

STUDY PROTOCOL

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Study protocol of a phase II study to evaluate safety and efficacy of neo-adjuvant pembrolizumab and radiotherapy in localized rectal cancer

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Abstract

Background: Reshaping the tumor microenvironment by novel immunotherapies represents a key strategy to improve cancer treatment. Nevertheless, responsiveness to these treatments is often correlated with the extent of T cell infiltration at the tumor site. Remarkably, microsatellite stable rectal cancer is characterized by poor T cell infiltration and, therefore, does not respond to immune checkpoint blockade. To date, the only available curative option for these patients relies on extensive surgery. With the aim to broaden the application of promising immunotherapies, it is necessary to develop alternative approaches to promote T cell infiltration into the tumor microenvironment of these tumors. In this regard, recent evidence shows that radiotherapy has profound immunostimulatory effects, hinting at the possibility of combining it with immunotherapy. The combination of long-course chemoradiotherapy and immune checkpoint inhibition was recently shown to be safe and yielded promising results in rectal cancer, however short-course radiotherapy and immune checkpoint inhibition have never been tested in these tumors.

Methods: Our clinical trial investigates the clinical and biological impact of combining pembrolizumab with short-course radiotherapy in the neo-adjuvant treatment of localized rectal cancer. This phase II non-randomized study will recruit 25 patients who will receive short-course preoperative radiotherapy (5 Gy × 5 days) and four injections of pembrolizumab starting on the same day and on weeks 4, 7 and 10. Radical surgery will be performed three weeks after the last pembrolizumab injection. Our clinical trial includes an extensive translational research program involving the transcriptomic and proteomic analysis of tumor and blood samples throughout the course of the treatment.

Discussion: Our study is the first clinical trial to combine short-course radiotherapy and immune checkpoint inhibition in rectal cancer, which could potentially result in a major breakthrough in the treatment of this cancer.

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Additionally, the translational research program will offer insights into immunological changes within the tumor and blood and their correlation with patient outcome. Taken together, our work will help optimizing future treatment combinations and, possibly, better selecting patients.

Trial registration: This study was registered with www.clinicaltrials.gov: NCT04109755. Registration date: June, 2020.

Keywords: Rectal cancer, Radiotherapy, Pembrolizumab, T cell infiltration

Background

Rectal cancer (RC) is one of the most frequently occurring cancers and leading cause of cancer death worldwide [1–3]. Apart from surgery, which has a massive impact on the quality of life of RC patients, management of RC largely relies on conventional methods such as chemotherapy (CT) and radiotherapy (RT). For tumors localized close to the anal margin or node positive tumors, which represent 45 to 60% of the newly diagnosed patients, European and American oncology guidelines recommend either five weeks of neoadjuvant chemo-RT (CRT) followed by an 8- to 12-week treatment-free interval before radical surgery, or a short-course preoperative RT (SCPRT) with surgery taking place either 1–2 or 8–10 weeks after RT [4, 5]. These two modalities of treatment are currently judged equivalent [6, 7].

The importance of an intact immune surveillance function in controlling tumor outgrowth has been known for decades [8]. In recent years, the field of cancer immunotherapy has seen considerable progress due to the discovery of new therapies targeting immune checkpoint molecules such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death 1 (PD1) and programmed cell death ligand 1 (PD-L1) [9]. Clinical trials investigating immune checkpoint inhibitors (ICIs) in advanced tumor settings have shown positive results in several cancers and these treatments have been integrated into standard of care [10, 11]. Although ICIs can provide deep and durable responses in some cancers, clinical benefit is usually limited to a subset of patients. In gastrointestinal cancers, impressive results have been obtained with the anti-PD1 antibody pembrolizumab in heavily pre-treated patients with advanced disease carrying mismatch repair deficient genes (dMMR) [12]. Tumors with dMMR are typically associated with microsatellite instability (MSI) and display a higher response rate to ICIs compared to microsatellite stable (MSS) tumors. Recently, Overman and colleagues published results of an anti-PD1 immunotherapy in second line treatment in MSI-H metastatic colorectal cancer (CRC) [13]. These early results showed 50% progression free survival (PFS) and 60% overall survival (OS) at two years, outperforming results obtained with second line chemotherapies [13]. Unfortunately, MSI-H tumors represent

only 1 to 3% of RCs, and, consequently, the majority of RC patients will not respond to ICIs.

Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) and favorable prognosis in various malignancies [14]. In particular, the presence of CD8⁺ T cells and the ratio of CD8⁺ T cells/FoxP3⁺ regulatory T cells (Tregs) correlate with improved prognosis and long-term survival in solid malignancies [15]. As T cell infiltration is typically dictated by the presence of tumor-specific antigens (neoantigens), it is not surprising that MSI-H tumors display a greater number of TILs as compared to MSS cancers [16]. In line with these observations, high “immunoscores” were found predictive of response to ICIs [17, 18].

In order to broaden the application of ICIs to MSS RC, novel therapeutic approaches aiming at reconditioning the tumor microenvironment (TME) by either promoting TIL activation or infiltration are required. To this end, RT is a promising option, as recent evidence suggests that ionizing radiation can induce important immunomodulatory effects in the TME allowing trafficking of T cells into the tumor [19]. These studies speak in the favor of combining RT and ICIs. Lately, a bulk of work investigating the effect of radio-immunotherapy in pre-clinical tumor models of various solid cancers, including CRC, highlighted the potential benefit of this approach [20–22]. Following these results, a few clinical trials are evaluating the combination of RT and ICIs in RC (NCT02948348, NCT04124601, NCT04262687, NCT04558684), but always in combination with CT and in advanced clinical settings. Encouraging safety and toxicity profiles from these studies indicate that radio-immunotherapy combinations could represent a valid opportunity for RC patients. For instance, in the VOLTAGE trial (NCT02948348) where patients received CRT followed by the anti-PD1 antibody nivolumab, only mild toxicity was reported [23]. Moreover, 30% of patients with locally advanced MSS RC reached pathological complete response (pCR). Remarkably, our clinical trial represents the first study where the impact of combining the anti-PD1 antibody pembrolizumab with short-course RT in the neo-adjuvant treatment of localized RC will be evaluated (NCT04109755). Alongside this clinical trial, a translational research project will provide a deep understanding of the dynamic changes in the immune and

tumor cell states that are associated with responses to treatment and patient outcome. Ultimately, these results will have profound implications for the treatment of RC patients and the design of future clinical trial protocols for other non-inflamed tumors.

Method and design

Hypothesis

We hypothesize that combining pembrolizumab with SCPRT can increase the histological response compared to the standard of care (RT alone) due to the increased immune infiltrate in the tumor (specifically CD8⁺ T cell and FOXP3⁺ T cell) in subjects with localized MSS and MSI RC.

Objectives

The primary objective of this study is the assessment of the *pathological Complete Response (pCR)* after radio-immunotherapy. In addition to pCR rate, tumor regression grade (TRG) will be assessed as part of the diagnostic procedure for the evaluation of the response to treatment. Secondary objectives include *Tolerability and Safety*, *OS*, *DFS*, *Locoregional Relapse-Free Survival (LRRFS)*, *Distant Metastasis-Free Survival (DMFS)*, *Quality of Life*, *Post-Operative Complication* and *Quality of Surgery*. Lastly, this clinical trial comprises an exploratory objective that overlaps with a translational research program aiming at providing new insights into the tumor and immune cell states that are associated with response to treatment and identifying novel prognostic and predictive biomarkers for clinical decision making.

Study endpoints: the clinical trial will assess the following parameters

1. *pCR* by a complete histological assessment in conjunction with TRG using the Mandard regression grade score. These parameters will be evaluated at the completion of the radio-immunotherapy treatment after the surgical procedure;
2. *Tolerability and Safety* using the common terminology criteria for adverse events (CTCAE) Version 4.0 and Dindo Clavien classification of surgical complication. These parameters will be evaluated at the completion of the radio-immunotherapy treatment (i.e. proportion completing the planned neoadjuvant treatment and proportion proceeding to surgery);
3. *OS*, defined as the time from study entry until death due to any cause. Subjects who have not died at the time of last known follow-up will be censored;

4. *DFS*, defined as the time from study entry until recurrence, second primary cancer, or death without evidence of recurrence or second primary cancer;
5. *LRRFS*, defined as the time from surgery until local or regional recurrence. LRRFS is evaluated in patients who had an R0 resection only;
6. *DMFS*, defined as the time from surgery to metastatic recurrence;
7. *Quality of Life* using EORTC QLQ-C30 questionnaire and EORTC QLQ-CR29;
8. *Post-Operative Complication* according to the Dindo-Clavien score;
9. *Quality of Surgery* according to Nagtegaal's recommendations;
10. *Tumor Immune* and *Peripheral Immune Responses* in relation to the clinical outcomes.
 - a) Comparison of the tumor immune microenvironment before (treatment-naïve) and after neo-adjuvant radio-immunotherapy using RNA sequencing and flow cytometry.
 - b) Quantitative and qualitative assessment of the immune responses in the periphery by analysis of peripheral blood (peripheral blood mononuclear cells (PBMC), circulating tumor DNA (ctDNA), serum and plasma).
 - c) Correlation of these findings with clinicopathological parameters and clinical outcomes.

Study design

This trial is a phase II, single arm study, including 25 patients with localized RC. Following diagnosis and workout, patients will receive SCPRT (5 Gy × 5 days) and four injections of pembrolizumab, on weeks 1, 4, 7 and 10. This phase of treatment will last 12 weeks, then surgery will be performed. Immune response will be monitored in the blood (specifically in PBMC) before, during radio-immunotherapy and after surgery and within the tumor before and after treatment. An outline of the study design is depicted in Fig. 1.

Study population

Inclusion criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

1. Male/female participants must be at least 18 years old, have adequate health conditions and be willing to comply with the study protocol.

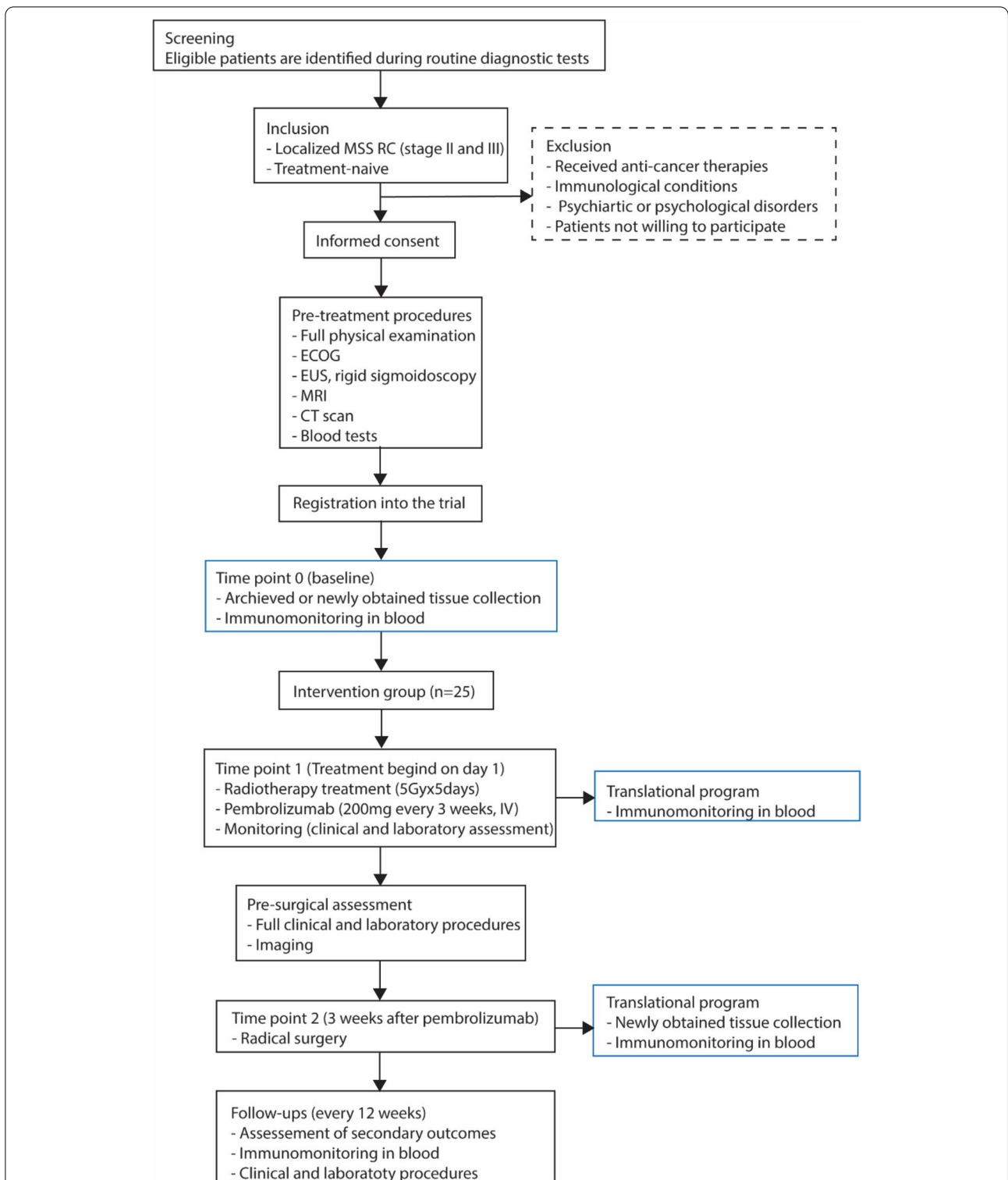


Fig.1 Flowchart of the study. RC: rectal cancer, MSS: microsatellite stable, ECOG: Eastern Co-operative Oncology Group, EUS: endoscopy ultrasound, MRI: magnetic resonance imaging, CT: computed tomography

2. Participants must have been diagnosed with localized RC and not being treated for this disease before the start of the clinical trial:
 - a. cT3a/b very low, levators clear, mesorectal fascia clear or cT3a/b in mid- or high rectum, cT1,2,3 and cN1-2 (not extranodal), no extramural vascular invasion;
 - b. cT3c/d or very low localisation levators threatened, mesorectal fascia clear;
 - c. cT3c/d mid-rectum, cT1,2,3 and cN1-N2 (extranodal), extramural vascular invasion present, limited cT4aNO.
3. A multi-disciplinary tumor board should recommend neo-adjuvant SCPRT and surgery as the optimal treatment option for these patients.

Exclusion criteria

Participants are excluded from the study if any of the following criteria apply:

1. Participants who have received anti-cancer therapy, live vaccine or have participated in other investigational trials in close proximity with the start of the clinical trial. If treated with RT, received major surgery or participated in another study, the participant has to be fully recovered.
2. Participant suffering from active autoimmune diseases, infections, immunodeficiency, other malignancies or health conditions that could confound the results of the study and/or interfere with the subject's participation for the full duration of the study.

Study interventions: the clinical trial will include the following therapeutic interventions

Biopsy

A treatment-naïve biopsy will be collected within a week before the start of the trial.

Pembrolizumab

Four injections of pembrolizumab will be administered intravenously (IV) at a fixed dose of 200mg, over a period of 9 weeks, starting on the first day of the SCPRT and repeated every three weeks. There is no maintenance treatment.

External beam radiotherapy

SCPRT will be administered using volumetric-modulated arc therapy (VMAT) to a prescription dose of 25 Gy in 5 fractions of 5 Gy to the planning target volume (PTV) over 5 days (Monday to Friday). RT starts on day 1 of treatment (always on a Monday).

Surgery

Surgical procedure will take place 12 weeks after the end of the RT, meaning three weeks after the last (fourth) injection of pembrolizumab.

Blood sampling

The collection of peripheral blood is scheduled throughout the clinical trial as described in Fig. 2.

An overview of the trial intervention is presented in Table 1.

Statistical analysis

The primary outcome of this study is pCR. The pCR rate, defined as the absence of residual invasive disease in the rectum and in the lymph nodes at the completion of the neoadjuvant treatment [24], reaches 12–16% in patients receiving treated with SCPRT with delayed surgery or CRT, respectively [5]. Remarkably, induction CT before CRT and surgery increase pCR to 30%-35% [25]. Our hypothesis is that the our study intervention results in a pCR rate that is superior to that of SCPRT. The null hypothesis assumes a 10% pCR rate, and the alternative a 30% pCR rate, which at the usual alpha (0.05) and beta (0.2) cutoffs, require a population of 25 patients, using an exact binomial sample size calculation. The null hypothesis will be rejected if at least 6 patients out of 25 meet the primary endpoint.

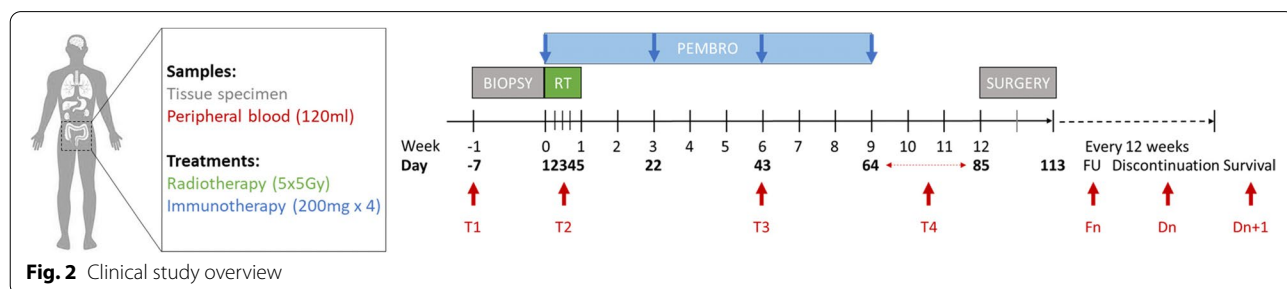


Fig. 2 Clinical study overview

Table 1 Trial interventions

Trial period	Screening Phase		Intervention Phase					Surgery Phase		Follow-up		Discontinuation							
	Pre-screening	Main Study Screening	1	2	3	4	5	6	7	8	9	10	11	12	Prior to surgery	Surgery	Follow-up	Baseline	Follow-up
Visits			1	2	3	4	5	6	7	8	9	10	11	12					
Scheduling Window (Days):	-28 to -7	-7 to 0			1	2	3	4	5	22	43	64	65-85 (+28 days)	85 (+28 days)				At time of event	Every 12 weeks
Radiotherapy						R1	R2	R3	R4										
Pembrolizumab						C1				C2	C3	C4							
Tissue collection		Biopsy																	
Immunomonitoring in blood		T1					T2				T3	T4			Surgical specimen		Fn	Dn	Dn+1

Discussion

Currently, the standard of care for localized RC consists of neo-adjuvant treatments such as long-course CRT or SCPRT. Both treatments have been shown to increase local control and pCR compared to surgery alone. Contrary to dMRR/MSI-H tumors, novel cancer immunotherapies have shown little clinical impact on MSS CRC. Therefore, the Food and Drug Administration (FDA) granted approval for the use of such ICIs exclusively in the first- and second-line treatment of unresectable or metastatic dMMR/MSI-H CRCs. Remarkably, on top of the direct cytotoxic effects on tumor cells, RT is able to reprogram the TME to exert a potent anti-tumor immune response, thus providing a window of opportunity for immune-modulation. Recently, the first combined approach associating CRT with immunotherapy (Nivolumab) in the VOLTAGE trial [NCT02948348] was shown to be safe, and yielded promising results (30% pCR) in the treatment of MSS RC, but similar combinations (such as SCPRT with ICIs) have never been tested on RC.

This project investigates a novel combination treatment strategy for the treatment of localized RC. Specifically, we will study the clinical and biological impact of combining immunotherapy (pembrolizumab) with SCPRT in the neo-adjuvant treatment of localized MSS and MSI RC. This phase II non-randomized clinical trial is currently open and will recruit 25 patients that will be treated with SCPRT (5 Gy × 5 consecutive days) and four injections of pembrolizumab followed by delayed surgery (NCT04109755). The study primary outcome is pCR evaluated in conjunction with TRG, which is assessed by the Mandard regression grade score. Secondary outcomes include tolerability, safety, survival responses and immune and cellular dynamics in the tumor and in the peripheral blood. We postulate that RT will induce immunogenic cell death followed by inflammation, neoantigen release, reprogramming of the TME, resulting in elicitation of adaptive antitumor immune responses. These immune responses, mediated by tumor specific T cells, will be further amplified by the addition of the immune checkpoint inhibitor pembrolizumab. Upon combination treatment, we anticipate an increase of CD3⁺, CD8⁺ and FoxP3⁺ T cells in the TME, a dynamic change of the immune cell populations in the peripheral blood and an increased tumor regression as compared to historical controls due to the increased immune infiltrate within the tumor. Our clinical trial will generate important clinical data on the safety and efficacy of the combination and an invaluable insight into immunological changes linked to this combined modality.

Abbreviations

CRT: Chemoradiotherapy; CT: Chemotherapy; ctDNA: Circulating tumor DNA; CT: Computed tomography; CTLA-4: Cytotoxic T lymphocyte-associated protein 4; dMMR: Deficient mismatch repair mechanisms; DMFS: Distant metastasis-free survival; ECOG: Eastern co-operative oncology group; EUS: Endoscopy ultrasound; ICI: Immune checkpoint inhibitor; IV: Intravenously; LRRFS: Locoregional relapse-free survival; MRI: Magnetic resonance imaging; MSI: Microsatellite instability; MSS: Microsatellite stable; OS: Overall survival; pCR: Pathological Complete Response; PBMC: Peripheral blood mononuclear cell; PTV: Planning target volume; PD1: Programmed cell death 1; PD-L1: Programmed cell death ligand 1; PFS: Progression free survival; RT: Radiotherapy; RC: Rectal Cancer; Tregs: Regulatory T cells; SCPRT: Short-course preoperative radiotherapy; TME: Tumor microenvironment; TIL: Tumor-infiltrating lymphocyte; TRG: Tumor regression grade; VMAT: Volumetric-modulated arc therapy.

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Authors' contributions

Principal investigator: TK. Drafting of the Protocol Manuscript: CC. Intellectual Content: CC, NCB, MT, ADF, PB, AJF, GP, TM, ZT, Ct, MDM, VD, PYD, FR, and TK. Study supervision: CC, CT, TK. Revision and Final Approval of the Article: CC, NCB, MT, ADF, PB, AJF, GP, TM, ZT, Ct, MDM, VD, PYD, FR, and TK. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This clinical study (registration number: NCT04109755) has received ethical approval from the Regional Research and Ethics Committee (CCER) and Swissethics (Protocol number: 2018–02346). A written informed consent is obtained from all participants upon participation.

Consent for publication

Not applicable.

Competing interests

TK discloses consulting or advisory role for MSD, BMS, Lilly, Roche, Boehringer Ingelheim and Servier.

TZ declares the following conflict of interests: Honoraria/Travel costs (institutional) — Janssen, Amgen, Ferring, Debiopharm, Bayer, Astellas; Research Grants (institutional) — Varian Medical Systems, Debiopharm; Advisory Boards (institutional) — Janssen.

All other authors declare that they have no competing interests.

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