Journal for ImmunoTherapy of Cancer

Suitability of tumor-associated antibodies as predictive biomarker for response to immune checkpoint inhibitors in patients with melanoma: a short report

Karlijn de Joode ,[1] Sharon Veenbergen,[2] Claudia Kransse,[2] Dian Kortleve,[1] Reno Debets ,[1] Ron H J Mathijssen ,[1] Arjen Joosse,[1] Marco W J Schreurs,[2] Astrid A M Van der Veldt[1,3]

To cite: de Joode K, Veenbergen S, Kransse C, *et al*. Suitability of tumor-associated antibodies as predictive biomarker for response to immune checkpoint inhibitors in patients with melanoma: a short report. *Journal for ImmunoTherapy of Cancer* 2023;**11**:e006467. doi:10.1136/jitc-2022-006467

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jitc-2022-006467).

Accepted 13 January 2023

[1]Department of Medical Oncology, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, The Netherlands
[2]Laboratory of Medical Immunology, Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands
[3]Department of Radiology & Nuclear Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Correspondence to
Dr Astrid A M Van der Veldt; a.vanderveldt@erasmusmc.nl

ABSTRACT

In 2019, Fässler *et al* showed in this journal that the presence of tumor-associated antibodies correlated with response to immune checkpoint inhibitor treatment in patients with metastatic melanoma. The results of this study suggested that tumor-associated antibodies directed against melanocyte-differentiation antigens and the cancer-germline antigen NY-ESO-1 should be further investigated as candidate biomarkers for response to immune checkpoint inhibitors. The aim of the current study was to validate and extend these previous findings. Therefore, we examined the correlation between serum levels of tumor-associated antibodies and tumor response after treatment with immune checkpoint inhibitors in patients with metastatic melanoma.

All patients included in this prospective study were diagnosed with advanced stage melanoma and treated with nivolumab or pembrolizumab monotherapy. Blood samples were collected before and during treatment. Serum levels of tumor-associated antibodies against the melanocyte differentiation antigen Melan-A and the cancer germline antigens NY-ESO-1, MAGE-C2, MAGE-A6 and ROPN1B were measured at baseline and during treatment. Differences between responders and non-responders were assessed using the Mann-Whitney U-test, and differences between different overall survival categories with the Kruskal-Wallis test. P values ≤0.05 were considered significant.

Serum samples of 58 patients with advanced melanoma with long-term follow-up (>3 years) were collected. In contrast to the findings of Fässler *et al*, for all antibodies tested, we found no significant differences between serum levels of responders and non-responders before or during treatment with immune checkpoint inhibitors. In addition, no significant differences were found in serum levels of tumor-associated antibodies for different overall survival groups.

Although our study included a larger and more mature cohort of patients with longer follow-up, we could not externally validate the findings of Fässler *et al*. In addition, we were not able to identify other cancer germline antigens as predictive biomarkers of response to immune checkpoint inhibitors in patients advanced melanoma. Based on the results of the present study, clinical applicability of tumor-associated antibodies directed against tumor antigens as predictive biomarkers for immune checkpoint inhibitors in patients with advanced melanoma is not feasible.

INTRODUCTION

Cutaneous melanoma is a malignant tumor of the skin derived from melanocytes that accounts for around 3% of all malignant skin cancers. The introduction of immune checkpoint inhibitors (ICIs) for the treatment of (metastatic) melanoma has significantly improved the survival outcomes for patients with melanoma.[1] However, only a subset of patients gain long-term clinical benefit, while many patients experience severe and even lifelong toxicity from treatment with ICIs.[2] For a substantial number of patients with advanced stage melanoma, there is still a need for new therapies. The introduction of new agents[3] for patients with melanoma will be accompanied by uncertainties regarding the most suitable treatment for individual patients. Predictive biomarkers of response to ICI treatment may guide the individualized treatment strategy for patients with advanced stage melanoma.

Melanoma is known for its immunogenic properties, as shown by the rate of spontaneous regression,[4 5] the durable tumor responses after treatment with ICIs[1] and the use of adoptive T cell therapies[6] and tumor-infiltrating lymphocytes[7] as treatment. In addition, immunogenicity is illustrated by the presence of tumor-associated antibodies,[8] which are directed against tumor antigens. For melanoma, tumor antigens can be

divided into melanocyte differentiation antigens (MDAs), for example, glycoprotein 100 (gp100), tyrosinase and Melan-A/MART-1, and cancer germline antigens (CGAs), such as members of the MAGE family and NY-ESO-1. These tumor antigens are known for their ability to induce spontaneous cellular and humoral immune responses in patients with melanoma, while expression of CGAs is normally silenced in adult tissue.[4 8 9] Therefore, (immune responses against) these antigens are often targets for the development of new immunotherapy-based treatments.[10–13]

Fässler *et al* showed in this journal that the presence of tumor-associated antibodies prior to treatment initiation correlated with response to ICI treatment in two cohorts of patients with metastatic melanoma, consisting of 20 and 21 patients, respectively.[14] Serum concentrations of antibodies directed against both CGAs and MDAs were compared between responders and non-responders before and during treatment with ICIs. Based on the results of this study, it was suggested that tumor-associated antibodies directed against different MDAs and the CGA NY-ESO-1 should be further explored as candidate (surrogate) biomarkers for response to ICIs.[14]

Here, we examined the correlation between the presence of tumor-associated antibodies and clinical outcome after ICI treatment in patients with metastatic melanoma. First, the aim was to validate the results of Fässler *et al*, in an independent cohort of patients with metastatic melanoma, to determine whether clinical implementation as a predictive biomarker could be feasible. To this end, our antigen selection partly overlapped with the selection of Fässler *et al*, allowing for the development of harmonized ELISA-based antibody detection and direct comparison of the results with the previous cohorts.[14] Moreover, an additional number of antigens were selected to expand research on the predictive value of tumor-associated antibodies as a surrogate marker for tumor response to ICI treatment.

MATERIALS AND METHODS

Patient and sample collection

All patients selected for this study were diagnosed with advance stage melanoma and treated with nivolumab or pembrolizumab monotherapy. Patients were prospectively included in the MULTOMAB trial (MEC2016-011) (International Clinical Trials Registry Platform (ICTRP), NTR7015) (see online supplemental appendix 2) after providing written informed consent. All patients were treated at the Erasmus University Medical Center in Rotterdam, the Netherlands. Blood samples were collected prospectively before and during ICI treatment. Patients who started anti-PD-1 treatment before May 2018 were included to ensure long-term (ie, at least 3 years) follow-up data were available. Only patients from whom blood was collected at different time points both before and during treatment were included. The on-treatment samples were withdrawn between 1 and 3 months after treatment initiation. To analyze whether differences in blood serum concentrations of tumor-associated antibodies were affected by treatment type, that is, pembrolizumab or nivolumab, treatment details were collected and potential differences between these two treatment types were examined. To determine response to ICI treatment, the best overall response was measured according to RECIST V.1.1.[15] Responders were defined as patients having complete response (CR) or partial response (PR) according to RECIST V.1.1.[15] Moreover, survival data was collected. Overall survival (OS) was defined as the time from initiation of ICI treatment to death (from any cause). To analyze differences in OS, data were categorically divided into the following groups: short-term survivors (OS <1 year), patients with an intermediate OS (1–3 years), and long-term survivors (OS >3 years).

Tumor antigen selection

To determine the tumor antigen selection and to develop the different ELISAs, the methods and results of the study by Fassler *et al*[14] were applied exactly for the current study. Most significant results in the previous study were found for the MDA Melan-A and a CGA NY-ESO-1.[14] To validate and reproduce these results, these antigens were both included in the current study and ELISAs were developed according to the same conditions and protocols of the previous study[14] (for detailed information regarding the ELISA development, see section 'Detection of antibodies against tumor antigens').

The selection of the remaining antigens for the current study was based on the potential relevance of antibodies directed against CGAs, as the most important results in the study by Fässler *et al* were shown for the CGA NY-ESO-1.[14] CGAs are known for their promotion of oncogenic processes and CGAs have often been associated with tumor evolution and clinical outcome.[9 10 16–18] This allows for the identification of potential predictive biomarkers and possible targets for new oncological treatments. In addition, the majority of CGAs are known to have high expression in metastatic melanoma.[19] Therefore, the following antigens were additionally selected for the current analysis: MAGE-C2, MAGE-A6 and ROPN1B.

Detection of antibodies against tumor antigens

For a direct comparison with the results of Fässler *et al*, the ELISA conditions were reproduced for both Melan-A and NY-ESO-1. Since the background signal of the antibodies directed against Melan-A was high, different dilutions were again tested in healthy control samples. Finally, our tested dilutions led to the same conditions as stated in the paper by Fässler *et al*, resulting in the application of harmonized ELISAs for both Melan-A and NY-ESO-1 as compared with Fässler *et al*.[14] Maxisorp 96-well clear polystyrene flat-bottom ELISA plates (ThermoFisher Scientific, Massachusetts, USA) were coated overnight at 4°C with recombinant tumor antigens Melan-A (Abcam, Cambridge, UK), NY-ESO-1 (Lifespan Biosciences, Seattle, Washington, USA), MAGE-A6 (Abnova,

Taipei, Taiwan), MAGE-C2 (Abnova, Taipei, Taiwan) and ROPN1B (Abnova, Taipei, Taiwan) diluted in 0.1M carbonate buffer (pH 9.5) (see online supplemental appendix 1).[14] The plates were washed six times with phosphate-buffered saline (PBS) (pH 7.4). Afterwards, non-specific binding was blocked with 5% non-fat dry milk (Santa Cruz Biotechnology, Dallas, TX) in PBS and incubated for 2 hours at room temperature, followed by six wash cycles with PBS. The patient sera were diluted in 5% non-fat dry milk/PBS (according to online supplemental appendix 1) and incubated for 2 hours at room temperature, followed by six wash cycles with PBS. The peroxidase-conjugated anti-human IgG (ELITECH group, Spankeren, The Netherlands) (1:2500) was incubated for 2 hours at room temperature, and followed by six wash cycles with PBS. The substrate solution consists of orthophenylenediamine (0.5 mg/mL; Sigma-Aldrich, St. Louis, Missouri, USA) in 0.1M citrate buffer (pH 5.6), containing 0.08% H_2O_2 (Sigma-Aldirch, St. Louis, MO) and the plates were incubated for 30 min in the dark at room temperature. Afterwards, the reaction was stopped using 1.25M H_2SO_4. The optical density (OD) was read at 492 nm with an automatic ELISA plate reader (BioTek, Winooski, Vermont, USA).

Statistical analysis

Significance between two groups was determined using Mann-Whitney U-test and differences between multiple groups by using the Kruskal-Wallis test. GraphPad Prism V.5.0 software (GraphPad Software, San Diego, California, USA) was used for all statistical analysis. P values of 0.05 or less were regarded as significant.

RESULTS

In total, serum samples of 58 patients with advanced melanoma were collected. Baseline characteristics of the patients are summarized in table 1. Overall, 33 (57%) patients were male, median age was 63.5 years (interquartile rang 52.5–72 years) and for most patients anti-PD-1 treatment was the first treatment line in metastatic setting. In total, 30 patients (52%) had response to treatment (PR or CR), 19 (33%) of patients had progressive disease and 8 (14%) had stable disease. Median OS was 576 days (27 days–not reached). At 3 years since treatment initiation, 34 (58%) patients were still alive, while 12 (21%) patients died within 1 year after treatment initiation.

Table 1 Clinical patient characteristics

Variable—n (%)	Total group (n=58)
Sex	
Male	33 (57)
Female	25 (43)
Age	
Median age in years (IQR)	63.5 (52.5–72)
BRAF status	
Mutated	28 (48)
Non-mutated	29 (50)
Unknown	1 (2)
Prior systemic treatment	
No	50 (86)
Yes	8 (14)
Type of anti-PD-1 treatment	
Nivolumab	34 (59)
Pembrolizumab	24 (51)
Presence of brain metastases at treatment initiation	
No	30 (52)
Yes	11 (19)
Unknown	17 (29)
LDH	
≤ ULN	31 (53)
>1 × ULN	22 (38)
>2 × ULN	3 (5)
Unknown	2 (3)
Best overall response to treatment	
Complete response	14 (24)
Partial response	16 (28)
Stable disease	8 (14)
Progressive disease	19 (33)
Non-evaluable	1 (2)
Overall survival (OS)	
OS <1 year	12 (21)
OS 1–3 years	12 (21)
OS >3 years	34 (58)

LDH, lactate dehydrogenase levels; ULN, upper limit of the normal range.

Tumor-associated antibodies in responders and non-responders at baseline

Serum levels for all antibodies directed against the different CGAs and Melan-A were measured at baseline, i.e. before initiation of ICI treatment (figure 1A–E). Although for most patients antibody levels could be detected above the set quantification limit, the concentrations were very variable. In addition, serum levels for the antibody directed against Melan-A (figure 1A) were higher compared with the levels of CGA-directed antibodies, that is, NY-ESO-1 MAGE-A6, MAGE-C2 and ROPN1B, respectively (figure 1B–E). For all antibodies tested, no significant differences were found between the serum levels of responders and non-responders before start of treatment. To determine whether differences in antibody concentrations could predict survival outcomes, differences in serum levels between the OS groups were also examined (figure 1A–E). No significant differences

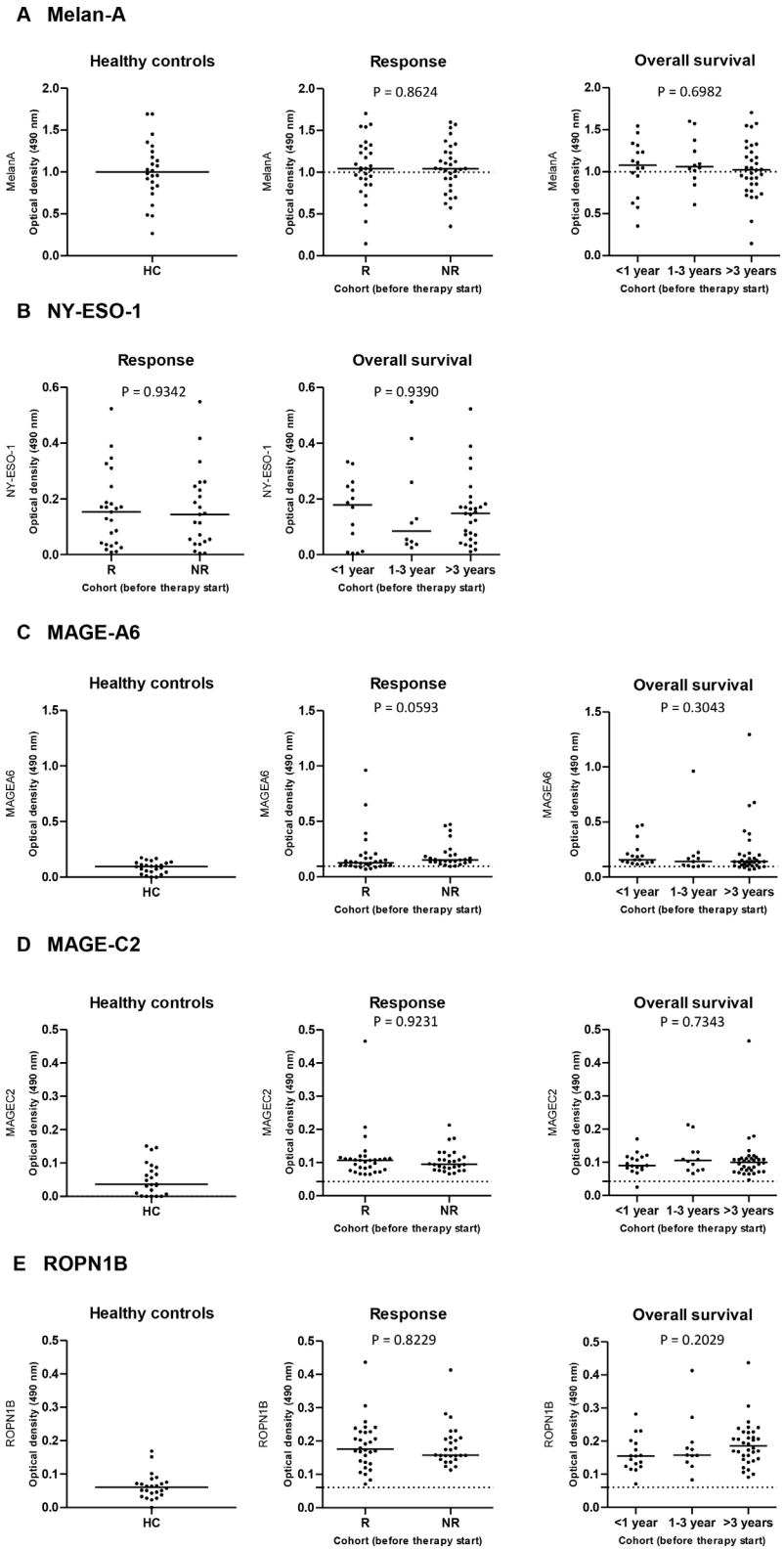


Figure 1 No difference in serum levels of tumor-associated antibodies at baseline. The figures show the optical density (OD) values of the different tumor-associated antibodies in serum of healthy controls, responders (R), non-responders (NR) and the different overall survival (OS) subgroups, that is, OS <1 year, OS 1–3 years and OS >3 years, respectively. The dotted lines represent the median serum levels of the healthy controls for the different antibodies. The following antibodies were measured: Melan-A (A), NY-ESO-1 (B), MAGE-A6 (C), MAGE-C2 (D) and ROPN1B (E). Differences between responders and non-responders were tested for significance using Mann-Whitney U test and differences between the overall survival groups by the Kruskal-Wallis test. P values are indicated in the different graphs. For all antibodies tested, no significant differences were found between the serum levels of different patient cohorts before treatment start. Figures were created with GraphPad Prism V.5.0 software.

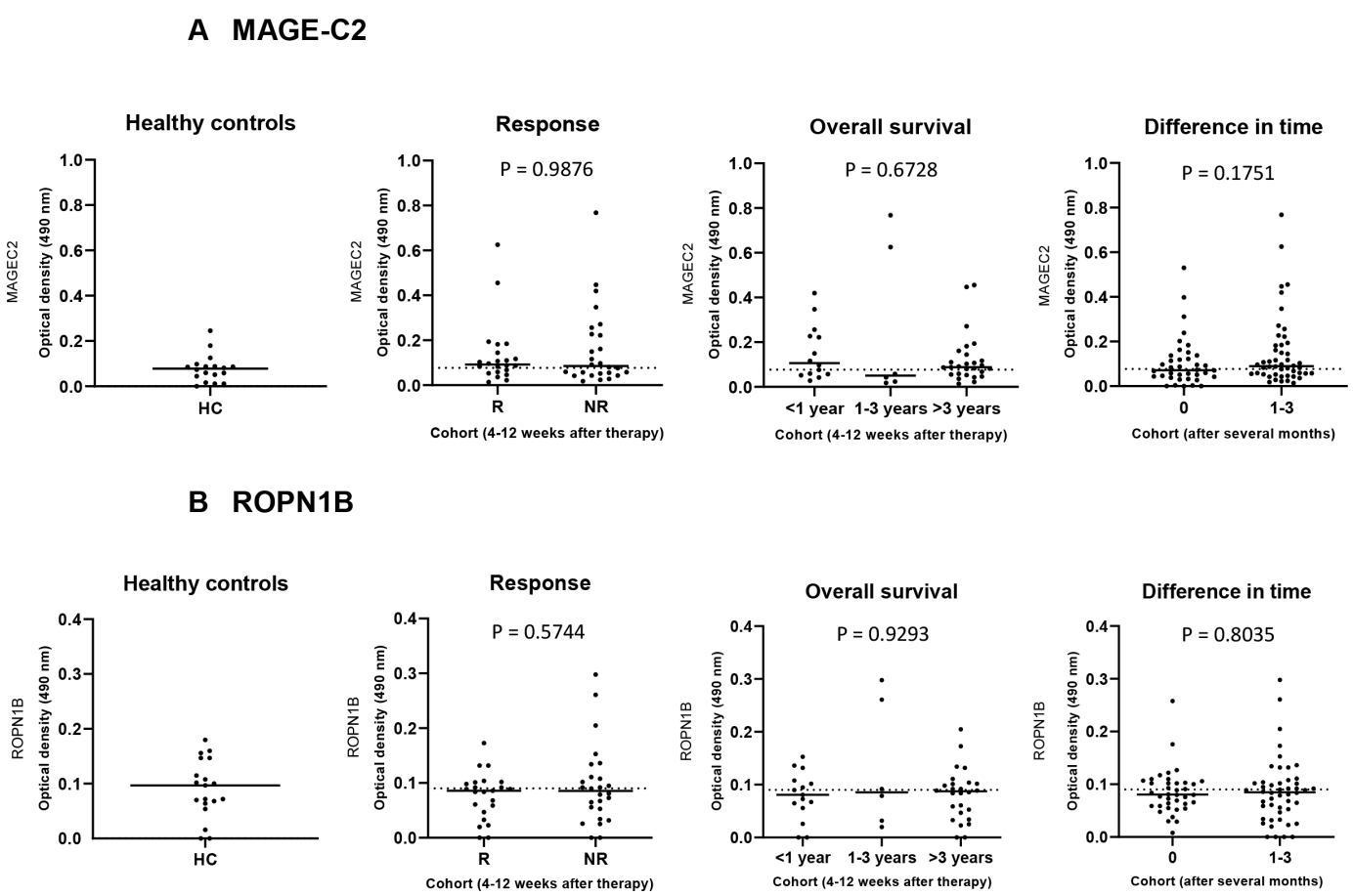


Figure 2 No differences in serum levels of tumor-associated antibodies during treatment with immune checkpoint inhibitors (ICIs). The figures show the optical density (OD) values of antibodies against MAGE-C2 (A) and ROPN1B (B) during treatment with immune checkpoint inhibitors in serum of healthy controls, responders (R), non-responders (NR) and the different overall survival (OS) subgroups, that is, OS <1 year, OS 1–3 years and OS >3 years, respectively. Blood samples for the on-treatment measurements were withdrawn 1–3 months after treatment initiation. The dotted line represents the median serum levels of the healthy controls for MAGE-C2 and ROPN1B, respectively. Differences between responders and non-responders and different time points were tested for significance using Mann-Whitney U test and differences between the OS groups by the Kruskal-Wallis test. P values are indicated in the different graphs. For both MAGE-C2 and ROPN1B, no significant differences were found between the serum levels of different patient cohorts during treatment. The figures were created with GraphPad Prism V.5.0 software.

were found for the different OS groups at baseline (figure 1A–E).

Tumor-associated antibody concentrations during treatment with ICIs

In the study by Fässler *et al*,[14] some antibody levels changed over time, resulting in differences between responders and non-responders. Therefore, in the current study, blood was also collected during treatment with ICIs to determine whether the antibody levels changed during treatment. Figure 2 shows the OD values of antibodies against MAGE-C2 (figure 2A) and ROPN1B (figure 2B) during ICI treatment. No significant differences were found between responders and non-responders, even when the different OS groups were compared. To determine whether the antibody levels changed over time, serum levels at baseline and during treatment were compared. However, no time differences could be detected for the antibodies directed against MAGE-C2 (figure 2A) or ROPN1B (figure 2B) between responders and non-responders nor in the different OS groups.

Last, although nivolumab and pembrolizumab are considered interchangeable in daily clinical practice, tumor-associated antibody levels could be selectively affected by the choice of drugs. As shown in figure 3, no significant antibody differences were found in the sera of patients treated with either nivolumab or pembrolizumab.

DISCUSSION

This study showed that antibody levels directed against the MDA Melan-A and against the CGAs NY-ESO-1, MAGE-C2, ROPN1B and MAGE-A6 cannot be used as predictive markers for the response to treatment with ICIs. More specific, no significant differences in the serum concentrations of these tumor-associated antibodies between responders and non-responders were demonstrated at

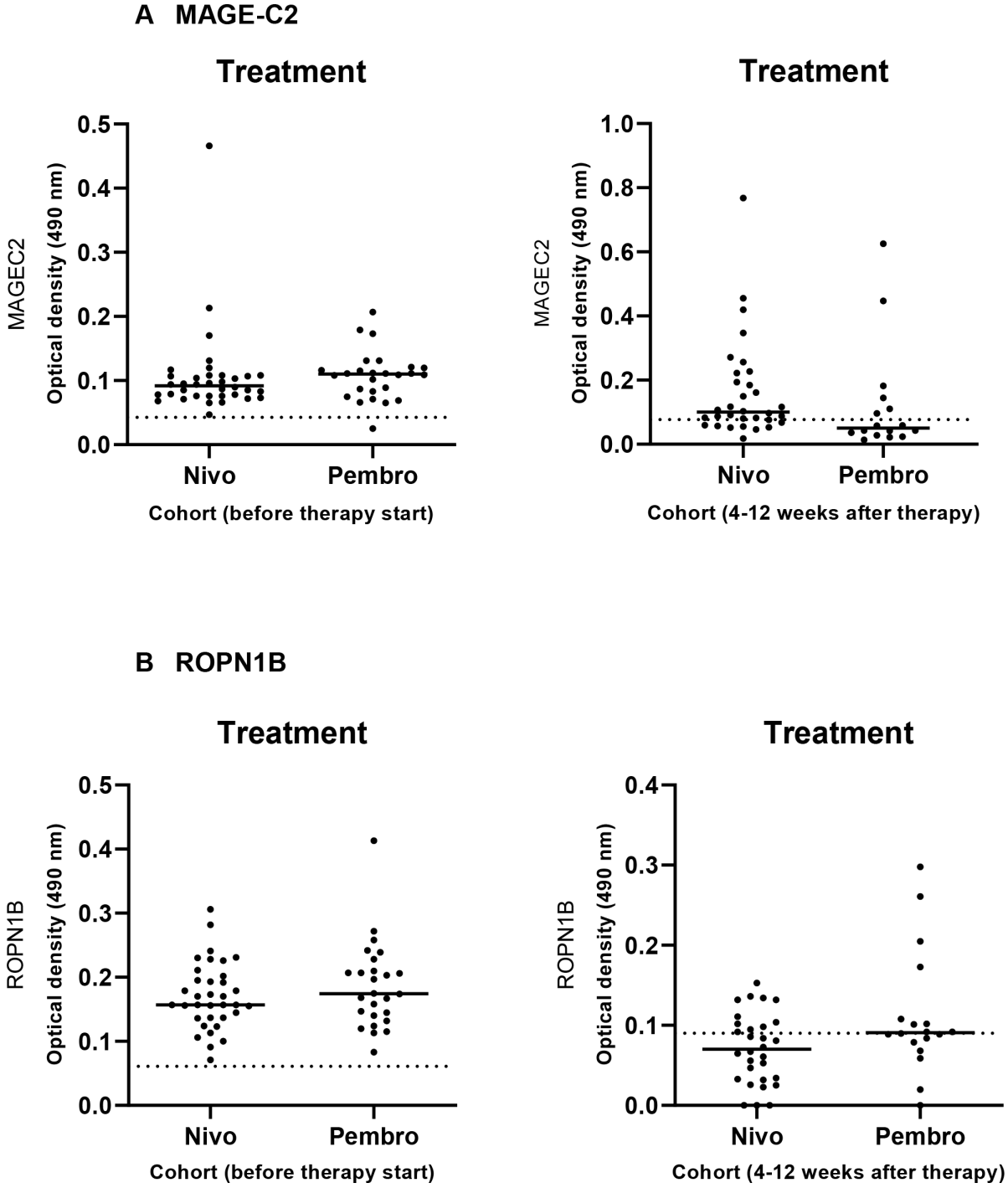


Figure 3 No differences in serum levels of tumor-associated antibodies in patients receiving different immune checkpoint inhibitors (ICIs). The figures show the optical density (OD) values of antibodies against MAGE-C2 (A) and ROPN1B (B) in serum of patients receiving either nivolumab or pembrolizumab. Antibodies against MAGE-C2 (A) and ROPN1B (B) were measured before and during treatment, that is, 1–3 months since treatment initiation. Figures were created with GraphPad Prism V.5.0 software.

baseline or during treatment. A homogeneous cohort of patients with metastatic melanoma was included for this study and long-term follow-up data were available for all patients. Therefore, it was possible to assess whether differences in serum antibody concentrations resulted in long-term OS differences. However, no differences in antibody concentrations at baseline or during treatment were found between the OS groups, that is, between short-term and long-term survivors.

Our results are in contrast with the study of Fässler *et al*, published in this journal in 2019, which demonstrated the potential of two of these antibodies as a surrogate marker for response to ICI treatment.[14] Because we included a larger, more homogeneous and more mature cohort of

patients with melanoma with longer follow-up, less significant results were not expected. Importantly, the antigen selection of the current study partly overlaps, but also differs significantly from the study by Fässler *et al*.[14] As the most promising results were found for NY-ESO-1 in their study, we decided to mostly include CGAs, while in their study mainly MDAs were included. Although this might explain some of the differences in the results, the promising results which were found in the previous study for NY-ESO-1[14] could not be reproduced in the current study.

One of the limitations of the current study is that only circulating antibodies have been taken into account, and the antigen expression of the tumor tissue was not examined. Previous studies have identified the expression of CGAs in tumor tissue as a poor prognostic marker and these antigens may play a role in tumor metastasis.[16–18] The circulating antibody levels might depend on the level of antigen expression of the tumor. In addition, the expression of CGAs or MDAs in tumor tissue has previously been correlated with tumor burden[19] and tumor burden has been associated with decreased OS rates.[20] Subsequently, it is conceivable that higher serum concentrations are associated with poorer response to treatment and decreased OS. For advanced melanoma, tumor burden is known to be associated with lactate dehydrogenase level (LDH) levels.[21] Since patients with variable LDH levels were included in the current study, this study cohort is representative of a real-world population of patients with melanoma with differences in tumor burden.

Antibodies directed against tumor antigens have been extensively studied in the past.[10 22] Immune responses against such antigens are often exploited for the development of new immunotherapy-based treatments,[23–25] for example, for therapeutic vaccines or adoptive T- cell treatment.[13] The results of the current study do not imply that these tumor antigens are not suitable as targets for new anticancer immunotherapies, since we did not investigate T-cell responses. However, based on the results of the current study, the clinical applicability of tumor-associated antibodies directed against tumor antigens as a predictive biomarker for ICI treatment does not seem feasible. This study emphasizes the importance of external validation of predictive biomarker studies, in order to determine their relevance for clinical practice.

Contributors Study concept and design: KdJ, AJ, DK, RD, RHJM, MWJS and AAMVdV. Acquisition, analysis or interpretation of data: KdJ, SV, CK, MWJS and AAMVdV. Statistical analysis: SV, CK and MWJS Drafting of the manuscript: KdJ, SV, CK, MWJS and AAMVdV. Critical revision of manuscript for important intellectual content: all authors.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests KdJ declares travel expenses from Ipsen, outside the submitted work; RD has received research support from MSD and Bayer, personal fees from Bluebird Bio, Genticel, other support from Pan Cancer T outside the submitted work (all paid to the Erasmus MC Cancer Institute), and is listed as inventor for European patent application no's. 21152822.9 and 21184727.2 (pending to Erasmus MC). RHJM declares funding for investigator-initiated research (all paid to institution) from Astellas, Bayer, Boehringer-Ingelheim, Cristal Therapeutics, Pamgene, Pfizer, Novartis, Roche, Sanofi and Servier, all outside the submitted work; AAMVdV reports advisory board (all paid to institution) of BMS, MSD, Merck, Pfizer, Ipsen, Eisai, Pierre Fabre, Roche, Novartis, Sanofi, all outside the submitted work. All other authors declare no competing interests.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study involves human participants and was approved by Medical Ethical Committee at the Erasmus Medical Center.Protocol ID: MEC2016-011. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as online supplemental information.

ORCID iDs
Karlijn de Joode http://orcid.org/0000-0001-5928-2899
Reno Debets http://orcid.org/0000-0002-3649-807X
Ron H J Mathijssen http://orcid.org/0000-0001-5667-5697

REFERENCES

1 Wolchok JD, Chiarion-Sileni V, Gonzalez R, *et al*. Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma. *J Clin Oncol* 2022;40:127–37.
2 Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *N Engl J Med* 2018;378:158–68.
3 Tawbi HA, Schadendorf D, Lipson EJ, *et al*. Relatlimab and nivolumab versus nivolumab in untreated advanced melanoma. *N Engl J Med* 2022;386:24–34.
4 Aung PP, Nagarajan P, Prieto VG. Regression in primary cutaneous melanoma: etiopathogenesis and clinical significance. *Lab Invest* 2017.
5 Tarhini AA, Lee SJ, Tan A-C, *et al*. Improved prognosis and evidence of enhanced immunogenicity in tumor and circulation of high-risk melanoma patients with unknown primary. *J Immunother Cancer* 2022;10:e004310.
6 Robbins PF, Kassim SH, Tran TLN, *et al*. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. *Clin Cancer Res* 2015;21:1019–27.
7 Rohaan MW, Holz Borch T, van denJH, *et al*. Tumor-infiltrating lymphocytes (TIL) therapy versus ipilimumab in advanced melanoma [in press]. *NEJM* 2022.
8 Stockert E, Jäger E, Chen YT, *et al*. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. *J Exp Med* 1998;187:1349–54.
9 Gnjatic S, Nishikawa H, Jungbluth AA, *et al*. Ny-Eso-1: review of an immunogenic tumor antigen. *Adv Cancer Res* 2006;95:1–30.
10 Kortleve D, Coelho RML, Hammerl D, *et al*. Cancer germline antigens and tumor-agnostic CD8+ T cell evasion. *Trends in Immunology* 2022;43:391–403.
11 Slingluff CL, Zarour HM, Tawbi HA-H, *et al*. A phase 1 study of NY-ESO-1 vaccine + anti-CTLA4 antibody ipilimumab (IPI) in patients with unresectable or metastatic melanoma. *Oncoimmunology* 2021;10:1898105.
12 Rohaan MW, Gomez-Eerland R, van den Berg JH, *et al*. Mart-1 TCR gene-modified peripheral blood T cells for the treatment of

metastatic melanoma: a phase I/iia clinical trial. *Immunooncol Technol* 2022;15:100089.
13 MAGE-C2 TCR T cell trial to treat melanoma and head and neck cancer (MC2TCR). n.d. Available: https://clinicaltrials.gov/ct2/show/results/NCT04729543
14 Fässler M, Diem S, Mangana J, *et al*. Antibodies as biomarker candidates for response and survival to checkpoint inhibitors in melanoma patients. *J Immunother Cancer* 2019;7:50.
15 Eisenhauer EA, Therasse P, Bogaerts J, *et al*. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228–47.
16 Zhao Q, Xu WT, Shalieer T. Pilot study on MAGE-C2 as a potential biomarker for triple-negative breast cancer. *Dis Markers* 2016;2016:2325987.
17 Yang F, Zhou X, Miao X, *et al*. MAGEC2, an epithelial-mesenchymal transition inducer, is associated with breast cancer metastasis. *Breast Cancer Res Treat* 2014;145:23–32.
18 Liu Q, Huang X, Li Q, *et al*. Rhophilin-associated tail protein 1 promotes migration and metastasis in triple negative breast cancer via activation of RhoA. *FASEB J* 2020;34:9959–71.
19 Tio D, Kasiem FR, Willemsen M, *et al*. Expression of cancer/testis antigens in cutaneous melanoma: a systematic review. *Melanoma Res* 2019;29:349–57.
20 Poklepovic AS, Carvajal RD. Prognostic value of low tumor burden in patients with melanoma. *Oncology (Williston Park)* 2018;32:e90–6.
21 Gershenwald JE, Scolyer RA, Hess KR, *et al*. Melanoma staging: evidence-based changes in the American joint Committee on cancer eighth edition cancer staging manual. *CA Cancer J Clin* 2017;67:472–92.
22 Salmaninejad A, Zamani MR, Pourvahedi M, *et al*. Cancer/Testis antigens: expression, regulation, tumor invasion, and use in immunotherapy of cancers. *Immunol Invest* 2016;45:619–40.
23 Robbins PF, Morgan RA, Feldman SA, *et al*. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol* 2011;29:917–24.
24 Lu Y-C, Parker LL, Lu T, *et al*. Treatment of patients with metastatic cancer using a major histocompatibility complex class II-restricted T-cell receptor targeting the cancer germline antigen MAGE-A3. *J Clin Oncol* 2017;35:3322–9.
25 Morgan RA, Chinnasamy N, Abate-Daga D, *et al*. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. *J Immunother* 2013;36:133–51.

Appendix 1. Concentrations of the tumor antigens and serum dilutions for the development of the ELISA

antigen	final amount/well (µg)	amounts/well (µg) tested during optimisation	final serum dilution	serum dilutions tested during optimisation
Melan-A	0.25	-	1:10	-
NY-ESO-1	0.0625	-	1:1280	-
MAGE-A6	0.0312	0.5, 0.25, 0.125, 0.0625, 0.0312	1:40	1:5, 1:10, 1:20, 1:40, 1:80
MAGE-C2	0.0312	0.5, 0.25, 0.125, 0.0625, 0.0312	1:40	1:5, 1:10, 1:20, 1:40, 1:80
ROPN1B	0.0625	0.5, 0.25, 0.125, 0.0625, 0.0312	1:20	1:5, 1:10, 1:20, 1:40, 1:80

PROTOCOL:

prospective saMpling in intravenoUsLy treaTed oncolOgy patients: Monoclonal AntiBodies (MULTOMAB)

NL55840.078.15 **MULTOMAB**

PROTOCOL TITLE 'prospective saMpling in intravenoUsLy treaTed oncolOgy patients: Monoclonal AntiBodies'

Protocol ID	**MEC2016-011**
Short title	**MULTOMAB**
EudraCT number	**Not applicable**
Version	**10**
Date	**18-07-2022**
Coordinating investigators/project leader	**Maaike Hofman, MD** **Department of Medical Oncology, Room EE1924/Be-451A** **Erasmus MC Cancer Institute Rotterdam** **Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands** **Tel: +31 618197784** **Email: m.hofman@erasmusmc.nl** **Karlijn de Joode, MD** **Department of Medical Oncology, Room EE1924/Be-451A** **Erasmus MC Cancer Institute Rotterdam** **Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands** **Tel: +31 10 70 38573** **Email: k.dejoode@erasmusmc.nl** **Edwin Basak, MD** **Department of Medical Oncology, Room EE1924/BE-451A** **Erasmus MC Cancer Institute Rotterdam – Josephine Nefkens Institute** **'s Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands** **Tel: +31 10 7037312** **Email: e.basak@erasmusmc.nl** **Stijn L.W. Koolen, PharmD PhD** **Department of Medical Oncology, Room Be-424**

NL55840.078.15 **MULTOMAB**

Erasmus MC Cancer Institute Rotterdam – Josephine Nefkens Institute

's Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands

Tel: +31 10 7041252

Fax: +31 10 7041003

Email: s.koolen@erasmusmc.nl

NL55840.078.15 **MULTOMAB**

Principal investigator(s)	**Erasmus MC:** **Prof. A.H.J. Mathijssen, MD/PhD** **Department of Medical Oncology, Room Be424** **Erasmus MC Cancer Institute Rotterdam** **'s Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands** **Tel: +31 10 7034897** **Fax: +31 10 7041003** **Email: a.mathijssen@erasmusmc.nl** **Amphia Ziekenhuis Breda:** **Dr. C.H. van der Leest, MD/PhD** **Department of Pulmonology** **Amphia Hospital Breda** **Molengracht 21, 4818 CK Breda, the Netherlands** **Tel: +31 76 5955349** **Email: cvanderleest@amphia.nl** **Kantonsspital St. Gallen:** **M. Joerger, MD/PhD** **Department of Medical Oncology & Hematology** **Cantonal Hospital, 9007 St.Gallen, Switzerland** **Tel.: +41 76 559 1070** **Email: markus.joerger@kssg.ch**
Writing commitee	**Prof. J.G.J.V. Aerts, MD/PhD** **Department of Pulmonology, Room V-116A** **Erasmus MC Rotterdam** **'s Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands** **Tel: +31 10 7030678** **Email: j.aerts@erasmusmc.nl** **Dr. J.E.M.A. Debets, PhD** **Department of Medical Oncology, Room Be-430B**

	Erasmus MC Cancer Institute, Rotterdam **'s Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands** **Tel: +31 107038307** **Fax: +31 107044365** **Email: j.debets@erasmusmc.nl** **Dr. Sander Bins, MD/PhD** **Department of Medical Oncology, Room Be451a** **Erasmus MC Cancer Institute Rotterdam – Josephine Nefkens Institute** **'s Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands** **Tel: +31 10 7041112** **Fax: +31 10 7041003** **Email: s.bins@erasmusmc.nl**
Sponsor (in Dutch: verrichter/opdrachtgever)	**Erasmus MC Cancer Institute**
Subsidising party	**Not applicable**
Independent expert (s)	**Not applicable**
Laboratory sites	**Laboratory of Translational Pharmacology, Erasmus MC Cancer Institute, The Netherlands**
Pharmacy	**Not applicable**

NL55840.078.15

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor or legal representative: **Head of Department ad interim:** Dr. M.P.J.K. Lolkema		
Principal investigator: Prof. dr. A.H.J Mathijssen		

NL55840.078.15

TABLE OF CONTENTS

NL55840.078.15

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
ctDNA	**Circulating Tumor DNA**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
GCP	**Good Clinical Practice**
IC	**Informed Consent**
MAB	**Monoclonal Antibody**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
PK	**Pharmacokinetics**
PBMC	**Peripheral blood mononuclear cell**
(S)AE	**(Serious) Adverse Event**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

NL55840.078.15

SUMMARY

Rationale: In oncology, monoclonal antibodies (mABs) have become key players. Still, their pharmacokinetics (PK) are largely unexplained. Since PK might predict for treatment outcome or toxicity, we aim to map the PK of mABs and factors that influence it.

Objective: To set up a bank of prospectively collected blood samples for pharmacokinetic analyses of monoclonal antibodies.

Study design: Prior to, during and after treatment with mABs, blood samples for pharmacokinetic analyses, peripheral blood immune cells (in PBMC), circulating tumor DNA (ctDNA), germline DNA, and factors related to the coagulation cascade will be withdrawn. In addition, feces will be collected before and during treatment with mABs. A geriatric assessment will be performed in a selected group of elderly patients. In a selected group of patients with esophageal cancer, fresh frozen tumor material is obtained from resected tissue.

Optionally, additional blood samples will be withdrawn in the first week after the first treatment cycle for pharmacokinetic analyses.

Study population: Adult patients planned to receive a monoclonal antibody as anti-cancer treatment.

Main study parameters/endpoints: The primary study endpoint is the trough level of different monoclonal antibodies over time. Secondary endpoints include the quantity of antibodies against the therapeutic monoclonal antibody, intrinsic characteristics of peripheral blood immune cells (in PBMC), in particular during aging, the role of ctDNA in immune response, the variations in germline DNA on efficacy and toxicity of immunotherapy, the effect of the gut microbiome on response to therapy, and the activation of the coagulation cascade in response to therapy.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The risk of blood withdrawals is negligible. In subset of patients, tumor material is derived from resected tissue, which has already been obtained during surgery as part of standard care. Therefore, there is no additional risk for these patients.

1. INTRODUCTION AND RATIONALE

Monoclonal antibodies (mABs) are key players in the treatment of cancer. Trastuzumab was one of the first registered targeted monoclonal therapies in oncology and has proven its value over the years in the treatment of HER2 positive breast and stomach cancer. Many others have come over the years and recently, promising anti-cancer activity has been reported from immune modulating antibodies such as pembrolizumab and nivolumab. Both target the mechanism that makes tumor cells inhibit the function of the immune system. This has led to rapid approval of nivolumab treatment for non-small cell lung cancer (NSCLC) and melanoma.[1, 2] However, the value of other mABs like bevacizumab is still under debate and even some nivolumab treated patients do not (really) benefit from this treatment. In addition, all mABs may cause serious toxicity, and right now it is unknown which patients are prone to experience side-effects.

Factors influencing the inter-individual differences in toxicity and benefit from these therapies are largely unknown.[3] For mABs used in the treatment of cancer this lack of knowledge is striking, whereas in rheumatology these factors have been partly unravelled for various antibodies. For example, in some therapies it is known that antibodies which are formed intrinsically and that target the exogenous antibodies, may lead to a rapid breakdown of the drug, to lower plasma concentrations, and may ultimately lower its clinical benefit.[4] For mABs used in the treatment of cancer we aim to map the factors that cause the inter-individual differences and we will focus on pharmacokinetics (PK), immunogenicity and peripheral blood immune cell characteristics prior to and during treatment. We will monitor whether differences in PK lead to different clinical outcome. Previous studies have shown that the immune system changes when patients age. These factors are associated with clinical frailty, which can be identified with geriatric assessments. To identify whether immunosenescence has an effect on the immune system in patients treated with monoclonal antibodies and whether it has an effect on the response to monoclonal antibodies, a geriatric assessment will be performed in the elderly patients (age 65 years and older)[5].

The analysis of circulating tumor DNA (ctDNA) has previously been used to investigate potential prognostic markers in patients who have or are being treated for their primary tumor with curative intent[6]. ctDNA comprises of DNA fragments derived from tumor cells, which enter the blood stream after apoptosis or by active shedding of DNA fragment by living tumor cells. As such, the presence and dynamics of genetic aberrations such as mutations in ctDNA prior to and during adjuvant therapy holds promise as a marker for minimal residual disease and prognosis. To enable tumor-specific monitoring of minimal residual disease, the primary tumor should be analyzed for genetic aberrations. To also study the relation between

ctDNA and ctDNA dynamics and prognosis, primary tumor tissue and repeated plasma samples will be collected for future analysis, including but not limited to RNA- and DNA-sequencing.

Studies have shown that the gut microbial composition could also affect the cancer immunity and therefore play a role in the response to therapy with mABs. To study the effect of the gut microbiome on cancer immunity, feces sampling will be performed prior and during treatment with mABs in patients with melanoma[7].

In addition, previous studies have demonstrated that patients who are treated with mAbs, specifically immune checkpoint inhibitors, have an increased risk to develop both venous and arterial thrombotic events[8,9]. However, the direct effects of this therapy on the hemostatic system and coagulation cascade have not yet been investigated. Therefore, plasma samples will be collected to analyze the direct effects on the hemostatic system, which will also be correlated to PK data to investigate inter-individual differences in the susceptibility for this adverse event.

2. OBJECTIVES

Primary Objective:

- To set up a bank of prospectively collected blood samples from patients treated with monoclonal antibodies.

Secondary Objectives:

- To correlate pharmacokinetic (PK) parameters with effectiveness and toxicity of monoclonal antibodies.
- To determine the influence of immunogenicity on PK.
- To determine the influence of peripheral blood immune cell characteristics on PK and effectiveness and toxicity of monoclonal antibodies.
- Explore the characteristics of exosomes before start of treatment, during treatment, and after disease progression.
- To assess the course of PK and PBMC characteristics shortly after the first treatment cycle, i.e. within 1 week.
- To validate an assay that can determine serum concentrations of monoclonal antibodies.
- Explore the characteristics of ctDNA before start of treatment, during treatment, and after disease progression
- To determine the effect of mABs on markers of the coagulation cascade activation and correlate with PK data.
- To determine characteristics of the gut microbiome of melanoma patients receiving mABs before and during treatment and the effect of the microbiome on treatment response.
- To study the relation between immunosenescence and geriatric parameters (assessment)
- To evaluate the effect of variations in germline DNA on immunotherapy efficacy and toxicity
- To correlate tumor DNA and RNA profiling with ctDNA characteristics and prognosis.

3. STUDY DESIGN

From patients treated with monoclonal antibodies, blood samples will be drawn from the IV catheter that is placed for the infusion of the specific drug or from the IV catheter that is used for standard of care blood withdrawal. Sampling will be performed just prior to start of the infusion of the drug or during standard of care blood withdrawal. For PK analysis this procedure will be performed in advance of every cycle of treatment and until the next treatment line, according to regular blood withdrawals. Time of blood withdrawal and start of

infusion will be registered on a specific form. Optionally, additional blood samples will be withdrawn at three time points for PK analyses: 2 hours, 48 hours and 7 days after the first cycle of treatment.

For PBMC characterization, exosome characterization, ctDNA isolation, and hemostasis measurements, blood samples will be withdrawn just prior to start of the treatment and during treatment.

In case of progression, PBMC characterization, exosome characterization, ctDNA isolation and hemostasis blood samples could be withdrawn optionally. The collection of samples for germline DNA will take place only once during treatment and only for a selected group of patients.

For patients with advanced melanoma, collection of feces samples will take place prior to treatment and on different time-points during treatment with mABs.

In patients with melanoma aged 65 years or older, a short geriatric assessment (10-15 minutes) will be performed by telephone. During follow-up, patients will receive short follow-up consults via telephone after 6 months, 1 year and 2 years.

In patients with esophageal cancer, tumor tissue will be collected as part of standard care. All patients with esophageal cancer will undergo surgical resection before treatment with mABs. From the resected material, biopsies will be taken and stored fresh frozen, e.g. for RNA sequencing purposes.

Table 1 depicts when each sample or assessment will be executed.

Material	**Before treatment start**	**Between cycle 1 and 2**	**During treatment**	**After progression**	**Until next treatment line**
PK	X	X (optional)	X	X	X
PBMCs	X	X (optional)	X	X	X
Exosomes	X (optional)		X (optional)	X (optional)	
ctDNA	X		X	X (optional)	
Germline DNA≠			X (once during immunotherapy treatment)		
Feces¥	X	X	X	X	
Geriatric assessment×	X		X (6 months, 1 year, 2 year)		
Fresh frozen biopsy*	X				
Hemostatic markers	x	x	X (3m, 1y, optional: 6m)		

≠Only applicable for patients treated outside the Erasmus MC and Amphia hospital Breda
¥ only applicable for patients with advanced melanoma.
Version number: 10, date 18-07-2022

NL55840.078.15

× only applicable for patients with melanoma ≥65 years.
* only applicable for patients with esopgahealcancer

de Joode K, *et al. J Immunother Cancer* 2023; 11:e006467. doi: 10.1136/jitc-2022-006467

4. STUDY POPULATION

4.1 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Age ≥18 years
- Able to understand the written information and able to give informed consent
- Planned treatment with (intravenous) monoclonal antibodies for any type of cancer according to standard of care. Monoclonal antibodies include, but are not limited to: cetuximab, nivolumab, ipilimumab, pembrolizumab, bevacizumab, trastuzumab, durvalumab, avelumab, and atezolizumab.

4.2 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Unable to draw blood for study purposes

4.3 Sample size calculation

Since all the analyses in this study have an exploratory character and no null hypothesis could be formulated, it was not possible to calculate a needed sample size. The planned number of included patients is 3000.

Based on the literature and clinical practice, it is not possible to perform a power calculation to calculate the number of study patients required for the analysis of ctDNA, tumor DNA and RNA. After obtaining 10 tissue samples, we will analyze the tumor samples and perform a power calculation based on this data if possible.

5. TREATMENT OF SUBJECTS

Treatment will be performed according to standard of care, i.e. evaluation of treatment effect, toxicity and other clinical parameters will be performed by the treating physician according to local practice. None of the study procedures will influence decision making in the treatment.

5.1 Investigational product/treatment

Not applicable

5.2 Use of co-intervention (if applicable)

Not applicable

5.3 Escape medication (if applicable)

Not applicable

6. INVESTIGATIONAL PRODUCT

Not applicable

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Pharmacokinetics – i.e. trough levels – of monoclonal antibodies.

8.1.2 Secondary study parameters/endpoints

- Quantification of antibodies against the monoclonal antibody
- Immune cell characteristics (PBMC analyses)
- Tumor response and clinical outcome (tumor relapse, death) (ctDNA and germline DNA)
- Hemostatic markers
- Characteristics of the gut microbiome
- Immunosenescence
- Transcriptomic profiling of tumor RNA and DNA

8.2 Randomisation, blinding and treatment allocation

Not applicable

8.3 Study procedures

8.3.1 Registration

All patients must personally sign and date the consent form before any study specific procedure is performed. The registration will be performed by one of the study coordinators or their delegates at the above mentioned contact address. Study IDs will be assigned in chronological order.

8.3.2 Blood withdrawal

To be eligible for this study, patients have to be treated with a monoclonal antibody according to standard of care. An exception is made for patients who undergo resection of primary esophageal cancer, since adjuvant treatment with monoclonal antibodies is based on the presence of vital tumor tissue in the resected material and analysis of this tissue will be incorporated in the study procedures. Hence, these patients will be asked prior to the esophageal resection and therefore might not be treated with monoclonal antibodies. Since these drugs are given intravenously, blood samples for the study can be withdrawn from the i.v. catheter via which the drug will be administered or from the i.v. catheter that is used for standard of care blood draw just prior to infusion. All samples will be drawn before the start of infusion of the drug. In advance of every cycle blood for pharmacokinetic analyses will be withdrawn in a 5 ml serum tube. If treatment is stopped, blood will be withdrawn until the next treatment line from the i.v. catheter that is used for standard of care blood withdrawal. Samples for PBMC characterization will routinely be collected before the first and second cycle in patients treated with mABs that interfere with the immune system. During treatment and until the next treatment line

it is optional to collect samples for PBMC characterization. Optionally, exosomes will be measured before the first cycle of treatment, after progression and during treatment from the i.v. catheter via which the drug will be administered or from the i.v. catheter that is used for standard of care blood withdrawal.

Additional blood samples will be withdrawn between the first and second cycle of treatment. Samples for PK analysis will be withdrawn at 2 and 48 hours, and after 7 days after the first treatment cycle. Samples for PBMC characterization will be withdrawn after 48 hours and 7 days. These patients will be asked to stay in the day care center for 2 hours after their first treatment, and to return to the hospital after 48 hours and 7 days. Blood samples will be obtained via venepuncture. The additional blood sampling in the first week is optional.

8.3.3 Pharmacokinetic sample

A 5 mL serum tube will be drawn and centrifuged at 2,000 G during 10 minutes. Approximately 2 mL serum will be stored at or below -80 °C.

8.3.4 PBMC characterization sample

Three 10 mL tubes of anti-coagulated blood will be drawn and processed according to Appendix A.

8.3.5 Markers of hemostasis

To evaluate the effects of mABs on the hemostatic system, two 6ml citrate tubes will be drawn and processed accordingly at different time points (Table 1).

8.3.6 Clinical Record Forms

Clinical parameters of each patient will be recorded anonymously in the main study file. The file will contain the following sections:

- Baseline: Patient demographics.
- Cancer history: Diagnosis, history of treatment.
- Treatment: Dose, date of every mAB infusion.
- Toxicities: Adverse events of CTCAE grade 3 or higher.
- Outcome: All assessments for evaluation of treatment effect as performed for standard of care.

8.3.7 Exosomes characterization sample

One 10 mL tube of anti-coagulated blood will be drawn and centrifuged at 3,000 rpm for 10 minutes, before 2 hours after sampling. The samples will be stored at -80 °C.

8.3.8 ctDNA characterization sample

Two 10 mL CellSave tubes will be drawn for obtaining ctDNA (circulating tumor DNA) and processed and stored accordingly.

8.3.8. Feces sampling

For patients with advanced melanoma, feces is collected in 3 tubes prior to treatment with anti-PD-1 antibody and in 2 tubes every 3 weeks for a total of 3 courses. After instructions, patients will collect their feces at home in a standardized manner. See appendix B for a detailed description of the stool collection for patients. Patients receiving anti-PD-1 therapy in cycles of 2 weeks will be asked to collect feces at home at the 3-weeks' time point, and store in the freezer until hospital visit. Immediately after the feces is collected it will be stored in a -80℃ freezer. We will also collect fecal material in tubes with 20% glycerol once, prior to treatment, for preservation of live bacteria. During routine visits when feces is collected, patients will be asked about stool frequency and form, and fill in a questionnaire about food and nutritional supplements. If patients are willing to participate in the study, but refuse to collect feces, they can still be included in the study.

8.3.9 Geriatric assessment

Only in patients aged 65 years and older with melanoma, a short geriatric assessment will be performed. This will include the G8-questionnaire, the 6-CIT, the KATZ-ADL, LAWTON-IADL and EQ-5D (appendix C). Altogether, the assessment will take 10-15 minutes. During follow-up, patients will be called by telephone for a short follow-up, using the EQ-5D and KATZ-ADL questionnaires. If one of these tests shows abnormalities, we will refer patients to the geriatrician for a more extensive geriatric assessment. If patients are willing to participate in the study, but refuse to undergo the geriatric assessment, they can still be included in the study.

8.3.10 Germline DNA analysis

To evaluate the effect of variations in germline DNA on immunotherapy efficacy and toxicity, blood for DNA isolation will be withdrawn for a selected number of patients once during immunotherapy treatment. Four EDTA tubes of 10ml will be withdrawn and stored and processed accordingly.

8.3.11 Transcriptomic tumor profiling

Patients with esophageal cancer will undergo a tumor resection in accordance with standard of care. Biopsies from the resected material will be taken by a pathologist, without manipulating the assessment of the radicality of resection for standard of care. The biopsies will be fresh frozen according to local standard procedures and stored for further analysis. Primarily, this transcriptomic analysis will be performed to facilitate ctDNA characterization as described in paragraph 8.3.7. Additionally, tumor DNA and RNA may be used to look for prognostic and predictive markers.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal (if applicable)

Not applicable

8.5 Replacement of individual subjects after withdrawal

Not applicable

8.6 Follow-up of subjects withdrawn from treatment

Not applicable

8.7 Premature termination of the study

As patients are treated according to standard-of-care and the results of the test do not influence clinical decision-making, no premature termination of the study is foreseen.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the blood draw. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that is a consequence of the blood withdrawal for this study and that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a

period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable

9.3 Annual safety report

Not applicable

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable

10. STATISTICAL ANALYSIS

10.1 Primary study parameter(s)

The trough concentrations of monoclonal antibodies will be described over time.

10.2 Secondary study parameter(s)

- The presence and quantity of antibodies against the monoclonal antibodies will be described over time.
- The quantity of antibodies will be correlated to the trough concentrations.
- Immune cell characteristics will be correlated to the trough concentrations.
- The relation of primary and secondary study parameters to clinical outcome, i.e. response and toxicity.
- Explore characteristics of exosomes before the first cycle of treatment, during treatment, and after progression.
- Optionally, the course of pharmacokinetics and PBMC characteristics will be assessed within 1 week after the first treatment cycle.
- To evaluate the effects of mAbs on hemostatic markers.
- Explore characteristics of ctDNA before the first cycle of treatment, during treatment, and after progression.
- The characteristics of the gut microbiome of patients receiving mABs before and during treatment and the effect of the microbiome on treatment response.
- The relation between geriatric parameters and immunosenescence in patients receiving mABs
- To evaluate the effect of variations in germline DNA on immunotherapy efficacy and toxicity
- To correlate tumor RNA and DNA profiling with ctDNA characteristics.
- To correlate tumor RNA and DNA with prognosis

Due to the heterogeneous characteristics of the study population, all parameters will be analysed under ceteris paribus conditions. For the primary endpoint, trough concentrations will be described per drug. Analyses for the secondary study parameters will also be stratified for drugs and – if deemed relevant – for tumor or patient characteristics. For example, response data will be analysed per drug, per tumor type and per treatment setting, e.g. first-line or second-line therapy.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The investigator will ensure that this study is conducted to the standards of Good Clinical Practice, in full conformance with the “Declaration of Helsinki” (latest amendment http://www.wma.net/en/30publications/10policies/b3/), the Dutch laws and regulations with the W.M.O. (“Wet Medisch-wetenschappelijk Onderzoek met mensen”) in particular.

11.2 Recruitment and consent

Patients will be asked to participate in this study by their treating physician when they meet our inclusion and exclusion criteria. If the patient is interested in participation, he/she will be informed about the study and will be given pertinent information as to the intended purpose of the study. The procedures and possible hazards, to which the patient will be exposed, will be explained. The patient will also receive information in writing (informed consent form). If the only study related interventions are blood draws from an i.v. catheter that was already necessary for standard care, patients are allowed to sign the informed consent form immediately, following careful reading. However, if a subject needs to consider their informed consent, this will be allowed as well. Prior to the blood draw, feces collection, geriatric assessment and obtaining the tissue biopsies for this study the informed consent statement must be signed by the patient and the person who has conducted the informed consent procedure. The patient will be provided with a second original of the signed informed consent statement. The patient may withdraw from the study at any time without having to give any reasons and without prejudicing future medical treatment. A selected group of patients will receive a separate informed consent form, in which the nature and burden of the additional sampling is explained.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable

11.4 Benefits and risks assessment, group relatedness

All blood samples for study purposes will be withdrawn from the i.v.) catheter that is placed for the infusion of the monoclonal antibody. The samples will be taken in advance of the infusion. Since the i.v. catheter has to be placed for standard of care, the extra risk of the study procedures is negligible. The risk of collecting feces samples is considered negligible.

When informed consent is given for the additional sampling, three extra venepunctures will be performed for the blood sampling, which is accompanied by a negligible additional risk of complications.

Tissue biopsies are derived from resected tissue at the laboratory, after the surgical

procedure have been performed conform of standard care. Hence, there is a no additional risk of complications.

11.5 Compensation for injury

The METC Erasmus MC has given exemption for the *WMO-proefpersonenverzekering*. The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

11.6 Incentives (if applicable)

Not applicable

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Each patient will be given a unique patient study number. All data and human materials collected from patients will be stored for at least 15 years. Any rest materials will be destroyed at the end of the study, unless the subject has given consent on the ICF to store rest materials for a maximum of 15 years.

12.2 Monitoring and Quality Assurance

This is a protocol with negligible risk and the study will be monitored accordingly. The monitoringplan and explanation of negligible risk can be found in section K6.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.4 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.5 Public disclosure and publication policy

We may submit trial results for publication in a peer reviewed scientific journal.

NL55840.078.15

13. STRUCTURED RISK ANALYSIS

Not applicable

14. REFERENCES

1. Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med. 2015 Jul 9;373(2):123-35.
2. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med. 2015 Jul 2;373(1):23-34.
3. Keizer RJ, Huitema AD, Schellens JH, Beijnen JH. Clinical pharmacokinetics of therapeutic monoclonal antibodies. Clin Pharmacokinet. 2010 Aug;49(8):493-507
4. Zandvliet ML, van Bezooijen JS, Bos MA, Prens EP, van Doorn M, Bijen I, et al. Monitoring antigen-specific biologics: current knowledge and future prospects. Ther Drug Monit. 2013 Oct;35(5):588-94.
5. Wildiers H, Heeren P, Puts M, et al. International Society of Geriatric Oncology consensus on geriatric assessment in older patients with cancer. J Clin Oncol. 2014 Aug; 32(24):2595-603
6. Schraa SJ, van Rooijen KL, van der Kruijssen DEW, Rubio Alarcón C, et al. PLCRC-MEDOCC group. Circulating tumor DNA guided adjuvant chemotherapy in stage II colon cancer (MEDOCC-CrEATE): study protocol for a trial within a cohort study. BMC Cancer. 2020 Aug 20;20(1):790.
7. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018; 359(6371): 97-103.
8. Moik F, Chan WE, Wiedemann S, et al. Incidence, risk factors, and outcomes of venous and arterial thromboembolism in immune checkpoint inhibitor therapy. Blood 2021; 137(12): 1669-78.
9. Gong J, Drobni ZD, Alvi RM, et al. Immune checkpoint inhibitors for cancer and venous thromboembolic events. Eur J Cancer 2021; 158: 99-110.

NL55840.078.15

Appendix A: Procedure summary for PBMC characterization

Isolation of mononuclear cells and plasma from clinical blood samples by density gradient separation using Leucosep™ tubes

Materials

- Leucosep™ tubes, 50 ml, sterile (nr 277 290; Greiner Bio One, GmbH); Alternative: pre-filled with Leucosep™ (nr 227288)
- Tubes, 50 ml, sterile (Greiner)
- Cryotubes, 1.8mL (Greiner)
- Lymphoprep ™ (Nycomed, Asker, Norway), or Ficoll™ (GE Healthcare) Density 1.007+/- 0.0001 g/mL, use at 18ºC-25ºC);
- Phosphate-buffered saline (PBS) ***without Ca and Mg!***;
- Fetal bovine serum (FBS), or Human serum Albumin (HSA, 20%)

Preparation

Loading Leucosep™ tube (nr 277290) with separation medium (Lymphoprep/Ficol)

(In case of pre-filled tube – skip this step)

- Warm-up separation medium to room temperature (RT) protected from light.
- Fill the 50 mL LeucosepTM tube with 15 ml separation medium.
- Close the tubes with the screw-cap and centrifugate for 30 seconds at 1000 x g and RT. The separation medium is now located below the porous barrier.
- The tubes are now ready for loading with anti-coagulated blood.

PBS diluting / washing buffer

- PBS 1%FBS: 500ml PBS, add 5 ml FBS,
- Or PBS + 1% HSA: 500 ml PBS, add 15 ml HAS 20%

Procedure

Step 1: Isolation of plasma

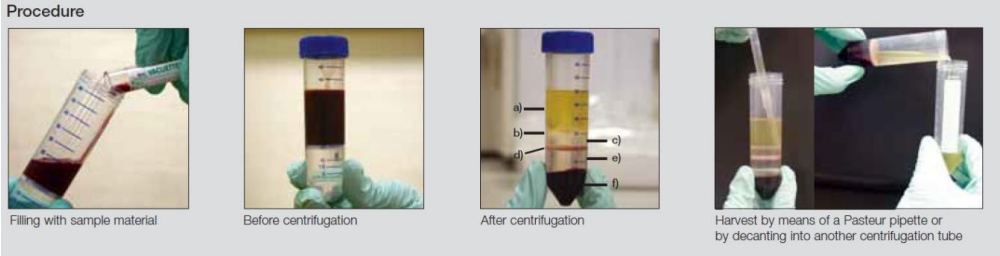
1) Pour the 15–30 ml anti-coagulated blood sample carefully into the 50 mL centrifuge tube
2) Centrifugate 10 minutes at 1000 x g and RT in a swinging bucket rotor.
3) After centrifugation the sequence of layers occurs as follows (seen from top to bottom): a) Plasma; b) white cells or buffy layer and c) red blood cells
4) Collect plasma, take care not to disturb the white and red cell layers, and prepare x5 1ml aliquots and store at -80°C.
5) Resuspend the remaining blood and dilute with PBS+1%BSA (or 1%HSA) to 50 mL

Step 2: Isolation of PBMC

1. Pipet 25 mL of diluted blood sample into two 50 mL Lymphoprep/Ficoll loaded Leucosep™ tubes.
2. Centrifugate 10 minutes at 1000 x g and RT in a swinging bucket rotor; brakes-off.

de Joode K, *et al. J Immunother Cancer* 2023; 11:e006467. doi: 10.1136/jitc-2022-006467

3. After centrifugation the sequence of layers occurs as follows (seen from top to bottom): a) Plasma; b) enriched cell fraction (interphase consisting of lymphocytes / PBMCs) ; c) separation medium; d) porous barrier; e) separation medium; f) pellet (erythrocytes and



granulocytes).

4. Collect and discard of the diluted plasma layer up to a minimum remnant of 5 to 10 mm above the interphase. This helps to prevent contamination of the enriched cells with platelets.
5. Harvest the enriched cell fraction (lymphocytes / PBMCs) by means of pouring the supernatant above the porous barrier from the LeucosepTM tube into another centrifugation tube; pool supernatant from both tubes in one 50 mL tube. The porous barrier effectively avoids recontamination with pelleted erythrocytes and granulocytes.
6. Wash the enriched cell fraction (lymphocytes / PBMCs) with PBS +1% FBS (or 1% HSA), add a volume up to 50 mL, subsequently centrifugate for 10 minutes at 250 x g.
7. Repeat washing step twice, resuspend the cell pellet with 5 ml of PBS +1% FBS (or 1% HSA).
8. Count viable cells, e.g., using trypan blue exclusion.

Step 3: Cryopreservation of PBMC

1. After isolation, washing and counting of PBMC centrigugate again or 10 minutes at 250 x g, aspirate supernatant, resuspend cell pellet and put the tube on melting ice.
2. Add serum; volume 0.5 ml per ampoule (see table below for recommended number of ampoules)
3. Slowly add the same volume of freezing medium (RPMI1640 + 20% DMSO) while gently resuspending.
4. Transfer 1.0 mL cell suspension to each ampoule (cryotube).
5. Transfer ampoules to the precooled (at 4°C) freezing container (Mr Frosty) and place freezing container overnight in a -70°C freezer.
6. Transfer frozen ampoules to gas liquid nitrogen (gas phase) prior to transport to centralized laboratory.

Yield PBMC	**Number of ampoules**	
0 - 7 x10^6	**1**	
8 - 11 x10^6	**2**	**4 - 6,5 x10^6 PBMC per ampoule**
12 - 24 x10^6	**3**	**4 – 8,0 x10^6**
25 - 32 x10^6	**4**	**6 – 8,0 x10^6**
> 33 x10^6	**5**	**> 6,5 x10^6**

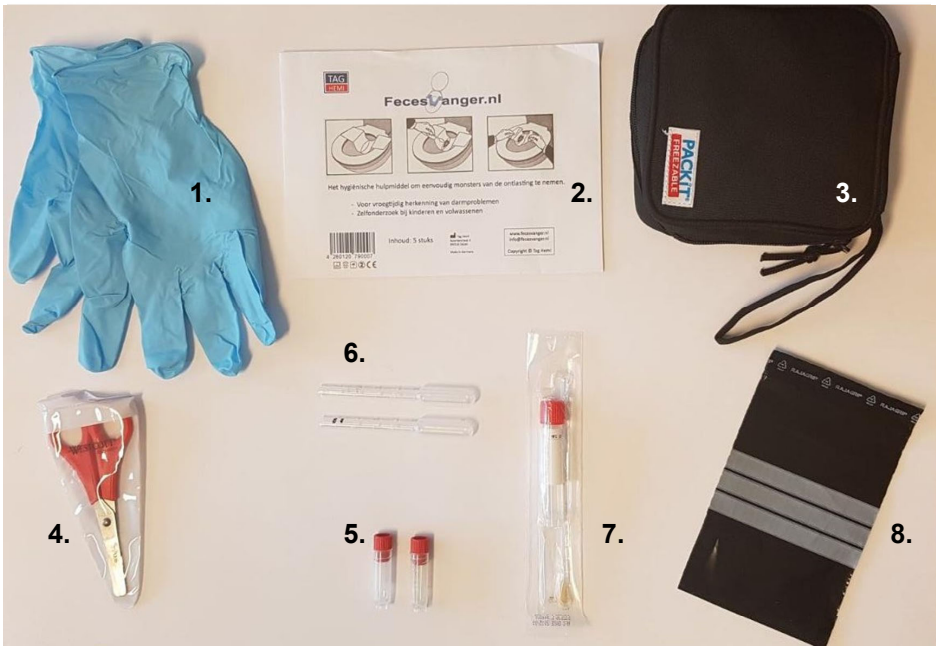
Appendix B: Patients instructions for collecting feces

Instructie voor het verzamelen van ontlasting

In deze instructie leest u welke materialen u van het ziekenhuis ontvangt voor het verzamelen van ontlasting en beschrijven we stap-voor-stap hoe u de ontlasting verzamelt. Wij raden u aan eerst de instructie helemaal door te lezen.
Heeft u vragen over het verzamelen van ontlasting? Neemt u dan gerust contact met ons op.

Welke materialen ontvangt u van het ziekenhuis?

Om ontlasting te verzamelen ontvangt u de volgende materialen (afb. 1):
1. Handschoenen
2. Ontlastingopvangers
3. Koeltasje
4. Schaartje
5. Kleine buisjes (rode dop)
6. Pipetjes
7. Grote buis met wattenstaafje (rode dop)
8. Zwarte zakjes



Afb. 1 – Materialen voor verzamelen

Hoe werkt het verzamelen van ontlasting?

Het opvangen en verzamelen van de ontlasting verloopt in zeven stappen.

Stap 1: Opvangen ontlasting
Stap 2: Verzamelen ontlasting met pipet
Stap 3: Overbrengen ontlasting naar buisje
Stap 4: Verzamelen ontlasting met wattenstaafje
Stap 5: Buisjes verzamelen in zwarte zakje
Stap 6: Bewaar het zwarte zakje met de buisjes in de vriezer

In dit document worden de stappen stuk voor stuk nader toegelicht.

Stap 1: Opvangen ontlasting

Om ontlasting op te vangen, gebruikt u de meegeleverde ontlastingopvanger (afb. 2 hieronder).

Afb. 2 – Ontlastingopvanger

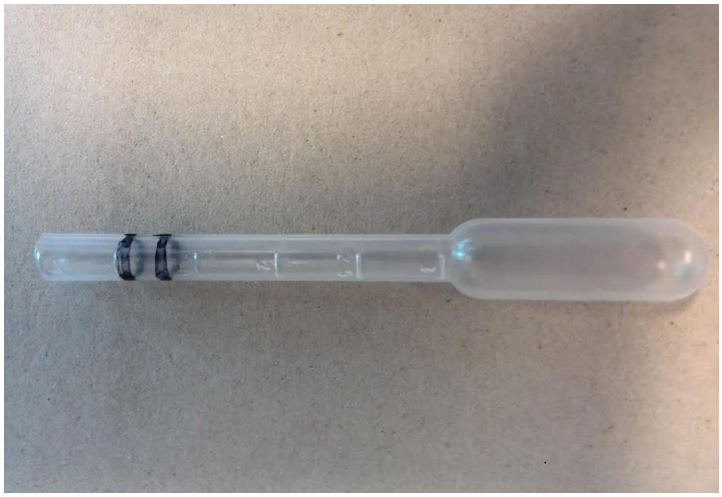
Vouw voorzichtig de ontlastingopvanger open in de richting van de pijltjes.
Bevestig deze met de plakstroken op het achterste deel van de wc-bril, zonder dat de plakstrip met het water in contact komt.
U kunt nu de ontlasting opvangen.

Als u klaar bent gaat u door naar stap 2 op de volgende pagina

Stap 2: Verzamelen ontlasting met pipet

Nu u de ontlasting heeft opgevangen, kunt u met de pipet (afb. 3 hieronder) de ontlasting verzamelen.
Knijp het ballonnetje bovenaan de pipet voorzichtig in en houd het uiteinde van de pipet in de ontlasting.
Druk het pipetje tot ongeveer het eerste zwarte streepje in de ontlasting en laat het ballonnetje voorzichtig los. Door het loslaten van het ballonnetje kan de ontlasting een beetje omhoog gezogen worden, dat is niet erg.
Veeg de buitenkant van de pipet af met wc-papier.

Vergeet niet de handschoenen te gebruiken.



Afb. 3 – Pipet met zwarte streepjes

U zult deze stap nog een keer uitvoeren. In totaal gebruikt u per keer ontlasting verzamelen twee pipetjes. De resterende pipetjes kunt u bewaren voor de volgende keer.

Als u klaar bent gaat u door naar stap 3 op de volgende pagina

Stap 3: Overbrengen ontlasting van pipet naar buisje

Breng nu het uiteinde van de pipet over in het kleine buisje met de rode schroefdop (afb. 4 hieronder). Probeer hierbij geen contact te maken met het schroefdraad. Knip het stukje pipetje dat in het buisje zit af, bij het tweede zwarte streepje (afb. 5 hieronder).
Het stukje pipet dat u over hebt, kunt u weggooien in de prullenbak.

Herhaal stap 2 en 3 nog een keer, zodat u in totaal in 2 kleine buisjes met een pipet ontlasting heeft verzameld.

De resterende buisjes en pipetjes kunt u bewaren voor de volgende keer.

Afb. 4 – Buisje met rode schroefdop

Afb. 5 – Knip een stukje van de pipet af

! U voert stap 2 en 3 twee keer uit !

Als u klaar bent gaat u door naar stap 4 op de volgende pagina

<u>Stap 4: Verzamelen ontlasting met wattenstaafje</u> (Alleen voorafgaand aan het starten met immunotherapie)

Voor deze stap gebruikt u het meegeleverde wattenstaafje én de grote buis. Het werkt als volgt: Open de verpakking van de buis en draai de dop eraf.
Let op: er zit vloeistof in het buisje, dus houd het buisje rechtop!
Haal het wattenstaafje uit de verpakking en zorg dat het wattenstaafje steriel (schoon) blijft en niet met ander materiaal in aanraking komt.
Draai het wattenstaafje eenmaal door de ontlasting op de plek waar u in stap 2 met de pipet ontlasting heeft verzameld.
Doe het wattenstaafje met de ontlasting in de buis. Probeert u daarbij zo min mogelijk de buis te raken.
Breek het wattenstaafje af bij het rode streepje (zie afb. 6 hieronder). Draai vervolgens de dop er weer op.
Schud de buis twee keer om.

Afb – 6 Afbreken bij het rode streepje

Het verzamelen van ontlasting is nu klaar en de ontlastingopvanger kan nu doorgespoeld worden. Wacht voor het doorspoelen een ogenblik, zodat het papier wat water opneemt en makkelijker door te spoelen is. Het grijze bakje kan in de afvalbak of container.

Let op: Er hoeft maar eenmaal een potje met een wattenstaafje te worden verzameld. Dit hoeft alleen de eerste keer, vooraf aan start met immunotherapie.

Als u klaar bent gaat u door naar stap 5 op de volgende pagina

Stap 5: Buisjes verzamelen in het zwarte zakje

Doe alle buisjes in het zwarte zakje.

De eerste keer zijn dit drie buisjes: 1 groot buisje met een rode dop en een wattenstaafje, en 2 kleine buisjes met een rode dop. Doe de buis met het wattenstaafje bij voorkeur rechtop in het zwarte zakje.

De andere keren zijn dit twee buisjes: 2 kleine buisjes met een rode dop.

Afb. 7 – De drie buisjes in het zwarte zakje

Als u klaar bent gaat u door naar stap 6 op de volgende pagina

Stap 6: Bewaar het zwarte zakje met de buisjes in de vriezer

Doe het zwarte zakje direct in de koeltas (afb. 8) en plaats deze in uw vriezer. Plaats het bij voorkeur zo in de vriezer dat de grote buisjes rechtop staan.

Afb. 8 – Doe het zwarte zakje in de koeltas

Stap 7 was de laatste stap, u bent klaar.

Bewaar het koeltasje in de vriezer totdat u het mee kunt nemen naar uw volgende polibezoek.

Vergeet niet om tijdens het bezoek het koeltasje direct af te geven.

Hartelijk dank voor uw medewerking.

Appendix C Geriatric assessment

TCIMM Geriatrisch assessment ≥ 65 jaar

Doel:

Onderscheid maken tussen kwetsbare en niet kwetsbare ouderen

Betere ondersteuning en follow up kwetsbare ouderen.

Relateren van kwetsbaarheidskenmerken aan immunologische veroudering

Door onderzoek betere kennis krijgen over wie er kwetsbaar wordt tijdens de behandeling.

Instructies:

Plak op dit setje een patiënt sticker of noteer naam en geboortedatum.

Vul bij iedere patiënt ≥ 65 jaar de aanhangende vragenlijsten in.

Bij afwijkingen op 1 van deze vragenlijsten wordt de patiënt verwezen voor een uitgebreider consult bij de ouderengeneeskunde. Vertel dit aan de patiënt.

Vertel dat de patiënt over 6 maanden en 1 jaar wordt gebeld om de kwaliteit van leven kort te bespreken

Laat dit pakketje zo spoedig mogelijk inscannen in Hix

NL55840.078.15 **MULTOMAB**

G8 vragenlijst Patiëntnummer:

Omcirkel het gegeven antwoord.

Items	Scores
Is de intake de afgelopen 3 maanden verminderd? Bijvoorbeeld door een vermindering in eetlust, maag/darm- bezwaren of problemen met slikken?	0 = ernstig verminderde intake 1= matig verminderde intake 2 = geen verandering in intake
Gewichtsafname gedurende de 3 afgelopen maanden	0 = gewichtsverlies > 3 kg 1 = ik weet het niet 2 = gewichtsverlies tussen 1-3 kg 3 = geen gewichtsverlies
Mobiliteit	0 = gebonden aan bed of stoel 1 = zelfstandig uit bed/stoel, maar niet naar buiten 2 = komt buiten
Bestaan er neuropsychologische problemen?	0 = ernstige dementie of depressie 1 = milde dementie 2 = Geen neuropsychologische problemen
BMI (gewicht in kg) / (lengte in m2)	0 = BMI <19 1 = BMI tussen 19 en 21 2 = BMI tussen 21 en 23 3 = BMI ≥ 23
Neemt de patiënt meer dan drie voorgeschreven medicijnen?	0 = Ja 1 = Nee
Vindt de patiënt dat hij gezonder is, of minder gezond, dan de meeste mensen van zijn leeftijd?	0 = minder 0,5 = Geen idee 1 = even goed 2 = beter
Leeftijd	0 = > 85 1 = 80-85 2 = < 80

Totaalscore 0-17. Een score van ≤ 14 wordt als afwijkend beschouwd.

ADL (Algemene Dagelijkse Levensverrichtingen)

Omcirkel het gegeven antwoord.

Wassen:
0 Geheel zelfstandig
1 Allen hulp bij wassen rug, één lichaamsdeel of voeten noodzakelijk
2 Onzelfstandig

Aankleden:
0 Geheel zelfstandig
1 Alleen hulp bij aantrekken van de schoenen
2 Onzelfstandig (blijft anders –gedeeltelijk- ongekleed)

Toiletgebruik:
0 Geheel zelfstandig
1 Alleen hulp nodig bij het gaan naar het toilet of toilethandelingen
2 Onzelfstandig, gaat niet naar het toilet

Transfers
0 Komt zelfstandig in/uit bed/stoel
1 Heeft hulp nodig bij in/uit bed/stoel komen
2 Komt niet uit bed

Continentie
0 Continent voor urine en ontlasting
1 Zo nu en dan kleine “ongelukjes”
2 Supervisie nodig, gebruikt catheter, of is volledig incontinent

Voeden
0 Eet zelfstandig
1 Alleen hulp nodig bij voorsnijden en smeren van brood
2 Onzelfstandig (moet geholpen worden, heeft voedingssonde of infuus)

Score totaal:	

Toelichting:

Een score van 2 of hoger is een afwijkende score.

IADL (Instrumentele Algemene Dagelijkse Levensverrichtingen)

Telefoneren
0 Telefoneert zelfstandig, zoekt nummers op, enzovoorts
1 Draait een aantal vertrouwde nummers zelf
2 Beantwoordt telefoon: niet in staat nummers te draaien of op te zoeken
3 Maakt geen gebruik van de telefoon

Inkopen doen
0 Doet zelfstandig alle inkopen
1 Doet enkele kleine inkopen zelfstandig
2 Moet begeleid worden bij het doen van alle inkopen
3 Niet in staat inkopen te doen

Maaltijd bereiden
0 Zelfstandig in het plannen, bereiden en opdienen van maaltijden
1 Heeft hulp nodig bij het bereiden van maaltijden
2 Verwarmd door anderen bereide maaltijden
3 Eten moet kant en klaar worden voorgezet

Huishoudelijke activiteiten of klusjes
0 Verricht zelfstandig lichte huishoudelijke activiteiten
1 Heeft hulp nodig bij alle huishoudelijke arbeid
2 Verricht geen enkel huishoudelijk werk
3 Nog nooit gedaan, altijd door anderen

Wassen van kleding en linnengoed
0 Doet zelfstandig de was
1 Doet kleine wasjes zelf
2 De was moet door anderen gedaan worden
3 Nog nooit gedaan, altijd door anderen

Openbaar vervoer en transport
0 Reist zelfstandig met openbaar- of eigen vervoer
1 Moet vergezeld worden bij reizen met openbaar vervoer; bestelt wel zelf een taxi
2 Reist uitsluitend onder begeleiding met taxi of auto; niet in staat met openbaar vervoer te reizen
3 Reist helemaal niet

Medicatiegebruik
0 Neemt zelfstandig medicijnen op juiste tijdstip en in juiste dosis
1 Neemt zelfstandig medicijnen in, indien tevoren klaargezet in juiste hoeveelheid
2 Niet in staat met medicijnen om te gaan
3 Nooit gedaan, omdat dit altijd voor hem/haar gedaan werd

Beheer van financiën
0 Regelt alle financiële zaken zelfstandig
1 Rekent boodschappen zelfstandig af; hulp nodig bij bank/giro zaken
2 Niet in staat met geld om te gaan
3 Nooit gedaan, omdat dit altijd voor hem/haar gedaan werd

Score:	

Voor deze vragenlijst bestaat geen afkapwaarde. De lijst wordt gebruikt om veranderingen in IADL te vervolgen.

Patient Health Questionnaire-2 (PHQ-2)

Hoe vaak hebt u *in de afgelopen 2 weken* last gehad van een of meer van de volgende problemen?

1. Weinig interesse of plezier in activiteiten
   - □ Helemaal niet (0)
   - □ Verscheidene dagen (1)
   - □ Meer dan de helft van de dagen (2)
   - □ Bijna elke dag (3)

2. Zich neerslachtig depressief of hopeloos voelen
   - □ Helemaal niet (0)
   - □ Verscheidene dagen (1)
   - □ Meer dan de helft van de dagen (2)
   - □ Bijna elke dag (3)

Totaal score:

Toelichting: een score van ≥ 3 is afwijkend.

Recente val

Bent u in de afgelopen 6 maanden gevallen?
- □ Ja
- □ Nee

EQ-5D

De EQ-5D is een vragenlijst die betrekking heeft op de kwaliteit van leven.

Zet bij iedere groep in de lijst hieronder een kruisje in het hokje achter de zin die het best past bij uw eigen gezondheidstoestand **vandaag**.**Error! Bookmark not defined.**

Mobiliteit
Ik heb geen problemen met lopen □1
Ik heb enige problemen met lopen □2
Ik ben bedlegerig □3

Zelfzorg
Ik heb geen problemen om mijzelf te wassen of aan te kleden □1
Ik heb enige problemen om mijzelf te wassen of aan te kleden □2
Ik ben niet in staat mijzelf te wassen of aan te kleden □3

Dagelijkse activiteiten
(bijv. werk, huishouden, gezins- en vrijetijdsactiviteiten)
Ik heb geen problemen met mijn dagelijkse activiteiten □1
Ik heb enige problemen met mijn dagelijkse activiteiten □2
Ik ben niet in staat mijn dagelijkse activiteiten uit te voeren □3

Pijn/klachten
Ik heb geen pijn of andere klachten □1
Ik heb matige pijn of andere klachten □2
Ik heb zeer ernstige pijn of andere klachten □3

Stemming
Ik ben niet angstig of somber □1
Ik ben matig angstig of somber □2
Ik ben erg angstig of somber □3

Gebruikershandleiding EQ-5D:
Een gezondheidstoestand op de EQ-5D kan worden uitgedrukt in een combinatie van 5 getallen. Bijvoorbeeld, toestand ‘21123’ staat voor enige problemen met lopen, geen problemen met zichzelf wassen en aankleden, geen problemen bij het uitvoeren van dagelijkse activiteiten, matige pijn of andere klachten en erg angstig of somberheid.
De uitslag graag zoals bovenstaand noteren.

Score: _ _ _ _ _

Six Item Cognitive Impairment Test (6-item CIT)

Item	Score		
1. In welk jaar zijn we nu?	Goed: 0	Fout: 4	
2. In welke maand zijn we nu?	Goed: 0	Fout: 3	

Herhaal dit adres: “Jan de Vries, Molenstraat 12, Groningen”(5 onderdelen)

Zometeen vraag ik u het adres nogmaals te herhalen.

3. Hoe laat is het nu?	Goed: 0	Fout: 3	
4. Tel terug van 20 naar 1	Goed: 0	Fout: 2	>1 fout: 4
5. Zeg de maanden in omgekeerde volgorde op	Goed: 0	Fout: 2	>1 fout: 4
6. Herhaal het adres	Goed: 0 3 fout: 6	1 Fout: 2 4 fout: 8	2 fout:4 5 fout: 10

Omcirkel de juiste score bij ieder item (alles goed: score 0; alles fout: score 28) en tel deze 6 items bij elkaar op.

Score:	

Totaalscore:
0-10: wijst op geen tot lichte verstoring van het cognitief functioneren.
≥ 11-28: wijst op matige tot ernstige verstoring van het cognitief functioneren.
LET OP: een score ≥8 wordt als afwijkend beschouwd.

Toelichting:
De 6-item CIT is een korte vragenlijst bestaande uit 6 items, welke niet beïnvloed wordt door opleidingsniveau van de proefpersoon. Afname duurt slechts 2-3 minuten. Deze Nederlandse versie is gebaseerd op een Nederlandse versie van de vragenlijst die gevalideerd is ten opzichte van de Mini-Mental State Examination (MMSE). Een optimale cutt-off score voor cognitieve problemen is een totale score van ≥ 11 (in overeenstemming met MMSE ≤ 23).