We thank the reviewers and editor for their time and efforts in reviewing our manuscript. The many helpful questions and suggestions have led to significant improvements in the manuscript. Below, we respond to each individual concern of the editor and reviewers. Original comments are in black text and our responses are in green.

**Editorial comments:**

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Checked

2. Figure 1: Please include a space between numbers and its corresponding units (i.e., 120 °C, etc.).

Updated

3. Please provide an email address for each author.

Added to title page.

4. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.

Updated

5. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.  
For example: Kryptofix®222, etc.

Added name Kryptofix 222 to materials table and replaced with more generic name in text.

Replaced most instances of ELIXYS and PURE/FORM with generic names throughout text.

6. JoVE policy states that the video narrative is objective and not biased towards a particular product featured in the video. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item. To this end, we ask that you please reduce the number of instances of "SOFIE", “ELIXYS”, within your text. The terms may be introduced but please use them infrequently and when directly relevant. Otherwise, please refer to the terms using generic language.

Updated

7. 1.3 and 1.4: Unclear what we can show here, please describe the actions. If there are no specific actions being performed I suggest unhighlighting these.

We have elected to remove the highlighting.

8. Please include single-line spaces between all paragraphs, headings, steps, etc.

Added.

9. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

We have checked that the length of highlighted region is within limits.

10. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

We have checked that the highlighted steps in sequence form a cohesive narrative.

We have verified that we have always highlighted complete sentences.

We have verified and made changes so that the highlighted part includes at least one action written in the imperative tense.

11. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

We have corrected our highlighting such that the substeps are also highlighted if they provide the detail of how to perform the ‘parent’ step

12. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:  
a) Critical steps within the protocol  
b) Any modifications and troubleshooting of the technique  
c) Any limitations of the technique  
d) The significance with respect to existing methods  
e) Any future applications of the technique

We have modified and reorganized the discussion to cover all of the points listed above.

**Reviewers' comments:**  
  
**Reviewer #1:**  
  
Manuscript Summary:  
The presented manuscript accurately presents the issue and the descriptions of each subsequent step are accurate. This manuscript may be presented as a fully sufficient guideline used in the implementation of newly developed radiotracers with keep of all the rules regarding their clinical use.  
  
Major Concerns:  
None  
  
Minor Concerns:  
None

We thank the reviewer for the positive assessment and remarks.

**Reviewer #2:**  
  
Manuscript Summary: This manuscript describes an automated radiosynthesis protocol for [18F]Clofarabine including computer-controlled synthesis sequence (labeling reaction, purification and formulation) as well as quality control (QC) procedures. The manuscript is well written and suitable for publication in JoVE.  
  
Minor Concerns:  
Comments:  
1. The Radiosynthesizer and PURE/FORM Module are united together as a single module with a single computer-controlled system, or two different modules with two computer-controlled systems?

We agree that in the current version the system description was confusing. Indeed, both the synthesizer and purification/formulation module are united together and operated as a single module with a single computer-controlled system. This has been clarified in the manuscript.

2. In the text, RESULTS section, it is better to give molar activity (MA, formerly known as specific activity, SA) data, because MA will significantly affect the clinical evaluation of an F-18 PET tracer. This reviewer noted that SA did appear in QC data table. Also, please change SA to MA, change Ci to GBq (SI unit).

We thank the reviewer for pointing this out. We have corrected specific activity in the results summary table for molar activity. We have further changed all radioactivity units to SI units, from Curie to Becquerel.

3. There are reports to indicate that QMA cartridge might decrease the MA (SA) of F-18 tracers. In ELIXYS module(s), is it able to eliminate the use of QMA cartridge?

We agree that the use of a trap and release cartridge can introduce cold fluoride that subsequently lowers the molar activity of the final product, either by contamination of the exchange resin, the solutions used for cartridge conditioning or the release solution. It is possible to omit the trap and release process of the F-18 ion, which could potentially further increase the molar activity. However, due to the larger amount of water, this may extend the time needed for the [18F]fluoride drying process. Furthermore, the use of the QMA allows recovery of O-18 enriched water for recycling and also has the effect of removing long-lived metal impurity radioisotopes (to avoid interference with reactions and contamination of final product), both of which would not be possible if the QMA is avoided.

4. Why in ELIXYS Radiosynthesizer huge amount of precursor was used in the F-18 radiosynthesis, here 6 mg? The big amount of the precursor can increase the radiochemical yield, but may decrease the MA/SA and chemical purity.

We have not discussed specific synthesis optimization details in this work as our goal was to have a generally applicable process description that fits most tracer productions. In the literature, many tracers are made with several mg of precursor up to 10s of mg of precursor. In this specific case of [18F]CFA, the established manual synthesis actually originally used 10mg of precursor dissolved in 1mL of acetonitrile. Further optimization studies have shown that the precursor concentration is important for high yields, but not the absolute amounts of precursor; thus precursor can be reduced by reducing the reaction volume. Though one of the drawbacks of many macroscale radiosynthesizers, including the ELIXYS system, is the difficulty of using very low volumes, we have been able to reduce the reaction scale to 600 µL (6mg of precursor) while still achieving reliable and robust results. These conditions resulted in a MA of ~350 GBq/µmol and an excellent chemical purity (no impurities detected) as described in the quality control data table.

5. There are some format issue in REFERENCES section, such as superscript issue of 18F.

We thank the reviewer for noticing some errors in the reference sections. We have revised this section and corrected all superscripts.

6. The protocol need to be further polished, because it includes numerous development, optimization and validation runs.

We thank the reviewer for this valuable input. We have thoroughly reconsidered our approaches, however in our opinion the protocol in its original version is the most appropriate that is descriptive for the majority of synthesis development processes. Omitting certain steps can potentially expedite the development process, but bear the risk of failure through programming errors or unforeseen challenges. This protocol is designed for both new and experienced users of automated radiosynthesizers. We feel that mock runs (“cold runs”) are always a good idea from a safety and efficiency point of view. Optimization runs are often needed when translating a literature protocol (either manual or on a different automated synthesizer) as typically the reaction conditions require slight modifications (temperatures, times, etc.) for the best performance. The validation runs are a required procedure (stipulated in regulatory requirements) if one is producing tracers for clinical use. We have revised the discussion part to better reflect and explain the critical steps within the protocol.