



Life Science Alliance

# Immunosuppressive Low-Density Neutrophils in the Blood of Cancer Patients Display a Mature Phenotype

Christophe Vanhaver, Frank Aboubakar Nana, Nicolas Delhez, Mathieu Luyckx, Thibault Hirsch, Alexandre Bayard, Camille Houbion, Nicolas Dauguet, Alice Brochier, Pierre van der Bruggen, and Annika Bruger

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Corresponding author(s): *Pierre van der Bruggen, de Duve Institute*

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## Review Timeline:

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Revision Received:	2023-10-26
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Scientific Editor: *Eric Sawey, PhD*

## Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

October 2, 2023

Re: Life Science Alliance manuscript #LSA-2023-02332-T

Prof. Pierre van der Bruggen  
Ludwig Institute for cancer research  
de DUVE Institute  
74 avenue Hippocrate  
UCL B1.74.03  
Brussels 1200  
Belgium

Dear Dr. van der Bruggen,

Thank you for submitting your manuscript entitled "Immunosuppressive Low-Density Neutrophils in the Blood of Cancer Patients Display a Mature Phenotype" to Life Science Alliance. The manuscript was assessed by an expert reviewer, whose comments are appended to this letter. We invite you to submit a revised manuscript addressing the Reviewer comments.

To upload the revised version of your manuscript, please log in to your account: <https://lsa.msubmit.net/cgi-bin/main.plex>

You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

While you are revising your manuscript, please also attend to the below editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

The typical timeframe for revisions is three months. Please note that papers are generally considered through only one revision cycle, so strong support from the referees on the revised version is needed for acceptance.

When submitting the revision, please include a letter addressing the reviewer comments point by point.

We hope that the comments below will prove constructive as your work progresses.

Thank you for this interesting contribution to Life Science Alliance. We are looking forward to receiving your revised manuscript.

Sincerely,

Eric Sawey, PhD  
Executive Editor  
Life Science Alliance  
<http://www.lsjournal.org>

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**A. THESE ITEMS ARE REQUIRED FOR REVISIONS**

-- A letter addressing the reviewers' comments point by point.

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-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, <https://www.life-science-alliance.org/authors>

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title and running title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

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We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

\*\*\*IMPORTANT: It is Life Science Alliance policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.\*\*\*

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Reviewer #1 (Comments to the Authors (Required)):

In their research manuscript, Vanhaver et al isolated neutrophils from blood or tumor of patients with NSCLC or ovarian cancer, as well as from blood of non-cancer patients, and investigated their propensity to act as T-cell immunosuppressive neutrophils in vitro. Importantly, they separated NDNs from LDNs for these functional tests. Their data elegantly concluded that not only LDN (agreeing with the literature) but also NDN from patients displayed immunosuppressive activity. This is an important finding, because it challenges the current view that only LDN suppress T cell proliferation.

Further, they identified two subsets of LDNs based on differential expression of CD45. They analysed differences in gene and protein expression between these two subsets, concluding that select genes were differentially expressed, which was not reflected at the protein level. This is important, as many studies use gene expression comparisons for innate immune cell subsets, not necessarily proteins.

They then compared transcriptomes of NDN, LDN(CD45high), LDN(CD45low) and TANs, uncovering a high degree of similarity and only few molecular changes, when considering bulk samples.

This is a good investigation that advances our knowledge about neutrophil phenotypic and functional diversity in cancer. The data are strongly supportive of each main point of the paper and I only have minor comments.

Minor comments:

1. For the T-cell immunosuppression part, the authors conclude that both LDN and NDN can be immunosuppressive. Reviewer notices (especially from the co-culture with autologous T cells) that NDN are invariably, and potently immunosuppressive, while LDN can be, but not always. Thus, it seems clear that NDN, at least in NSCLC, are more potent at Tcell immunosuppression in comparison to LDN. Although the authors cautiously state that "In fact, the suppressive activity of NDN was often more pronounced." (Page 5), could they more firmly conclude and discuss their findings in relation with current literature, in the Discussion?
2. Do the authors know if the protein expression of PDL1, ARG1, NOX2 and ORL1 is different comparing NDN to LDN? Maybe this information can be found in the literature, and would be nice to comment on, especially that there could be discrepancies between transcript and protein, as the authors highlighted when comparing LDN(CD45high) to LDN(CD45low).
3. Figure 1 legend: Remove "Data using autologous T-cells are shown in Sup." in "Data using autologous T-cells are shown in Sup. Data using autologous T-cells are shown in Sup. Figure 2 D."
4. Text p. 5. Reviewer feels that the following sentence should be split into two: "These frequencies are consistent with the frequencies reported (Cassetta et al., 2020) LDN yields from ovarian cancer blood samples were often too low (1,000-10,000 cells) for further functional experimentation."

Brussels, the 6th October 2023

Dear Editors,

We are submitting for your consideration a **revised** manuscript entitled “**Immunosuppressive Low-Density Neutrophils in the Blood of Cancer Patients Display a Mature Phenotype**” by Christophe Vanhaver et al.

The questions/comments of reviewers are written in black, our answers are written in blue. The modifications are indicated as track changes in the text.

Reviewer #1

Minor comments:

1. For the T-cell immunosuppression part, the authors conclude that both LDN and NDN can be immunosuppressive. Reviewer notices (especially from the co-culture with autologous T cells) that NDN are invariably, and potently immunosuppressive, while LDN can be, but not always. Thus, it seems clear that NDN, at least in NSCLC, are more potent at Tcell immunosuppression in comparison to LDN. Although the authors cautiously state that "In fact, the suppressive activity of NDN was often more pronounced." (Page 5), could they more firmly conclude and discuss their findings in relation with current literature, in the Discussion?

We have replaced “often” by “systematically” in order to more firmly conclude.

We added also a few sentences to the discussion to compare our data with the literature.

2. Do the authors know if the protein expression of PDL1, ARG1, NOX2 and ORL1 is different comparing NDN to LDN? Maybe this information can be found in the literature, and would be nice to comment on, especially that there could be discrepancies between transcript and protein, as the authors highlighted when comparing LDN(CD45high) to LDN(CD45low).

A supplementary figure ( Sup. Figure 4) has been added containing the data requested. We have also described these new data in the text

3. Figure 1 legend: Remove "Data using autologous T-cells are shown in Sup." in "Data using autologous T-cells are shown in Sup. Data using autologous T-cells are shown in Sup. Figure 2 D."

The modification has been introduced.

4. Text p. 5. Reviewer feels that the following sentence should be split into two: "These frequencies are consistent with the frequencies reported (Cassetta et al., 2020) LDN yields from ovarian cancer blood samples were often too low (1,000-10,000 cells) for further functional experimentation."

The modification has been introduced.

Best regards,



Prof. Pierre van der Bruggen

October 11, 2023

RE: Life Science Alliance Manuscript #LSA-2023-02332-TR

Prof. Pierre van der Bruggen  
de Duve Institute  
TILS  
avenue Hippocrate 75  
Brussels 1200  
Belgium

Dear Dr. van der Bruggen,

Thank you for submitting your revised manuscript entitled "Immunosuppressive Low-Density Neutrophils in the Blood of Cancer Patients Display a Mature Phenotype". We would be happy to publish your paper in Life Science Alliance pending final revisions necessary to meet our formatting guidelines.

Along with points mentioned below, please tend to the following:

- please upload all figure files as individual ones, including the supplementary figure files; all figure legends should only appear in the main manuscript file after the references section
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- figure 6 has only one panel; there is no need to label it as A. Please correct
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- please add callouts for Figures 5A; S2D; S4A,B; S5A-D and S8 to your main manuscript text
- please include the GEO accession number for the RNA-seq data in the Data Availability statement at this point

If you are planning a press release on your work, please inform us immediately to allow informing our production team and scheduling a release date.

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To upload the final version of your manuscript, please log in to your account: <https://lsa.msubmit.net/cgi-bin/main.plex>  
You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

#### A. FINAL FILES:

These items are required for acceptance.

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-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, <https://www.life-science-alliance.org/authors>

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**\*\*Submission of a paper that does not conform to Life Science Alliance guidelines will delay the acceptance of your manuscript.\*\***

**\*\*It is Life Science Alliance policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.\*\***

**\*\*The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements.\*\***

**\*\*Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.\*\***

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days.

Thank you for this interesting contribution, we look forward to publishing your paper in Life Science Alliance.

Sincerely,

Eric Sawey, PhD  
Executive Editor  
Life Science Alliance  
<http://www.lsajournal.org>

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October 27, 2023

RE: Life Science Alliance Manuscript #LSA-2023-02332-TRR

Prof. Pierre van der Bruggen  
de Duve Institute  
TILS  
avenue Hippocrate 75  
Brussels 1200  
Belgium

Dear Dr. van der Bruggen,

Thank you for submitting your Research Article entitled "Immunosuppressive Low-Density Neutrophils in the Blood of Cancer Patients Display a Mature Phenotype". It is a pleasure to let you know that your manuscript is now accepted for publication in Life Science Alliance. Congratulations on this interesting work.

The final published version of your manuscript will be deposited by us to PubMed Central upon online publication.

Your manuscript will now progress through copyediting and proofing. It is journal policy that authors provide original data upon request.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

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Again, congratulations on a very nice paper. I hope you found the review process to be constructive and are pleased with how the manuscript was handled editorially. We look forward to future exciting submissions from your lab.

Sincerely,

Eric Sawey, PhD  
Executive Editor  
Life Science Alliance  
<http://www.lsajournal.org>