SUBSTANTIAL AMENDMENT TO THE CLINICAL TRIAL PROTOCOL "myDAvIpNi"

CLINICAL TRIAL PROTOCOL
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Original Study Title:

Phase I clinical trial on intratumoral administration of autologous CD1c (BDCA-1)$^+$ myeloid dendritic cells plus avelumab and ipilimumab in combination with intravenously administered nivolumab

AMENDMENT DATE: FEBRUARY 15TH 2021

AMENDMENT TITLE: Phase I clinical trial on intratumoral administration of autologous CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myeloid dendritic cells plus ipilimumab and AS01 in combination with intravenously administered nivolumab

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ACADEMIC STUDY SPONSOR

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1. Contents

2. Amendment Protocol Version/Date: v.3.0_15FEB2021

2.1. Rational for this amendment

A pivotal role has been attributed to myeloid dendritic cells (myDC) in regulating the activity of antitumoral cytotoxic T-lymphocyte (CTL) activity within the tumor microenvironment (TME) (Broz, Binnewies et al. 2014). In animal models, myDC have been demonstrated to play an essential role in "licensing" antitumoral CTL to eradicate tumor cells. Spranger et al showed in a mouse model that effector T cells fail to migrate to a non- T cell-inflamed tumor resulting from a lack of chemokine CXCL9/10 produced by Batf3-driven myDC which are present in an inflamed TME. Activation of oncogenic signaling pathways such as the WNT/β-catenin pathway can lead to the exclusion of myDC from the TME (Spranger, Bao et al. 2015, Spranger and Gajewski 2016). Absence of myDC at the invasive margin and within metastases has been correlated with defective CTL activation allowing the metastasis to escape the antitumoral immune response (Salmon, Idoyaga et al. 2016). These myDC also migrate to tumor-draining lymph nodes and present tumor antigens to T cells in these secondary lymphoid organs (Roberts, Broz et al. 2016). Presence of myDC was more strongly correlated with T-cell infiltration into tumors as compared to neo-antigen load in 266 melanomas from The Cancer Genome Atlas (Spranger, Luke et al. 2016). More recent insight has shown that successful anti-PD-1 cancer immunotherapy (e.g. pembrolizumab) requires crosstalk between T cells and cDC1 involving the antitumoral cytokines IFN-γ and IL-12 (Garris et al. Cell 2019). CD141 $(BDCA\text{-}3)^+$ (cDC1) myDC are the most specialized dendritic cells in detection and uptake of tumor cells and cross-presentation of derived antigens to $CD8^+$ T cells.

At the time the myDAvIpNi trial was initiated, only clinical grade isolation of CD1c $(BDCA\text{-}1)^+$ myDC was available. We recently published the results on the first nine patients who were treated in Cohort-1 of this clinical trial (Schwarze, Awada et al. 2020). We have shown that our combinatorial therapeutic approach, including intratumoral injection of CD1c $(BDCA\text{-}1)^+$ myDCs in combination with the immune checkpoint inhibitors ipilimumab and avelumab plus intravenous nivolumab is feasible and safe and that it resulted in encouraging signs of antitumor activity in patients with advanced solid tumors.

More recently, a clinical grade isolation procedure for CD141 $(BDCA\text{-}3)^+$ myDC has been made available by Miltenyi Biotech (Bergisch Gladbach, Germany) and during a clinical grade isolation procedure $BDCA\text{-}1^+$ and $BDCA\text{-}3^+$ myDCs can now be isolated together. This CD1c $(BDCA\text{-}1)^+$ / CD141 $(BDCA\text{-}3)^+$ myDC cell co-product is currently under investigation in our phase I clinical trial myDCTV (NCT03747744), our adaptive phase I clinical trial Glitipni (NCT03233152) in patients with recurrent glioblastoma, and our phase II clinical trial LuSCID (Eudract 2019-003668-32) in patients with non-small cell lung cancer (NSCLC).

With this amendment, we now want to adapt the myDAvIpNi clinical trial protocol and combine intratumoral injection of the autologous CD1c $(BDCA\text{-}1)^+$ / CD141 $(BDCA\text{-}3)^+$ myDC cell product with intratumoral injection of ipilimumab (a CTLA-4 blocking monoclonal antibody) plus the adjuvants AS01, and systemic administration of nivolumab (a PD-1 blocking monoclonal antibody).

Encouraging results have been obtained in the myDCTV phase I study. Patients treated in the first three cohorts of the myDCTV phase I clinical trial (conducted in parallel with the myDAvIpNi trial) have been presented at the SITC annual meeting 2019 (Schwarze et al, abstract #P458). In these cohorts, patients were treated with intratumoral injection of increasing numbers of CD1c $(BDCA\text{-}1)^+$ myDCs. Two patients were treated in cohort-1 (0.5×10^6 myDC), and cohort-2 (1×10^6 myDC), respectively, and 3 patients were treated in cohort-3 (10×10^6 myDC). The treatment was feasible and safe with mainly low-grade treatment-related adverse events. No grade 4 adverse events were observed. Two patients treated in cohort-3 achieved a durable complete remission with pathologically confirmed complete response. Four patients obtained a partial response in injected lesions, while developing new lesions or progressing in non-injected pre-existing lesion(s), and therefore were considered to have progressive disease as best tumor response according to irRECIST criteria.

In cohort-4, 6 patients were treated with the CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC cell co-product. Also, in this cohort, study treatment was well tolerated with mainly low-grade constitutional and local (injection-site) adverse events. One patient developed an unconfirmed partial response in injected as well as non-injected lesions. A second patient obtained a complete response of injected and non-injected in-transit lesions on the left leg. At the same time, he progressed in distant lesions (mixed response) but derived palliative benefit from the experimental therapy. Three patients had progressive disease at first tumor response assessment (incl. one patient with mixed response). A final manuscript is currently in preparation. A powerpoint presentation summarizing the results of this study will be provided as an addendum (appendix C).
Additionally, we have observed that patients who respond to the treatment display a significant increase in tumor-resident CD8$^+$ T lymphocytes and PD-L1 upregulation after the first injection of T-VEC and the injection of the CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC. Therefore, a combined one single injection of T-VEC plus CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC was demonstrated to be sufficient to reinvigorate the cancer-immunity cycle.

The “Adjuvant System 01 (AS01)” (GSK) is a liposome-based (liposome components made from dioleoylphosphatidylcholine and cholesterol) adjuvant containing monophosphoryl lipid A (MPL) and QS21 (a purified saponin isolated from *Quillajasaponaria Molina* bark) as immunostimulants (Wang and Xu 2020). MPL is the biologically active portion of the gram-negative bacterial cell wall constituent lipopolysaccharide (LPS) of *Salmonella minnesota* strain R595. The two immunostimulants MPL and QS21 have a synergistic effect. The role of cholesterol in the liposomes is to reduce the hemolytic toxicity of QS21, which enhances the safety of the adjuvant. The AS01 adjuvant has been shown to induce activation of the Toll-like receptor-4 (TLR-4) and immune cell recruitment. AS01 is currently part of the recombinant subunit vaccine Shingrix® (GlaxoSmithKline (GSK)), a prophylactic vaccine preventing shingles. It has been shown that Shingrix is more effective than another shingles vaccine, the live attenuated vaccine Zostavax® (Merck), suggesting a pivotal role of the used adjuvant AS01 (Wang and Xu 2020). Its safety has been thoroughly investigated (see SmPC Shingrix).

Early (unpublished) preclinical data indicate a maturation of myDCs upon exposure to AS01 that is comparable to other maturing agents including T-VEC.

We hypothesize that the intratumoral injection of an autologous CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC cell co-product in with AS01 pretreated metastases will allow a more efficient maturation of myDC *in vivo* leading to a more effective antitumoral T-cell response following cross-presentation of tumor associated antigens. The administration of ipilimumab (IT) and nivolumab (IV) will be continued as these monoclonal antibodies will block CTLA-4 expressing regulatory T cells in the tumor microenvironment and circulating PD-1 expressing T lymphocytes, respectively.

The concept of our treatment strategy remains the same as in the previous version of this protocol except that cohort-2 will be treated with intratumoral injection of AS01 on day 1, followed by injection of autologous CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDCs on day 2, and will omit IT administrations of avelumab.

2.1.1. Overview of adapted treatment scheme

Treatment protocol for cohort-2 is will be adapted for the following items:

- Exchanging the CD1c (BDCA-1)$^+$ myDC cell product with the CD1c (BDCA-1)$^+$ plus CD141 (BDCA-3)$^+$ myDC cell product. There will be no dose escalation of the number of myDC injected intratumorally. The number of myDC injected per lesion will be according to the size of the lesion. For further information regarding the autologous CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC product, please consult the *investigational medicinal product* dossier (IMPD). The CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC cell product will be injected on day 2 of the study protocol.
- Instead of an intratumoral injection of avelumab on day 1, one injection of maximum 0.5 ml AS01 (containing 50 μg QS21 and 50 μg MPL) on day 1 will be performed, dosing will be adapted to injected lesion volume according to maximal diameter:
  - >5cm: 0.5ml AS01
  - 2.5cm – 5cm: 0.25ml AS01
  - 1.5cm – 2.5cm: 0.125ml AS01
  - 0.5cm – 1.5 cm: 0.075ml AS01
- Dosing of the study drugs ipilimumab and nivolumab will be administered as described in the original protocol.
- Patients will undergo an (if necessary, ultrasound-guided) tissue biopsy of the injected lesion on day 3.

2.1.2. Sample size

For this amendment we will recruit six study patients to cohort-2. There will be no dose escalation regarding the number of myDC injected. The number of cells (and corresponding volume of cell suspension) will be determined by the size of the injected metastasis (see table). Patients with a tumor volume of ≥4cm (: sum of longest diameters of injected lesions) will receive all isolated CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC.

3. Study Synopsis

Study Title	Phase I clinical trial on intratumoral administration of autologous CD1c (BDCA-1)+ / CD141 (BDCA-3)+ myeloid dendritic cells plus ipilimumab and AS01 in combination with intravenously administered nivolumab
Study Design	Open label, single-center, phase I clinical trial
Study Sponsor	Universitair Ziekenhuis Brussel (UZ Brussel)
Principle Investigator	Professor Bart Neyns, MD PhD Medische Oncologie Universitair Ziekenhuis Brussel (UZ Brussel) Laarbeeklaan 101, 1090 Brussel België Tel.: +32 2 477 5447 Email: Bart.Neyns@uzbrussel.be
Study rational	Cancer cells can be recognized by the patient's own immune system, a process that is referred to as the "cancer immunity cycle" (Chen and Mellman 2013, Mellman 2013, Chen and Mellman 2017). Remarkable anti-tumor activity has been achieved by blocking the inhibitory T-cell receptor CTLA-4 and/or the PD-1/-L1 axis. Immune checkpoint inhibition by monoclonal antibody (mAb) therapy has become a standard of care in patients with advanced melanoma, renal cell carcinoma, non-small cell lung carcinoma, Hodgkin's lymphoma and bladder cancer. Indications are continuously expanding. Activity of PD-1/-L1 and CTLA-4 inhibition has been correlated with hallmarks of pre-existing anti-tumor T-cell response (presence of activated cytotoxic T lymphocytes (CTL) in the tumor microenvironment (TME) as evident from transcription profiles) and PD-L1 expression by tumor cells (in response to T-cell secreted IFN-γ). Mutational load of the cancer cells and presence of highly immunogenic neo-epitopes in the cancer cell genome underlies the capacity for cancer cells to elicit an immune response (Tumeh, Harview et al. 2014). Responsiveness to treatment with CTLA-4 has been correlated with the expression of HLA class I molecules by the cancer cells (Rodig, Gusenleitner et al. 2017). In immune-evasive tumors a pivotal role has been attributed to the elimination of myeloid dendritic cells (myDC) from the TME. Myeloid dendritic cells play a pivotal role in the initiation and coordination of the activity of anti-tumor CTL activity within the TME (Broz, Binnewies et al. 2014). In animal models, myDC have been demonstrated to play an essential role in "licensing" anti-tumor CTLs to eradicate tumor cells. Activation of oncogenic signaling pathways such as the WNT/β-Catenin pathway can lead to the exclusion of myeloid DCs from the TME (Spranger, Bao et al. 2015, Spranger and Gajewski 2016). Absence of myDCs at the invasive margin and within metastases has been correlated with defective CTL activation allowing the metastasis to escape the anti-tumor immune response (Salmon, Idoyaga et al. 2016). These myDCs also migrate to tumor-draining lymph nodes and present tumor antigens to T-cells in these secondary lymphoid organs (Roberts, Broz et al. 2016). In mouse models, tumor-residing Batf3 dendritic cells were shown to be required for effector T Cell trafficking and success of adoptive T-cell therapy (Spranger, Dai et al. 2017). Presence of myDCs was more

	strongly correlated with a "T-cell inflamed TME signature" as compared to neo-antigen load in 266 melanomas from The Cancer Genome Atlas (Spranger, Luke et al. 2016). Two important human myDCs subsets exist that are differentiated by expression of either the BDCA-1 or BDCA-3 surface marker. The CD1c (BDCA-1)+ antigen is specifically expressed on human dendritic cells, which are $CD11c^{high}CD123^{low}$ and represent the major subset of myDCs in human blood (about 0.6 % of all peripheral blood mononuclear cells (PBMCs)). CD1c (BDCA-1)+ myDC have a monocytoid morphology and express myeloid markers such as CD13 and CD33 as well as Fc receptors such as CD32, CD64, and FceRI. Furthermore, myDC are determined to be CD4+, Lin (CD3, CD16, CD19, CD20, CD56)-, $CD2^+$, $CD45RO^+$, CD141 (BDCA-3)-low, CD303 (BDCA-2)-, and CD304 (BDCA-4/Neuropilin-1)-. A proportion of CD1c (BDCA-1)+ myDC co-expresses CD14 and CD11b. These dual positive cells for CD14+ and CD1c (BDCA-1) have immunosuppressive capacity and inhibit T-cell proliferation in vitro. Depletion of this cell type is preferred prior to using CD1c (BDCA-1)+ cells for immune-stimulatory purposes (Bakdash, Buschow et al. 2016, Schroder, Melum et al. 2016). CD141 $(BDCA-3)^+$ myDC (cDC1) are the most specialized dendritic cells in detection and uptake of tumor cells and cross-presentation of derived antigens to $CD8^+$ T cells. $CD1c\ (BDCA-1)^+$ myDC play an important role in the cross-presentation of tumor antigens following immunogenic cell death (Di Blasio, Wortel et al. 2016). Under conditions of tumor growth, myDC will be poorly recruited to the tumor microenvironment, do not get activated and thereby fail to efficiently coordinate anti-tumor immunity within the tumor micro-environment and present tumor associated antigens within tumor-draining lymph nodes. When activated appropriately, human CD1c (BDCA-1)+ dendritic cells secrete high levels of IL-12 and potently prime CTL responses (Nizzoli, Krietsch et al. 2013). In vitro, IL-12 production by CD1c (BDCA-1)+ myDC can be boosted by exogenous IFN-γ. (Nizzoli, Krietsch et al. 2013) CD1c (BDCA-1)+ myDC spontaneously "partially mature" within 12 hours following their isolation. Optimal maturation with secretion of IFN-γ as well as the orientation of stimulated T-lymphocytes towards a Th1 phenotype is only achieved following Toll-like receptor stimulation.(Skold, van Beek et al. 2015) Animal models have established the safety and efficacy of intra-tumoral administration of ipilimumab. An intratumoral dose of CTLA-4 blocking mAb administered at a ratio of [1:100] compared to intravenous dosing was found to result in equivalent anti-tumor effect and was associated with less systemic toxicity.(Fransen, van der Sluis et al. 2013, Marabelle, Kohrt et al. 2013) In an ongoing clinical trial for patients with recurrent glioblastoma, conducted by our research group, intratumoral injection of the CTLA-4 blocking mAb ipilimumab plus the PD-1 blocking mAb nivolumab have been proven feasible and safe. Intratumoral administration of an anti-PD-L1 IgG_1 mAb may increase the potential for antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The Adjuvant System 01 (AS01) is a liposome-based (liposome components made from dioleoylphosphatidylcholine and cholesterol) adjuvant containing monophosphoryl lipid A (MPL) and QS21 (a purified

	saponin isolated from *Quillajasaponaria Molina* bark) as immunostimulants. MPL is the biologically active portion of the gram-negative bacterial cell wall constituent lipopolysaccharide (LPS) of *Salmonella minnesota* strain R595. The two immunostimulants MPL and QS21 have a synergistic effect. The AS01 adjuvant has been shown to induce activation of the Toll-like receptor-4 (TLR-4) and immune cell recruitment. AS01 is currently part of the recombinant subunit vaccine Shingrix® (GlaxoSmithKline (GSK)), a prophylactic vaccine preventing shingles. It has been shown that Shingrix is more effective than another shingles vaccine, the live attenuated vaccine Zostavax® (Merck), suggesting a pivotal role of the used adjuvant AS01. Its safety has been thoroughly investigated. Preclinical data (unpublished) indicate a better maturation of myDCs upon exposure to AS01. We hypothesize that the intratumoral injection of an autologous CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC cell co-product in with AS01 pretreated metastases will allow a more efficient maturation of myDC *in vivo* leading to a more effective antitumoral T-cell response following cross-presentation of tumor antigens. The administration of ipilimumab (IT) and nivolumab (IV) will be continued as these monoclonal antibodies will block CTLA-4 expressing regulatory T cells in the tumor microenvironment and PD-1 expressing T lymphocytes in the peripheral blood circulation, respectively.	
Study hypothesis	We hypothesize that intratumoral injection of autologous CD1c (BDCA-1)$^+$ myeloid dendritic cells (myDC) and CD141 (BDCA-3)$^+$ myDC (isolated from PBMC) in combination with intratumoral (IT) injection of AS01, ipilimumab (anti-CTLA-4 IgG_1 mAb) and intravenous (IV) administration of nivolumab (anti-PD-1 IgG_4 mAb) will create a T-cell inflamed tumor microenvironment allowing for a more effective systemic adaptive anti-tumor immune response.	
Target patient population	Patients with injectable metastases from histologically confirmed solid tumors who have failed standard-of-care life prolonging therapeutic options will be invited to participate in this clinical trial.	
Summary of key eligibility criteria	• Histologically confirmed solid tumor • Metastatic disease that is progressive following standard of care • Skin, lymph node or other soft tissue metastases that can be injected by manual palpation, or by US- or CT-guidance • Availability of a pre-treatment tumor tissue biopsy (either as a representative archival sample or from a new tissue sample obtained by core needle biopsy or surgically excision) • No contra-indication for the collection of 500 ml of venous blood	
Primary Objectives and Endpoints	• *Objective*: To document the safety of intratumoral injection of autologous CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC co-product (isolated from PBMC) plus AS01, and ipilimumab (anti-CTLA-4 IgG_1 mAb) in combination with IV administration of nivolumab (anti-	• *Endpoint*: Adverse events occurring in patients treated in this phase I clinical trial (collected on a continuous basis). Type, frequency and severity (graded according to the CTCAEv5.0) will be reported descriptively.

	PD1 IgG_4 mAb).	
Secondary Objectives/ Endpoint	• Feasibility	• Percentage of study patients that can receive the planned CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC intratumoral injection.
	• Treatment disposition	• Number/volume of administered CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC • Administered dose of AS01, ipilimumab, and nivolumab
	• Anti-tumor activity	• Tumor response (ORR) according to RECISTv1.1 and iRECIST • Tumor response and duration of response of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC, AS01, and ipilimumab injected and non-injected metastases (reported descriptively)
	• Progression-free survival (PFS)	• Time from first study treatment administration to progression of disease according to RECISTv1.1 and iRECIST (and possibly itRECIST)
	• Overall survival	• Time from first first study treatment administration to death
Translational Objectives and Endpoints	• Effect on cellular- and molecular characteristics of the tumor microenvironment during/following study treatment	• Immunohistochemical analysis (CD3, CD8, CD4, PD-L1), multiplexed immunofluorescent imaging, and RNA-expression profiling (NanoString PanCancer IO 360 gene expression panel) of repetitive on-treatment tissue biopsies of injected metastases • T-cell receptor repertoire in metastases assessed by ImmunoSEQ analysis
	• Effect of study treatment on blood lymphocytes	• Peripheral blood differential white blood cell counts • Immunocytochemical differential cell counts (incl. CD3+, CD4+ and CD8+ lymphocytes) • T-cell receptor repertoire assessed by ImmunoSEQ analysis • Flow cytometric analysis of

		effector/naïve/memory T cells

Study plan	• **Patient identification:** patients who are considered potential candidates for study participation based on their prior disease history will be identified and invited to consider study participation
	• **Eligibility screen:** following written informed consent, candidate patients will be screened for their eligibility. A representative archival (or new [: optional or mandatory in case there is no archival tumor material available]) metastatic tumor tissue sample will be obtained as part of the eligibility screen procedure.
	• **Enrollment:** patients who meet all eligibility criteria will be enrolled to this clinical trial.
	• **Treatment phase:**
	• Day1: Isolation of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC:
	  • (a; 9-13h): patients will undergo a leukapheresis after which isolation of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC from the collected PBMC will be started. First, CD14$^+$ and CD19$^+$ cells will be depleted from the PBMC followed by isolation of CD1c (BDCA-1)$^+$ myDC and CD141 (BDCA-3)$^+$ myDC using the CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ Dendritic Cell Isolation Kit on the CliniMACS Prodigy® or on the immunomagnetic CliniMACS Plus isolation system (Miltenyi Biotec, Bergisch Gladbach, Germany). Isolated CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC will be available for intratumoral injection on day 2.
	  • (b; 16-17h): intratumoral administration of AS01 and ipilimumab: Up to 0.5 mL of AS01 will be injected in selected metastatic lesion(s) followed by Ipilimumab (Yervoy®, 50mg/10mL solution) to be injected at a maximum total dose of 10 mg (= 2 ml of a 50mg/10ml solution).
	  • (c; 13-17h): intravenous administration of nivolumab: 10 mg of nivolumab (Opdivo®, 40 mg/4mL solution) will be administered by a 15 minutes intravenous infusion
	• Day 2: intratumoral administration of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC: Following their isolation on day 2, CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC will be made available for intratumoral administration (*see also:* administration scheme for CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC).
	• Day 15 + every 14 days thereafter (Q2W):
	• Intratumoral injection of up to 0.5 ml AS01 will be performed up to week 12.
	• Intratumoral injection of Ipilimumab (Yervoy®, 50mg/10mL solution) at a maximum total dose of 10 mg (= 2 ml of a 50mg/10ml solution) will be performed.
	• 10 mg of nivolumab (Opdivo®, 40 mg/4mL solution) will be administered by a 15 minutes IV infusion.
	• Safety assessments will be made on a continuous basis during the treatment phase (incl. physical and blood analysis at every planned visit)
	• Tumor response assessments by whole body PET/CT will be scheduled in week 12 and every 12 weeks thereafter during the treatment phase; at earlier time points additional medical imaging will be performed if considered necessary for safety reasons or assessment of disease status	

	• Procurement of tumor tissue using a VacuCut® biopsy needle (or punch biopsy) will be performed on day 3 and before every intratumoral study drug administration (Q2W).
	• **End-of the study treatment phase**
	  • Study treatment will be discontinued at any time during the study at the diagnosis of progressive disease per iRECIST response assessment criteria, unacceptable adverse event(s), or patient withdrawal of consent to continue study treatment, lost-to-follow-up or death.
	  • Patients will be considered "off-study treatment" and enter the follow-up phase after the safety-follow-up visit 30 (+7) days after the date of the last administration of study treatment).
	• **Follow-up phase:**
	  • A safety follow-up visit will be performed approximately 30 (+7) days after the last administration of study treatment.
	  • Patients who have ended study treatment and experience no ongoing treatment related AEs of grade >2 will be followed-up for survival for 3 years following enrollment to the study.
	  • Patients who end study treatment without progression of disease will also be followed-up for their disease status for 3 years following enrollment to the study.
	  • During the follow-up phase the frequency and nature of investigations will be at the discretion of the treating physician and data will be captured from the medical file of the patient or through inquiry of their treating physician.

Assessments during the treatment-phase	• Safety will be monitored on a continuous basis. • A tumor biopsy (VacuCut® needle or punch biopsy) will be performed before every injection of AS01 and ipilimumab (if feasible). • Blood analyses (incl. routine chemistry- and hematology parameters) will be obtained on day 15 and prior to every administration of the study treatment. • Tumor response will be assessed by total body PET/CT in week 12 and every 12 weeks thereafter.
CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC cell product	Following transfer of the PBMC collected by leukapheresis, CD1c (BDCA-1)$^+$ myDC and CD141 (BDCA-3)$^+$ myDC will be isolated after depletion of CD14$^+$ and CD19$^+$ cells from the apheresis product using the CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ Dendritic Cell Isolation Kit on the CliniMACS Prodigy® isolation system (Miltenyi Biotec, Bergisch Gladbach, Germany). First, B cells are depleted using magnetic bead-coupled CD19 antibodies, followed by positive selection of CD1c (BDCA-1)$^+$ myDC and CD141 (BDCA-3)$^+$ myDC with biotin-coated CD1c and CD141 antibodies and magnetic bead-coupled anti-biotin antibodies. This procedure is expected to result in purified myDC, with an average purity of 63% and a yield between 20x10^6 and 70x10^6 cells. Following their isolation, CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC will be transferred for administration to the patient. CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC cell products will need to meet the following release criteria: a minimal yield of 10 x 10^6 CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC (total number of cells), >50% viability and >50% purity. The CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC cell products will be provided as a cell suspension for intratumoral injection at a minimal

	concentration of 0,25 x 10^6 myDC cells/ml and a maximal concentration of 10 x 10^6 myDC cells/ml. A minimal total of 1x10^6 CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC (= 4 ml of a 0,25 x10^6 myDC cells/ml suspension) and a maximum total of 40 x 10^6 myDC (= 4 ml of a 10 x10^6 CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC cells/ml suspension) will be injected.
CD1c (BDCA-1)$^+$ /CD141 (BDCA-3)$^+$ myDC administration	• Total administered CD1c (BDCA-1)$^+$ /CD141 (BDCA-3)$^+$ myDC volume will be up to 4.0 mL per patient. Injected volume per lesion will range from 0.1 mL for lesions ≤ 0.5 cm to 4.0 mL for lesions ≥ 5 cm in longest diameter. • Injection of all lesions in individual patients will not be required. • Determination of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC injection volume based on lesion size o > 5 cm up to 4 mL o > 2.5 cm to 5 cm up to 2 mL o > 1.5 cm to 2.5 cm up to 1 mL o > 0.5 cm to 1.5 cm up to 0.5 mL
AS01 dosing for intratumoral administration	• AS01 will be administered at up to a maximum total volume of 0.5 mL. Dosing will be adapted to injected lesion volume according to the maximal diameter of the metastasis: o >5cm: 0.5 ml AS01 o >2.5cm – 5 cm: 0.25 ml AS01 o >1.5cm – 2.5 cm: 0.125 ml AS01 o 0.5cm – 1.5 cm: 0.075 ml AS01 • Injection of all lesions in individual patients will not be required.
Ipilimumab dosing for intratumoral administration	• Total administered dose of ipilimumab (Yervoy®, 50mg/10mL solution) will be up to 2.0 mL per treatment. Injected volume per lesion will range from 0.05 mL for lesions ≤ 0,5 cm to 2.0 mL for lesions ≥ 5 cm in longest diameter. • Injection of all lesions in individual patients will not be required. • Determination of ipilimumab (Yervoy®, 50mg/10mL solution) injection volume based on lesion size o > 5 cm up to 2 mL o > 2.5 cm to 5 cm up to 1 mL o > 1.5 cm to 2.5 cm up to 0.50 mL o > 0.5 cm to 1.5 cm up to 0.25 mL
Nivolumab dosing	• Nivolumab (Opdivo®) will be administered at a fixed dose of 10 mg IV (q2w).
Statistical design and number of patients	• In cohort-2, a maximum of 3+3 patients will be recruited.
Study Period	• Recruitment Start Date: April 1, 2021 • Estimated recruitment End Date: December 31, 2021

4. Background

4.1. Autologous monocyte derived dendritic cell therapy for cancer

See more information in original protocol version.

4.2. Physiological role of myeloid dendritic cells in cancer immunity

See more information in original protocol version.

4.3. Role of myeloid dendritic cells in anti-tumor immunity

See more information in original protocol version.

4.4. Experience with plasmacytoid- and myeloid derived dendritic cell vaccines

See more information in original protocol version.

4.5. Isolation of CD1c (BDCA-1)+ / CD141 (BDCA-3)+ myDC

The isolation starts from peripheral blood mononuclear cells (PBMC) that are obtained by a leukapheresis. First, $CD14^+$ and $CD19^+$ cells are depleted from the PBMC followed by isolation of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC using the CD1c (BDCA-1)$^+$ and CD141 (BDCA-3)$^+$ Dendritic Cell Isolation Kit on the immunomagnetic CliniMACS Prodigy (Miltenyi Biotec, Bergisch Gladbach, Germany).

Various isolation procedures for CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC have been conducted at the stem cell laboratory of UZ Brussel (prof. Ivan Van Riet); this includes three test runs in healthy donors, and 11 patients within other clinical trials. The median of purity of the cell product (BDCA-1 plus BDCA-3) was 63% in these patients.

4.6. Adjuvant System 01 (AS01)

The Adjuvant System 01 (AS01) is a liposome-based (liposome components made from dioleoylphosphatidylcholine and cholesterol) adjuvant containing monophosphoryl lipid A (MPL), QS21 (a purified saponin isolated from *Quillajasaponaria Molina* bark) as immunostimulants. In AS01, MPL is a detoxified product derived from the lipopolysaccharide (LPS) extract of *Salmonella minnesota* strain R595. The two immunostimulants MPL and QS21 have a synergistic effect. The role of cholesterol in the liposomes is to reduce the hemolytic toxicity of QS21, which enhances the safety of the adjuvant. The way of action of QS21 is that it accumulates in the lymph nodes where it stimulates caspase-1, thereby releasing high-mobility group protein B1 (HMGB1), and the activation of the TLR4-MyD88 pathway. QS21 also accumulates in lysosomes which results in destruction of lysosomal enzymes and hence activation of the downstream immune pathways. AS01 activates the Toll-like receptor-4 (TLR-4) and stimulates immune cell recruitment. AS01 is part of the recombinant subunit vaccine Shingrix® (GlaxoSmithKline (GSK), a prophylactic vaccine preventing shingles. It has been shown that Shingrix is more effective and safer than another shingles vaccine, the live attenuated vaccine Zostavax® (Merck), suggesting a pivotal role of the used adjuvant AS01. Its safety has been thoroughly investigated.

4.7. PD-1 and CTLA-4 T-lymphocyte immune checkpoint inhibition

See more information in original protocol version.

4.8. Preclinical models on intratumoral CTLA-4 inhibition

See more information in original protocol version.

4.9. Clinical experience with intratumoral CTLA-4 inhibition

See more information in original protocol version.

5. Study rationale, research hypothesis and study objectives

5.1. Study Rationale and Aim

Currently, there are no satisfactory treatment options for patients with metastatic solid tumors who have failed curative and life-prolonging standard of care treatment options. Moreover, the life-expectancy in general is short (< 1 year in most cases) and accompanied by clinical deterioration and suffering from disease progression.

Recent insight into the interplay of myeloid and lymphoid cells in the TME has indicated an important role for "myeloid dendritic cells" as key-mediators in the "re-licensing" of anti-tumoral CTL within the metastases. Even when present in very low numbers, these cells play an essential role in re-licensing of anti-tumoral CTL within the TME. (Broz, Binnewies et al. 2014, Salmon, Idoyaga et al. 2016, Spranger, Dai et al. 2017)

CD1c (BDCA-1)+ myDC that are resident in the inflamed tumor microenvironment of the injected metastases could capture tumor antigens *in vivo*. Through the cross-presentation of these tumor antigens CD1c (BDCA-1)+ myDC could coordinate an effective anti-tumoral T-cell response.

Intratumoral administration of the anti-CTLA-4 IgG_1 mAb ipilimumab may increase the potential for antibody-dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). Thereby, tumor cell lysis may occur creating an inflamed TME and leading to the release of tumor antigens that may be captured by CD1c (BDCA-1)+ myDC. In addition, systemic PD-1 blockade by nivolumab will concomitantly release this physiological break on anti-tumor T-lymphocytes that do not reside in the TME at the time of intratumoral injection of study treatment.

The Adjuvant System 01 (AS01) is a liposome-based (liposome components made from dioleoylphosphatidylcholine and cholesterol) adjuvant containing monophosphoryl lipid A (MPL) and QS21 (a purified saponin isolated from *Quillajasaponaria Molina* bark) as immunostimulants. MPL is the biologically active portion of the gram-negative bacterial cell wall constituent lipopolysaccharide (LPS) of *Salmonella minnesota* strain R595. The two immunostimulants MPL and QS21 have a synergistic effect. The role of cholesterol in the liposomes is to reduce the hemolytic toxicity of QS21, which enhances the safety of the adjuvant. The AS01 adjuvant has been shown to induce activation of the Toll-like receptor-4 (TLR-4) and immune cell recruitment. AS01 is currently part of the recombinant subunit vaccine Shingrix® (GlaxoSmithKline (GSK)), a prophylactic vaccine preventing shingles. It has been shown that Shingrix is more effective than the live attenuated vaccine Zostavax® (Merck), suggesting a pivotal role of the used adjuvant AS01. Its safety has been thoroughly investigated.

Unpublished preclinical data indicate a maturation of myDCs upon exposure to AS01.

Therefore, we propose to investigate the effect of intratumoral injection of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDCs plus AS01 and ipilimumab in combination with IV administration of nivolumab.

5.2. Overall Risk/Benefit Assessment

Giving the already extensively documented safety profile of intravenously administered nivolumab and the "local" nature of intratumoral injection of AS01, myDCs and ipilimumab, we expect that this investigational therapy will be tolerable under the defined conditions outlined in this protocol.

Only patients who have failed all standard curative and life-prolonging standard therapy options will be eligible for participation in this study. In general, these patients will have a short “natural” life expectancy.

5.3. Research Hypothesis

We hypothesize that the intratumoral injection of an autologous CD1c (BDCA-1)$^{+}$ / CD141 (BDCA-3)$^{+}$ myDC cell co-product in with AS01 pretreated metastases will allow a more efficient activation of intratumoral myDC *in vivo* leading to a more effective antitumoral T-cell response following cross-presentation of tumor antigens. The administration of ipilimumab (IT) and nivolumab (IV) will be continued as these monoclonal antibodies will block CTLA-4 expressing regulatory T cells in the tumor microenvironment and PD-1 expressing T lymphocytes in the peripheral blood circulation, respectively.

5.4. Study objective(s) and endpoint(s)

Primary Objective and Endpoint	• *Objective*: To document the safety of intratumoral injection of autologous CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC co-product (isolated from PBMC) plus AS01, and ipilimumab (anti-CTLA-4 IgG_1 mAb) in combination with IV administration of nivolumab (anti-PD1 IgG_4 mAb).	• *Endpoint*: Adverse events occurring in patients treated in this phase I clinical trial (collected on a continuous basis). Type, frequency and severity (graded according to the CTCAEv5.0) will be reported descriptively.
Secondary Objectives/ Endpoint	• Feasibility	• Percentage of study patients that can receive the planned CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC intratumoral injection.
	• Treatment disposition	• Number/volume of administered CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC • Administered dose of AS01, ipilimumab, and nivolumab
	• Anti-tumor activity	• Tumor response (ORR) according to RECISTv1.1 and iRECIST • Tumor response and duration of response of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC,AS01 and ipilimumab injected and non-injected metastases (reported descriptively)
	• Progression-free survival (PFS)	• Time from first study treatment administration to progression of disease according to RECISTv1.1 and iRECIST (and possibly itRECIST)
	• Overall survival	• Time from first study treatment administration to death
Translational Objectives and Endpoints	• Effect on cellular- and molecular characteristics of the tumor microenvironment during study treatment	• Immunohistochemical analysis (CD3, CD8, CD4, PD-L1), multiplexed immunofluorescent imaging, and RNA-expression profiling (NanoString PanCancer IO 360 gene expression panel) of repetitive on-treatment tissue biopsies of injected metastases • T-cell receptor repertoire in metastases assessed by ImmunoSEQ analysis
	• Effect on blood lymphocytes following study treatment	• Peripheral blood differential white blood cell counts • Immunocytochemical differential cell counts (incl. CD3+, CD4+ and CD8+ lymphocytes)

		• T-cell receptor repertoire assessed by ImmunoSEQ analysis • Flow cytometric analysis of effector/naïve/memory T cells

6. Subject eligibility

For recruitment to the study, the following criteria must be met. Eligibility criteria for this study have been carefully considered to ensure the safety of the study subjects and that the results of the study can be used. It is imperative that subjects fully meet all eligibility criteria. All inclusion/exclusion criteria should be assessed during screening period and confirmed prior to starting treatment at Day 1.

6.1. Inclusion Criteria

1) Subject has provided informed consent prior to initiation of any study-specific activities/procedures.
2) Male or female age ≥ 18 years at the time of informed consent
3) All subjects must have histologically confirmed advanced cancer that cannot be completely surgically resected and have failed all standard curative and life prolonging therapy.
4) All subjects must have non-visceral metastatic disease localizations that are amenable to intra-tumor injection by clinical and ultrasound (US) guidance. These metastases should be amenable to a safe post-injection biopsy (partial or complete).
5) ECOG performance status of 0 or 1
6) Candidate for intralesional therapy defined as either one of the following:
   a) At least 1 injectable cutaneous, subcutaneous, or solid tumor lesion ≥ 10 mm in longest diameter
   b) Multiple injectable solid tumor lesions that in aggregate have a longest diameter of ≥ 10 mm injectable disease
7) Adequate organ function determined within 28 days prior to enrollment, defined as follows:
   a) Hematological
      i) Absolute neutrophil count ≥ 1500/mm3 ($1.5x10^9$/L)
      ii) Platelet count: ≥ 75.000/mm^3 ($7.5x10^9$/L)
      iii) Hemoglobin: ≥ 8 g/dL (without need for hematopoietic growth factor or transfusion support)
   b) Renal
      i) Serum creatinine: 1.5 x upper limit of normal (ULN), OR 24-hour creatinine clearance ≥ 60 mL/min for subject with creatinine levels > 1.5 x ULN. (Note: Creatinine clearance need not be determined if the baseline serum creatinine is within normal limits. Creatinine clearance should be calculated per institutional standard).
   c) Hepatic
      i) Serum bilirubin: 1.5 x ULN OR direct bilirubin ≤ ULN for a subject with total bilirubin level > 1.5 x ULN
      ii) Aspartate aminotransferase (AST): 2.5 x ULN OR ≤ 5 x ULN for subject with liver metastases
      iii) Alanine aminotransferase (ALT): 2.5 x ULN OR ≤ 5 x ULN for subject with liver metastases
   d) Coagulation
      i) International normalization ratio (INR) or prothrombin time (PT): 1.5 x ULN unless the subject is receiving anticoagulant therapy as long as PT and partial thromboplastin time (PTT)/ activated PTT (aPTT) is within therapeutic range of intended use of anticoagulants
      ii) PTT or aPTT: 1.5 x ULN unless the subject is receiving anticoagulant therapy

as long as PT and PTT/aPTT is within therapeutic range of intended use of anticoagulants

8) Female subject of childbearing potential should have a negative serum pregnancy test within 72 hours prior to enrollment.
9) Subject has a tumor sample (archival sample obtained within 3 months prior to study participation or newly obtained biopsy). Subject must submit the tumor sample during screening. Subjects with a non-evaluable archival sample may obtain a new biopsy and subjects with a non-evaluable newly obtained biopsy may undergo re-biopsy at the discretion of the investigator.
10) Adequate vascular access to undergo a leukapheresis.

6.2. Exclusion Criteria

1) Known active central nervous system (CNS) metastases. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids >10 mg/day of prednisone or equivalent. The exception does not include leptomeningeal metastasis which is excluded regardless of clinical stability.
2) History or evidence of active autoimmune disease that requires systemic treatment (ie, with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
3) History or evidence of cancer associated with immunodeficiency states (e.g., hereditary immune deficiency, organ transplant, or leukemia)
4) History of other malignancy within the past 5 years with the following exceptions:
   i) Malignancy treated with curative intent and with no known active disease present and has not received chemotherapy for $\geq$ 5 years before enrollment and felt to be at low risk for recurrence by the treating physician
   ii) Adequately treated non-melanoma skin cancer without evidence of disease at the time of enrollment
   iii) Adequately treated cervical carcinoma in situ without evidence of disease at the time of enrollment
   iv) Adequately treated breast ductal carcinoma in situ without evidence of disease at the time of enrollment
   v) Prostatic intraepithelial neoplasia without evidence of prostate cancer at the time of enrollment
   vi) Adequately treated superficial or in-situ carcinoma of the bladder without evidence of disease at the time of enrollment
2) Prior treatment of another tumor vaccine
3) Receive live vaccine within 28 days prior to enrollment
4) Prior chemotherapy, radiotherapy, biological cancer therapy, targeted therapy, or major surgery within 28 days prior to enrollment or has not recovered to CTCAE grade 1 or better from adverse event due to cancer therapy administered more than 28 days prior to enrollment.
5) Currently receiving treatment in another investigational device or drug study, or less than 28 days since ending treatment on another investigational device or drug study
6) Expected to require other cancer therapy while on study with the exception of local radiation treatment to the site of bone and other metastasis for palliative pain management
7) Other investigational procedures while participating in this study are excluded.
8) History or evidence of symptomatic autoimmune pneumonitis, glomerulonephritis, vasculitis, or other symptomatic autoimmune disease, or active autoimmune disease or syndrome that

has required systemic treatment in the past 2 years (ie, with use of disease modifying agents, corticosteroids or immunosuppressive drugs) except vitiligo or resolved childhood asthma/atopy. Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

9) Evidence of clinically significant immunosuppression such as the following:
    i) Diagnosis of immunodeficiency
    ii) Concurrent opportunistic infection
    iii) Receiving systemic immunosuppressive therapy (> 2 weeks) or within 7 days prior to the first dose of study treatment, including oral steroid doses > 10 mg/day of prednisone or equivalent except for management of adverse events and central nervous system (CNS) metastases during the course of the study. Subjects that require intermittent use of bronchodilators or local steroid injection will not be excluded from the study.
10) Known human immunodeficiency virus (HIV) disease
11) Known acute or chronic hepatitis B or hepatitis C infection
12) Known syphilis infection
13) Female subject is pregnant or breast-feeding, or planning to become pregnant during study treatment and through 5 months after the last dose of study treatment
14) Female subject of childbearing potential who is unwilling to use acceptable method(s) of effective contraception during study treatment and through 5 months after the last dose of study treatment. Note: Women not of childbearing potential are defined as:
    a) Postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.) OR
    b) Have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening; OR
    c) Has a congenital or acquired condition that prevents childbearing. Note: Acceptable methods of effective contraception are defined in the informed consent form.
15) Male subject who is unwilling to use acceptable method of effective contraception during trial participation and through 5 months after the last dose of study treatment. For this trial, male subjects will be considered to be of non-reproductive potential if they have azoöspermia (whether due to having had a vasectomy or due to an underlying medical condition). Note: Acceptable methods of effective contraception are defined in the informed consent form.
16) Subject has known sensitivity to any of the products or components to be administered during dosing
17) Subject likely to not be available to complete all protocol-required study visits or procedures, and/or to comply with all required study procedures to the best of the subject and investigator's knowledge
18) History or evidence of psychiatric, substance abuse, or any other clinically significant disorder, condition or disease (with the exception of those outlined above) that, in the opinion of the investigator, if consulted, would pose a risk to subject safety or interfere with the study evaluation, procedures or completion
19) Is or has an immediate family member (e.g., spouse, parent/legal guardian, sibling, or child) who is investigational site or sponsor staff directly involved in this trial, unless prospective institutional review board (IRB)/independent ethics committee (IEC) approval (by chair or designee) is given allowing exception to this criterion for a specific subject
20) Sexually active subject who is unwilling to use a barrier method (male or female condom) to avoid potential viral transmission during sexual contact during and within 30 days after treatment

21) Has undergone prior allogeneic hematopoietic stem cell transplantation within the last 5 years. (Subjects who have had a transplant greater than 5 years ago are eligible as long as there are no symptoms of Graft versus Host Disease.)
22) Has a known history of active Bacillus tuberculosis

7. Study Design

This study will consist of the following phases: eligibility screening-/enrollment-, treatment-, and follow-up phase.

7.1. Eligibility screen and enrollment phase

For more information see original protocol version.

7.2. Treatment Phase

- **Day1**: Leukapheresis, isolation of CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC, and IT administration of AS01, and ipilimumab, and IV administration of nivolumab:
    - (9 – 13h): Isolation of CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC: patients will undergo a leukapheresis after which isolation of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC from the collected PBMC will be started. First, CD14$^+$ and CD19$^+$ cells will be depleted from the PBMC followed by isolation of CD1c (BDCA-1)$^+$ myDC and CD141 (BDCA-3)$^+$ myDC using the CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ Dendritic Cell Isolation Kit on the CliniMACS Prodigy® or on the immunomagnetic CliniMACS Plus isolation system (Miltenyi Biotec, Bergisch Gladbach, Germany). Isolated CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC will be available for intratumoral injection on day 2.
    - (16 – 17h): Intratumoral administration of AS01 and ipilimumab: up to 0,5 ml AS01 will be administered by intratumoral injection followed by Ipilimumab (Yervoy®, 50mg/10mL solution) at a maximum total dose of 10 mg (= 2 ml of a 50mg/10ml solution).
    - (13-17h): Intravenous administration of nivolumab: 10 mg of nivolumab (Opdivo®, 40 mg/4mL solution) will be administered by a 15 minutes intravenous infusion
- **Day 2**: Intratumoral administration of CD1c (BDCA-1)$^+$ / CD141 (BDCA-3)$^+$ myDC co-product:
    - Following the end of the isolation procedure on day 2, CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC will be made available for administration to the patient (see administration scheme for CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC).
- **Day 3:** A tumor biopsy (VacuCut® or core needle biopsy or surgical resection) will be performed 1 day after injection of the myDC cell-product.
- **Day 15 + every 14 days thereafter:**
    - Intratumoral injection of AS01 up to maximum week 12.
    - Intratumoral injection of ipilimumab (Yervoy®, 50mg/10mL solution) at a maximum total dose of 10 mg (= 2 ml of a 50mg/10ml solution)
    - Before every intratumoral injection, we consider taking a tumor biopsy (with a Vacu-Cut® needle or a punch-biopsy) if feasible (e.g. depending on size and location of the lesion).

- **Day 15 + 14 days up to day 365:** 10 mg of nivolumab (Opdivo®, 40 mg/4mL solution) will be administered by a 15 minutes intravenous infusion.
- **Safety assessments** will be made on a continuous basis during the treatment phase (incl. physical and blood analysis at every planned visit)
- **Tumor response assessments** by whole body PET/CT will be scheduled in week 12 and every 12 weeks thereafter during the treatment phase. At earlier time points additional medical imaging will be performed if considered necessary for safety reasons or assessment of disease status
- **Procurement of tumor tissue** by VacuCut® needle (or punch biopsy) will be performed before every intratumoral study drug administration (on day 15 and every + 14 days thereafter).
- **End-of the study treatment phase:** Study treatment will be discontinued at any time during the study at the diagnosis of progressive disease per iRECIST response assessment criteria, unacceptable adverse event(s), or patient withdrawal of consent to continue study treatment, lost-to-follow-up or death.

7.3. End-of study treatment safety-visit

For more information see original protocol version.

7.4. Follow-up phase

For more information see original protocol version.

8. Investigational products

8.1. The CD1c (BDCA-1)+ myDC / CD141 (BDCA-3)+ myDC autologous cell product

In this clinical trial, the CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC product will be isolated from peripheral blood and will be used without *ex vivo* manipulation for injection into a metastasis (skin, subcutaneous, lymph-node, or other soft tissue). As CD1c (BDCA-1)$^+$ cells were shown to physiologically partially maturate within 24 hours after isolation from the blood, this trial will investigate the capacity of non-manipulated CD1c (BDCA-1)$^+$ myDC and CD141 (BDCA-3)$^+$ myDC to capture and cross-present tumor antigen following intratumoral injection. In order to create a Th1 “cytokine” inflamed tumor microenvironment where CD1c (BDCA-1)$^+$ myDC and CD141 (BDCA-3)$^+$ myDC are expected to mature and coordinate an effective antitumoral CTL-response, AS01 will be co-injected into the metastasis prior to the CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC product.

8.2. Isolation of CD1c (BDCA-1)+ myDC / CD141 (BDCA-3)+ myDC

First, CD14$^+$ and CD19$^+$ cells will be depleted from the PBMC product using murine anti-CD14 and anti-CD19 monoclonal antibodies conjugated to superparamagnetic iron dextran particles. This step is followed by the simultaneous isolation of CD1c (BDCA-1)$^+$ and CD141 (BDCA-3)$^+$ myDC using a combination of a BDCA-1 specific monoclonal antibody and a BDCA-3 specific monoclonal antibody, both biotin-conjugated (Miltenyi Biotec, Bergisch Gladbach, Germany). After labeling with the CliniMACS Anti-Biotin Reagent, the target cells are immunomagnetically enriched. In a final step, the isolated BDCA-1$^+$/ BDCA-3$^+$ dendritic cell fraction will be concentrated by centrifugation and resuspended in PBS/EDTA (Miltenyi) containing 0.5%

human albumin to obtain a cell suspension at the concentration (cells/ml) desired for clinical administration. *See also the IMPD file*.

Following their isolation, CD1c(BDCA-1)$^+$ / CD141(BDCA-3)$^+$ myDC will be transferred for administration to the patient. CD1c(BDCA-1)$^+$ / CD141(BDCA-3)$^+$ myDC cell products will need to meet the following release criteria: >50% cell viability and >50% cell purity.

8.3. Intratumoral injection of CD1c (BDCA-1)+ myDC / CD141 (BDCA-3)+ myDC product

The CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC product will be administered by intratumoral injection in the lesions that were injected with AS01 on day 1 (approximately 24 hours after intratumoral injection of AS01).

The total administered CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC volume will be up to 4.0 mL per intratumoral treatment session. Injected volume per lesion (skin-, soft tissue or lymph-node metastase(s)) will range from 0.1 mL for lesions < 0,5 cm to 4.0 mL for lesions > 5 cm in longest diameter. Injection of all lesions in an individual patient will not be required.

The determination of CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ injection volume is based on lesion size:

- > 5 cm up to 4 mL
- > 2.5 cm to 5 cm up to 2 mL
- > 1.5 cm to 2.5 cm up to 1 mL
- > 0.5 cm to 1.5 cm up to 0.5 mL

When lesions are clustered together, they are injected as a single lesion according to the table for determination of CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC injection volume based on lesion size.

Intratumoral injections of CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC will be performed under sterile conditions. At the end of the procedure the injected site will be covered with an absorbent pad and dry dressing.

8.3.1. Retreatment

Patients will not be considered for retreatment with CD1c (BDCA-1)$^+$ myDC / CD141 (BDCA-3)$^+$ myDC in this clinical trial.

8.4. Study Drugs

Study drugs consist of the following:

- AS01 (containing 50 μg QS21 and 50 μg MPL; subunit of Shingrix® (GSK))
- Ipilimumab (Yervoy®, 50mg/10mL)
- Nivolumab (Opdivo®, 40 mg/4mL)

8.5. Adjuvant System 01 (AS01)

Intratumoral administration

Adjuvant system AS01 will be administered by intratumoral injection into (sub-)cutaneous, and lymph node metastases or other soft tissue metastases, with or without guidance by ultrasound imaging. No visceral metastases will be injected. AS01 must be prepared and administered by a qualified healthcare professional.

Subjects should be assessed clinically for adverse events/toxicity prior to each dose. Complete blood count with differential and chemistry panels including liver function laboratory tests (ALT, AST, and total bilirubin) should be obtained according to the study protocol and the results

should be verified before each treatment. Dosing will occur only if these test values are acceptable.

Dosage and schedule

AS01 will be intratumorally injected every 14 days until week 12 (time of tumor response assessment by PET/CT).

Dosing will be adapted to injected lesion volume according to maximal diameter:

>5cm: 0.5ml AS01

2.5cm – 5cm: 0.25ml AS01

1.5cm – 2.5cm: 0.125ml AS01

0.5cm – 1.5 cm: 0.075ml AS01

Lesions should be injected until the maximum volume per day (0,5mL) has been reached or there are no further injectable lesions, whichever comes first.

Adjustment-, Delay-, or Permanent Discontinuation of AS01 dosing

Dose reductions of AS01 are not indicated at the exception of a reduction in the volume injected in an individual lesion due to a disease response.

Dose reductions with regards to changes in the concentrations of AS01 are not permitted. However, patients may require a reduction in the volume injected due to a disease response or due to local toxicity at the injection site.

If in the course of administration of AS01 the subject cannot tolerate the full dose due to an injection-related adverse event such as pain, the total volume given should be recorded, and the reason for intolerance should be documented as an adverse event.

Pre- and Post-medications

Pre- or post-medications should not be routinely administered prior to or after administration of the investigational products.

In case of mild acute systemic inflammatory response (e.g. pyrexia, chills) following administration of the investigational products, patients will receive paracetamol at a dose of 1g intravenously every 8 hours and diclofenac 75 mg intravenously every 12 hours iv until the resolution of symptoms to grade 0-1.

In case of acute severe systemic inflammatory response (e.g. hypotension, anaphylaxis) following administration of the investigational products, patients need to be managed according to local standards of care for management of shock and anaphylaxis.

8.6. Ipilimumab (Yervoy®)

For more information see original protocol version.

8.7. Nivolumab (Opdivo®)

For more information see original protocol version.

8.8. Dose Omission Criteria

For more information see original protocol version.

8.9. Pre- and Post-medications

For more information see original protocol version.

8.10. Concomitant Therapy

For more information see original protocol version.

8.11. Other Treatment Procedures

For more information see original protocol version.

8.12. Excluded Treatments and/or Procedures During Study Period

For more information see original protocol version.

8.13. Permitted Therapy During Study Period

For more information see original protocol version.

8.14. Discontinuation of Subjects Following any Treatment with Study Drug

For more information see original protocol version.

9. Study procedures

The Schedule of Assessments is summarized in Table 1

Table 1 Assessments made during the study

	Screening	Treatment phase (week number & day)					Follow-up phase	
Study Procedures	≤ -28 Days [1]	Day 1	Day 2	Day 3	Day 15 + and every other +14 days up to day 71 (=d1W11)	Day 15, and every other +14 days up to day 365	Safety [2]	Survival [3]
General Assessments								
Informed Consent	x							
Review of Eligibility Criteria	x							
Demographics, Medical, Surgical and Medication History	x							
Recording of Concomitant Medication	↦	↦	↦	↦	↦	↦	x	
Vital Signs [4]	x						x	
Physical Exam Including Body Weight	x						x	
ECOG Performance Status	x						x	
12-lead electrocardiogram (ECG) [5]	x						x	
Review of AEs and SAEs [6]	↦	↦	↦	↦	↦	↦	x	x
Survival Assessment								x
Treatment Administration								
AS01 and Ipilimumab intratumoral[7]		x			x			
Nivolumab IV[7]		x				x		
CD1c(BDCA-1)$^+$/CD141(BDCA-3)$^+$ myDC [8]			x					

[1] Procedures to be performed ≤ 28 days prior to enrollment.
[2] Safety follow-up will be performed approximately 30 (+7) days after the last dose of study treatment.
[3] Subjects will be followed for survival from the date of the safety follow-up visit until up to 36 months after the date of recruitment of the last subject.
[4] Vital signs (systolic/diastolic blood pressure, heart beat, and temperature) must be performed prior to the study treatment (AS01, Ipilimumab, and Nivolumab and/or CD1c(BDCA-1)$^+$/CD141(BDCA-3)$^+$ myDC administration), and at the safety follow-up.
[5] A single 12-lead ECG will be performed ≤ 28 days before enrollment and the safety follow-up visit.
[6] All SAEs that occur after the subject has signed the informed consent through 90 (+7) days after the cessation of all study treatment or 30 (+7) days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, whichever is later, will be reported to the sponsor and recorded in the CRF. SAEs must be reported to the sponsor within 24 hours of the investigator knowledge of the event. All non-serious AEs that occur and concomitant medications that are administered after enrollment through 30 (+7) days after the last dose of study treatment will be recorded in the case report form.
AEs and concomitant medications should be assessed on an ongoing basis and recorded at each subject visit.
[7] AS01 and Ipilimumab will be administered by intratumoral injection on day 1 of week 1, day 15, and every 2 weeks (±3 days) thereafter up to day 71 (= day 1 of week 11). AS01 (derived from Shingrix®, 0.5mL solution) will be administered by intratumoral injection of a maximum total volume of 0,5 ml (see drug administration section for guidance on volume according tumor dimension) followed by intratumoral injection of Ipilimumab (Yervoy®, 50mg/10mL solution) at a maximum total dose of 10 mg (= 2 ml of a 50mg/10ml solution). Ten mg of nivolumab (Opdivo®, 40 mg/4mL solution) will be administered by a 15-minute intravenous infusion, repeated every 2 weeks up to day 365 (: 1 year of study treatment). Dosing of study drugs should be continued until CR, disappearance of injected lesion, confirmed PD per iRECIST, unacceptable intolerance of study treatment, 12 months from the first dose of Nivolumab, or end of study, whichever occurs first. Due to the mechanism of action, subjects may experience growth in existing tumors or the appearance of new tumors prior to maximal clinical benefit of study treatment. Therefore, dosing should be continued provided that the subject has no evidence of confirmed PD per iRECIST and is able to tolerate the treatment. Assessments and procedures are to be performed within 3 days of the planned visit and results available prior to study drug administration, unless otherwise specified. It is recommended that dosing occur on the same day of the week (e.g., if first dose is administered on a Monday, all subsequent doses should be administered on a Monday), however, a ± 3-day dosing window is allowed.
[8] CD1c(BDCA-1)$^+$/CD141(BDCA-3)$^+$ myDC will be administered by intratumoral injection upon isolation from PBMC obtained by leukapheresis.

	Screening	Treatment phase (week number & day)					Follow-up phase	
Study Procedures	≤ 28 Days [9]	Day 1	Day 2	Day 3	Day 15 + and every other +14 days up to day 71 (=d1W11)	Day 15, and every other +14 days up to day 365	Safety	Survival
Local Laboratory Tests								
Hematology, chemistry and endocrine tests [10]	x	x			x	x	x	
PT (INR) and aPTT [11]	x							
Urine [12]	x	x			x	x	x	
Serum Pregnancy Test [13]	x				x	x	x	
Viral and bacterial serology [14]	x							
ImmunoSEQ blood sample [15]		x				x [16]		
IHC analysis of WBC [17] & flow cytometry of lymphocytes		x			x			
Special tests and procedures								
Archived Tumor Tissue for Biomarker Analyses [18]	x							
On-treatment tumor biopsy [19]				x		x		
Leukapheresis		x						

[9] Procedures to be performed ≤ 28 days prior to enrollment.
[10] Blood chemistry should include glucose, Na, K, Cl, bicarbonate, Anion Gap, Creatinine, Blood Urea Nitrogen, LDH, AST, ALT, gamma-GT, Alkaline Phosphatase, Bilirubin, amylase, lipase, albumin, Ca, P, Mg, CRP; endocrine tests: TSH, FT4, (FSH, testosterone/estradiol, ACTH, and cortisol at screening/baseline and when clinically indicated).
[11] Blood sample for coagulation will be collected at screening. During treatment, blood samples should be collected for coagulation as clinically indicated.
[12] Urine samples for urinalysis will be collected at screening. During treatment, urine samples should be collected and analyzed as clinically indicated.
[13] Serum pregnancy tests should be performed in female study participants of childbearing potential. A serum pregnancy test should be performed within 72 hours prior to enrollment, monthly during study treatment and at the end of systemic exposure, and highly effective contraception should be foreseen. In addition, contraception measures should be continued for at least 5 months following the last dose of nivolumab as indicated in the SmPC.
[14] Viral serology for the HIV, hepatitis B and C viruses (incl. serum anti-HCV antibodies and HBV surface, core and HBeAg & antibodies). Bacterial serology for syphilis.
[15] 10 ml of blood on EDTA will be collected for the purpose of ImmunoSEQ analysis on day 1, prior to treatment with AS01 and Ipilimumab, and in week 12.
[16] Only in week 12
[17] Only in week 12
[18] Formalin-fixed paraffin-embedded tumor tissue from a metastatic lesion (block or unstained tumor slides) and the associated pathology reports must be submitted prior to enrollment to the central laboratory for biomarker analyses. Alternatively, a new biopsy will be obtained as part of the screening procedure.
[19] VacuCut® Disposable Aspiration Biopsy Needle biopsy or punch biopsy or surgical tumor biopsy will be performed on day 3 after the first administration of AS01 and Ipilimumab.

	Screening	Treatment phase (week number & day)					Follow-up phase	
Study Procedures	≤ 28 Days	Day 1	Day 2	Day 3	Day 15 + and every other +14 days up to day 71 (=d1W11)	Day 15, and every other +14 days up to day 365	Safety	Survival
Tumor response assessments								
Clinical Tumor Assessment [20]	x	x		x	x	x		
Photographs of Visible Cutaneous & Subcutaneous Tumor Lesions [21]	x	x		x	x	x	x	X
Radiographic (CT, PET/CT, MRI, or US) Scans & Tumor Assessment [22]	x					x[23]		

[20] Clinical measurement of cutaneous, subcutaneous, and palpable nodal tumor lesions by caliper and response assessment per iRECIST. The screening clinical measurements must be done within 28 days prior to enrollment. During treatment, the clinical tumor assessments will performed independent of treatment cycle at day 1 of administration of AS01 and Ipilimumab and prior to administration of CD1c(BDCA-1)$^+$/CD141(BDCA-3)$^+$ myDC, week 12 (+1 week) and every 12 weeks (+1 week) thereafter or more frequently if clinically indicated until confirmed PD per iRECIST or start of new anticancer treatment. The schedule of clinical assessment of tumor lesions should not be adjusted for cycle initiation delays and performed according to the calendar. Response or progression should be by repeated clinical assessment ≥ 4 weeks after the first indication of response or progression. Tumor assessment is required at the safety follow up visit if the subject ended treatment prior to confirmed PD and has not had clinical tumor assessments performed within 4 weeks (+1 week) of the visit. For subjects who discontinued treatment for any reason other than confirmed PD, every effort should be made to complete clinical tumor assessments every 12 weeks (+1 week) or more frequently if clinically indicated during the long-term follow-up until documentation of confirmed PD per iRECIST, start of new anticancer treatment, if present, whichever occurs first. Note: When a lesion can be accurately evaluated by both radiographic imaging and clinical examination, radiographic imaging evaluations should be undertaken.

[21] Photographs of up to 5 visible (i.e., visible protrusion from skin surface) cutaneous and subcutaneous measurable tumor lesions which are selected as index lesions will be performed at screening within 28 days prior to enrollment. During treatment, the photographs of visible cutaneous and subcutaneous index tumor lesions and of new visible lesion cutaneous and subcutaneous lesions, if present, will performed at day 1, week 12 (+1 week) and every 12 weeks (+1 week) thereafter or more frequently if clinically indicated until confirmed PD per iRECIST or start of new anticancer treatment. The schedule of photograph of tumor lesions should not be adjusted for cycle initiation delays and performed according to the calendar. Response or progression should be confirmed by repeated photographs ≥ 4 weeks after the first indication of response or progression. Photographs of visible cutaneous and subcutaneous index tumor lesions and of new visible cutaneous and subcutaneous lesions, if present, is required at the safety follow up visit if the subject ended treatment prior to confirmed PD and has not had photographs performed within 4 weeks (+1 week) of the visit. For subjects who discontinued treatment for any reason other than PD, every effort should be made to complete photographs of visible cutaneous and subcutaneous index lesions and of new visible cutaneous and subcutaneous lesions, if present, every 12 weeks (+1 week) or more frequently if clinically indicated during the long-term follow-up until documentation of confirmed PD per iRECIST, start of new anticancer treatment, or end of study, whichever occurs first. The photographic images will be collected and held for possible supportive retrospective review of tumor response by an independent central review committee. Note: When a lesion can be accurately evaluated by both radiographic imaging and photographs, radiographic imaging evaluations should be undertaken.

[22] Radiographic imaging (whole body PET/CT, plus MRI of the brain if clinically indicated) is required at screening. Tumor assessments must include all known sites of disease. The screening scans must be done within 28 days prior to enrollment. During treatment, radiographic imaging (whole body PET/CT, plus MRI of the brain if clinically indicated), will be performed at week 13 and every 12 weeks thereafter or more frequently if clinically indicated until confirmed PD per iRECIST or start of new anticancer treatment. Imaging should not be adjusted for cycle initiation delays and performed according to the calendar. The imaging modality selected (e.g., PET/CT or MRI) should remain constant for any individual subject. Response or progression should be confirmed by repeated radiographic imaging ≥ 4 weeks after the first indication of response or progression. Radiographic imaging is not required at the safety follow up visit. For subjects who discontinued treatment for any reason other than confirmed PD, every effort should be made to complete radiographic assessments every 12 weeks (+1 week) or more frequently if clinically indicated during the long-term follow-up until documentation of confirmed PD per iRECIST, start of new anticancer therapy, or end of study, whichever occurs first. Subjects who have reached a confirmed CR may increase their interval of radiographic assessments up to 6 months after the first 2 years beyond confirmed CR.

[23] Tumor response assessment by whole body PET/CT and complementary medical imaging as indicated should be performed in week 13 and every 12 weeks thereafter until the end of the study treatment phase.

9.1. General Study Procedures

For more information see original protocol version.

9.2. Screening, Enrollment

The following procedures are to be completed during the screening period within 28 days of enrollment/randomization (unless otherwise noted) at time points designated in the Schedule of Assessments (Table 1):

- Confirmation that the Informed Consent Form has been signed
- Review of eligibility criteria
- Demographic data including sex, age or date of birth, race, and ethnicity will be collected in order to study their possible association with subject safety and treatment effectiveness.
- Vital signs (systolic/diastolic blood pressure, heart rate): Record all measurements on the vital signs electronic data record.
- Medical, surgical and medication history
- Physical examination including body weight as per standard of care
- Documentation of concomitant medications
- ECOG performance status assessment
- A 12-lead electrocardiogram (ECG): The ECG must include the following measurements: heart rate, PR, QRS, QT and QTc intervals. The investigator or designated site physician will review all ECGs. Once signed, the original ECG tracing will be retained with the subject's source documents.
- Local Laboratory Assessments within ≤ 14 days prior to enrollment:
  - Hematology panel: hemoglobin, hematocrit, white blood cell (WBC) count with 5-part differential (3-part differential if 5-part unable to be performed), red blood cell (RBC) count, platelets
  - Chemistry panel: sodium, potassium, chloride, calcium, magnesium, phosphorous, uric acid, total protein, albumin, blood urea, creatinine, total bilirubin, alkaline phosphatase, AST, ALT, glucose, LDH, CRP
  - Coagulation: PT or INR and PTT or aPTT
  - Thyroid function tests: fT4, TSH
  - Urinalysis (blood, glucose, protein, specific gravity) and reflexive microscopic exam only for any abnormal urinalysis results.
  - Serum pregnancy test for female subjects of childbearing potential within 72 hours prior to enrollment.
  - Radiographic tumor imaging (including whole body positron emission tomography [PET]/CT scan, and as indicated clinically magnetic resonance imaging [MRI]) of the brain, to be used as baseline imaging
  - Recording of serious adverse events that occur after subject signs informed consent.
- Clinical tumor assessments, including clinical measurement of skin and/or lymph node tumor lesions by caliper to be used as baseline assessment. Note: When a tumor lesion can be accurately evaluated by both, radiographic imaging and clinical examination, radiographic imaging evaluations should be undertaken.
- Photographs of visible (ie, visible protrusion from skin surface) skin, soft tissue, and/or lymph node tumor lesions:
  - Photographs of visible and measurable skin and/or lymph node tumor lesions (up to 5) which are selected as index lesions will be performed.
  - Note: When a tumor lesion can be accurately evaluated by both, radiographic imaging and photographs, radiographic imaging evaluations should be undertaken.
- Archived tumor tissue or new tumor biopsy for translational research.

- A representative archival (or new [: optional or mandatory in case there is no archival tumor material available]) metastatic tumor tissue sample will be obtained as part of the eligibility screen procedure.

10. Treatment Period

10.1. Treatment phase

Treatment begins when the first dose of protocol-defined therapies is administered to a subject.

Local laboratory assessments: Screening laboratory values may be used for treatment initiation assessment if completed within 3 days of study treatment initiation. On treatment tests can be performed within 3 days of the planned visit. Results should be reviewed prior to the administration of study treatment:

- Coagulation: PT or INR and PTT or aPTT – as clinically indicated
- Serum pregnancy test for female subjects of childbearing potential will be performed monthly

Subject should record (serious) adverse events at each visit.

Documentation of concomitant medications at each visit:

- Vital signs (systolic/diastolic blood pressure, heart rate, temperature (if clinically indicated)): documented on the vital signs electronic data record.
- Physical examination including body weight, as per standard of care
- ECOG performance status

Local laboratory assessments:

- Hematology panel: hemoglobin, hematocrit, WBC with 5-part differential, RBC, platelet
- Chemistry panel: sodium, potassium, chloride, calcium, magnesium, phosphorus, uric acid, total protein, albumin, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, ALT, glucose
- Thyroid function tests: FT4, TSH
- Urinalysis (blood, glucose, protein, specific gravity) and reflexive microscopic exam only as clinically indicated
- Central laboratory assessments:
- Tumor biopsy for biomarker analysis according to the following schedule: injected lesions will be biopsied before every study drug administration if feasible

- Radiographic tumor imaging assessments must include whole body PET/CT (MRI of the brain will only be performed if signs or symptoms suggestive of CNS metastasis are present). Imaging will be performed independent of treatment in week 13 and every 12 weeks thereafter or more frequently if clinically indicated until confirmed PD per iRECIST or start of new anticancer treatment. Imaging should not be adjusted for cycle initiation delays and performed according to the calendar. The imaging modality selected (eg, CT or MRI) should remain constant for any individual subject.
- Photographs of visible cutaneous and subcutaneous:
  - Photographs of visible cutaneous and subcutaneous index tumor lesions and of new visible lesion cutaneous and subcutaneous, if present, will be performed independent of treatment cycle at day 1 of week 0, day 1 of week 12 (+1 week) and every 12 weeks (+1 week) thereafter or more frequently if clinically indicated until confirmed PD per iRECIST criteria or start of new anticancer treatment. The scheduled photography of tumor lesions should not be adjusted for cycle initiation delays and performed according to the calendar.
  - Note: When a tumor lesion can be accurately evaluated by both, radiographic imaging and photographs, radiographic imaging evaluations should be undertaken.

- Clinical tumor assessments must include clinical measurement of cutaneous, subcutaneous, or palpable nodal tumor lesions by caliper if feasible. Note: When a tumor lesion can be accurately evaluated by both, radiographic imaging and clinical examination, radiographic imaging evaluations should be undertaken.
- Tumor response will be assessed using the iRECIST, in week 12 and every 12 weeks (+1 week) thereafter or more frequently if clinically indicated until confirmed PD or start of new anticancer treatment.
- Leukapheresis of approximately 15 liters of venous blood on day 1.
- Intratumoral injection of CD1c (BDCA-1)+ and CD141 (BDCA-3)+ myDC on day 2.
- Intratumoral ipilimumab administration:
  - Will be done on day 1 and repeated every 14 days.
- Intravenous nivolumab administration:
  - Day 1 and repeated every 2 weeks thereafter until the end of the first year of treatment (day 365 after the date of first drug administration).

10.2. Safety Follow-up Visit

Upon permanent discontinuation from the study treatment for any reason, the following procedures will be performed approximately 30 (+7) days after the last dose of IT ipilimumab, and IV nivolumab:

- Vital signs (systolic/diastolic blood pressure, heart rate, temperature): should be documented on the vital sign electronic data record.
- Physical examination including body weight as per standard of care
- ECOG performance status assessment
- Local laboratory Assessments
  - Hematology panel: hemoglobin, hematocrit, WBC count with 5-part differential (3-part differential if 5-part unable to be performed), RBC count, platelets
  - Chemistry panel: sodium, potassium, chloride, calcium, magnesium, phosphorus, uric acid, total protein, albumin, ureum, creatinine, total bilirubin, alkaline phosphatase, AST, ALT, glucose
  - Thyroid function tests: FT4, TSH
  - Serum pregnancy test for female subjects of childbearing potential
  - Urinalysis (blood, glucose, protein) and reflexive microscopic exam only for any abnormal urinalysis results
- Recording of adverse events
- Recording of serious adverse events: Serious adverse events that occur within 90 (+7) days after the cessation of all study treatment or 30 (+7) days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, will be reported within 24 hours following the investigator's knowledge of the event.
- Documentation of concomitant medications
- Radiographic tumor imaging, clinical tumor assessment, photographic assessment, and tumor response assessments are to be performed if the subject ended study treatment prior to confirmed PD per iRECIST and has not had assessments performed within 4 weeks (+1 week) of the visit.
- Radiographic tumor imaging assessments must include whole body PET/CT. MRI of the brain will only be performed if signs or symptoms suggestive of CNS metastasis are present.
- Photographic imaging assessments of visible cutaneous and subcutaneous:
  - Photographs of visible cutaneous and subcutaneous index tumor lesions and of new visible lesion cutaneous and subcutaneous, if present.
  - Note: When a tumor lesion can be accurately evaluated by both, radiographic imaging and photographs, radiographic imaging evaluations should be undertaken.
- Clinical tumor assessments must include clinical measurement of cutaneous, subcutaneous, or palpable nodal tumor lesions by caliper. Note: When a tumor lesion can be accurately

evaluated by both, radiographic imaging and clinical examination, radiographic imaging evaluations should be undertaken.

- Tumor response will be assessed using the iRECIST. Response (CR or PR) or PD to be confirmed by second consecutive response assessment no less than 4 weeks from the date of the first documented response or PD.

10.3. Long-term Follow-up

For more information see original protocol version.

11. Biomarker Development

For more information see original protocol version.

12. Withdrawal from study treatment

12.1. Subjects' Decision to Withdraw

For more information see original protocol version.

12.2. Investigator or Sponsor Decision to Withdraw or Terminate Subjects' Investigator Participation Prior to Study Completion

For more information see original protocol version.

13. Safety data collection, recording, and reporting

Assessment of adverse events will include the type, incidence, severity (graded by NCI CTCAE version 5.0). In general, grades of severity are corresponding to:

GRADE	Clinical Description of Severity
0	No change from normal or reference range (This grade is not included in the Version 4.03 CTCAE document but may be used in
1	MILD Adverse Event
2	MODERATE Adverse Event
3	SEVERE Adverse Event
4	LIFE-THREATENING consequences: urgent intervention indicated
5	DEATH RELATED TO Adverse Event

13.1. Adverse Events

For more information see original protocol version.

13.2. Reporting of Adverse Events

The term adverse event covers any unfavorable and unintended sign, symptom, syndrome, or illness that develops or worsens during the period of observation in the clinical study. Clinically relevant abnormal results of diagnostic procedures, including abnormal laboratory findings are considered to be adverse events.

No causal relationship with the study treatment or with the clinical study itself is implied by the use of the term “adverse event”. Planned diagnostic or surgical procedures that are permitted by the clinical study protocol are not adverse events. Planned diagnostic or surgical procedures related to conditions that existed at baseline are not adverse events. In the latter case the condition should be reported as medical history.

Adverse events will be reported in the electronic data record according to the Common Terminology Criteria for Adverse Events v5.0 (CTCAEv5.0). Additional descriptive reporting will be provided if useful for a better understanding or interpretation of the reported AE. Safety monitoring will rely on clinical observations, laboratory and radiology evaluations (see study plan). Individual pre-study values and laboratory normal value limits will be used as references.

All adverse events will be reported to Belgian competent authorities and local ethics committee according to the standard procedures applying to the conduct of clinical trials in Belgium.

13.3. Reporting of serious adverse events (SAE)

For more information see original protocol version.

13.4. Pregnancy and Lactation Reporting

If a pregnancy occurs in a female subject, or female partner of a male subject, while the subject is taking protocol-required therapies report the pregnancy to the competent authorities and ethics committee.

In addition to reporting any pregnancies occurring during the study, investigators should monitor for pregnancies that occur through 5 months after the last dose of study treatment. Within this period, highly effective contraception should be foreseen.

The pregnancy should be reported to the competent authorities and ethics committee within 24 hours of the investigator’s knowledge of the event of a pregnancy.

If a lactation case occurs while the female subject is taking protocol-required therapies report the lactation case to the competent authorities and ethics committee.

In addition to reporting a lactation case during the study, investigators should monitor for lactation cases that occur after the last dose of protocol-required therapies through 5 months after the last dose of nivolumab.

14. Statistical considerations and patient sample size

In total, a maximum of 6 patients (one cohort of maximum 6 patients) will be treated in this clinical trial. Taken into account the novelty of the proposed investigational regimen, this small sample size is considered adequate to provide first-in-man safety data and an adequate sample size for obtaining first data from translational research that will be useful to guide further development.

14.1. Statistical analysis

For more information see original protocol version.

15. Regulatory obligations

For more information see original protocol version.

16. Administrative and legal obligations

For more information see original protocol version.

17. References

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18. Appendices

18.1. Appendix A. Additional Safety Assessment Information

Adverse Event Grading Scale

The Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 is available at the following location:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

18.2. Appendix B. iRECIST

The iRECIST criteria, modified RECIST guideline for immunotherapy, are available at the following location: http://www.eortc.org/recist/introducing-modified-recist-guideline-for-immunotherapy/