, targeted agents if BRAF mutation or anti PD1 alone or combined with Ipilimumab whatever mutational status. Local therapy (surgery or more frequently radiosurgery) is regularly discussed during the course of the disease.
Our aim is to gain further insight on the feasibility and interest of BBB transient disruption in melanoma brain metastasis treated with immune checkpoint inhibitors. Therefore, we will enroll melanoma patients with brain metastasis, BRAF mutated or wild type for whom the tumor board decision is Nivolumab alone or combined with Ipilimumab, without immediate need for surgery of radiotherapy.
In our center 2 to 3 patients with brain metastasis are discussed in our tumor board every week, 2 are treated with immunotherapy

2.5 Name and brief description of the investigational medicinal device(s)

The SonoCloud® System is an active implantable medical device developed by CarThera (France). The SonoCloud® is indicated to locally and transiently increase the permeability of the blood brain barrier to facilitate the passage of substances or physician-specified agents including chemotherapy, into the cerebral parenchyma of patients with brain disease.

The SonoCloud System consists of four principle components:

(1) an implantable ultrasound transducer,

(2) a needle connection device,

(3) an external radiofrequency generator, and

(4) an ultrasound resonator.

The SonoCloud® Implant is designed to be fixed to the skull and is compatible with follow-up magnetic resonance (MR) imaging. The device is placed in a burr hole or in place of a bone flap and ultrasound energy is delivered directly to the brain tissue, without traversing the skull bone. The device is activated by connecting the implant to the external generator system using the transdermal needle. Once connected to the external generator, the implant delivers low-intensity pulsed ultrasound for duration of 120-270 seconds. The delivery of ultrasound energy to the brain tissue, which is performed in combination with systemic injection of an ultrasound resonator, temporarily disrupts the blood-brain barrier. The SonoCloud® System is designed to be activated repeatedly, following a typical schedule for administration of common drug therapies.

The SonoCloud system is for investigational use only.

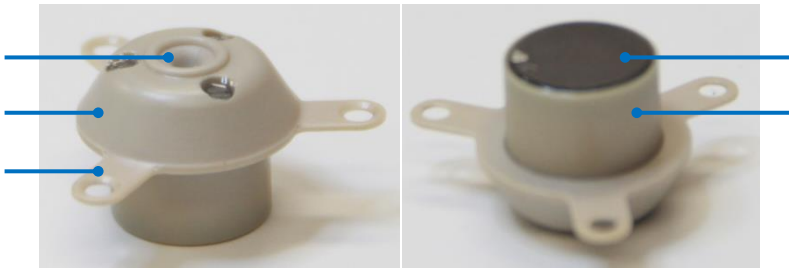
2.6 Description and justification of the method for using the device(s) and length of treatment

The SonoCloud-1 Implant consists of a 1 MHz, 10-mm diameter ultrasound transducer that is encapsulated in an 11.5-mm diameter biocompatible housing. The implant is placed in a 12-mm diameter burr hole during a surgical procedure and fixed to the skull bone using three standard titanium surgical screws. The ultrasound transducer is in direct contact with the dura mater, thus there is no absorption or distortion of the acoustic field by the skull bone.

The implant consists of a top hat, in contact with the tissue of the scalp, and which has a small hole that is designed to receive the bipolar needle connection when the implant is connected to the generator for activation. The three fins on the top hat each contain a hole, which is used to fix the implant to the skull bone using standard titanium surgical screws.

The bottom face of the implant is in contact with the dura mater. The bottom part includes an internal connection chamber that contains the electrical contacts for connecting the implant to the bipolar needle during each activation. A self-sealing septum inside the implant allows for the repeated passage of the transdermal needle and guarantees that the internal chamber (containing electrical contacts) remains free of any body fluid. The bottom face of the implant contains the 10-mm diameter 1 MHz ultrasound transducer that generates pulsed ultrasound to disrupt the BBB.

SonoCloud-1 Implant



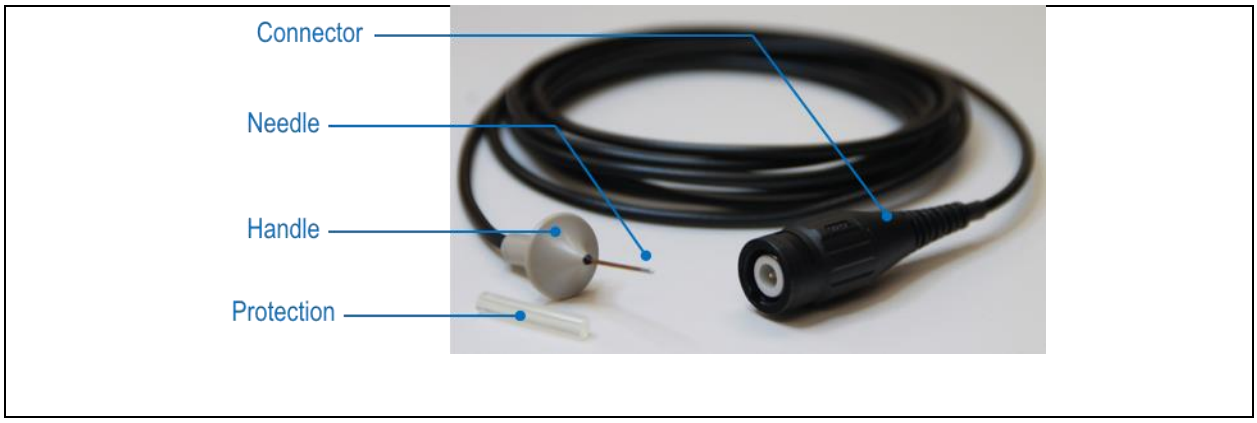
The SonoCloud-1 Implant does not contain any power source. It is activated only when connected to the generator through the use of the transdermal needle. When the SonoCloud-1 Implant is activated, pulsed ultrasound is delivered to the tissue.

SonoCloud-1 Transdermal Needle

The transdermal needle is designed for single-use and allows for the implant to be connected to the external generator. The transdermal needle connects to the generator via a connector on the front face of the system. The generator connector is attached to a cable with a length of 2.5-meters. An ergonomic grip facilitates handling of the needle by the practitioner, and a bipolar needle with an exterior diameter of 0.84 mm and a length of 20 mm is located at the distal end.

The connection between the needle and the implant is achieved by first passing the scalp of the patient, then by traversing the self-sealing septum of the implant. Once the septum is traversed, a connection between the electrical contacts in the interior chamber of the implant and the contacts at the distal tip of the needle is established. The self-sealing membrane located inside the implant ensures that no body fluids are in contact with the zone of electrical contacts in the interior of the implant. The total duration of contact of the transdermal needle with the patient is typically less than 15 minutes.

SonoCloud-1 Transdermal Needle



SonoCloud-1 Generator

The SonoCloud-1 Generator is a radiofrequency generator that provides the electrical stimulation to the implant. Since the SonoCloud-1 implant contains no internal power source, the implant is powered using the external generator system during each procedure. The radiofrequency energy sent from the generator via the transdermal needle is converted into ultrasound energy by the 10-mm diameter, 1 MHz circular piezoceramic transducer that is located in the SonoCloud-1 Implant.

The SonoCloud-1 Generator contains a 7" touchscreen display that guides the practitioner through the treatment process. In addition, the connection between the generator and the implant via the transdermal needle is continuously verified throughout the treatment process.

SonoCloud-1 Resonator

The ultrasound resonator that is used with the SonoCloud® device, a phospholipid micro-bubble produced according to GMP with extensive human pharmacokinetics and safety data available and commercialized under Sonovue's brand in Europe by Bracco. It consists of a kit containing a vial of sulphur hexafluoride gas and phospholipid powder, a prefilled syringe of solvent (sodium chloride solution) and a transfer and ventilation system (mini spike).

Utilisation of Sonocloud

Each sonication step consists of generation of pulsed ultrasound in combination with administration of the ultrasound resonator. The procedure is started at the same time as the intravenous injection of the acoustic resonators. The resonators are injected as a bolus, at a dose of 0.1 mL/kg, over a period of 50 seconds. After the injection of the acoustic resonators, sonication continues, for total sonication duration of 270 seconds.

See section 7 and refer to "USER MANUAL OU INVESTIGATOR BROCHURE FOR FURTHER DETAILS".

2.7 Summary of the known and foreseeable benefits and risks for the participants

Benefits

Rationale for the use of the proposed conditioning

The combination of repeated opening of the BBB with the SonoCloud® pulsed ultrasound system (pulsed US in combination with systemic microbubble injection) and systemic anti PD-1+/- anti CTLA4 monoclonal antibodies is worth exploring in brain metastases of melanoma to improve the care of these patients with a very poor prognosis. We anticipate that BBB opening will increase brain penetration of monoclonal antibodies and will help at boosting immunity in the brain, with the goal to control brain disease with the same magnitude as for systemic disease. This would also open avenues for the treatment of brain metastases in other tumor

types such as lung and breast cancers as well as for GBM in combination with checkpoint inhibitors.
The expected individual benefit is prolonged survival of patients with brain metastasis

Risks Risks

- Risks associated with the SonoCloud® device (implantation/explantation):
  - ✓ pain: during device implantation/explantation, while the device is implanted/explanted or during the needle puncture : so far only transient local pain has been observed during needle puncture in some of the 20 patients implanted and treated in the ongoing GBM study
  - ✓ infection : not reported so far

- Risks associated with the US cures
  - ✓ blood effusion on the sonication beam
  - ✓ brain edema
  - ✓ local epilepsia
  - ✓ faintness
  - ✓ facial placy

- Risks associated with immunotherapy and advanced melanoma:
  - ✓ melanoma progression and immune adverse event related to immunotherapy are inherent to clinical practice (real-life). Among immune related adverse events occurring after immunotherapies, the incidence of neurological manifestations is estimated to 3.8% with anti-CTLA4 antibodies, 6.1% with anti-PD1 antibodies, and 12.0% with the combination of both (Cuzubo 2017). Most are grade 1 or 2 and consist of non-specific symptoms such as headache (55%). The incidence of neurological grade 3 or 4 AE are below 1% for all types of treatment. Headaches, encephalopathies and meningitis were the most commonly reported (21%, 19% and 15%, respectively) with median time of onset of 6 weeks. In most cases, drug interruption and steroids led to neurological recovery. Since we use low dose ipilimumab and since anti PD1 related toxicity does not seem to be dose dependant we do not expect an increased incidence of neurological toxicities with use of BBB ; however an independant DSMB will review regularly all safety issues.

- Risks associated with 18F-FDOPA PET/CT
  - ✓ 1/Risks associated with tracer (FDOPA) extravasation. The volume of injected tracer is very small. If extravasation should occur, patients should be informed and monitored to detect potential adverse effects, such as erythema, or burns. These side effects are very rare with radiopharmaceuticals for diagnostic use.
  - ✓ 2/Risks associated with FDOPA injection. No reaction to FDOPA injection has been described. However, any allergic reaction (pruritus, rash…) to the injection should be reported and documented. Prior history of reaction to iodine contrast IS NOT a contraindication to the injection of FDOPA.
  - ✓ 3/Risks associated with radiation. FDOPA PET/CT delivers a dose of 4,4 mSv (to be compared with the radiation of a conventional brain CT 1,83 mSv and of a thoraco-abdomino-pelvic CT 7,5 mSv). The increased risks of stochastic events due to these low radiation doses are difficult to quantify. However, the risk appears very low compared with the severity of the disease (melanoma patients with brain metastasis), so the risk/benefit ratio is in favor of the use of this imaging procedure.

Appendix 19.2 Rationale for the use of FDOPA PET/CT for brain metastasis imaging

18F-FDOPA is a radiolabelled amino acid, a substrate for the enzyme aromatic amino acid decarboxylase in dopaminergic neurons resulting in a physiological uptake in the basal ganglia (Cicone, Filss, et al. 2015).
FDOPA is relevant for the initial diagnosis of primary brain tumours, with a probable prognostic value of intensity of tumour uptake on pretherapeutic PET (Bell et al. 2015; Fueger et al. 2010; Filss et al. 2017).
It has also been used after radiotherapy for the differentiation between progressive/recurrent brain tumours, and radionecrosis, in primary and secondary brain tumours (Filss et al. 2017; Yu et al. 2018).
For primary brain tumours, it can accurately detect recurrences (Karunanithi et al. 2013)
Lizarraga et al. demonstrated in 32 patients that FDOPA PET could between progression and radionecrosis with a high diagnostic accuracy of 83%(sensitivity, 81%; specificity, 84%) in patients with a MRI suggestive of progression/recurrence (Lizarraga et al. 2014). Also, this evaluation with FDOPA PET was prognostic of progression-free survival. Semi-quantitative indices and visual analysis provided similar results.
Cicone et al. evaluated 50 lesions in 42 patients treated by stereotaxic radiosurgery. Excellent diagnostic performance was obtained using a cut-off value of SUVmaxlesion/SUVmaxbackground, with a sensibility of 90% and a specificity of 92%, performing possibly better than MRI (Cicone, Minniti, et al. 2015).

Benefit/Risk balance

- So far no significant toxicity has been observed in the ongoing phase 1 study and there is a potential to improve local immunity in the brain with the goal to obtain the same control of the metastases as in the extra-cranial setting with the combination of US and nivolumab.
- So far no toxicity has been observed with pulsed US delivered every 4 weeks through the SonoCloud® device in the ongoing phase 1 study. A total of 89 sonication sessions have been delivered, at the doses of 0.5 to 1.40 MPa, in a 4-week interval (SonoCloud Investigator's Brochure) and up to 9 sonications for the same patient, every 4 weeks, with good tolerance. There is also experience in monkeys with 7 sonications administered on an every-2-week regimen without any safety issue (Horodyckid C, JNS June 2016)
- Patients will receive their infusion of nivolumab (flat dose: 240mg 30 minutes infusion) or nivolumab (1mg/kg) combined with ipilimumab (3mg/kg) total of 2h.
- Sonications will be performed after antibodies infusion in the Saint Louis Clinical Investigation Center or "CIC" (dedicated early phase I platform labelized by INCA) under strict medical and paramedical surveillance.
- MRI will be performed when indicated just after sonication within 30 minutes
- Patients will be monitored during 6 hours before going back home. Patients will be kept in the unit and under medical monitoring in case of seizures suspicion (an EEG will be performed in this case) or in case of chest discomfort, with adapted management (an ECG will be performed in that case).
- Patients will kept be aware of the immunotherapy toxicity profile (irAEs), so as to promptly identify and report symptoms related to immune related adverse events. In addition, oustside the visits in CIC, patients will be followed by remote monitoring using a standardized questionnaire as used in daily practice for all patients treated by immunotherapies (Patients will be contacted by phone 1 or 2 days before the visit in Saint Louis hospital. This questionnaire will verify that the patient don't have any toxicity since the last visit).
- Recommendations and guidelines for the management of irAEs have been published and are now available for clinicians.

The balance is in favor of the benefits, as so far the safety of the procedure seems excellent, as close monitoring of patients will be done and as this procedure proposed to patients at high risk of mortality, could improve patients' outcome.
An independant Data Safety Monitoring Board (IDSMB) will be overlooking at the safety data all along the study period.

Expected patient and public health benefit
Patients with advanced melanoma and brain metastasis are most often excluded from clinical trials. Therefore clinical trials in this high unmet medical need population are awaited.
Safety and feasibility of opening the BBB in patients with advanced melanoma and brain metastases treated with Nivolumab +/- Ipilimumab would pave the way to (1) change SOC and prognostic of patients with advanced melanoma when brain metastases occur, (2) test other checkpoints or combination of checkpoint inhibitors in brain metastases of melanoma, (3) provide rationale for evaluation of antiPD-1 agents in other cancers where immunotherapy has been demonstrated efficient such as lung cancer.

3. Objectives

3.1 Primary objective

The main objective will be the determination of the most successful dose (MSD), defined as the dose with the highest probability of efficacy of opening BBB without toxicity directly related to the ultrasound emission.
A total of 3 dose levels will be evaluated (0.78, 0.9 and 1.03 MPa); each cohort will include a minimum of 3 patients treated with the same pressure dose all along the study.

As of October 3, 2022, 3 patients were treated at level 1 (0.78MPa) and 1 patient was at level 2 (0.9MPa). No side effects or toxicity were observed (very good tolerance).
Since the CRM design recommends that a new patient could be treated at the higher level (1.03MPa), the patient n°5 will receive the 1.03MPa dose.

3.2 Secondary objectives

- Clinical Efficacy of antiPD1 +/- antiCTLA4 with BBB opening in the study population
- Safety of Nivolumab alone or combined to Ipilimumab in the context of BBB opening

3.3 Objective of any future ancillary study

A pharmacokinetic study will be conducted to investigate the pharmacokinetic/pharmacodynamics relationship between antiPD1 +/- antiCTLA4 plasma exposure at BBB opening and efficacy as the interindividual variability in nivolumab and ipilimumab pharmacokinetics in patients with advanced melanoma has been described: 32% variability of plasma exposure after 3mg/kg repeated doses of ipilimumab (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125377Orig1s000ClinPharmR.pdf) , and 26.0% variability of plasma exposure after 10mg/kg repeated doses of nivolumab (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/125554Orig1s000ClinPharmR.pdf).
Ipilimumab and Nivolumab plasma quantification methods will be developed and validated in Saint-Louis Pharmacology department according to the general recommendations for the immunoassays.

Biobanking :
Blood samples will be taken for biobanking (plasma, PBMC) at J1, and before treatment (perfusion and sonication) at week 6, week 12 and week 16.

It consists in 4 EDTA 7mL and 6 lithium heparin 7mL tubes, addressed whithin 3 hours at room temperature to the Pharmacology Department of Saint-Louis.

Blood samples will be taken for ancillary study (such as cell free DNA monitoring…) and be collected into:

- 4 EDTA 7 mL before BBB opening at least 30 min after Nivolumab +/- Ipilimumab end of administration (for Nivolumab : Week 2, Week 4, Week 8, Week 10 and for Ipilimumab + Nivolumab : Week 3, Week 9).

- 4 EDTA 7mL after sonication (for Nivolumab : Week 2, Week 4, Week 8, Week 10 and for Ipilimumab + Nivolumab : Week 3, Week 9).

Plasma Banking: Blood from EDTA tubes will be centrifuged (3000 rpm, 10', at room temperature), then plasma will be collected and transferred in low binding microtubes, and stored at -20°C until analysis

Ficolls Heparinate Lithium tubes will be centrifuged (3082g, 10 min, 4° C) within 2h after collection; then plasma was transferred to propylene tubes and stored −20 °C until analysis.

PBMC Banking: Mononuclear cells will be isolated from peripheral blood by Ficoll separation on lithium heparin blood samples. Cells will be cryopreserved at least in 10 Million cells DMSO/unit, then stored in liquid nitrogen until analysis.

18F-FET PET/CT ancillary study

18F-FDOPA PET/CT ancillary study

18F-FDOPA PET/CT will be performed for all patients to gather more information on tumor behavior with this innovative treatment. As explained in Appendix 19.2, this can provide data on:
1/Tumor aggressiveness before treatment
2/Metabolic response to therapy and
3/Discrimination between post-therapeutic modifications and disease recurrence after treatment.

18F-FDOPA PET/CT procedure

Baseline 18F-FDOPA PET is to be performed before or at the latest 8 days after the first treatment with sonication and nivolumab+/- Ipilimumab. Evaluation 18F-FDOPA PET is to be performed 12 weeks after this first treatment course (±15 days).
18F-FDOPA PET procedure will take place in the nuclear medicine department of Saint Louis Hospital. Patients are placed on PET/CT device after vein catheterization and infusion with saline solution. A CT of the skull is acquired for correction attenuation and lesions localizations. 18F-FDOPA (3 MBq/kg according to the AMM) is injected intravenously, when the patient is on the PET table. A 40 minutes dynamic acquisition over the skull is performed.

18F-FDOPA PET analysis

Imaging analysis
Visual and quantitative analysis (of dynamic and static data) will be performed.
In particular, for static analysis SUVmax and SUVmean of brain lesions, of the striatum and brain background, as well as visual score, will be determined on summed images between 10 and 30 minutes.

18F-FDOPA PET diagnosis value

In this particular clinical setting several criteria can be assessed:

1/Predictive value of initial 18F-FDOPA PET for therapeutic response
2/Predictive and prognosis value of metabolic response observed between baseline and evaluation 18F-FDOPA PET
3/Discrimination between post-therapeutic modifications and disease recurrence in case of persisting morphological lesions.

4. Description of the research

4.1 Study endpoints

Primary endpoint

Primary Endpoint:
The main objective will be the determination of the most successful dose (MSD), defined as the dose with the highest probability of efficacy of opening BBB without toxicity directly related to the ultrasound emission. Thus, the primary endpoint will be the success defined as efficacy without toxicity. To assess the MSD, we will use for that the continual reassessment design proposed by O' Quigley (2001) adapted for the identification of the dose leading to the greatest percentage of successes.

- **Toxicity evaluation**: Safety will be assessed clinically and using brain MRI. An electroencephalogram will be performed only if clinically needed. Dose-limiting-toxicities (DLTs) evaluation will be done during the first 4 weeks of treatment for Nivolumab treatment and 6 weeks for Nivolumab + Ipilimumab treatment. DLTs directly related to the ultrasound emission are defined as occurrence of an adverse effect during the first administration of treatment, such as: :
  - neurological deficit within 2 days after the procedure and persistent at day 15;
  - localized brain edema not preexisting to the procedure;
  - occurrence of cerebral median line deviation not controlled by routine treatment or requiring salvage surgical procedure;
  - partial epilepsy induced or enhanced after the procedure and not controlled by routine therapy;
  - focal encephalopathy in the area of the BBB opening and not reversible;
  - bleeding or ischemia of more than 1cm diameter, in the area of the BBB opening occurring within 2 days of the procedure;
  - brain herniation requiring salvage surgery

- **Efficacy evaluation**: Efficacy in terms of blood barrier disruption will be estimated after the first and the second ultrasound treatment and immunotherapy administration. A brain MRI exam will be done immediately after the first and second US treatment (~30 minutes after US-induced BBB disruption). At each exam, standard fluid attenuated inversion recovery (FLAIR), T1w contrast enhanced (0.2cc/kg, Dotarem®), susceptibility-weighted angiography (SWAN), and diffusion sequences will be obtained. T1w MR Images will be analyzed to grade the type of BBB opening observed. Four different grading stages will be assessed, with the following definitions:
  - Grade 0 no BBB opening,
  - Grade 1 contrast enhancement in sub-arachnoid space,
  - Grade 2 contrast enhancement in sub-arachnoid space and grey matter,
  - Grade 3 contrast enhancement in sub-arachnoid space, grey matter and white matter.

  Efficacy will be defined as grade 2 or 3 BBB opening at the first session of sonication.

All MRIs performed will be reviewed in real time by the radiologist and the neurosurgeon in order to grade the type of BBB opening observed. This analyze will be performed at each : Day 1 – Week 2 for Nivolumab alone or Week 3 for Nivolumab an association with Ipilimumab – Week 12 .

Secondary endpoints

Secondary endpoint :
Best overall response rate and ORR at 3 months
Best intracranial overall response rate (BICORR) and ICORR at 3 months
Best extracranial overall response rate (BECORR) and ECORR at 3 months
Progression free survival (PFS) at 3 months
Intracranial progression free survival (PFS) at 3 months 6 months, 12 – 18 and 24 months

Extracranial progression free survival (PFS) at 3 months 6 months, 12 – 18 and 24 months
Overall survival (OS) at 4 months 6 months, 12 – 18 and 24 months
Safety using CTCAE version 5.0

Clinical activity will be assessed using TEP/TDM Scans (extracranial lesions) and MRI (intracranial metastases). Clinical objective response rate (ORR) (defined with the RANO and iRANO and the RECIST version 1.1 and the Immune-Related Response Criteria) and PFS defined as time from day 1 of immunotherapy perfusion and sonication to disease progression (according to the RANO and RECIST version 1.1) and the immune related Response Criteria- or death, whichever is first.

4.2 Description of research methodology

Design of the study:

Patients will be prospectively enrolled in the center during 24 months and followed during 24 months.

A prospective, multicenter (St Louis Hospital for the patient's treatment and US sonication and la Pitié Salpétrière for the implantation, and explantation of the device) (n=21 patients treated), non-randomized, open label study. Patients will be sequentially included in cohorts of three patients.

Prior to trial entry, during a consultation, the investigators (or designated assistant) will explain the nature of the trial, its purpose, procedures, expected duration, alternative therapies, as well as the benefits and risks. Each patient will be given the opportunity to ask questions and will be informed on the right to withdraw from the trial at any time without prejudice.
Written informed consent will be obtained from the patient after 2 days of reflection. Functions of organ systems must be documented before inclusion, as outlined in the inclusion criteria, including: physical exam, ECG, biological function…
Patients will be included if:
- they fulfill the inclusion criteria defining eligibility;
AND
- the evaluation of organ functions has not revealed any non-inclusion criteria as defined above.

All inclusions will be reviewed by the neurosurgeon, who will assess implantation feasibility.

Proposed treatment / Study Plan : after inclusion, patients will be implanted 48h to 7 days after consultation with anesthetist and neurosurgeon (SonoCloud® device). Patients will receive 1 week minimum after Sonocloud implantation their first US treatment (a period of 15 days is possible if the patient's clinical condition allows it and if the investigator considers it necessary). An 18F-FDOPA PET/CT will be performed +/-8days before the first sonication. Patients will receive Nivolumab alone or in combination with Ipilimumab depending on the St Louis tumor board decision and previously to the sonication. Treatment by Nivolumab combined with Ipilimumab will be preferred for the patients no previously treated by Ipilimumab and considering the toxicity and general status.
PDL1 expression will be done and it's taken consideration in tumor board discussion. Although the sensitivity and specificity of these marker is weak.
Patients treated by Nivolumab alone will receive at maximum of 7 sonications sessions over twelve weeks with immunotherapy.
Patients treated by Nivolumab combined by Ipilimumab will receive at maximum of 5 sonications sessions over twelve weeks.

Nivolumab alone will be continued in case of tumor control afterwards, per investigator's decision.

Number of participating sites

This is a single-center prospective interventional study.

- Recruitment center

Recruitment will be performed on Dermatology department, and Centre d'Investigation Clinique (CIC), St Louis Hospital – APHP Paris.

Inclusion, treatment by Nivolumab alone or an association with Ipilimumab, imaging and US sonication will be performed at St Louis Hospital – APHP Paris.

- Non-recruiting center

The implantation of the device Sonocloud®, and explantation will be performed at La Pitié Salpêtrière Hospital –APHP Paris (Neurochirurgy department – Pr Carpentier).

4.3 Avoiding and reducing bias

Participant identification

The participants in this research will be identified as follows:
Site number (3 digits) - Sequential selection number for the site (4 digits) - surname initial - first name initial
This reference number is unique and will be used for the entire duration of the study.
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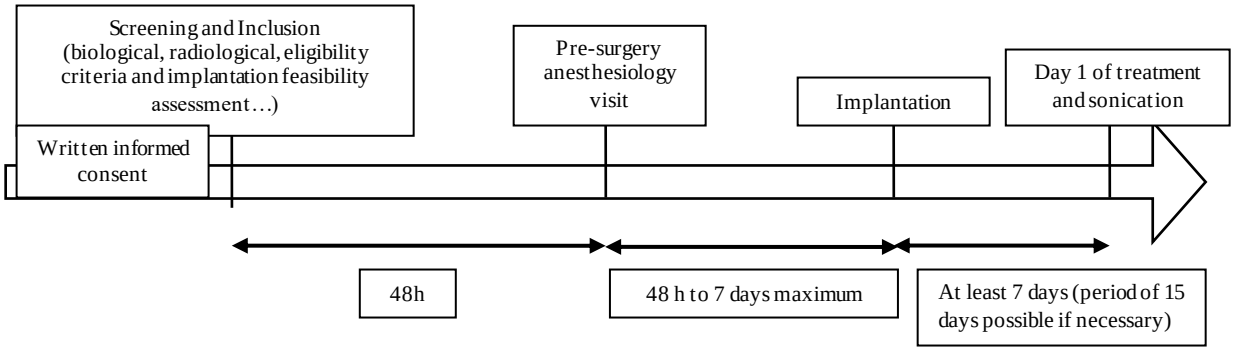
5. Procedure for the trial



Whose consent must be obtained	Who informs the individuals and collects their consent	At what point the individuals are informed	At what point the consent is obtained
Patients with advanced brain metastases melanoma	Investigator of dermatologic department	Routine and screening visit	During the screening visit after having had at least 2 days of reflection

5.1 Screening and Inclusion visit

Prior to trial entry, the investigators (or designated assistant) will explain the nature of the trial, its purpose, procedures, expected duration, alternative therapies, as well as the benefits and risks. Each patient will be given the opportunity to ask questions and will be informed on the right to withdraw from the trial at any time without prejudice.
Study proposal to a patient during a consultation. After time of reflexion of 2 days written informed consent will be obtained from the patient.
Functions of organ systems must be documented before inclusion, as outlined in the inclusion criteria, including: physical exam, ECG, biological function…

During the screening visit (duration 21 days maximum), assessments will be performed in the Centre d'Investigation Clinique (Hospital Saint Louis) as day-hospital sessions. The following procedures are to be completed

1- Height, Weight, vital signs (temperature, blood pressure, oxygen saturation, pulse measurements) and a complete physical examination will be performed and will include the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, peripheral vascular system and neurologic system. If indicated based on medical history and/or symptoms, rectal, external genitalia, breast, and pelvic exams will be performed.
2- A TEP scan (+/- intravenous (i.v.) contrast) is required for all patients**.** A brain MRI is required (with the basic MR sequences: FLAIR (before gado injection) T1-weighted contrats enhanced (0.2ml/kg, Doterem) SWAN (or a more standard T2 sequence can be

used, to detect potential microhemmorhages), Diffusion). Radiological assessement should not be older than 21 days before first dose of treatment

3- A 18F-FDOPA PET/CT will be performed +/-8 days before the first sonication
4- Colour photography of any skin lesions (if applicable).
5- Following blood samples will be assessed:

- haematology (WBC count plus differential (total neutrophil including bands, lymphocyte, monocyte, eosinophil, basophil and other counts), RBC count, haemoglobin, haematocrit and platelet count),
- clinical chemistry (Sodium, potassium, chloride, bicarbonate, total calcium, inorganic phosphate, magnesium, urea or blood urea nitrogen (BUN), glycemia, creatinine, bilirubin (total, indirect and direct), AST, ALT, albumin, alkaline phosphatase, LDH, CPK, GGT), US Troponin.
- Endocrinology (Cortisol, TSH, Free T4, Free T3)
- All females of childbearing potential will have a serum pregnancy test at selection/inclusion visit. If positive, the patient must be excluded from the study.

6- Cardiac assessment:

A Subsequent 12-lead ECG will be performed (and if clinically needed for the others visits).
Cardiac ultrasound with LVEF measure

7- Concomitant treatments
8- PDL1 expression

Patients will be included if:
- they fulfill the inclusion criteria defining eligibility;
AND
- the evaluation of organ functions has not revealed any non-inclusion criteria as defined above.

All inclusions will be reviewed by the coordination team (during a phone call) including the dermato-oncologist, the radiologist and the neurosurgeon, who will assess implantation feasibility.

5.2 Pre-surgery anesthesiology visit

Subjects that have met all eligibility criteria will be ready to begin study and a pre-visit with anesthetist and surgeon will be performed 48h maximum after the verification of criteria.
During this visit anesthetist and surgeon will explain to the patient the procedure to implant the device.
If implantation of the SONOCLOUD is not possible according to neurosurgeon (any patient morphological characteristics (e;g skin characteristics, bone thickness, other…), which, from neurosurgeon's opinion, prevent implantation of the device or may impair the ability of the patient to receive treatment with Sonocloud, patient would be excluded.

5.3 Surgery visit

The patients will be hospitalized (outpatient) to be implanted in the Neuro-Surgery department (hospital Pitié Salpêtrière) 48 hours to 7 days after the pre-surgery visit.

The US device is implanted within the skull bone overlying the metastasis area (contrast-enhancing region or high-signal FLAIR region). Importantly, when the metastasis is next to or within eloquent regions, the device is implanted in this critical area in an attempt to prevent tumoral progression and to have the highest potential enhancement to patient's quality of life.
If implantation of the SONOCLOUD is not possible according to neurosurgeon (any patient morphological characteristics (e;g skin characteristics, bone thickness, other…), which, from neurosurgeon's opinion, prevent implantation of the device or may impair the ability of the patient to receive treatment with Sonocloud, patient would be excluded.

The device is implanted during a dedicated surgical procedure in an ambulatory fashion under local anesthesia (xylocaine).

- Consultation of neurosurgery and preoperative anesthesia
- Hospitalization in neurosurgery around 8:00
- 10:00 am Implantation of the device under local anesthesia in the operating room with intravenous injection of antibiotic (Cefazolin) as is the usual surgical protocol when implanting foreign material
- 10:30 am Supervision in the recovery room for 6 hours
- Post-implantation brain scan
- 4.30 pm Return to hospitalization of neurosurgery
- Home delivery around 18h00
- Antibiotic for 72 hours by Orbénine if no allergy

In all case, prophylactic Intravenous injection of Cefazoline is required (if no allergy)

The recommended 15 mn surgery procedure is as followed:

- 3-cm skin opening (semicircular incision to prevent the future scar to be located on the zone of transdermal connection),
- Creation of a 12-mn burr hole without dura matter opening,
- Pose of the transducer in contact with the external face of the dura matter with no residual bone in between to have no distortion and no attenuation of emitted US
- Secure the device with 3 titanium screws
- Closure of the skin.

Neuronavigation systems could be used to position the device in the desired location.

The complete duration of the hospitalization is around 10 hours, including 30 minutes for the surgical procedure and around 6 hours for the post implantation monitoring. At the end of the day patients will return home.

5.4 Baseline and subsequent visits: Day 1 to week 9 (for combinaison treatment) or to Week 12 (for nivolumab alone)

Subjects will be ready to begin study.

DAY 1 TO WEEK 9 (for combinaison treatment) or DAY 1 TO WEEK 12 (for nivolumab alone)

The following assessment will be performed:

- Physical examination :

A complete physical examination will be performed and will include the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, peripheral vascular system and neurologic system. If indicated based on medical history and/or symptoms, rectal, external genitalia, breast, and pelvic exams will be performed.

Colour photography of any skin lesions (if applicable).

- Vitals signs :

Weight, Pulse, Saturation, Blood pressure, Temperature, ECOG

- Biological assessment:

- haematology (WBC count plus differential (total neutrophil including bands, lymphocyte, monocyte, eosinophil, basophil and other counts), RBC count, haemoglobin, haematocrit and platelet count),

- clinical chemistry (Sodium, potassium, chloride, bicarbonate, total calcium, inorganic phosphate, magnesium, urea or blood urea nitrogen (BUN), glycemia, creatinine, bilirubin (total, indirect and direct), AST, ALT, albumin, alkaline phosphatase, LDH, CPK, GGT), US Troponin.
- Endocrinology (Cortisol, TSH, Free T4, Free T3)
    - Blood samples (Plasma, PBMC…) for biobanking, PK and future ancillary study. Pregnancy test: urinary test during treatment visit. A positive urine pregnancy test requires a serum HCG test
    - Toxicity (using CTC AE version 4.0)
    - Treatment :
        - Immunotherapy :

Immunotherapy perfusion (Nivolumab alone or an association with Ipilimumab) followed by sonication.
Nivolumab and Ipilimumab will be used according to their approved dose and management (As indicated in the CPRs for the two treatments). They will be delivered by the local Pharmacy Departement under the control of Pr Madelaine:
Nivolumab alone: flat dose 240mg infusion (30 minutes infusion). During their participation of study patient will received 7 perfusions of Nivolumab (Day 1 to Week 12). Nivolumab alone will be continued in case of tumor control afterwards, per investigator's decision.
Combination strategy: Nivolumab 1mg/kg (30 minutes infusion) followed by Ipilimumab 3mg/kg (90 minutes infusion) or Nivolumab 3mg/kg (30 minute infusion) followed by Ipilimumab 1mg/kg (30 minute infusion). During their participation of study patient will received 4 combination treatment perfusions (Day 1 to Week 9). Nivolumab alone will be continued in case of tumor control afterwards, per investigator's decision.

- Sonication :

SonoVue is injected as a bolus, at a dose of 0.1 mL/kg, over a period of 30 seconds. Insertion of the transdermal needle could be done through CT scanning guidance in order to visualize the axis of entry of the needle. This option will be left to the choice of the investigator.
Then, the sonication starts at the acoustic pressure dose of either 0.78 or 0.9 or 1.03 MPa according to the cohort they have been assigned to.

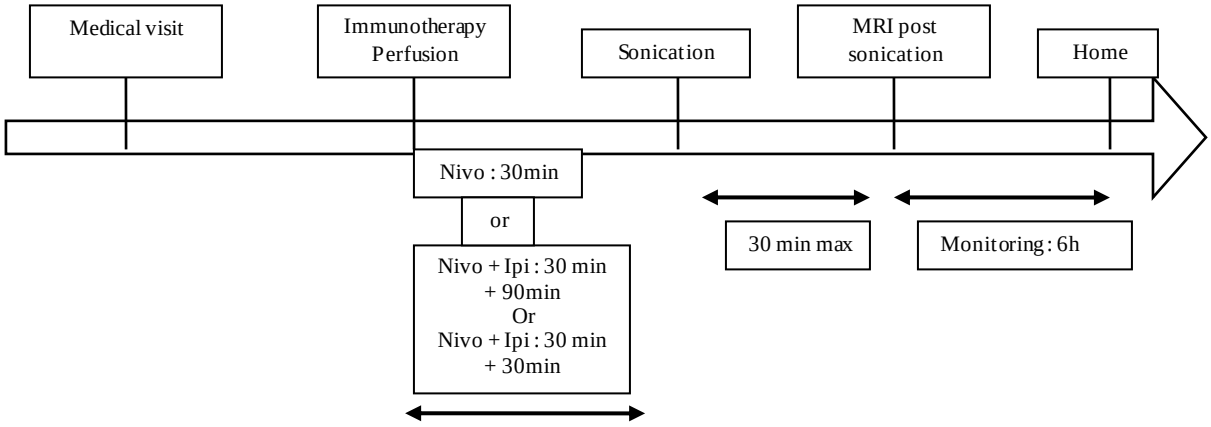
As of October 3, 2022, 3 patients were treated at level 1 (0.78MPa) and 1 patient was at level 2 (0.9MPa). No side effects or toxicity were observed (very good tolerance).
Since the CRM design recommends that a new patient could be treated at the higher level (1.03MPa), the patient n°5 will receive the 1.03MPa dose.

Brain RMI post sonication after first and second sonication (30 min maximum after the sonication -Saint Louis Hospital radiology department) to ensure (1) radiological safety of the procedure (absence of brain edema, bleeding or ischemia), (2) accurate BBB opening.
First sonication will be performed at minimum 7 days after implantation.
Sonication will be performed 1h maximum after the end of perfusion

- Documentation of Concomitant treatments

Patients will be monitored during 6 hours after completion of sonication before going back home. Patients will be kept under surveillance in case of seizures suspicion (an EEG will be performed in this case) or in case of chest discomfort with adapted management including electrocardiogram or Cardiac ultrasound (LVEF measure).

Adverse event will be discussed in St Louis RCP (Tumor board) according to our usual practice. All neurological adverse event will be discussed between the onco-dermatology and the neuro surgery teams; if needed the patient will have further exploration and/or adequate surgery procedures and the patient will be clinically monitored in the neuro-Surgery department (La Pitié Salpêtrière), to ensure no post sonication neurological disturbances including neurological deficit or partial epilepsia or brain bleeding.



WEEK 6
The following procedures will be added:

- ECG

During the Week 12, the following procedures will be added:

- Biobanking
- electrocardiogram or Cardiac ultrasound (LVEF measure) if clinically need
- a TEP/TDM scan (+/- intravenous (i.v.) contrast) (and 18F-FDOPA PET/CT: +/- 15 days after the first treatment) of chest abdomen and pelvis
- A brain MRI is required (with the basic MR sequences : FLAIR(before gado injection) T1-weighted contrast enhanced (0.2ml/kg, Doterem) SWAN (or a more standard T2 sequence can be used, to detect potential microhemorrhages), Diffusion)

After clinical examination and if relevant, review of the scans and /or MRI data (< 7 days) to assess the tumor status, patients will receive Nivolumab alone or an association with Ipilimumab infusion according to the standard mode of delivery in the Centre d'Investigation Clinique (Hospital Saint Louis as day-hospital sessions),

During all study, oustside the visits in CIC, patients will be followed by:
- remote monitoring using a standardized questionnaire as used in daily practice for all patients treated by immunotherapies (annexe 19.5). Patients will be contacted by phone 1or 2 days before the visit in Saint Louis Hospital. This questionnaire will verify that the patient don't have any toxicity since the last visit

7 sonications will be performed for the patient with Nivolumab alone.
5 sonications will be performed for the patient with Nivolumab combination with Ipilimumab.

Warning:

In case of delayed of Immunotherapy perfusion the sonication and Brain MRI should be delayed too.

5.5 Follow-up visits

During the second part of the study (Follow up: Week 14 to week 16) the following assessment will be performed:

- Physical exam:

A complete physical examination will be performed and will include the examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen,

back, lymph nodes, extremities, peripheral vascular system and neurologic system. If indicated based on medical history and/or symptoms, rectal, external genitalia, breast, and pelvic exams will be performed.
Color photography of any skin lesions (if applicable).

- Vitals signs :

Weight, Pulse, Saturation, Blood pressure, Temperature, ECOG

- Biological assessment :

- haematology (WBC count plus differential (total neutrophil including bands, lymphocyte, monocyte, eosinophil, basophil and other counts), RBC count, haemoglobin, haematocrit and platelet count),
- clinical chemistry (Sodium, potassium, chloride, bicarbonate, total calcium, inorganic phosphate, magnesium, urea or blood urea nitrogen (BUN), glycemia, creatinine, bilirubin (total, indirect and direct), AST, ALT, albumin, alkaline phosphatase, LDH, CPK, GGT), US Troponin.
- Endocrinology (Cortisol, TSH, Free T4, Free T3)

- Pregnancy test : urinary test
- Toxicity (using CTC AE version 4.0)
- Biobanking (at Week 16)
- electrocardiogram or Cardiac ultrasound (LVEF measure) if clinically need
- Treatment :

Nivolumab alone in case of tumor control afterwards, per investigator's decision.

- Brain MRI (+/- 2 days to week 16):

After completion of sonications or in case of progression with initiation of new therapy or DLT directly related to the ultrasound emission or toxicity related to immunotherapy, explantation of the device will be proposed to the patient in order to limit the infectious risks (Implantation duration until the end of sonication, 16 weeks maximum after inclusion). The Pre-surgery anesthesiology visit will be performed and the procedure of explantation will be done by the same team as the one having performed the implantation.
If the patient dies before the end of the study, the explantation will be carried out as soon as possible in relation to the patient's death, which will be agreed upon when it is included in the trial. If this is the wish / will of the person of trust, reporting a recent will of the patient not communicated to the investigators, this explantation will not be carried out.

In case of any adverse event, the patient case will be discussed between the onco-dermatology and the neuro surgery teams; if needed the patient will have further exploration and/or adequate surgery procedures, the patient will be clinically monitored in the neuro-Surgery department (La Pitié Salpêtrière).

During all study, patients will be followed by:
- remote monitoring using a standardized questionnaire as used in daily practice for all patients treated by immunotherapies (annexe 19.5) Patients will be contacted by phone 1or 2 days before the visit in Saint Louis Hospital. This questionnaire will verify that the patient don't have any toxicity since the last visit

During the third part of the study (Follow up at 6, 12, 18 and 24 months) the assessment of Survival status and systemic treatment, response will be collected.

5.6 End of study visit

The end of study visit will be done at month 24. The assessment of Survival status and systemic treatment, response) will be collected.

5.7 Expected length of participation, chronology and duration of the study.

Inclusion period	39 months	
Duration of participation for each participant, of which: • Maximum length of the screening period (verification of eligibility criteria): • Length of use of the device: • Follow-up period:	24 months 3 weeks Use of the device: 4mn max *7 (nivolumab) or *5 (nivo +ipi) over a period of 12 weeks. explantation: at the end of sonication, 16 weeks maximum after inclusion 81 weeks	
Total study duration:	63 months	

5.8 Table or diagram summarizing the chronology of the research

Treatment by Nivolumab alone :

		Screening / inclusion Visit	Pre-visit anesthetist and surgeon (2 days after screening visit)	Implantation device visit (2 days to 7 days after previsit with anesthetist and surgeon)	Day 1	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
Consent		X									
Medical History		X									
Physical exam		X	R	R	X	X	X	X	X	X	X
Vitals signs		X	R	R	X	X	X	X	X	X	X
Medical procedure (Ecg,…)		X			X[2]	X[2]	X[2]	X	X[2]	X[2]	X[2]
Cardiac ultrasound (LVEF measure)		X			X[2]	X[2]	X[2]	X[2]	X[2]	X[2]	X[2]
PDL1 Expression		X									
Pregnancy test (βHCG and urinary test)		R[5]			R[5]	R[5]	R[5]	R[5]	R[5]	R[5]	R[5]
Biological assessment		R	R		R	R	R	R	R	R	R
Radiological assessment	**MRI**	X			R [1]	R [1]	X[2]	X[2]	X[2]	X[2]	X
	CT-scan TEP/TDM scan	X									X
Radiological assessment (ancillary study)	18F-FDOPA PET/CT				R[3]						R[4]
Treatment	**Sonocloud**				R	R	R	R	R	R	R
	Immuno therapy				X	X	X	X	X	X	X
Biobanking (plasma, PBMC…)					R	R	R	R	R	R	R
Concomitant treatment		X		X	X	X	X	X	X	X	X
Toxicity using CTC AE version 5.0					X	X	X	X	X	X	X

		Week 14	Week 16	Pre-visit anesthetist and surgeon / Explantation device visit (between week 12 and week 16 *)	Follow Up (at 6, 12, 18 and 24 months)
Physical exam		X	X	X	
Vitals signs		X	X	X	
Medical procedure (Ecg,...)		X[2]	X[2]		
Cardiac ultrasound (LVEF measure)		X[2]	X[2]		
Pregnancy test (βHCG and urinary test)		X	X		
Biological assessment		X	X		
Radiological assessment	**MRI**		R		
Treatment	**ImmunoTherapy**	X[6]	X[6]		
Biobanking (plasma, PBMC…)			R		
Concomitant treatment		X	X	X	
Toxicity using CTC AE version 5.0		X	X	X	
Survival status (systemic treatment, response)					X

R : Procedures added for the study

[1] : Brain MRI after the first and second sonication

[2] : if clinically need

[3] : performed at baseline or +/- 8 days of Day 1 if patient include in clinical trial

4 : performed at Week 12 or +/- 15 days of week 12

[5] : βHCG at Selection / inclusion visit and urinary test from Day 1 A positive urine pregnancy test requires a serum HCG test

[6] : investigators' decision

* : as soon as possible in case of patient's death or progression with initiation of new therapy or DLT directly related to the ultrasound emission or toxicity related to immunotherapy

Treatment by Nivolumab combined with Ipilimumab:

		Screening Visit	Pre-visit anesthetist and surgeon (2 days after screening visit)	Implantation device visit (2 days to 7 days after previsit with anesthetist and surgeon)	Day 1	Week 3	Week 6	Week 9	Week 12
Consent		X							
Medical History		X							
Physical exam		X	R	R	X	X	X	X	X
Vitals signs		X	R	R	X	X	X	X	X
Medical procedure (Ecg,…)		X			X[2]	X[2]	X	X[2]	X[2]
Cardiac ultrasound (LVEF measure)		X			X[2]	X[2]	X[2]	X[2]	X[2]
PDL1 expression		X							
Pregnancy test (βHCG and urinary test)		R[5]			R[5]	R[5]	R[5]	R[5]	R[5]
Biological assessment		R	R		R	R	R	R	R
Radiological assessment	**MRI**	X			R [1]	R [1]	X[2]	X[2]	X
	TEP/TDM Scan	X							X
Radiological assessment (ancillary study)	18F-FDOPA PET/CT				R[3]				R [4]
Treatment	**Sonocloud**				R	R	R	R	R
	Immuno therapy				X	X	X	X	X
Biobanking (plasma, PBMC…)					R	R	R	R	R
Concomitant treatment		X		X	X	X	X	X	
Toxicity <u>using CTC AE version 5.0</u>					X	X	X	X	

		Week 14	Week 16	Pre-visit anesthetist and surgeon / Explantation device visit (between week 12 and week 16*)	Follow Up (at 6, 12, 18 and 24 months)
Physical exam		X	X	X	
Vitals signs		X	X	X	
Medical procedure (Ecg,…)		X[2]	X[2]		
Cardiac ultrasound (LVEF measure)		X[2]	X[2]		
Pregnancy test (βHCG and urinary test)		X	X		
Biological assessment		X	X		
Radiological assessment	**MRI**		R		
Treatment	**ImmunoTherapy**	X[6]	X[6]		
Biobanking (plasma, PBMC…)			R		
Concomitant treatment		X	X	X	
Toxicity using CTC AE version 5.0		X	X	X	
Survival status (systemic treatment, response)					X

R : Procedures added for the study

[1] : Brain MRI after the first sonication

[2] : if clinically need

[3] : performed at baseline or +/- 8 days of Day 1 if patient include in clinical trial

4 : performed at Week 12 or +/- 15 days of week 12

[5] : βHCG at Selection / inclusion visit and urinary test from Day 1 A positive urine pregnancy test requires a serum HCG test

[6] : investigators' decision

* : as soon as possible in case of patient's death or progression with initiation of new therapy or DLT directly related to the ultrasound emission or toxicity related to immunotherapy

5.9 Distinction between standard care and research

TABLE: "Standard care" vs. "Added interventions" required specifically for the research

Procedures and treatments to be provided during the study	**Procedures and treatments associated with standard care**	**Procedures and treatments added for the study**
Sonocloud® Implantation		Neuro oncologist consultation
Sonocloud® Implantation		Pre surgery anesthesiology visit
Sonocloud® Implantation		Implantation of the device (day hospital)
Treatment	Immunotherapy treatment : Nivolumab or Nivolumab combined with Ipilimumab	BBB opening with pulsed US Post sonication brain MRI
Treatment		BBB opening with pulsed US
Sonocloud® Explantation		Neuro oncologist consultation Pre surgery anesthesiology visit. Explantation of device (day hospital).
Radiological assessment	At screening and Week 12 : Brain MRI, TEP/TDM	Insertion of the transdermal needle could be done through CT scanning guidance in order to visualize the axis of entry of the needle. This option will be left to the choice of the investigator Post sonication brain MRI at day 1, w2 or w3 (according treatment) and w16 18F-FDOPA PET : before or at the latest 8 days after the first treatment with sonication and nivolumab and w 12
Biological assessment		Haematology, clinical chemistry and Endocrinology at: visit of screening, Pre surgery, post surgery and during treatment visit Pregnancy test : βhcg at screening and Urinary during treatment

Biobanking		Blood samples plasma, PBMC): 4 EDTA 4ml and 6 lithium heparin 7ml tubes at day 1, w6, w12 and w16 Blood samples :5 mL lithium heparinized Vacutainer before each BBB opening
Medical procedure	ECG at visit of screening and during treatment visit	
PDL1 Expression	At screening	

5.10 Biological samples

The samples that are taken during the study (plasma, PBMC) will be stored in a biological sample bank.

The collection(s) will be stored at the Pharmacology Department of Saint-Louis related to the CRB of Saint Louis Hospital. At the end of the study, the samples will be preserved. At the end of the study, the samples may be used for further analysis not described in the initial protocol but which may be useful for our investigation in light of developments in scientific knowledge on the pathology of melanoma, its diagnosis and treatment, provided the participant is informed and gives consent, as stated in the information consent form and in accord with the CPP advice.

If the samples are kept at the end of the trial, the sample bank will be declared to the minister responsible for research (Article L. 1243-3 of the CSP (French Public Health Code))

Type of sample	**Volume**	**Storage location** (name and organization)	**Collection supervisor** (name and organization)	**Purpose of the sample bank**	**Storage duration**	**End use (e.g. destruction)**
Plasma	4 EDTA 4ml tube	-20°C Pharmacology Department Saint-Louis	Pr Samia MOURAH Pharmacology Department Saint-Louis	Ancillary translational studies	15 years	destruction
PBMC	6 lithium heparin 7ml tubes	Liquid Nitrogen Pharmacology Department Saint-Louis	Pr Samia MOURAH Pharmacology Department Saint-Louis	Ancillary translational studies	15 years	destruction
PBMC	5mL Lithium heparin	Liquid Nitrogen Pharmacology Department Saint-Louis	Pr Samia MOURAH Pharmacology Department Saint-Louis	Future ancillary translotional studies	15 years	destruction

For samples taken from deceased persons, the Biomedicine Agency was requested and considered that it was not necessary to make a declaration in accordance with Articles L. 1232-2 and L. 1241-6 of the Code. of public health.

6. ELIGIBILITY CRITERIA

6.1 Inclusion criteria

Inclusion Criteria

- Patients with histologically confirmed metastatic melanoma
- Patients must have recovered from all side effects of their most recent systemic or local treatment for metastatic melanoma (grade ≤ 1).
- At least one measurable brain metastasis between 5 mm and 35 mm in diameter, not previously treated with surgery and/or radiosurgery and located less than 5 cm from the skull
- Patients may have received -or not- prior radiosurgery and/or surgery for brain metastases; if they have received prior local treatment, they must have at least 1new RANO and RECIST assessable brain metastases.
- BRAF status wild or mutated (and in that case previous treatment with BRAF inhibitor +/- MEK inhibitor allowed)
- Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤ 1.
- Age>18year
- Hemoglobin ≥10g/dl
- Platelets ≥ 100000mm3
- Neutrophils ≥1500/mm3
- Creatinine Clearance ≥ 50 ml/mn
- AST <3N
- ALT<3N
- Total bilirubin <1.5N
- Alkaline phosphatase <3N
- INR < 1.5
- Prothrombin ≥70%
- TCA <1.2
- No Hepatocellular insufficiency
- No injury (unhealed) to the head
- No allergy to poly isoprene
- Signed informed consent
- Patient with health insurance coverage
- Life expectancy > 3 months

6.2 Non-inclusion and Exclusion criteria

Non-Inclusion Criteria

- Ocular melanoma
- Symptomatic lepto-meningeal involvement.
- Symptomatic hemorrhagic brain metastases.
- Symptoms of incoercible intracranial pressure; patients receiving corticosteroids and patients presenting intermittent seizures can be enrolled if they have a stable dose of corticosteroids (≤ 30mg/day corticotherapy) and anti-epileptic treatment since at least 2 weeks before enrolment.
- Indication for urgent neurosurgery or radiotherapy
- Prior malignancy active within the previous 2 years except for locally curable cancers that have been apparently cured or stage I untreated Chronic Lymphoid Leukemia.
- Known HIV infection.
- Concurrent administration of any anticancer therapies other than those administered in this study.

- Treatment with any cytotoxic and/or investigational drug, antiCTLA4 or targeted therapy ≤ 4 weeks or < 5 half lives for targeted therapies or chemotherapy, prior to day 1 of study
- Prior whole brain radiotherapy
- Pregnant or lactating women

Investigators shall counsel WOCBP, and male subjects who are sexually active with WOCBP, on the importance of pregnancy prevention and the implications of an unexpected pregnancy. Investigators shall advise on the use of highly effective methods of contraception (Addenda 19.11), which have a failure rate of < 1% when used consistently and correctly.

- Contraindications to Nivolumab and Ipilimumab as defined in SPC (https://www.vidal.fr/substances/24410/nivolumab/)
- Serious or uncontrolled medical disorders that, in the opinion of the investigator, may increase the risk associated with study participation or study drug administration impair the ability of the patient to receive protocol therapy, or interfere with the interpretation of study results.
- Allergy to iodine, gadolinium, lidocaine
- Contra-indications to SonoVue® :
  hypersensibility to sulfur hexafluoride,
  recent acute coronary syndrome or unstable ischemic heart disease
  congestive heart failure≥ Class III or IV as defined by New York Heart Association
  concurrent treatment with Dobutamine
  severe pulmonary arterial hypertension
  uncontrolled systemic hypertension
  respiratory distress syndrome
- Concurrent treatment considered of unknown toxicity for the SNC in context of BBB opening such as
- Benzodiazepin (or any other sedative/hypnotic will be excluded (interrupted for at least 10 half lives before BBB opening) since their neurological tolerance after BBB opening is unknown)
- Antihistaminic
- Pro convulsing drug
- Butyrophenons, phenothiazin or any other “conventional” antipsychotic
- Barbituric
- OAM inhibitor
- Anticholinergic
- Any other drug according investigator to cause cerebral toxicity due to BBB opening
- Concurrent anticoagulant or antiplatelet therapy
- Uncontrolled epilepsy
- MRI contra indication (claustrophobia, intracorporal metallic material…)
- Phlebitis, active pulmonary embolism
- Prisoners or subjects who are involuntarily incarcerated
- Psychological, familial, sociological, or geographical conditions that potentially hamper compliance with the study protocol and follow-up schedule; those conditions should be discussed with the subject before registration in the trial

Drugs authorized under special conditions of stable dosage for at least 1 month prior inclusion:
• Inhibitors of acetylcholine esterase (i.e., donepezil Aricept, galantamine, rivastigmine) Memantine
• Atypical antipsychotics (eg Quetiapine)
• Antidepressants (other than MAO inhibitors and anticholinergics) Dose < 20 mg / kg
• Thyroid hormones

Exclusion criteria:

- Implantation of the SONOCLOUD not possible according to neurosurgeon

Any patient morphological characteristics (e.g. skin characteristics, bone thickness, other), which, from neurosurgeons’ opinion, prevent implantation of the device or may

impair the ability of the patient to receive treatment with SonoCloud®, would be excluded

6.3 Enrolment procedure

	Number of participants
Total number of participants to be included	*21*
Number of sites	*1*
Enrolment period (months)	*39*
Number of participants/site	*21 ou 23/24*
Number of participants/site/month	***1 / month***

6.4 Termination and exit rules

Criteria and methods for early termination of the study interventions or the product administration:

There are a number of possible situations:

- Temporary suspension: the investigator must document the reason for suspending and resuming the treatment in the participant's source file and the case report form (CRF)
- Premature termination, but the participant remains enrolled in the study until the end of his/her participation: the investigator must document the reason
- Early termination and exit from the study: the investigator must document the reason or reasons

Criteria and procedure for early withdrawals and exits from the study

- Participants may exit the study at any time and for any reason.
- If, during the course of his/her participation in the study, the participant presents a DLT, then the study product/procedure must be discontinued but the participant will continue to be monitored for the study.
- The investigator can temporarily or permanently withdraw a participant from the study for any safety reason or if it is in the participant's best interests.
- Participant lost to follow-up: the participant cannot be located. The investigator must make every effort to reconnect with the participant (and document his attempts in the source file), at least to determine whether the participant is alive or dead
- If a participant exits the trial prematurely or withdraws consent, any data collected prior to the date of premature exit may still be used.
- If a participant exits the study prematurely, and if the participant agrees OS and PFS will still be collected

- In case of serious adverse events, see the corresponding section on vigilance
- In case of adverse events, see section bellow “Discontinuation Criteria”

- The case report form will list the various reasons why the participant exited or was withdrawn from the study:

☐ Lack of efficacy
☐ Adverse reaction
☐ Another medical issue
☐ Personal reasons of the participant
☐ Explicit withdrawal of consent
☐ Lost to follow-up

Any subject can withdraw from participating in the research at any time and for any reason. In such case the patient will be replaced to assure that the design could be applied.

AH-HP (the sponsor) or the Competent Authority (ANSM) may prematurely discontinue all or part of the trial, temporarily or permanently, upon the recommendation of the Data Safety Monitoring Board in the following situations:
- first, if suspected unexpected serious adverse reactions (SUSAR) are observed in one of the research arms or if there is a discrepancy in the serious adverse reactions between the two arms, requiring a reassessment of the benefit-risk ratio for the trial.

Similarly, AH-HP, as the sponsor, or the Competent Authority (ANSM) may prematurely cancel the trial due to the discovery of unexpected facts or new information about the procedure used, in light of which the objectives of the study or clinical program are unlikely to be achieved.

AP-HP, as the sponsor, reserves the right to permanently suspend enrolment at any time if the enrolment targets have not been met.
If the study is cancelled prematurely for safety reasons, AP-HP will inform the Competent Authority (ANSM) and the Ethics Committee of its decision within 15 days, together with justification for the decision and any recommendations from the Data Safety Monitoring Board in the case of a Substantial Amendment.

Dose Modifications for sonication :
Dose reductions or dose escalations are not permitted.

Dose Delay for sonication :
In case of delayed of immunotherapy perfusion, the sonication should be delayed

Discontinuation criteria for Sonication:
Sonication will be stopped for the following:
- DLT
- Stop treatment by immunotherapy (due to toxicity)
- Disease progression with change of treatment
- the end of sonication, 12 weeks maximum after Day 1 of immunotherapy perfusion. During a sonication session, the Investigator can stop the procedure at any time in case of any abnormal behavior or pain from patient. The ultrasound session must not be resumed at that visit even if the signs or symptoms that justified discontinuation of the procedure disappear. The reason for suspending the sonication should be documented in the participant's source file and the eCRF. The decision to resume SC1 treatment at the next cycle should be discussed with the DSMB.

Treatment Beyond Disease Progression (immunotherapy and sonication)
Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of Progressive Disease (PD).
Subjects will be permitted to continue treatment beyond initial RANO and RECIST 1.1 defined PD as long as they meet the following criteria:

- Investigator-assessed clinical benefit, and
- Subject is tolerating study drug.

The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment.

- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, CNS metastases)

A radiographic assessment/scan should be performed within 6 weeks of initial investigator-assessed progression to determine whether there has been a decrease in the tumor size or continued PD. The assessment of clinical benefit should be balanced by clinical judgment as to whether the subject is clinically deteriorating and unlikely to receive any benefit from continued treatment with nivolumab.
If the investigator feels that the subject continues to achieve clinical benefit by continuing treatment, the subject should remain on the trial and continue to receive monitoring according to the Time and Events Schedule.

For the subjects who continue study therapy beyond progression, further progression is defined as an additional 10% increase in tumor burden from time of initial PD. This includes an increase in the sum of diameters of all target lesions and/ or the diameters of new measurable lesions compared to the time of initial PD. Treatment with study medication should be discontinued permanently upon documentation of further progression.
New lesions are considered measureable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). Any new lesion considered non-measureable at the time of initial progression may become measureable and therefore included in the tumor burden if the longest diameter increases to at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). In situations where the relative increase in total tumor burden by 10% is solely due to inclusion of new lesions which become measurable, these new lesions must demonstrate an absolute increase of at least 5 mm.

Discontinuation of Subjects following any Treatment with investigational product

Subjects MUST discontinue sonication (and non-investigational product at the discretion of the investigator) for any of the following reasons:

- Subject's request to stop study treatment
- Any clinical adverse event, laboratory abnormality or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Termination of the study
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness
- Pregnancy.

All subjects who discontinue sonication should comply with protocol specified follow-up procedures. Subjects who discontinue sonication must continue to be followed for collection of outcome and/or survival follow-up data as required until death or the conclusion of the study.

The only exception to this requirement is when a subject withdraws consent for all study procedures including post-treatment study follow-up or loses the ability to consent freely (ie, is

imprisoned or involuntarily incarcerated for the treatment of either a psychiatric or physical illness).
If sonication is discontinued prior to the subject's completion of the study, the reason for the discontinuation must be documented in the subject's medical records and entered on the appropriate case report form (CRF) page.

Management of immunotherapy toxicity:
Will be followed according to irAE as indicated annexe 19.6

Withdrawal of Consent
Subjects who request to discontinue sonication and immunotherapy will remain in the study and must continue to be followed for protocol specified follow-up procedures. The only exception to this is when a subject specifically withdraws consent for any further contact with him/her or persons previously authorized by subject to provide this information. Subjects should notify the investigator of the decision to withdraw consent from future follow-up **in writing**, whenever possible. The withdrawal of consent should be explained in detail in the medical records by the investigator, as to whether the withdrawal is from further treatment with study drug only or also from study procedures and/or post treatment study follow-up, and entered on the appropriate CRF page. In the event that vital status (whether the subject is alive or dead) is being measured, publicly available information should be used to determine vital status only as appropriately directed in accordance with local law.

7. Medical device used for the study - Treatments, procedures and strategies associated with the use of the device

7.1 Investigational medical device(s) being studied

Description of the device

The SonoCloud® system is developed by CarThera, Institut du Cerveau et de la Moelle épiniere, boulevard de l'hôpital, Hôpital de la Pitie Salpetrière, 75646 Paris cedex 13, France.

The device is manufactured by CarThera's selected providers according to the Essential Requirements of the Directive 90/385 .

The implant, generator and the kit containing the single-use needle (needle +connector) and ultrasound resonator are provided to the investigational site by CarThera separately. All the material is labelled "for investigation use only".

The model, version, including any software version, serial number, batch number, composition of the medical device, description of materials, are described in the updated INVESTIGATOR BROCHURE.

Intended purpose of the device

The SonoCloud system is a non-CE marked implantable device intended to locally and transiently increase the permeability of the blood brain barrier to facilitate the passage of substances or physician-specified agents including chemotherapy, into the cerebral parenchyma of patients with brain disease.

Instructions for using the device

To activate the device for 4 min, the transdermal needle is connected to the implant and connected to an external radiofrequency generator.
The US sonication is initiated after injection of an intravenous bolus injection of SonoVue® microbubbles (Bracco, Geneva, Switzerland) at the dose of 0.1 mL/kg.
The connection procedure and sonication are guided by an interactive software interface through a touchscreen on the generator system. During the procedure, the practitioner is guided through a series of steps in the generator software in which the operator enters the patient information, physician information, verifies that the needle is properly connected to the implant, and then begins the treatment.

The transducer is operated with a burst length of 25,000 cycles (23.8 ms) at a pulse repetition frequency of 0.5-1 Hz (1.19-2.38% duty cycle) for a total duration of 120-240 seconds
The total time to perform a sonication procedure is less than 15 minutes.
See USER MANUAL FOR DETAILED INSTRUCTIONS for USE

Summary of the training and experience needed to use the device

In addition to the set-up visit, a specific training, performed by CarThera is planned for people participating to the clinical research and habilitated to use the SonoCloud system.
The user manual is distributed to each training participant of the training.
This specific training will be performed during the presentation of the study and during the first sonications.
For any question or technical issue, the technician of CarThera could be contacted during the working hours at the following telephone number:

CarThera: + 33 4 72 62 62 68

Whenever required, the presence of a duly habilitated CarThera's technician during the procedure of sonication is possible.

Description of medical, laboratory and surgical procedures involved in using the device

The SonoCloud® US (CarThera, Paris, France) device is implanted within the skull bone if possible overlying the tumor area (contrast-enhancing region or high-signal FLAIR region). In case the patient is eligible for debulking surgery under general anesthesia, the device will be implanted during the surgical procedure within a burr hole after dura matter closing and before skin closure. If surgical resection is not indicated (most of the cases), the device will be implanted during a dedicated surgical procedure in an ambulatory fashion under local anesthesia. This procedure consists of a 3-cm skin opening, creation of a burr hole without dura-matter opening, implantation of the device and closure of the skin as described in section 2.6.

After completion of sonications or in case of progression, explantation of the device will be proposed to the patient. This procedure will be done by the same team as the one having performed the implantation.

7.2 Description of the Investigational medicinal products

Nivolumab and ipilimumab will be used according to their approved dose and management.
They will be delivered by the local Phamacy Department under the control of Dr I. Madelaine.
Nivolumab alone: flat dose 240mg infusion every 2 weeks until 12 weeks or progression or toxicities.
Nivolumab alone could be given at week 14 and week 16 in case of tumor control per investigator's decision.

Combination strategy: nivolumab 1 mg/kg and ipilimumab 3 mg/kg or nivolumab 3 mg/kg and ipilimumab 1 mg/kg for 4 infusions every 3 weeks until 9 weeks or progression or toxicities. Nivolumab alone could be given at week 12-14 and week 16 in case of tumor control per investigator's decision.

In general, Ipilimumab 3mg/kg-Nivolumab 1mg/kg is preferred for patients with metastatic melanoma.
However in certain situation, elderly patient, patients with past history of immune related AE is previously expose to immune checkpoint inhibitor, , and after disucssion in multidisciplinary tumor board, ipilimumab 1mg/kg Nivolumab 3mg/kg can be proposed instead of ipilimumab mg/kg3 Nivolumab 1mg/kg.

Sonovue (sulfur hexafluroride) will be used for the sonication.
It is injected as a bolus, at a dose of 0.1 ml/kg, over a period of 30 seconds.Then, the sonication starts at the acoustic pressure dose of either 0,78 or 0,9 or 1,03 MPa according to the cohort they have been assigned to.
Sonication will be performed 1h maximum after the end of perfusion of Nivolumab (if Nivolumab alone) or Ipilimumab (if Nivolumab in combination with Ipilimumab).
Sonovue will be labelled according to the regulatory requirements in France (Good Manufacturing Practices), and will be supplied by Clinical Trial Department of the Agence Générale des Produits de Santé (DEC AGEPS), Assistance Publique-Hôpitaux de Paris, for the sonication to the local Pharmacy Department.

7.3 Authorized and prohibited treatments (physical, medicinal, non-medicinal, surgical), including rescue medication

In this trial patients are treated according to real life recommended procedures. Apart from benzodiazepine and other sedative agents (see paragraph 2.2)

Authorized treatments	Prohibited treatments
- Antidepressant SRI (other than MAO inhibitor or anticholinergic) <20mg/kg - Corticoids (≤ 30mg/day corticotherapy) - Thyroid hormone - Morphinic, Antalgic (palier 1-2-3) - Stereotactic radiotherapy of brain metastasis outside the sonocloud field (no radiotherapy during the DLT period)	- Benzodiazepin (or any other sedative/hypnotic) - Antihistaminic - Pro-convulsing drug - Butyrophenons, Phenothiazin or any other “conventional” antipsychotic - Barbituric - MAO inhibitor - Anticholinergic - Any other drug according investigator to cause cerebral toxicity due to BBB opening - Concurrent anticoagulant or antiplatelet therapy - Radiotherapy during the DLT period

8. PERFORMANCE AND EFFICACY ASSESSMENT

Primary endpoint:
Success defined as grade 2 or 3 BBB opening/disruption with pulsed US using the SonoCloud® system without toxicity related to the device assessed clinically and using brain MRI (DLT evaluated during the first 4 weeks of treatment by Nivolumab alone and during the first 6 weeks of treatment by Nivolumab in combination with Ipilimumab). The primary endpoint will be the success defined as efficacy without toxicity.

Secondary endpoint:
Best overall response rate and ORR at 3 months
Best intracranial overall response rate (BICORR) and ICORR at 3 months
Best extracranial overall response rate (BECORR) and ECORR at 3 months
Progression free survival (PFS) at 3 months
Intracranial progression free survival (PFS) at 3 months 6 months, 12 – 18 and 24 months
Extracranial progression free survival (PFS) at 3 months 6 months, 12 – 18 and 24 months
Overall survival (OS) at 4 months 6 months, 12 – 18 and 24 months
Safety using CTCAE version 5.0

Clinical activity will be assessed using TEP/TDMScans (extracranial lesions) and MRI (intracranial metastases). Clinical objective response rate (ORR) (defined with the RANO and iRANO and the RECIST version 1.1 and the Immune-Related Response Criteria) and PFS defined as time from day 1 of immunotherapy perfusion and sonication to disease progression (according to the RANO and RECIST version 1.1) and the immune related Response Criteria- or death, whichever is first.

8.1 Proposed methods and timetable for measuring, collecting and analyzing the efficacy and performance data

The efficacy data must include a chronological record of the performance and efficacy assessment parameters.

Also describe the procedural circuits for the trial (circuit for additional examinations or functional tests, sample circuit, treatment unit circuit, etc.) and name the persons responsible.

Patients will be monitored at each cycle for efficacy assessment (clinical assessment and photographs if applicable) (see section 5).
Peripheral blood will be collected at the 4 times points (before the first dose, at week 6, week 12 and week 16) in 4 EDTA 4ml and 6 lithium heparin 7ml and 1 lithium heparin 5mL tube (Pr S. Mourah Pharmacology Department of Saint-Louis).

9. SPECIFIC COMMITTEES FOR THE TRIAL

9.1 Scientific Committee

- Role: determine the objective of the study, write the protocol, recommend changes to the protocol during the study, will meet (physically, TC Meeting or exchange by mail) as frequently as necessary.
- Members of the Committee:

Investigator Coordonator Pr Céleste LEBBE	Dermatologist	AP-HP, Hôpital Saint-Louis	33-1-42-49-46-79 celeste.lebbe@aphp.fr
Scientific investigator Pr Carpentier	Neuro surgeon	AP-HP, Hôpital La Pitié Salpêtrière	33-1-1-42-16-34-05 alexandre.carpentier@aphp.fr
Methodologist Matthieu RESCHE-RIGON	Methodologist	AP-HP, Hôpital Saint-Louis	33-1-42-49-97-47 matthieu.resche-rigon@univ-paris-diderot.fr
Others			
Moufida DABBECH	Project manager	AP-HP, Hôpital Saint-Louis	33-1-44-84-17-32 moufida.dabbech-jourdain@aphp.fr
Laetitia DA MEDA	Clinical Studies Coordinator	AP-HP, Hôpital Saint-Louis	33-1-42-49-93-92 Laetitia.da-meda@aphp.fr
Chafia ABBOU-BENIHADDADENE	Project manager	DRCI-URC AP-HP, Hôpital Saint-Louis	33-1-42-38-53-22 chafia.benihaddadene@univ-paris-diderot.fr
Sarra DALIBEY	Head of safety department	AP-HP, Hôpital Fernand-Widal	33-1-40-27-57-85 Sarra.dalibey@aphp.fr
Guest : Carole DESSEAUX	Carthera		carole.desseaux@carthera.eu

10. SAFETY ASSESSMENT - RISKS AND CONSTRAINTS ADDED BY THE STUDY

10.1 Definitions according to *the Regulation (EU) 2017/745*:

- **Adverse event:**

Any untoward medical occurrence, unintended disease or injury, or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the investigational medical device. (MDR Article 2(57))

Note:

a. This definition includes events that are anticipated as well as unanticipated events

b. This definition includes events occurring in the context of a clinical investigation related to the investigational device, the comparator or the procedures involved.

- **Serious Adverse reaction:**

Any adverse event that led to any of the following:

a) led to death,

b) led to serious deterioration in the health of the subject, that either resulted in :

1) a life-threatening illness or injury,

2) a permanent impairment of a body structure or a body function,

3) in-patient or prolonged hospitalization, or

4) medical or surgical intervention to prevent life-threatening illness or injury permanent impairment to a body structure or a body function,

5) chronic disease

c) led to foetal distress, foetal death or a congenital abnormality or birth defect.

- **Adverse device effect/ serious adverse device effect:**

An adverse device effect is an adverse event related to the use of an investigational device. A serious adverse device effect is an adverse device effect that has resulted in any of the consequence characteristics of a serious adverse event.

- **Unexpected adverse reaction to an investigational medicinal product:**

Any adverse reaction to a product for which the nature, severity, frequency or outcome is inconsistent with the safety information described in the Reference Safety Information (summary of product characteristics, or the investigator's brochure if the product is not authorised).

- **Device deficiency (DD)**

· Any inadequacy in the identity, quality, durability, reliability, safety or performance of an investigational device, including malfunction, use errors or inadequacy in information supplied by the manufacturer. (MDR Article 2(59))

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<u>According to Article R.1123-46 of the Code de la Santé Publique and the guidelines for sponsors of clinical trials conducted on medical devices and *in vitro* diagnostic medical devices (ANSM)</u>:

- **Emerging safety issue**

Any new information that may lead to a reassessment of the risk/benefit ratio of the study or the product subject to the study, to modifications to the use of the product, the conduct of the study, or the study documents, or to a suspension, interruption or modification of the protocol of the study or other similar studies. For studies involving the first administration or use of a health product in healthy volunteers, any serious adverse reaction.

Examples:

a) any clinically significant increase in the frequency of an expected serious adverse reaction;
b) suspected unexpected serious adverse reactions in participants who have terminated their participation in the clinical trial and of which the sponsor has been notified by the investigator, in addition to any possible follow-up reports;
c) any new fact relating to the conduct of the clinical trial or the use of the medical device, that may impact the safety of the study participants.

Examples:

- a serious adverse event likely to be related to the trial's interventions and diagnostic procedures and which may impact the conduct of the clinical trial
- a significant risk for the trial participants, such as the ineffectiveness of the medical device in treating a life-threatening illness under investigation
- significant results from a recently completed preclinical study that may impact the risk/benefit ratio (such as a biomechanical study)
- the premature termination, or temporary suspension, of a trial conducted on the same medical device in another country, for safety reasons
- an unexpected serious adverse reaction associated with a non-investigational health product required for carrying out the trial, (e.g. challenge agents, emergency treatment)

d) recommendations from the Data Safety Monitoring Board (DSMB), if applicable, that may affect the safety of the study participants
e) any unexpected serious adverse reaction reported to the sponsor by another sponsor of a clinical trial carried out in a different country but relating to the same medical device

10.2 The role of the investigator

The investigator must **assess:**
- the seriousness of each adverse event and record all serious and non-serious adverse events in the case report form (eCRF)
- if the device deficiency might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate. The investigator must record all deficiency that might have led to a serious adverse or not in the case report form (eCRF)

The investigator must **document** serious adverse events **as thoroughly as possible** and provide a definitive medical diagnosis, if possible.

The investigator must **assess the intensity** of the adverse events:

- Common Terminology Criteria for Adverse Events [National Cancer Institute]

The investigator must **assess the causal relationship** between the serious adverse events and each investigational medical device and/or the investigation procedure and/or investigational medicinal products.

The method used by the investigator is based on the following causality terms (extract from MDCG 2020-10/1):

- Not related,
- Possible,
- Probable,
- Causal relationship

Their definition are presented in the following table.

Causality term	Assessment criteria
Not related	Relationship to the device, comparator or procedures can be excluded when:

Causality term	Assessment criteria
	- the event has no temporal relationship with the use of the investigational device, or the procedures related to application of the investigational device; - the serious adverse event does not follow a known response pattern to the medical device (if the response pattern is previously known) and is biologically implausible; - the discontinuation of medical device application or the reduction of the level of activation/exposure - when clinically feasible - and reintroduction of its use (or increase of the level of activation/exposure), do not impact on the serious adverse event; - the event involves a body-site or an organ that cannot be affected by the device or procedure; - the serious adverse event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risk factors); - the event does not depend on a false result given by the investigational device used for diagnosis[1], when applicable; In order to establish the non-relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious adverse event.
Possible	The relationship with the use of the investigational device or comparator, or the relationship with procedures, is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment). Cases where relatedness cannot be assessed, or no information has been obtained should also be classified as possible.
Probable	The relationship with the use of the investigational device or comparator, or the relationship with procedures, seems relevant and/or the event cannot be reasonably explained by another cause.
Causal relationship	The serious adverse event is associated with the investigational device, comparator or with procedures beyond reasonable doubt when: - the event is a known side effect of the product category the device belongs to or of similar devices and procedures; - the event has a temporal relationship with investigational device use/application or procedures; - the event involves a body-site or organ that o the investigational device or procedures are applied to; o the investigational device or procedures have an effect on; - the serious adverse event follows a known response pattern to the medical device (if the response pattern is previously known); - the discontinuation of medical device application (or reduction of the level of activation/exposure) and reintroduction of its use (or increase of the level of activation/exposure), impact on the serious adverse event (when clinically feasible); - other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/and an effect of another device, drug or treatment) have been adequately ruled out; - harm to the subject is due to error in use; - the event depends on a false result given by the investigational device used for diagnosis[2], when applicable; In order to establish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/procedures and the serious adverse event.

1: If an investigational device gives an incorrect diagnosis, the patient might, for example, receive an unnecessary treatment and incur all the risks that accompany that treatment, or might be incorrectly diagnosed with a serious disease. In other cases, the patient might not receive an effective treatment (thereby missing out on the benefits that treatment would confer) or might not be diagnosed with the correct disease or condition.

2: If an investigational device gives an incorrect diagnosis, the patient might, for example, receive an unnecessary treatment and incur all the risks that accompany that treatment, or might be incorrectly diagnosed with a serious disease. In other cases, the patient might not receive an effective treatment (thereby missing out on the benefits that treatment would confer), or might not be diagnosed with the correct disease or condition.

The investigator will state whether the serious adverse event follows a deficiency related to the investigational device.

The investigator will state whether the serious adverse event follows on from a medication error.

Procedures for notifying the sponsor are presented in the section 10.2.4

10.2.1 Serious adverse events that require a notification without delay by the investigator to the sponsor

The investigator notifies the sponsor immediately, but not later than 3 calendar days after investigation site study's personnel's awareness of:

a) any SAE (see section 10.1) occurred during clinical investigation with the exception of any event which is listed in the protocol (see relevant section) and, if applicable, in the investigator's brochure as not requiring immediate notification,

b) any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate,

c) Any new findings in relation to any event referred to in point a) and b)

10.2.2 Specific features of the protocol

10.2.2.1 Other events that require the investigator to notify the sponsor without delay

- Adverse events judged as "medically significant"
  - blood effusion on the sonication beam
  - brain edema within 72 hour after sonication
  - local epilepsia
  - faintness
  - facial placy

  The investigator should clarify whether the serious adverse event is the result of a device deficiency related to the investigational device.

  The investigator must notify the sponsor without delay on the day when the investigator becomes aware of these adverse events, with the same modalities and within the same timeline as for serious adverse events (see section 10.2 P57).

- ***In utero*** exposure
  The investigator must notify the sponsor without delay on the day when the investigator becomes aware of any pregnancy that occurs during the trial, even if not associated with an adverse event. The pregnancy is reported using a special form, appended to the protocol.

- **Exposure via breastfeeding** Exposure via breastfeeding occurs if an infant or a child could have been exposed via the breast milk of a mother for whom study procedure had been realized.
  Even if such exposure is not associated with an adverse event, the investigator must always notify the sponsor without delay on the day when the investigator becomes aware of any exposure via breastfeeding.

10.2.2.2 Serious adverse events/device deficiency (that would not led to a serious adverse event) that do not require the investigator to notify without delay the sponsor

These serious adverse events are simply recorded in the case report form (e-CRF). A data retrieval of the case report forms will be implemented for serious adverse events every 12 months by Clinical Trial Unit and transmit to Safety Department at expertisecsi.drc@aphp.frand to DSMB members if they whish.

- *Normal and natural course of the condition*:
  - *scheduled inpatient hospitalization for monitoring the condition under investigation*
  - *inpatient hospitalization for routine treatment or for monitoring the condition under investigation, not associated with a deterioration in the trial subject's condition*
  - *worsening of the condition under investigation Disease progression grade ≤ 3,Edema managed by corticotherapy without sequelae after 72 hours of sonication*

- *Device deficiency that would not led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate*

- *Special circumstances*
- *Hospitalization for a pre-existing illness or condition*
- *Hospitalization for a medical or surgical treatment arranged prior to the trial*
- *Admission for social or administrative reasons*
- *Transfer to the emergency ward (<12 hours)*

- *Adverse events during the trial possibly related to treatments/acts prescribed as a part of the patient's standard care*

The investigator as health care professional must notify these events to the appropriate health surveillance institution according to the situation. Examples: Agence Régionale de Santé, quality department of your hospital, Centre Régional de Pharmacovigilance, local correspondent of the materiovigilance unit (ANSM), etc.

- Adverse events which occur far away from the use of the medical device: the serious adverse event occurring after four weeks of explanation of investigational medical device do not require the investigator to notify the sponsor. An e-CRF extraction of these serious adverse events will be realized every 6 months by clinical research unit and transmitted to safety department at expertisecsi.drc@aphp.fr.

10.2.3 Period during which SAEs/device deficiencies must be notified without delay by the investigator to the sponsor

The investigator notifies the sponsor immediately, but not later than 3 calendar days after investigation site study's personnel's awareness of:

- trial subject begins the first investigational procedure (Immunotherapy perfusion)
- up to 4 weeks after the explanation of the investigational device
- indefinitely, if the SAE is likely to have been caused by the medical device and/or investigational medicinal products and/or the specific investigation procedures.

  NB: In this case, the investigator does not have to collect indefinitely in the in the case report form (eCRF) all SAEs / defects possibly related to the clinical investigation, but must only notify them, to the sponsor, as soon as he becomes aware of them

10.2.4 Procedures and deadlines for notifying the sponsor

The initial notification of an SAE/device deficiency must be provided in a written report signed by the investigator using a notification form specific to the study and intended for this purpose (in the case report form):

- for each SAE: provide the SAE form,
- for each device deficiency (that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate) : provide the device deficiency form,
- for device deficiency that has led to established SAE : provide device deficiency form **and** SAE form.

Each item in this form must be completed by the investigator so that the sponsor can carry out the appropriate analysis.

The initial notification to the sponsor of a serious adverse event/deficiency must be quickly followed by an additional detailed written report (or reports) so that the case outcome may be monitored by the Safety Department or to provide further information.

Whenever possible, the investigator will provide the sponsor with any documents that may be useful (medical reports, laboratory test results, results from additional examinations, etc.). These documents must be non-identifying. In addition, the documents must include the following: study acronym, number and participant's initials.

Any adverse event will be monitored until fully resolved (stabilisation at a level considered acceptable by the investigator, or return to the previous state) even if the participant has left the study.

The initial notification, the SAE/deficiency follow-up reports and all other documents must be sent to the sponsor's Safety Department, by email only (eig-vigilance.drc@aphp.fr). It should be noted that it is possible to send SAE/deficiency reports to the Safety Department by fax to +33 (0)1 44 84 17 99, only in the event of a failed attempt to send the SAE report by email (in order to avoid duplication).

For studies which use e-CRFs:

- the investigator completes the SAE/deficiency notification form in the e-CRF, then validates, prints, and signs the form before sending it by email;
- if it is not possible to connect to the e-CRF, the investigator should complete, sign, and send the SAE/deficiency notification form to the Safety Department. As soon as the connection is restored, the SAE/deficiency notification form in the e-CRF must be duly completed.

The investigator must respond to all requests from the sponsor for additional information.
For all questions relating to the notification of an adverse event, the Safety Department can be contacted via email: vigilance.drc@aphp.fr.

For cases of *in utero* exposure, the investigator will complete the Notification and Follow-up form for a pregnancy occurring during participation in a study".
The investigator must monitor the pregnant woman throughout her pregnancy or until the pregnancy is terminated, and must notify the sponsor of the outcome of the pregnancy using this form.
If the outcome of the pregnancy falls within the definition of a serious adverse event (miscarriage, pregnancy termination, foetal death, congenital abnormality, etc.), the investigator must follow the procedure for reporting SAEs.

The initial pregnancy notification, the SAE follow-up reports, and any other documents will be sent to the sponsor according to the same procedures specified herein.

Specific case of studies conducted with the administration of a radioactive product (e.g. PET): in case of the onset of cancer or development of a hereditary deficiency linked to the exposure of ionising radiation, the investigator completes the specific form for secondary cancers.

10.3 Role of the sponsor

The sponsor, represented by its Safety Department, shall continuously assess the safety of each investigational medical device and/or investigational medicinal products and/or study procedures throughout the trial.

10.3.1 Analysis and declaration of serious adverse events

The sponsor assesses:

- the **seriousness criteria** of all reported adverse events,
- **the causal relationship** with each investigational device and/or investigational medicinal products and/or investigation procedures, and/or any other treatments.
- all serious adverse events where the investigator and/or sponsor believe that a causal relationship with *the investigational medical device and/or its implementing gesture* and/or investigational medicinal products *and/or the study procedures* is reasonably possible are considered as suspected serious adverse reaction.
- the Unanticipated Serious Adverse Device Effect
  It is an effect which by its nature, incidence, severity or outcome has not been identified in the current risk assessment. Procedures associated with the use of a device should be addressed in the risk assessment, which makes it possible to determine whether the procedure related SAEs are Unanticipated Serious Adverse Device Effect or not. SAEs related to procedures imposed by the clinical investigation plan but not with the use of the device should not be considered Serious Adverse Device Effects. (extract from MDCG 2020-10/1)
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- the **expected or unexpected nature** of the serious adverse reactions concerning investigational medicinal products.
  Any serious adverse reaction for which the nature, severity, frequency or outcome is inconsistent with the safety information described in the summary of product characteristics, is considered as unexpected.

  The sponsor, acting through its Safety Department, assesses the unanticipated (investigational device) or expected/unexpected nature (investigational medicinal products) of the serious adverse reaction based on the information described below**.**

Serious adverse events likely to be related to the investigational device - refer to the Investigator's Brochure of ***Sonocloud***®. Serious adverse events likely to be related to the investigation procedure: -Implementing gesture (implantation, sonication and explanation): refer to the Investigator's Brochure of ***Sonocloud***®. Risks associated with the implantation/explantation of ***Sonocloud***® device: - Infection, - Hemorrhage - Pain

Serious adverse events likely to be related to the investigational medicinal products:
- refer to SmPC of Nivolumab
- refer to SmPC of Ipilimumab
- refer to SmPC of the *of Sonovue*®

- for post-operative Céfazoline/Orbenine (cloxacilline) administration (to be replaced by another antibiotic in case of allergia or in case of different antibiotic standard of care of the participating centers): refer to SmPC of speciality administered.
-for lidocaine administration: refer to SmPC of speciality administred.

-for MRI, CT-scan, and PET-scan: claustrophobia (anxiety, feeling of being locked up, panic, sweats and breathing difficulties), anxiety attack, nausea, vomiting, panic attack, dizziness, discomfort without traumatic consequences, allergic shock, renal failure related to iodine, gadolinium agent.

-for 18F-FDOPA PET/CT: erythema, or burns related to extravasation

The sponsor will report to National Competent Authoritie:

a) any serious adverse event that has a causal relationship with the investigational device, the comparator or the investigation procedure or where such causal relationship is reasonably possible;

b) any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate;

c) any new findings in relation to any event referred to in points a) and b).

The reporting is made by the sponsor, or its legal representative in the EU when the sponsor is established outside the EU, or the person/body to whom the sponsor or its legal representative has delegated the declaration [to be adapted].
The serious adverse events and the device deficiency that are the subject of the above-mentioned clinical investigation must be reported to the ANSM and, if applicable, at the same time to all the competent national authorities of the Member States of the European Union where the study is carried out [to be adapted].
NB: The CPPs are not recipients of this information.

Reporting timelines to National Competent Authorities:

The sponsor must report to all NCA where the clinical investigation is authorised to start:
For all reportable events which indicate an imminent risk of death, serious injury, or serious illness and that requires prompt remedial action for other patients/subjects, users or other persons or a new finding to it:
Immediately, but not later than 2 calendar days after awareness by sponsor of a new reportable event or of new information in relation with an already reported event.
This includes events that are of significant and unexpected nature such that they become alarming as a potential public health hazard. It also includes the possibility of multiple deaths occurring at short intervals.
These concerns may be identified by either the NCA or the manufacturer.

Any other reportable events or a new finding/update to it: Immediately, but not later than 7 calendar days following the date of awareness by the sponsor of the new reportable event or of new information in relation with an already reported event.
The promoter reports to ANSM by email, at EC.DM-COS@ansm.sante.fr, the safety data mentioned, as described, in the form of a table such as that presented in the appendix of the recommendation entitled "Investigation summary safety report form" (MDCG-2020-10 / 2).

The table gives a cumulative overview of the reportable events per clinical investigation and will be updated and transmitted to participating NCAs each time a new reportable event or a new finding to an already reported event is to be reported. More detailed information has to be provided on request of an NCA, if so requested by using the individual study specific reporting form.

• When Eudamed will be available but not yet compulsory (pending the end of the 6-month period following the date of publication of the opinion of the European Commission referred to in article 34.3 of the RDM), the sponsor may declare either in table form or in Eudamed;

• From the moment when Eudamed becomes compulsory, the reporting will have to be made in Eudamed only.

Any suspected unexpected serious adverse reaction relating to a medicinal product must also be reported electronically using the Eudravigilance European adverse drug reactions database managed by the European Medicines Agency (EMA).

The sponsor must notify all the investigators involved about any information that could adversely affect the safety of the research participants.

Analysis and declaration of other safety data

According to Article 1123-46 of the Code de la Santé Publique and the guidelines for sponsors of clinical trials conducted on medical devices and in vitro diagnostic medical devices (ANSM), an emerging safety issue is defined by any data which could significantly led to re-evaluation of the benefit-risk ratio of the trial or the product under investigation, modifications in the use of this product, in the conduct of the trial or in the trial documents, or to suspension, interruption or modification in the protocol of the clinical trial or other similar trials. For trials involving the first administration or use of a health product in healthy volunteers: any serious adverse reaction.

The sponsor will report to the competent authority and the Ethics Committee without delay upon knowledge any emerging safety issues and, if applicable, describe what urgent safety measures have been taken by the sponsor.

Following the initial declaration of any emerging safety issue, the sponsor will report any additional relevant information about the emerging safety issue in the form of a follow-up report, which must be sent to ANSM and CPP no later than 15 days upon knowledge of the sponsor.

If the suspected unexpected serious adverse reaction meets the definition of an emerging safety issue, the sponsor will report both the SUSAR and the emerging safety issue to the ANSM according to the appropriate modalities and within the regulatory timelines as previously described.

10.3.2 Analysis and declaration of other safety data

Pursuant to Article 1123-46 of the *Code de la Santé Publique* (French Public Health Code) and the guidelines for sponsors of clinical trials on medical devices and *in vitro* diagnostic medical devices (ANSM), an emerging safety issue is defined by any new data that may lead

to a reassessment of the benefit-risk ratio of the trial or of the product under investigation, to modifications in the use of this product, in the conduct of the trial, or documents pertaining to the trial, or to suspend, halt or modify the study protocol or other similar trials. For trials involving the first administration or use of a health product in healthy volunteers: any serious adverse reaction.

The sponsor will inform the competent authority and the Research Ethics Committee without delay upon knowledge of any emerging safety issues and, if applicable, describe what measures have been taken.

Studies focussing on healthy volunteers **[include or delete as applicable]**:

The sponsor will inform the competent authority and the Research Ethics Committee without delay upon knowledge of any emerging safety issues and, if applicable, describe what measures have been taken. The sponsor will inform without delay the regional healthcare authority (*Agence Régionale de Santé*) of any emerging safety issues relating to healthy volunteers taking part in a clinical study and of any measures, if applicable, that have been taken.

Following the initial declaration of any emerging safety issue, the sponsor will report to competent authorities any additional relevant information about the emerging safety issue in the form of a follow-up report, which must be sent no later than 15 days upon knowledge of the information.

10.4 Independent Data and Safety Monitoring Board (DSMB)

An independent Data Safety Monitoring Board (DSMB) can be set up by the sponsor. Its primary mission is to monitor safety data. It can have other missions, such as monitoring efficacy data (especially if the protocol includes interim analyses).
The sponsor is responsible for justifying the creation or absence of a supervisory committee to the Competent Authority (ANSM) and to the Ethics Committee.
A DSMB will be set up for this trial. *The DSMB must hold its preliminary meeting ideally before the protocol is submitted to the competent authority and the Ethics Committee.*

The members of the DSMB are:

Dr. GERBER Sophie	*Service de Neuroradiologie* *1850 rue Raymond Losserand* *75014 PARIS*
Dr. EFTEKHARI Pirayeh	*CRPV* *Hopital Fernand Widal* *PARIS*
Dr. LESIMPLE Thierry	*Centre Eugène Marquis* *Service d'Onco dermatologie* *RENNES*
Stéphanie ALLASSONNIERE, Associate Pr Ecole Polytechnique,	*Pr of Applied Mathematics Université Paris Descartes, France*
Pr Sophie COLNAT – COULBOIS	*Service de Neurochirurgie* *Hôpital Central – CHU de Nancy*

	29 Avenue du Maréchal de Lattre de Tassigny *54000 NANCY* *s.coulbois@chru-nancy.fr*

All missions as well as the precise operating procedures of the DSMB are described in the DSMB charter of the clinical trial.

The DSMB has a consultative role. The final decision concerning the conduct of the clinical trial relies on the sponsor.

11. Data management

The investigator will permit the sponsor‘s representatives to monitor the study at the frequency defined in the contract, depending on enrolment at the centre. Case Report Forms (CRFs) and related source documents will be reviewed in detail during monitoring visit (completeness, adherence to the guidelines, accuracy compared to source documents). The sponsor‘s representative will also review regulatory documents, drug storage and accountability.
The investigator must maintain a comprehensive and centralized filing system of all study related documentation that is suitable for inspection by sponsor‘s monitors or representatives of other regulatory agencies.

11.1 Data collection

All data will be recorded in patients’ medical records. All data can be monitored.

11.2 Identification of data recorded directly in the CRFs which will be considered as source data

11.3 Not applicable Right to access source data and documents

Data access
In accordance with GCP:
- the sponsor is responsible for ensuring all parties involved in the study agree to guarantee direct access to all locations where the study will be carried out, the source data, the source documents and the reports, for the purposes of the sponsor's quality control and audit procedures or inspections by the competent authority
- the investigators will ensure the persons in charge of monitoring, auditing and inspecting the clinical trial and of quality control have access to the documents and personal data strictly necessary for these tasks, in accordance with the statutory and regulatory provisions in force (Articles L.1121-3 and R.5121-13 of the French Public Health Code)

Source documents:
The source documents are any original document or item that proves the existence or accuracy of a data-point or fact recorded during the trial. Source documents will be kept by the investigator, or by the hospital in the case of hospital medical records, for the statutory period.

Source documents (medical record, laboratory tests results, medical imaging reports, sonication results) are defined as any original document or object that can prove the existence or accuracy of a piece of information or a fact recorded during the research. These documents will be kept for 15 years by the investigator or by the hospital in the case of a hospital medical file.

Data confidentiality:

The persons responsible for the quality control of clinical studies (Article L.1121-3 of the French Public Health Code) will take all necessary precautions to ensure the confidentiality of information relating to the investigational medicinal products, the study, the study participants and in particular the identity of the participants and the results obtained.
These persons, as well as the investigators themselves, are bound by professional secrecy (in accordance with the conditions set out in Articles 226-13 and 226-14 of the French Criminal Code).
During and after the clinical study, all data collected about the study participants and sent to the sponsor by the investigators (or any other specialised collaborators) will be anonymised.
Under no circumstances will the names and addresses of the subjects be shown.
Only the subject's initials will be recorded, along with an identification code specific to the study indicating the order of enrolment.

The sponsor will ensure that each subject has agreed in writing for any personal information about him or her which is strictly necessary for the quality control of the study to be accessed.

11.4 Data processing and storage of research documents and data

Identification of the data processing manager and the location(s)
Data will be collected on an E-CRF, with data entry performed in each centre by Clinical research assistants (CRA) and/or physicians.
Monitoring of the data will be performed by CRA under the superivison of the URC and DRCI.
Statistical analysis will be performed by Pr Matthieu Resche-Rigon, Saint Louis hospital, Paris.

Data entry

Data will be entered electronically via a web browser (Cleanweb).

Archiving:

Documents specific to a study involving human participants not concerning a health product will be archived by the investigator and the sponsor for *15 years* near the end of the study.

This indexed archiving applies to:

- A sealed envelope for the investigator, containing **one copy of all information sheets and consent forms signed by all individuals at the site who participated in the research**;
- A sealed envelope for the sponsor, containing one copy of all information sheets and consent forms signed by all individuals at the site who participated in the research;
- "Study" binders for the Investigator and the sponsor, containing (non-exhaustive list):
  - all successive versions of the protocol (identified by version no. and date), and its appendices
  - the ANSM authorizations and Ethics Committee decisions
  - correspondence
  - the enrolment list or register
  - the appendices specific to the research
  - Final report
- The case report forms

11.5 Ownership of the data

AP-HP is the owner of the data. The data cannot be used or disclosed to a third party without its prior permission.

12. Statistical aspects

12.1 Proposed statistical methods, including the timetable for any planned interim analyses

The main objective will be the determination of the most successful dose (MSD) among 3 potential doses (d1=0.78, d2=0.9 and d3=1.03 MPa), defined as the dose with the highest probability of BBB opening efficacy without toxicity directly related to the ultrasound emission. The design applied for this trial corresponds to a Continual Reassment Method (CRM) proposed by O'Quigley in 2001 [ref]. Consider a trial in which $j \in \{1,...,n\}$ patients may be entered, n being the greatest number of patients that we are prepared to enter. The dose level for the jth patient, Xi, can be viewed as random taking discrete values xj, where $x \in$ {d1, d2, d3}. Let Yj be a binary random variable {0,1}, where 1 denotes a toxic response. Let Vj be a binary random variable {0, 1}, where 1 denotes a satisfactory efficacy for the jth patient $j \in \{1,...,n\}$. We define the true probability of toxic response at $Xj = xj$ by $R(xj) = Pr(Yj = 1 \mid Xj = xj)$. The probability of satisfactory efficacy, given no toxicity, at $Xj = xj$ is given by $Q(xj) = Pr(Vj = 1 \mid Xj = Xj, Yj = 0)$, so that $P(di) = Q(di)\{1 - R(di)\}$ is the probability of success at dose di. A successful experiment would identify the dose level l such that $P(dl) > P(di)$ for all i not equal to l.
In this work, we used the following power models $R(di)=\square i\hat{}a$ and $Q(di)=\square i\hat{}b$. Having treated j patients and observed their responses, we can obtain estimates of $R(di)$, $Q(di)$ of the true unknown probabilities at the k dose levels. The $(j + 1)th$ included patient will be treated at level $x(j+1) \in$ {d1, d2, d3} such that $P(xj+1) > P(di)$ (i = 1, .. , k; x(i+1) != di).
Here we will include patients by cohort of three. Analyses will be performed after each cohort of three.
All patient will be analysed in intention to treat, i.e., at the dose level recommended by the design.

As of October 3, 2022, 3 patients were treated at level 1 (0.78MPa) and 1 patient was at level 2 (0.9MPa). No side effects or toxicity were observed (very good tolerance).
Since the CRM design recommends that a new patient could be treated at the higher level (1.03MPa), the patient n°5 will receive the 1.03MPa dose.

Descriptive statistics will be used to summarize patient characteristics. In general, quantitative variables will be described by their median and first and third quartiles, and qualitative variables will be described by the frequencies of the modalities and associated percentages.

The epidemiological and clinical characteristics of patients at inclusion will be described by dose, without statistical testing. Protocol violations, causes of abandonment and loss of patients and the characteristics of these patients will be detailed.

The final estimates of probality of success for the MSD will be given with its 95% Confidence interval.
Best overall response rate and ORR at 3 months will be estimated with its 95% Confidence interval.
Best intracranial overall response rate (BICORR) and ICORR at 3 months will be estimated with its 95% Confidence interval.
Best extracranial overall response rate (BECORR) and ECORR at 3 months will be estimated with its 95% Confidence interval.
Progression free survival, 6 months and 1 year PFS will be estimated using Kaplan Meier estimator.
Intracranial progression free survival, 6 months and 1 year intracranial PFS will be estimated using Kaplan Meier estimator.

Extracranial progression free survival, 6 and 1 year months extracranial PFS will be estimated using Kaplan Meier estimator.
Overall survival (OS) at 6 months and 1 year will be estimated using Kaplan Meier estimator.
Safety using CTCAE version 5.0

12.2 Hypotheses for calculating the required number of participants, and the result

Continual Reassessment Method has been shown to accurately estimate the MSD based on small samples of less than 25 patients. Owing to the number of potential evaluated doses, 21 (7x3) patients will be enrolled. This will allow an accurate estimation of the response rate at the estimated MSD.
In case of missing data, complete case analyses will be performed.
Any subject can withdraw from participating in the research at any time and for any reason. In such case the patient will be replaced to assure that the design could be applied. The investigator can temporarily or permanently end a subject's participation in the research for any reason that affects the subject's safety or which would be in the subject's best interests. Considering that a maximum of 10% would be able to withdraw from participating in the research a budget for 21+2=23 patients will be considered.

13. QUALITY CONTROL AND ASSURANCE

Every interventional study managed by AP-HP is ranked according to the projected risk incurred by the participants, using a classification system specific to AP-HP-sponsored clinical trials.

13.1 General organization

The sponsor must ensure the safety and respect of individuals who have agreed to participate in the study. The sponsor must have a quality assurance system for monitoring the implementation of the study at the research centers.

For this purpose, the sponsor shall appoint Clinical Research Associates (CRA) whose primary role is to carry out regular follow-up visits at the study sites, after completing their initial visits. The purpose of monitoring the study, as defined in the Good Clinical Practices (GCP section 5.18.1), is to verify that:

- the trial participants are safe, protected and their rights are being met
- the data being recorded is accurate, complete and consistent with the source documents
- the study is carried out in accordance with the current version of the protocol, with GCP and with all statutory and regulatory requirements.

Strategy for site opening
The strategy for opening the sites is determined using the tailored monitoring plan.
The opening of the different participating center will be performed on site by the CRA from the URC-DRCI from Saint Louis hospital.

Scope of site monitoring
For this study, the appropriate monitoring level has been determined based on the complexity, the impact and the budget for the study.
Therefore the sponsor, in agreement with the coordinating investigator, has agreed on a logistical score and impact and the corresponding study monitoring level of: High Risk level D.

The various levels are described in the Human Research Trial monitoring charter

13.2 Quality control

A Clinical Research Associate (CRA) appointed by the sponsor will be responsible for the proper running of the study, for collecting, documenting, recording and reporting all handwritten data, in accordance with the Standard Operating Procedures applied within the DRCI and in accordance with Good Clinical Practice as well as with the statutory and regulatory requirements.

The investigator and the members of the investigator's team agree to make themselves available during regular Quality Control visits by the Clinical Research Associate.
During these visits, the following elements will be reviewed:

- written consent
- compliance with the study protocol and its procedures
- quality of the data collected in the case report forms: accuracy, missing data, consistency of the data with the "source" documents (medical files, appointment books, original copies of laboratory results, etc.)
- management of the devices used

13.3 Case report forms

Electronic CRF:
All information required by the protocol must be entered in the case report forms. The data must be collected as and when they are obtained, and must be written clearly and legibly. Any missing data must be coded.
Every site will have access to the electronic case report forms via a web-based data collection system. Investigators will be given instructions for using this tool.
Using on-line case report forms means the CRA can view the data quickly and remotely. The investigator is responsible for the accuracy, quality and relevance of all the data. In addition, there are consistency checks to ensure the data are verified immediately upon being entered. The investigator must validate any changes to the values in the case report form. An audit trail will be kept of all changes. A justification can be added when applicable, as a comment.
A print-out, authenticated (signed and dated) by the investigator, will be requested at the end of the research. The investigator must archive a copy of the authenticated document that was issued to the sponsor.

13.4 Management of non-compliances

Any events that occur as a result the investigator or any other individual involved in conducting the study failing to comply with the protocol, standard operating procedures, good clinical practice or statutory and regulatory requirements must be recorded in a declaration of non-compliance and sent to the sponsor.

The sponsor has its own procedures for managing these non-compliances.

13.5 Audits/inspections

The investigators agree to accept the quality assurance audits carried out by the sponsor as well as the inspections carried out by the competent authorities. All data, documents and reports may be subject to regulatory audits and inspections. These audits and inspections cannot be refused on the basis of medical secrecy.

An audit can be carried out at any time by independent individuals appointed by the sponsor. The aim of the audits is to ensure the quality of the study, the validity of the results and compliance with the legislation and regulations in force.

The individuals who lead and monitor the study agree to comply with the sponsor's requirements and with the competent authority regarding study audits or inspections.

The audit may encompass all stages of the study, from the development of the protocol to the publication of the results and the storage of the data used or produced as part of the study.

13.6 Principal Investigator's declaration of responsibility

Before starting the trial, each investigator will give the sponsor's representative a signed and dated copy of his/her recent curriculum vitæ and RPPS number (Répertoire Partagé des Professionnels de Santé, Collective Database of Health Professionals). The CV must describe any previous participation in clinical research and related training.

Each investigator will agree to comply with legislation and to conduct the trial in line with GCP, in accordance with the Declaration of Helsinki.

The Principal Investigator at each participating site will sign a declaration of responsibility (standard DRCI document) which will be sent to the sponsor's representative.
The investigators and their co-workers will sign a delegation form specifying each person's role.

14. ETHICAL AND LEGAL CONSIDERATIONS

14.1 Methods for informing and obtaining consent from the research participants

In accordance with Article L.1122-1-1 of the Code de la Santé Publique, no interventional research can be carried out on a person without his/her free and informed consent, obtained in writing after the person has been given the information specified in Article L.1122-1 of said Code.

The person will be given a reflection period of 2 days between receiving the information and being asked to sign the consent form.

The person's free and informed written consent will be obtained by the principal investigator, or by a doctor representing the investigator, before the person is enrolled on the trial at selection visit.

The information sheet and a copy of the consent form signed and dated by the trial participant and by the investigator or the doctor representing the investigator, will be sent to the individual prior to being enrolled on the trial.

In addition, the investigator will specify in the research participant's medical file the methods used for obtaining their consent as well as the methods used for providing information with a view to obtaining consent. The investigator will retain the original signed and dated consent form.

14.2 Prohibition of concomitant clinical studies participation and exclusion period after the study, if applicable

The participants can however participate in other non-interventional studies.

14.3 Authorization of research site

Surgery is performed in care units according to standard of care by a trained team: a site authorization is not required

The study requires interventions other than those usually performed at the care unit:

Units participating to this step of the study must have specific authorization: sonication sessions are performed in the “Centre d’Investigation Clinique (CIC) – Hôpital Saint Louis-Paris: CLIP^2 i.e. Inca labeled early phase center which has a specific authorization

15. Legal obligations

15.1 The sponsor's role

Assistance Publique Hôpitaux de Paris (AP-HP) is the sponsor of this study and by delegation, the Clinical Research and Innovation Delegation (DRCI) carries out the study's missions in accordance with Article L.1121-1 of the Code de la Santé Publique. Assistance Publique Hôpitaux de Paris reserves the right to halt the study at any time for medical or administrative reasons. In this case, notification will be sent to the investigator

15.2 Request for approval from the CPP

AP-HP, as sponsor, obtains prior approval from the Ethics Committee for its human interventional studies of medical devices or in vitro diagnostic medical devices, within the scope of the Board's authority and in accordance with statutory and regulatory requirements.

15.3 Request for approval from the ANSM

AP-HP, as sponsor, obtains prior approval from the ANSM for its human interventional studies of medical devices or in vitro diagnostic medical devices, within the scope of the Board's authority and in accordance with statutory and regulatory requirements.

15.4 Procedures relating to IT and freedoms regulation

The computer file used for this research is implemented in accordance with the French (CNIL amended) and European (*General Data Protection Regulation* – GDPR) regulations.

Declaration of compliance with the MR 001 "Reference Method"

This research is governed by the CNIL "Reference Method for processing personal data for human interventional studies" (MR-001, amended). AP-HP, the sponsor, has signed a declaration of compliance with this "Reference Method”

The data processing implemented for this research will be registered in the AP-HP register, under the responsibility of the AP-HP Data Protection Officer, in accordance with GDPR (General Data Protection Regulation)

15.5 Modifications to the study

Any substantial modification to the protocol by the coordinating investigator must be sent to the sponsor for approval. After approval is given, the sponsor must obtain, prior to starting the study, approval from the CPP and authorization from the ANSM within the scope of their respective authorities.
The information sheet and the consent form can be revised if necessary; in particular, if there is substantial amendment to the study or if adverse reactions occur.

15.6 Final study report

The final study report referred to in Article R.1123-60 CSP is written and signed by the sponsor and the investigator. A report summary, meeting the competent authority's guidelines, will need to be sent to the competent authority and Ethics Committee within one year of the end of the trial i.e. the end of the participation of the last research participant.

16. FUNDING AND INSURANCE

16.1 Sources of monetary support

- *The study was funded by a grant from Programme Hospitalier de Recherche Clinique - PHRC 2016 (Ministry of Health) and by a grant from Bpifrance, Financing in the framework of the "DOME" project: Support for the Structural Research and Development Project for Competitiveness*

16.2 Insurance

For the duration of the study, the Sponsor will take out an insurance policy covering the sponsor's own third party liability as well as the third party liability of all the doctors involved in the study. The sponsor will also provide full compensation for any damages caused by the study to the study participants and their beneficiaries, unless the sponsor can prove that the harm is not the fault of the sponsor or any agent. Compensation cannot be refused on the grounds of a third party act or the voluntary withdrawal of the person who initially consented to participate in the study.

Assistance Publique- Hôpitaux de Paris (AP-HP) has taken out insurance from HDI-GERLING through BIOMEDIC-INSURE for the full study period, covering its own civil liability and that of any agent (doctor or research staff) for the duration of the study, in accordance with Article L.1121-10 of the Code de la Santé Publique.

17. Publication rules

The author(s) of any publication relating to this study must include the AP-HP among their affiliations and name the sponsor AH-HP (DRCI) and the source of funding, if funded by a call for tenders (e.g. national or regional PHRC); a copy of the publication must be sent to the sponsor (see below for rules governing affiliation, and naming the sponsor and funders).

17.1 Mention of AP-HP affiliation for projects sponsored by AP-HP

- If an author has several affiliations, the order in which the institutions are mentioned (AP-HP, University, INSERM, etc.) is not important
- However, if the research is funded by an internal call for tenders at the AP-HP, the first affiliation must be "AP-HP"
- Each of these affiliations must be identified by an address and separated by a semicolon
- The AP-HP institution must feature under the acronym “AP-HP” first in the address, specifically followed by: AP-HP, hospital, department, city, postcode, France

17.2 Mention of the sponsor AP-HP (DRCI) in the acknowledgements of the text

- "The sponsor was *Assistance Publique – Hôpitaux de Paris* (Clinical Research and Innovation Department)"

17.3 Mention of the financial backer in the acknowledgements of the text

The study was funded by a grant from Programme Hospitalier de Recherche Clinique - PHRC 2016 (Ministry of Health) and by a grant from Bpifrance, Financing in the framework of the "DOME" project: Support for the Structural Research and Development Project for Competitiveness

This research program will be registered on the website http://clinicaltrials.gov/ : ***NCT04021420***

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19. List of addenda

Every addendum and the log of addenda versions are attached, independently of the protocol. Every addendum can be modified (change of addendum version) without modifying the version of the protocol.

19.1 List of investigators

19.2 18F-FDOPA PET for therapeutic response of brain metastasis

19.3 Serious Adverse Events report form

19.4 CE marking, user manual (if CE marked) and/or Investigator's Brochure

19.5 Questionnaire or scale

19.6 Management of immunotherapy toxicity

19.7 RANO criteria

19.8 Response Criteria (RECIST 1.1

19.9 Performance status scales

19.10 AJCC Melanoma Staging

19.11 Methods of contraception