Author’s response to reviews

Title: AMLVaran: A software approach to implement variant analysis of targeted NGS sequencing data in an oncological care setting

Authors:
Christian Wünsch (c.wuensch@uni-muenster.de)
Henrik Banck (banckhenrik@gmail.com)
Carsten Müller-Tidow (direktor.med5@med.uni-heidelberg.de)
Martin Dugas (dugas@uni-muenster.de)

Version: 1 Date: 13 Dec 2019

Author’s response to reviews:

Dear Sir,

thank you for considering our manuscript for publication, and thank you for your comments and your valuable input to our work.

We hereby resubmit our revised manuscript entitled "AMLVaran: A software approach to implement variant analysis of targeted NGS sequencing data in an oncological care setting" (with change of one word in the title, to clarify the scope of our tool, as recommended by Dr. Glaab).

We provide point-by-point responses regarding the reviewer’s comments below.

We addressed all reviewer comments in the revised version of our manuscript. Therefore, we hope, that the revised manuscript will now be acceptable for publication in BMC Medical Genomics.

With kind regards,
Christian Wünsch

Reviewer 1 (Enrico Glaab)

Major comments:

1.) We thank the reviewer for pointing out the missing GitHub URL.
   The source code of the project was kept private during the course of AMLVaran's development due to concerns regarding plagiarism. Therefore, access to the Git repository had been restricted to the demo account that was mentioned under "Availability of data and materials" in the manuscript.

   Changes to the manuscript:
   The Git repository has been switched to "public" now, and both, manuscript and Quick Start Guide,
were updated with the public link to the source code repository. The source code is now publicly available at https://github.com/cwuensch/AMLVaran.

2.) Thanks to the reviewer for bringing the importance of documentation regarding setup and best practices to our attention.
As AMLVaran was intended to be operated by clinical personnel, the process of uploading a sample for processing has been designed to be as easy-to-use as possible. Apart from the chosen target assay, there are not many configuration options that a standard user has to specify.
The selection and configuration of the variant calling tools and filter settings are intended to be done by the system's administrator (with bioinformatics background), not by the end-users.
Given, that the software is initialized with well-tried default settings of a reliable and established pipeline that have been carefully validated, we believe, that the necessity for changes during a standard use case will be minimal.
In order to account for more complicated analyses from advanced users, the documentation was improved to put more emphasis on customization options.

Changes to the manuscript:
The software installation process was simplified by providing some Docker scripts, as well as an installation script, which performs the complete process of installation and configuration with recommended default settings.
The Quick Start Guide was updated and chapters 7 and 9 were added, which provide less experienced users with a detailed and easy-to-use guideline towards software installation and with recommended best practice settings, as well as advice for advanced customization.

3.) We thank the reviewer for this promising suggestion.
A non-binary classification is indeed highly likely to give a better impression of the sureness of a variant’s rating.
Actually, the appeti8 filtering scheme already generates non-binary scores for a variant being an artifact resp. a polymorphism. These scores have been added to AMLVaran’s output, and we gave an outline, how a probabilistic measure could be derived from these scores.

Changes to the manuscript:
We provided some additional statistics and added a detailed discussion of this aspect to the manuscript’s supplement (paragraph 4).

4.) We appreciate the reviewer's question about applying the software on non-cancer applications.
AMLVaran has been designed with a focus on a specific clinical diagnostic workflow, where single tumor probes of cancer patients are analyzed with a standardized procedure, and lists of somatic variants are created that provide comprehensive annotation and rule-based diagnostic recommendations for known therapy-relevant variants.
Basic research, e.g. statistical studies to investigate new mutations in a larger cohort of patients, and family-based germline trio studies would require substantial modifications of AMLVaran, and are therefore not in the main focus of this tool.
Apart from these limitations, basically any disease involving known therapy-relevant mutations can be examined with AMLVaran. The software was tested on a set of germline samples from a local project regarding male infertility. The results were promising, but validation is still pending and may be published at a later point of time.
However, the variant calling and filtering algorithms in the current software version are primarily optimized for highly sensitive tumor variant calling, at the cost of slower performance. For the
detection of germline variants, a single variant calling tool might be sufficient and faster, so the appreciable cancer pipeline seems to be not the most efficient choice for germline sequencing. Thus, we recommend the current release of AMLVaran only for somatic variant calling.

Changes to the manuscript:
Title, abstract and objectives of the manuscript were updated to emphasize the focus of AMLVaran regarding somatic mutations from tumor data. Additionally, a paragraph was added to the Limitations section to clarify the scope of this tool.

Minor comments:
1.) Thank you for the note on missing images. This effect is due to the LaTeX template of BMC Medical Genomics. It explicitly disallows the placement of images within the text and requires them to be included separately.

Changes to the manuscript:
The "scaling" error was removed, but the graphics are still included separately at the end, as required by the journal’s Submission guidelines.
For an impression of how the manuscript should look like, please refer to the included reference PDF that has been rendered as intended by the authors.

2.) The reviewer is addressing WES sequencing - a relevant aspect, which, given increasingly lower sequencing costs, will only gain more importance in the future. From the start, the development of AMLVaran was focused on targeted sequencing, because it is meant to improve diagnostic decisions by inspecting variants in well-known hotspot-regions that are known to be relevant for therapy. At present, in WES data there are many variants of unknown significance (VUS), which have limited value in the clinical setting. However, we recognize the need to analyze not only targeted samples, but to process also WES data.

Changes to the manuscript:
Some optimizations have now been incorporated into AMLVaran, to make the software capable of performing WES analyses with a reasonable performance, and a set of WES samples was processed as a proof-of-concept. A section with recommended adaptations and the generated results was added to the supplement of the manuscript (paragraph 6). WGS data, however, is out of scope for this tool.

Reviewer 2 (Syed Haider)

General (target audience):
Thank you for sharing this important and valid perspective. We do not dispute, that biomedical researchers might find value in AMLVaran and the analyses that the tool supports. In fact we would be delighted to develop the software further to reach a possible second user group. However, according to our experience, professional bioinformaticians would put even more emphasis on flexibility and customizations than AMLVaran can offer, and that corresponding full bioinformatics use cases often incorporate additional data types or require more complex tests and
specific analyses, which make a more custom and specialized approach appear as a more viable and practical solution in these cases.

After careful consideration of the above-mentioned argument, we'd still expect the main target audience of AMLVaran to be medical professionals, and not biomedical researchers. The software is intended to improve clinical routine care, with a very controlled and predictable input and output:
The software is capable of processing single samples, as they occur in routine care, and it offers a clear overview on the mutational state of defined hotspot areas that are associated with a therapeutic relevance for a specific disease. Rule-based diagnostic recommendations can be given out as well.

Given the technical challenges that AMLVaran's design might impose for physicians in daily care, we agree, that the main audience for this tool is likely to consist mainly of molecular pathologists or laboratory staff, who have a more technical background, are specially trained to interpret the results correctly, and who will then send a written overall assessment to the physician in care. However, our user studies indicate, that a regular physician is also able to use the software in order to create a clinical report, which contains a quick therapy-related overview, as well as more detailed information in case of more specialized research questions. This opportunity for direct, clinical incorporation of NGS results from a standardized pipeline seems to be highly valuable.

Specific queries:

1.) Thanks to the reviewer for pointing out the aspect of data security and legal/ethical questions.
Naturally, both, data security and ethical and legal issues, are of the high relevance when dealing with sequencing data.
We have therefore taken care to ensure data security during development by ensuring encrypted data transfer, server-sided filtering of user input, prevention of SQL injections, user management with session authentication and password encryption and more. Of course, also the system’s administrator must take measures to secure his webserver. We have improved the tool's documentation to underline these aspects.
Since the manuscript is focusing primarily on technical aspects, the ethical and legal implications cannot be discussed in full length here. So we refer to previous discussions of this topic and added references to previous publications. Hopefully, this will suffice to do this highly relevant topic justice.

Changes to the manuscript:
A section regarding data security measures and mentioning also ethical and legal aspects, has been included into the supplement of the manuscript (paragraph 2).

2.) The reviewer is addressing an important point regarding the pipeline's central design.
We do admit that we could have emphasized it better, but AMLVaran offers way more than just a web-interface to appreci8. The core of the application is a flexible, generic pipeline, with no dependence on any specific variant calling tool. Any variant calling approach that is based on the combination of one or more standalone variant calling tools can be supported, and any possible filtering scheme can be implemented, as long as its respective annotations are supplied. appreci8 is a particularly sophisticated pipeline, using 8 callers and an elaborated filtering strategy, and has therefore been chosen as a proof-of-concept, showing, that its results can be met by AMLVaran's generic pipeline. Given, that AMLVaran can successfully reproduce results from this complex pipeline, it stands to reason, that the implementation of other variant pipelines will be equally successful.
To ensure, that the AMLVaran pipeline functions correctly, it had to be verified, that it generates the same results as the reference implementation, or that differences can be explained by factors which lie outside the calculations of the algorithm (e.g. database changes).
It is of course desirable to compare AMLVaran and appreci8 with matching databases, however, we
were also very interested in keeping AMLVaran's internal annotation database constantly updated. A return to an old status is, while possible, usually not intended, since AMLVaran’s central database can hold only one version of an annotation source at a time.

In addition, it also does not seem to be feasible for a regular user to deliberately perform the analysis process with older annotation data (e.g. dbSNP 138 instead of 150, which has been outdated for 6 years). In clinical routine, the current annotation versions should usually be used, and therefore we saw the necessity to prove that the results will still be comparable in this case.

With this in mind, we believe, that the performed validation is sufficient to show, that the results are extremely similar, while slight differences can be fully explained by factors that lie outside of the software.

Changes to the manuscript:
We updated the manuscript in order to emphasize the generic aspect of the pipeline even stronger.