PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The MUKnine OPTIMUM protocol: A screening study to identify high risk multiple myeloma patients suitable for novel treatment approaches combined with a phase II study evaluating optimised combination of biological therapy in newly diagnosed high risk multiple myeloma and plasma cell leukaemia
AUTHORS	Brown, Sarah; Sherratt, Debbie; Hinsley, Samantha; Flanagan, Louise; Roberts, Sadie; Walker, Katrina; Hall, Andrew; Pratt, Guy; Messiou, Christina; Jenner, Matthew; Kaiser, Martin

VERSION 1 – REVIEW

REVIEWER	ROUSSEL Murielle IUC ONCOPOLE TOULOUSE FRANCE
REVIEW RETURNED	22-Nov-2020

GENERAL COMMENTS	how will you evaluate the role of cyclophosphamide within the induction regimen? by comparison to the griffin trial?

REVIEWER	Roberto Mina University of Turin, Italy
REVIEW RETURNED	02-Dec-2020

GENERAL COMMENTS	The authors reported the study design of a clinical trial investigating an intensive treatment strategy for HR multiple myeloma patients. Given the dismal outcome of HR patients with MM, there is great interest in the development of new therapeutic approaches to such patients to improve their outcomes. Therefore, clinical trials focusing primarily on HR patients are of great importance and the auhtors' effort very much appreciated. 1) What is the biological/clinical rationale for the addition of cyclophosphamide in the induction phase in patients already receiving a 4-drug regimen? The evolution trial did not support this choice. Could you please comment on that? 2) Available data suggest that tandem autologous SCT improves PFS/OS of HR patients; Can you please explain why you did choose not to pursue this path? 3) in the introduction the authors state that ASCT is a common salvage approach for RRMM; I don't quite get reference since we are discussing about NDMM patients. 4) do the authors have any clinical data to justify the use of early bortezomib after ASCT? 5) in the introduction the authors state that lenalidomide maintenance is also effective in HR patients; this is surely true

	based on the data of the English group, but the pooled analysis published by McCarthy did not confirm this; this should be also reported in the introduction. 6) is there any age cut-off to define transplant eligibility? 7) I understand the rationale of allowing a couple induction cycles while molecular tests are run; what about though primary refractory patients not responding/progressing during the standard of care induction cycles? will they be excluded from the trial or kept in and will they receive DVRCd? the selection of responding patients may represent a patient selection bias.

REVIEWER	Antonio Giovanni Solimando Bari University, Italy
REVIEW RETURNED	14-Dec-2020

GENERAL COMMENTS	This is a good protocol and balanced assessment considering the status of diagnosing the genetic changes early and identify more effective first-line treatment options for high-risk MM patients. The article highlights important data that might have been overlooked when promulgating the clinical value of high-risk MM and the related trials. An attempt to describe MM and the tumor niche genomic landscape in a patient was performed by Walker et al. with a pragmatic approach: they tried identifying the potential targetable mutations. As described, more than 40 genetic lesions were druggable, but only three of them have already been targeted in clinical practice. In more details, new technologies for multidimensional measurement (for instance combination of single-cell RNA sequencing, genomic, immunophenotyping) of immune cells and proteins might help to build an “immunogram” to evaluate immune status and cancer-immune interactions in individual patients and thereby predict capacity to respond to immunotherapeutic strategies. Therefore, it is reasonable to design tailored clinical trials aimed to stratify patients differentially according to disease risk. Remarkable efforts have been attempted in order to translate these unmet clinical needs to bedside-approaches. As recently reported, TP53 mutational status and 1q amplification evaluation harbor a significant prognostic impact that can be overcome by a more aggressive therapeutic approach. In this frame of thinking, new investigational treatments that incorporate genomic-directed stratification in both NDMM and RRMM and techniques, such as single-cell RNA-seq analysis and mass cytometry (CyTOF), hold great promise in incorporating a comprehensive immune-microenvironment characterization into the individualized trial design and randomization. Can the author comment on this, and expand the discussion? The underlying message here is that more precision and individualized approaches need to be tested also in well-designed clinical trials taking into account the immune-signature – a challenge, but I would be interested in their perspective of how this might be done.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: ROUSSEL Murielle
Institution and Country: IUC ONCOPOLE, TOULOUSE, FRANCE
Please state any competing interests or state 'None declared': None Declared

Comments to the Author
How will you evaluate the role of cyclophosphamide within the induction regimen? by comparison to the griffin trial?
We thank the reviewer for the comment. The trial will formally compare outcomes with genetically matched ultra high-risk patients treated with intensive therapy on NCRI Myeloma XI (NCT01554852), all of whom received cyclophosphamide in combination therapies with carfilzomib/revlimid/dexamethasone (KCRD), revlimid/dexamethasone (CRD) or thalidomide dexamethasone (CTD) during induction therapy. This analysis is outlined in supplementary file 1 which has now been uploaded.
At the time of trial design GRIFFIN results had not been reported. Since the GRIFFIN trial has now reported on genetic high risk subgroups, albeit not ultra high-risk, in abstract form, an exploratory comparison for a matched ultra high-risk group may become a possibility in the future when more comprehensive data is fully published. So far comprehensive genetics have not been analysed in GRIFFIN, specifically no information on double-hit tumours or gene expression high risk tumours has been released.

Reviewer: 2
Reviewer Name: Roberto Mina
Institution and Country: University of Turin, Italy
Please state any competing interests or state 'None declared': None

Comments to the Author
The authors reported the study design of a clinical trial investigating an intensive treatment strategy for HR multiple myeloma patients. Given the dismal outcome of HR patients with MM, there is great interest in the development of new therapeutic approaches to such patients to improve their outcomes.
Therefore, clinical trials focusing primarily on HR patients are of great importance and the auhtors' effort very much appreciated.
We thank the reviewer for the comment.

1) What is the biological/clinical rationale for the addition of cyclophosphamide in the induction phase in patients already receiving a 4-drug regimen? The evolution trial did not support this choice. Could you please comment on that?
Addition of cyclophosphamide was considered specifically to address the challenges of treating high-risk disease. At the time of trial design (late 2016), GRIFFIN results and in particular results on high-risk patients were not yet available. Results of the EVOLUTION trial available at time of trial design did not provide specific information on high risk genetic subgroups. As EVOLUTION did not report particular adverse effects, we opted to include cyclophosphamide to ensure the maximum treatment intensity for high-risk and ultra-high risk patients accessible at the time.
MUKnine OPTIMUM will formally compare outcomes in a 'digital comparator' approach with matched high-risk and ultra high-risk patients treated in NCRI Myeloma XI (NCT01554852). All patients received cyclophosphamide in combination therapies such as carfilzomib/revlimid/dexamethasone (KCRD), revlimid/dexamethasone (CRD) or thalidomide dexamethasone (CTD) during induction

therapy.
In addition, recent evidence suggests potential synergy between daratumumab and cyclophosphamide based on enhanced immune activation (https://doi.org/10.1182/bloodadvances.2019000010). Although published after design of the trial, this data provides further support for inclusion of cyclophosphamide in the daratumumab combination regimen used in MUKnine OPTIMUM.
We plan to discuss the rationale as well as up to date literature for cyclophosphamide when the results of the trial are reported. As this is a protocol paper, we understand the format is restricted to portraying the design of the study.

2) Available data suggest that tandem autologous SCT improves PFS/OS of HR patients; Can you please explain why you did choose not to pursue this path?
At the time of trial design (late 2016), evidence on tandem autologous SCT for high-risk and ultra high-risk MM was equivocal. In particular its value in the context of modern combination therapy, consolidation and maintenance was unknown. The STaMINA trial did not report benefit of tandem transplant, including for the high-risk population at ASH 2016. Results from EMN02/HO95 data on tandem transplant were not yet available at the time. We will discuss up to date published evidence once results for MUKnine OPTIMUM are reported, but feel it may not be adequate to discuss further in this protocol paper, which focuses on design of the study.

3) in the introduction the authors state that ASCT is a common salvage approach for RRMM; I don't quite get reference since we are discussing about NDMM patients.
We agree with the reviewer. This is a clerical error, we have corrected this section to replace ‘salvage’ with ‘induction consolidation’ therapy.

4) do the authors have any clinical data to justify the use of early bortezomib after ASCT?
Peri-melphalan bortezomib was based on the 2010 IFM phase 2 trial results and the design of the IFM 2014-02 trial, although results of the latter were not available at the time of the design of the trial (late 2016).
Early post-ASCT bortezomib had, to our knowledge, not been reported before. In analogy to tandem autologous SCT, where second transplant is frequently performed already after 2 months (e.g. in EMN02/HO95), early velcade was included for enhanced tumour control during hematopoietic recovery, and to avoid the high-risk malignant clone outpacing haematopoietic reconstitution in high-risk and ultra-high risk disease. Safety will be reported as part of Secondary Trial Objectives and is listed as an endpoint.

5) in the introduction the authors state that lenalidomide maintenance is also effective in HR patients; this is surely true based on the data of the English group, but the pooled analysis published by McCarthy did not confirm this; this should be also reported in the introduction.
The lenalidomide maintenance meta-analysis by McCarthy et al was published in JCO in July 2017, after design of MUKnine OPTIMUM was completed. The analysis reported a PFS benefit for the high-risk group, but no OS benefit. The authors specifically highlighted missing data for genetic risk markers for a larger proportion of patients and expressed caution regarding interpretation of sub-group analyses for OS. We feel that positive data on PFS from this analysis rather supports the MUKnine OPTIMUM design. We will discuss up to date published evidence once results for MUKnine OPTIMUM are reported.

6) is there any age cut-off to define transplant eligibility?
There was no numeric age cut-off for transplant but clear wording in the inclusion/exclusion criteria to only include patients that were fit for intensive therapy. This approach is in line with recruitment criteria for Myeloma XI, which safely recruited >4,400 patients across the UK, as well as standard practice in the UK for which no age restriction is imposed on accessibility of transplant for patients

with myeloma. A sentence has been added to clarify this.
Recent results from the Myeloma XI trial (https://doi.org/10.3324/haematol.2020.262360) and other studies such as GMMG-HD5 (https://doi.org/10.1038/s41375-020-0724-1) published after start of MUKnine OPTIMUM demonstrated that patients over 65 selected based on investigator assessed fitness assessment are safely treated and benefit from high dose melphalan and ASCT, supporting this approach.

7) I understand the rationale of allowing a couple induction cycles while molecular tests are run; what about though primary refractory patients not responding/progressing during the standard of care induction cycles? will they be excluded from the trial or kept in and will they receive DVRCd? the selection of responding patients may represent a patient selection bias.
The trial was conducted under two separate protocols, the screening protocol and the treatment protocol. Primary endpoint for the screening protocol was to investigate whether central risk status could be determined within 8 weeks, equivalent to 2 cycles of standard induction therapy. The most effective standard of care induction therapy available for high-risk myeloma in the UK at time of recruitment, VTD as per IFM2013-04, was explicitly recommended as per protocol. The maximum cut-off of 8 weeks was determined to include even technically challenging cases for which repeat testing would be required. Turnaround time for the majority of patients was anticipated to be shorter, equivalent to 1 cycle of VTD or less. Any patients experiencing issues during induction will be reported when full results of the screening protocol are published.
Any patients showing signs of progression during the maximum 2 cycles standard of care induction were allowed to be included in the treatment protocol, minimising potential selection bias. However, as most patients were anticipated to only require 1 cycle of VTD whilst central results were generated, the proportion of patients was anticipated to be low to very low.

Reviewer: 3
Reviewer Name: Antonio Giovanni Solimando
Institution and Country: Bari University, Italy
Please state any competing interests or state ‘None declared’: None declared

Comments to the Author
This is a good protocol and balanced assessment considering the status of diagnosing the genetic changes early and identify more effective first-line treatment options for high-risk MM patients. The article highlights important data that might have been overlooked when promulgating the clinical value of high-risk MM and the related trials.

We thank the reviewer for the comment.

An attempt to describe MM and the tumor niche genomic landscape in a patient was performed by Walker et al. with a pragmatic approach: they tried identifying the potential targetable mutations. As described, more than 40 genetic lesions were druggable, but only three of them have already been targeted in clinical practice.
In more details, new technologies for multidimensional measurement (for instance combination of single-cell RNA sequencing, genomic, immunophenotyping) of immune cells and proteins might help to build an “immunogram” to evaluate immune status and cancer-immune interactions in individual patients and thereby predict capacity to respond to immunotherapeutic strategies.

Therefore, it is reasonable to design tailored clinical trials aimed to stratify patients differentially according to disease risk. Remarkable efforts have been attempted in order to translate these unmet clinical needs to bedside-approaches. As recently reported, TP53 mutational status and 1q amplification evaluation harbor a significant prognostic impact that can be overcome by a more aggressive therapeutic approach. In this frame of thinking, new investigational treatments that

incorporate genomic-directed stratification in both NDMM and RRMM and techniques, such as single-cell RNA-seq analysis and mass cytometry (CyTOF), hold great promise in incorporating a comprehensive immune-microenvironment characterization into the individualized trial design and randomization. Can the author comment on this, and expand the discussion?

The underlying message here is that more precision and individualized approaches need to be tested also in well-designed clinical trials taking into account the immune-signature – a challenge, but I would be interested in their perspective of how this might be done.

We thank the reviewer for the comments and agree that a thorough translational research program including profiling of the tumour and its microenvironment are highly relevant to understand more about patients who benefit from treatment, in particular in a trial like MUKnine OPTIMUM. We would like to reassure the reviewer that a range of exploratory translational research projects are currently underway to deepen the insights generated by the trial. However, as the current manuscript is a protocol paper and inherently has to focus on design, primary and secondary endpoints of the study, space is very limited to detail exploratory research aims. These will be detailed in future publications, when results from the trial are available.

VERSION 2 – REVIEW

REVIEWER	Roberto Mina University of Torino
REVIEW RETURNED	23-Jan-2021

GENERAL COMMENTS	The authors nicely addressed all the issues raised.

REVIEWER	Antonio Giovanni Solimando Guido Baccelli Unit of Internal Medicine, Department of Biomedical Sciences and Human Oncology, School of Medicine, Aldo Moro University of Bari, Bari, Italy
REVIEW RETURNED	08-Feb-2021

GENERAL COMMENTS	The authors have clarified several of the questions I raised in my previous review. Most of the major problems have been addressed by this revision.