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REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The current manuscript "Exploiting human immune repertoire transgenic mice to identify protective monoclonal antibodies against an extensively antimicrobial resistant nosocomial bacterial pathogen." by Baker et al. The overall theme, to develop alternative treatment modalities to treat multi-drug resistant bacterial infections is highly relevant. The study presents an impressive amount of work leading to a large number of mAbs that were cloned using a kymouse immunized with OMVs and a systematic high-throughput screen algorithm. The obtained results in the present study are for the most part not unexpected. Although the approach is agnostic and sophisticated from a technical stand point the identified targets are expected. The only mAb that shows some even though moderate efficacy in in vivo infection binds to the KL49 polysaccharide capsule of *Acinetobacter*.

Major comments: the paper is very long and could be shortened significantly

Abstract

1. The abstract is well written but too descriptive and does not include any concrete data it is written like a lay abstract and not a scientific abstract. The authors need to restructure the abstract and provide details of the present study.

Introduction

1. The introduction is well-written but superficial. The authors should provide a few more literature references that discuss the antigenic composition of outer membrane vesicles (OMVs). It needs to be clarified that neutralizing mAbs usually target the live bacteria in vivo and not the OMVs even though the latter may contain vaccine targets and can be used to vaccinate. Also it should be discussed how OMVs are different from the outer surface of the bacteria, which has polysaccharide attached on the outside. Line 58-59 The effect the covid pandemic had on promoting the spread of *Acinetobacter* data is not relevant because now the number of covid patients on vents are over. It should be stated that *Acinetobacter* prevalence differs indifferent parts in the world

2. Line 52-54. The authors claim that *A. baumannii* showed resistance to antimicrobials. These kinds of sentences require proper citations in the text for better validation.

3. Line 76-77 "there are no validated target antigens" is not correct highly protective Abs to *Acinetobacter* have been described Travis Nielsen et al *Inf and Immun* 2021

Material & Methods

1. The authors need to provide constant units for centrifugation throughout the manuscript. It is better to present the centrifugal units in g as RPM differs for different centrifugation instruments.

2. The authors used the wrong abbreviation of "mins" throughout the manuscript. Please replace it with "min, " which is the correct minute abbreviation. Also, the authors suggested either using abbreviations for all terms like hours and others or writing full terms and not using inconsistent units' representation.

3. The authors should provide the manufacturer's origin country for every consumable and instrument used in the current study.

4. The authors should provide details or sequences of primers used to amplify VH and VL sequences in material & methods or supplementary files.

5. Line 362: The authors use the wrong symbol of degree. Correct it with a valid symbol of degree.

6. Line 435 & 436: The authors should provide the reference from where the method was adopted.

7. Line 473: What do authors mean by culture normalised to OD600 nm of 1.0? Is the culture grown to

an OD of 1 or diluted to 1? If diluted, then in what solution?

Results

Overall would have helped to show a schemata of the AB screening strategy. The results should be shortened considerably

4. Why did they pick only strains from a Vietnam ICU? Unclear the logic to do WGS on these strains and boot strap analysis. How diverse is the polysaccharide capsule in *Acinetobacter* and are the capsule types associated with clonal types? line 102 to reflect the temporal genetic diversity...unclear what that rationale means

5. Omv vaccination how many mice? It seems 5. I don't think Fig 1b needs to be in a figure. There is nothing unusual. It is nice but also expected that the mice all show similar response

6. Line155 unclear rationale why VH/VL from BM plasma cells were included

7. Complement binding assays done with heat killed serum?

8. Target identification of 10kDA only by inference no direct target identification

9. In vivo testing done in this study is very limited. Unclear how valuable the mAbs are even the KL49 specific Ab is not highly protective. No opsonophagocytosis assays presented. They are a standard assay. Murine experiments only with intra nasal infection, no iv infection. No experiments performed with immunocompromised mice (neutropenic) and no experiments results performed where the Ab is given after infection. The reduction of CFU in the lung is not impressive (1-2 log reduction). Also the mAb should be tested as adjunct given to the infected mouse in combination with an antibiotic.

10. Line288 prophylactically treated? Intranasal? Needs to be clarified

Figures

1. In Fig. 1a, the value of this figure is unclear. The authors need to better describe the parameters in the figure legend, like the parameters for bootstrapping, substitutions, etc. The tree shows the bootstrap value of 100% at nodes, but what value did the authors set during creating a tree? The same follows for the other parameters used when generating a tree.

2. In Supplementary Fig. 1a, the authors also need to provide SEM images of reference or standard OMVs along with their OMVs structure for better analysis of structure integrity, or authors can also correlate their OMVs SEM data with already reported SEM images of OMVs from other reports. The authors should provide a brief protocol for preparing the OMVs sample for SEM analysis in a supplementary file. In Supplementary Fig. 1b, what authors run in Lane 2 & 3? What is the purpose behind this? From Lane 3, it is observed that the standard ATCC 17978 strain's OMVs do not contain any antigenic proteins. However, the authors mentioned that the OMVs isolated from the strain used in the current study have multiple antigenic proteins. Can authors clarify?

3. What do authors mean by naïve serum antibody signal? Can authors provide the bar of the same in Fig. 1 a-d? The authors label the Y-axis of Fig. 1a-d as Relative Antibody Titre. Then, they need to provide the bar in the figure to which they relate the antibody titre and the unit of this in the bracket for proper representation of data.

4. In indirect ELISA of mAbs, The ideal experiment sequence is all mAbs binding with intact bacterial cells, followed by binding of positive mAbs with OMVs or all mAbs binding with OMVs. Then, correlate how many mAbs bind individually with bacterial cells or OMVs and mutually with both factors. This may provide a better correlation of mAbs binding with bacterial cells than its proxy OMVs. Authors must also provide color code descriptions of dots in Fig. 2c & d.

5. From Fig. 3d, it was observed that almost all the mAbs targeted the KL49 capsular antigen. Also, the

authors use the BAL 191 that has KL49 capsule antigen for further experiments in the current study. However, they used BAL 084 and BAL276 to generate phage expression libraries, which might not have KL49-type capsule antigens. Does this selection impact the finding of suitable mAbs targets? The authors need to justify the choice of these isolates for the phage expression library and provide proper interpretation in correlation with other results.

6. The labelling of the SDS-PAGE image in Supplementary Fig. 4 & 5 is insufficient. The authors should provide proper and consistent figures labelling for better data representation. Also, in Supplementary Fig. 4b, the mAbs 1348 and 1349 showed binding with a protein with a molecular weight of ~30 kDa in western blotting; however, the authors did not interpret or discuss this protein antigen. The authors validate the mAbs binding with ~10 kDa protein antigen.

7. For Fig. 5a & b: The authors wrote in figure legends that mice were infected with BAL191 & BAL276, whereas, in the results section, they mentioned that they infected the mice with BAL191. Please cross-check for the error and provide the correct condition. Also Fig 5b they say no effect and show significance

8. Fig 6 a unclear why this data is shown they should just test binding and opsonophagocytosis

9. On a whole collection of strains with KL49 and without KL49 type

Discussion

The discussion lacks depth and insight regarding how useful this mAb would be in the clinical arena where you do not necessarily know the clonal type of the Acinetobacter

Does not properly discuss challenges of capsule specific mAb .

Does the capsule change?

How prevalent in this specific capsule type and is it linked to a clonal type

There are many highly effective capsule specific mAb some even in clinical trials

They are not discussed

Also not discussed how one would use a prophylactic Ab? During an outbreak situation?

Most importantly it is not clear why this very labour intensive sophisticated approach is better at identifying highly protective Abs. I don't see any paradigm shifting approach.

Other targeted approaches using purified cell walls or polysaccharide have generated high affinity Abs that are a lot more protective. The screening approach is limited if you only consider complement binding and ELISA binding.

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3 (Remarks to the Author):

The authors utilized human immune repertoire transgenic mice to identify human antibodies to A.

baumannii, a antibiotic resistant pathogen. For good reason, this is a priority organism for development of therapeutics. The use of OMV from multiple clinical isolates directed a target agnostic campaign. Several human monoclonals were identified by binding both OMV and intact bacteria which is a strength. A subset was then shown to be functional antibodies - including in vitro and in vivo efficacy.

It is agreed that there are few monoclonal antibodies to this bacteria, let alone human but there is at least one humanized antibody. How do these compare and can anything be concluded by the comparison?

The serum response to OMV includes all IgG subclasses - almost in equal amounts. Usually a protein response is heavily IgG1 driven - that is not seen here. Are carbohydrates with the OMV driving IgG2, IgG4 - or is this a typical response of Kymab mice?

How many mice were used as source of B cells - that contributes to whether there was good representation of the response. The frequency of sequenced paired VH/VL seems low or this as expected? Is there any skewing of the repertoire?

For in vivo studies - was there any protection treating with mAb after challenge?

It may be an overstatement that this study identifies a path for the use of monoclonal antibodies as prophylaxis. That has been proposed - and developed - for other bacteria.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The current manuscript “Exploiting human immune repertoire transgenic mice to identify protective monoclonal antibodies against an extensively antimicrobial resistant nosocomial bacterial pathogen.” by Baker et al. The overall theme, to develop alternative treatment modalities to treat multi-drug resistant bacterial infections is highly relevant. The study presents an impressive amount of work leading to a large number of mAbs that were cloned using a kymouse immunized with OMVs and a systematic high-throughput screen algorithm. The obtained results in the present study are for the most part not unexpected. Although the approach is agnostic and sophisticated from a technical stand point the identified targets are expected. The only mAb that shows some even though moderate efficacy in in vivo infection binds to the KL49 polysaccharide capsule of *Acinetobacter*. Major comments: the paper is very long and could be shortened significantly.

We have made major alterations throughout to address these points resulting in reduction of the text. Most notably in the results section (see below).

Abstract

1. The abstract is well written but too descriptive and does not include any concrete data it is written like a lay abstract and not a scientific abstract. The authors need to restructure the abstract and provide details of the present study.

The abstract has been rewritten and restructured to add additional details of the present study.

Introduction

1. The introduction is well-written but superficial. The authors should provide a few more literature references that discuss the antigenic composition of outer membrane vesicles (OMVs). It needs to be clarified that neutralizing mAbs usually target the live bacteria in vivo and not the OMVs even though the latter may contain vaccine targets and can be used to vaccinate.

New text has been added (L80-L91) of the revised manuscript and new references added to address these points.

Also it should be discussed how OMVs are different from the outer surface of the bacteria, which has polysaccharide attached on the outside. Line 58-59 The effect the covid pandemic had on promoting the spread of *Acinetobacter* data is not relevant because now the number of covid patients on vents are over. It should be stated that *Acinetobacter* prevalence differs indifferent parts in the world

Lines L57-59 in the original manuscript have been removed. New text and reference added to address global *A. baumannii* prevalence (L55-56) of the revised manuscript.

2. Line 52-54. The authors claim that *A. baumannii* showed resistance to antimicrobials. These kinds of sentences require proper citations in the text for better validation.

We have now added a reference to line 55 to cover mechanisms of acquisition of antimicrobial resistance of *A. baumannii*.

3. Line76-77 “there are no validated target antigens” is not correct highly protective Abs to *Acinetobacter* have been described Travis Nielsen et al Inf and Immun 2021

New text and the suggested reference have been added (L76-L78 in the revised manuscript) to address this.

Material & Methods

1. The authors need to provide constant units for centrifugation throughout the manuscript. It is better to present the centrifugal units in g as RPM differs for different centrifugation instruments.
Units stated as rpm now changed to g units.

2. The authors used the wrong abbreviation of "mins" throughout the manuscript. Please replace it with "min, " which is the correct minute abbreviation. Also, the authors suggested either using abbreviations for all terms like hours and others or writing full terms and not using inconsistent units' representation.

Term 'mins' changed to 'min' for all abbreviations.

3. The authors should provide the manufacturer's origin country for every consumable and instrument used in the current study.

Now added in all cases.

4. The authors should provide details or sequences of primers used to amplify VH and VL sequences in material & methods or supplementary files.

The primer sets used to amplify VH and VL sequences fall under company proprietary knowledge, have been designed to work with the Kymouse cDNA alone and have not been used to amplify VH and VL sequences from human cDNA. We kindly request the option not to publish sequences of the primer sets used.

5. Line 362: The authors use the wrong symbol of degree. Correct it with a valid symbol of degree.

Corrected.

6. Line 435 & 436: The authors should provide the reference from where the method was adopted.

Text changed to: *As described above (Indirect ELISA of live bacterial isolates and outer membrane vesicles).*

7. Line 473: What do authors mean by culture normalised to OD600 nm of 1.0? Is the culture grown to an OD of 1 or diluted to 1? If diluted, then in what solution?

Text changed to *100 µl of overnight bacterial cultures normalised by dilution in fresh LB broth to an OD600nm of 1.0...*

Results

Overall would have helped to show a schemata of the AB screening strategy.

We considered doing this but felt that because the process of identifying the mAbs was not fully systematic from the outset, such a schema would result in oversimplification that could be misleading and the approach is generally better outlined by following the text and figures.

The results should be shortened considerably

We have reduced the word count of the results section from 2665 to 2309 in the revised manuscript.

4. Why did they pick only strains from a Vietnam ICU? Unclear the logic to do WGS on these strains and boot strap analysis. How diverse is the polysaccharide capsule in *Acinetobacter* and are the capsule types associated with clonal types ? line 102 to reflect the temporal genetic diversity...unclear what that rationale means

Clarifying text has been added to explain the rationale for selecting strains from a (L101-L112 in the revised manuscript). Full details of the capsule diversity among the strains are given in the cited study (Schultz et al. Repeated local emergence of carbapenem-resistant *Acinetobacter baumannii* in a single hospital ward. *Microb Genom* 2, e000050 2016)

5. Omv vaccination how many mice? It seems 5. I don't think Fig 1b needs to be in a figure. There is nothing unusual. It is nice but also expected that the mice all show similar response

We now indicate number of Kymouse platform mice were immunised (n=5). We agree that Fig 1d showing similar responses across mice is redundant and have removed it from the figure 1.

6. Line155 unclear rationale why VH/VL from BM plasma cells were included

More clarifying text has been added to L146-152.

7. Complement binding assays done with heat killed serum?

We did not perform the presented screening assays involving C3b deposition with heat-killed serum as a control. Instead we compared signal in each case using a human IgG1 isotype antibody control.

8. Target identification of 10kDa only by inference no direct target identification

We inferred that the 10kDa target bound by specific mAbs in western blots was LOS as i) binding in western blotting using a carbohydrate-only preparation still showed detectable 10kDa band ii) Specific mAbs bound to OCL1-typed LOS producing strains only and ii) Specific mAbs failed to bind any phage expression library plaques (Acinetobacter polypeptide expressing) in multiple screens.

9. In vivo testing done in this study is very limited. Unclear how valuable the mAbs are even the KL49 specific Ab is not highly protective. No opsonophagocytosis assays presented. They are a standard assay.

We have added additional panels to Fig. 4 to show capsule binding of mAb 1416 to the KL49-producing strain CM10420 (new Fig. 4c) but not ATCC17978 (KL3-producing, new Fig. 4d). We have performed opsonophagocytic assays on two KL49 producing strains showing effect on opsonophagocytic uptake on addition of mAb 1416 (new Fig. 4e) as well as no effect on opsonophagocytic uptake using the same conditions with a non-KL49 producing strain. These data are contextualised in the revised manuscript with additional text (L233-L240).

Murine experiments only with intra nasal infection, no iv infection. No experiments performed with immunocompromised mice (neutropenic) and no experiments results performed where the Ab is given after infection. The reduction of CFU in the lung is not impressive (1-2 log reduction). Also the mAb should be tested as adjunct given to the infected mouse in combination with an antibiotic.

10. Line288 prophylactically treated? Intranasal? Needs to be clarified

There are limitations with all murine models for *A. baumannii* in the context of human infection as infection of mice is not established. We selected intranasal challenge as we could assess ability of a mAb given prophylactically to reduce CFU in the lung and to prevent systemic dissemination to the spleen as useful correlates to assess potential efficacy, critically using the same clinical strains that had caused infection in humans. We agree that a comprehensive data package to address in vivo efficacy for the three mAbs we selected would be highly desirable. However, our intention was to generate in vivo data as a PoC in this instance as our manuscript aims to outline target agnostic methods to identify mAbs to carbapenem-resistant clinical strains of *A. baumannii*. We agree that the suggested experiments could be included in subsequent manuscripts to further evaluate functional activity of the mAbs we have identified but would require a substantial amendment of our protocols for animal use that would fall outside of the intended scope of the current work. We have amended the text (L264-265) to *tested whether mAb 1416 protected given prophylactically could protect mice after intranasal challenge with CM10420* to clarify how mAb 1416 was administered to mice and how they were subsequently challenged.

Figures

1. In Fig. 1a, the value of this figure is unclear. The authors need to better describe the parameters in the figure legend, like the parameters for bootstrapping, substitutions, etc. The tree shows the

bootstrap value of 100% at nodes, but what value did the authors set during creating a tree? The same follows for the other parameters used when generating a tree.

We have added new text to the figure legend 1 to address these comments.

2. In Supplementary Fig. 1a, the authors also need to provide SEM images of reference or standard OMVs along with their OMVs structure for better analysis of structure integrity, or authors can also correlate their OMVs SEM data with already reported SEM images of OMVs from other reports.

We have added two citations to the figure legend Supplementary Fig. 1a for comparison of the OMV SEM images generated for previously published reports.

The authors should provide a brief protocol for preparing the OMVs sample for SEM analysis in a supplementary file.

We have now added details of preparation of the OMVs for SEM analysis in the file: Supplementary Methods and Figure Legends.

In Supplementary Fig. 1b, what authors run in Lane 2 & 3? What is the purpose behind this? From Lane 3, it is observed that the standard ATCC 17978 strain's OMVs do not contain any antigenic proteins. However, the authors mentioned that the OMVs isolated from the strain used in the current study have multiple antigenic proteins. Can authors clarify?

For added clarity we have amended Fig. 1b to show only lanes containing OMV preparations from strains BAL 191, BAL 215 and BAL 276 with indicated positions of molecular markers. These samples were run together on the same gel as an OMV prep isolated from ATCC 17978 that was erroneously run and the gel and contained no OMV material. This is the reason why no protein bands appeared in the lane containing the erroneous ATCC 17978 preparation.

3. What do authors mean by naïve serum antibody signal? Can authors provide the bar of the same in Fig. 1 a-d? The authors label the Y-axis of Fig. 1a-d as Relative Antibody Titre. Then, they need to provide the bar in the figure to which they relate the antibody titre and the unit of this in the bracket for proper representation of data.

We have calculated antibody titres directed at OMVs by calculating the endpoint dilution required to match the indirect ELISA signal observed for serum added from non-immunised mice (which are referred to as naïve). Indirect ELISA signal for non-immunised mice was measured at 1/100 in all cases which was the lowest dilution of serum tested for indirect ELISA signal in all cases. In all cases, the titres from immunised mice would be represented on the graph at a level of 10^0 or 1. As an alternative, we could have calculated all relative antibody titres against the indirect ELISA signal for an isotype antibody, but we felt that measuring relative antibody titre of immunised mice relative to non-immunised mice was a more meaningful metric. To add clarity, we amended text to state that relative antibody titre measured by indirect ELISA of sera from immunised Kymouse platform mice (n=5) was measured versus non-immunised Kymouse platform mice in figure legends 1b-d and changed 'naïve' to 'non-immunised mice' in the manuscript text.

4. In indirect ELISA of mAbs, The ideal experiment sequence is all mAbs binding with intact bacterial cells, followed by binding of positive mAbs with OMVs or all mAbs binding with OMVs. Then, correlate how many mAbs bind individually with bacterial cells or OMVs and mutually with both factors. This may provide a better correlation of mAbs binding with bacterial cells than its proxy OMVs. Authors must also provide color code descriptions of dots in Fig. 2c & d.

We tested all mAbs for binding against intact bacterial cells and OMVs, therefore sequence that this was done is a moot point. We have adjusted text (L158-L162) to avoid misinterpretation and to emphasise that all mAbs for subsequent studies were selected from those binding intact bacterial cells. To avoid confusion, all colour coding has been removed from dots in Fig. 2c and 2d.

5. From Fig. 3d, it was observed that almost all the mAbs targeted the KL49 capsular antigen. Also, the authors use the BAL 191 that has KL49 capsule antigen for further experiments in the current study. However, they used BAL 084 and BAL276 to generate phage expression libraries, which might not have KL49-type capsule antigens. Does this selection impact the finding of suitable mAbs targets? The authors need to justify the choice of these isolates for the phage expression library and provide proper interpretation in correlation with other results.

BAL 084 and BAL 276 do not produce KL49 capsule, however, the phage expression library can only be screened for mAbs targeting protein antigens rather than polymeric carbohydrate antigens. We selected BAL 084 and BAL 276 as representatives of the immunising panel and since we were screening for mAbs that bound protein present on all the strains used to generate the OMVs, it should not be necessary to construct libraries based on all strains used. We were only able to identify mAbs that bound Oxa-23 using the library screen (present in all strains used to generate OMVs for immunisation). We further contextualised use of the library by the fact that mAbs unable to bind any expressed sequence in the expression library was able to bind a 10 kDa fragment that ended up being LOS (continued to bind non-proteinaceous 10 kDa band).

6. The labelling of the SDS-PAGE image in Supplementary Fig. 4 & 5 is insufficient. The authors should provide proper and consistent figures labelling for better data representation.

We have relabelled western blot images for Supplementary Fig. 4 and Fig 5. for better interpretation. This includes consistent lane and molecular weight marker information throughout. Because we have performed western blotting on both resolved bacterial lysates and proteinase K-treated bacterial lysates (to resolve polymeric carbohydrate antigens) we have included labelling on each image to indicate what type of sample was run. For proteinase K-treated bacterial lysates we have indicated typical positions for capsule (K) and lipooligosaccharide (LOS) antigens. We have split Supplementary Fig 5 into two, one showing only proteinase K-treated bacterial lysate western blots (Supplementary Fig 5) and a new supplementary Fig 6 showing resolved bacterial lysates with detection of capsule by anti-KL49 mAbs to aid interpretation.

Also, in Supplementary Fig. 4b, the mAbs 1348 and 1349 showed binding with a protein with a molecular weight of ~30 kDa in western blotting; however, the authors did not interpret or discuss this protein antigen. The authors validate the mAbs binding with ~10 kDa protein antigen.

We have amended text in the manuscript to make more explicit mention of the protein with a molecular weight of ~30 kDa protein by western blotting (L258-263 in the revised manuscript).

7. For Fig. 5a & b: The authors wrote in figure legends that mice were infected with BAL191 & BAL276, whereas, in the results section, they mentioned that they infected the mice with BAL191. Please cross-check for the error and provide the correct condition. Also Fig 5b they say no effect and show significance

We have deleted reference to challenge with BAL 276 in the figure legend for Fig 5a. We have amended text to refer to significant differences in spleen CFU for mice receiving mAbs 1042 and 1349.

8. Fig 6 a unclear why this data is shown they should just test binding and opsonophagocytosis

9. On a whole collection of strains with KL49 and without KL49 type

We use Fig 6a to indicate that *A. baumannii* strains predominating in the Vietnamese ICU and causing neonatal sepsis were related to BAL 191 and produce KL49 capsule. Therefore, testing mAb 1416 on a related strain cultured 10 years after culture of BAL 191 offers important information on stability of the epitope mAb 1416 is targeting.

We now include new data for bacterial binding (TEM) and opsonophagocytosis for mAb 1416 with two KL49 producing strains and a non KL49-producing strain (New Figure 4c, d and e)

Discussion

The discussion lacks depth and insight regarding how useful this mAb would be in the clinical arena where you do not necessarily know the clonal type of the Acinetobacter

Agree that this should be addressed but depends on level of cross-reactivity. Our study aims at increasing the number of targets available that have potential for cross-reactivity. Agree that targeting polymeric carbohydrate is not original but we also identify carbapenamase as membrane targetable by a mAb. Although Carbapenamse enzymes show a degree of diversity in Acinetobacter, Oxa-23 is dominant among CRAB clones and could represent a useful target.

Does not properly discuss challenges of capsule specific mAb .

Here we identify a KL-49 specific mAb as protective. We mention that capsule is clone specific and have attempted to cover a range of K types in our screens.

Does the capsule change?

Because mAb 1416 binds strains isolated 10-years apart in the same ICU, this suggests that the capsule epitope it targets remains stable. Addressed in L315-L321

How prevalent in this specific capsule type and is it linked to a clonal type

New text added to address KL49 linkage to clonal type L326-L329

There are many highly effective capsule specific mAb some even in clinical trials

They are not discussed

Addressed with citation in L323-L326

Also not discussed how one would use a prophylactic Ab? During an outbreak situation?

Addressed now in L327-L331

Most importantly it is not clear why this very labour intensive sophisticated approach is better at identifying highly protective Abs. I don't see any paradigm shifting approach.

Other targeted approaches using purified cell walls or polysaccharide have generated high affinity Abs that are a lot more protective. The screening approach is limited if you only consider complement binding and ELISA binding.

As an end-to-end approach, we do not believe this approach is particularly labour-intensive. An important contribution that our approach makes is the ability to both identify human mAbs (ie. removes the need for humanisation), provides an assessment of broad human repertoire engagement in a target agnostic manner and provides multiple related clones generated by affinity maturation. Mining these clone clusters after identifying a single member with protective potential can provide additional candidates that could show i) improved protective capacity and ii) developability advantages when selecting a mAb for subsequent clinical development. We highlight these advantages while toning down the suggestion of a paradigm shift (L333-344).

Reviewer #2 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #3 (Remarks to the Author):

The authors utilized human immune repertoire transgenic mice to identify human antibodies to *A. baumannii*, an antibiotic resistant pathogen. For good reason, this is a priority organism for development of therapeutics. The use of OMV from multiple clinical isolates directed a target agnostic campaign. Several human monoclonals were identified by binding both OMV and intact bacteria which is a strength. A subset was then shown to be functional antibodies - including in vitro and in vivo efficacy.

It is agreed that there are few monoclonal antibodies to this bacteria, let alone human but there is at least one humanized antibody. How do these compare and can anything be concluded by the comparison?

For a meaningful comparison, the humanised mAb in question would need to be studied in a head-to-head in vitro or in vivo study. This falls outside the scope of the current work.

The serum response to OMV includes all IgG subclasses - almost in equal amounts. Usually a protein response is heavily IgG1 driven - that is not seen here. Are carbohydrates with the OMV driving IgG2, IgG4 - or is this a typical response of Kymab mice?

This is a very interesting point. It should be mentioned that we are using transgenic mice producing fully human antibodies and there may be differences with how IgG class-switching occurs in transgenic mice vs the human. Therefore, conclusions on antigen-specific driving of IgG subclass are difficult to draw.

How many mice were used as source of B cells - that contributes to whether there was good representation of the response. The frequency of sequenced paired VH/VL seems low or this as expected? Is there any skewing of the repertoire?

We used B cells deriving from 4 different mice for VH/VL sequencing. The frequency of VH/VL paired sequences were in line with what we typically achieve with the single B cell plate-based method we used for VH/VL sequencing. Human germ line VH and VL usage was relatively diverse, but we would need to compare with VH/VL gene usage from a similarly immunised human response to assess degree of skewing.

For in vivo studies - was there any protection treating with mAb after challenge?

We did not perform protection in a therapeutic setting. This would be interesting to address in subsequent studies.

It may be an overstatement that this study identifies a path for the use of monoclonal antibodies as prophylaxis. That has been proposed - and developed - for other bacteria.

An important contribution that our approach makes is the ability to both identify human mAbs (ie. removes the need for humanisation), provides an assessment of broad human repertoire engagement in a target agnostic manner and provides multiple related clones generated by affinity maturation. Mining these clone clusters after identifying a single member with protective potential can provide additional candidates that could show i) improved protective capacity and ii) developability advantages when selecting a mAb for subsequent clinical development. We highlight these advantages while toning down the suggestion of a paradigm shift (L333-344).

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

Experiments outlining the protective efficacy of mAb 1416 continue to be insufficient in this review hence the authors' claim they identified a protective mAb cannot stand like this even if they only look at prophylaxis which is not really that valuable you should show that mAb also works when given afterwards

in Figure 6

they only show one animal experiment with 2-8 mice

they use an isotope specific treatment control

in Fig 6 b and in Fig 6d the CFU of the isotope specific control is higher than that of PBS the infection control they only compare the mAb to the isotope control not the PBS control that is the only reason they generate statistical significance. The higher CFU relative to PBS is a problem and suggests an non specific binding and uptake into the spleen of the isotope control they either need to find a better isotope control or show at least real significance compared to the PBS control

in Fig 6E the numbers are not adequate PBS and Isotope should have each 5 mice to judge significance . For spleen the CFU are very low 10^2 is barely detection level of CFU so again difficult to judge with one experiment

also animal experiments need to all have the same numbers and they should explain why some groups have 2 and some 8 what happened to the rest of the mice

Also they still have now shown how many different *Acinetobacter* strains the mAb 1416 would bind how strain specific is the target and or how common is this KL49 producing *Acinetobacter* strain type. It is my understanding that there are 100 distinct capsules. Is this beyond that one ICU a common capsule type? It is my understanding that Of these 100 capsule types, the most common three are KL6, KL10, and KL47, which altogether occur in more than 10% of all nosocomial infections based on Rosesathorn Soontarach et al 2022

does the mAb not bind to any other capsule type?

Reviewer #2 (Remarks to the Author):

"I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts."

Experiments outlining the protective efficacy of mAb 1416 continue to be insufficient in this review hence the authors' claim they identified a protective mAb cannot stand like this even if they only look at prophylaxis which is not really that valuable you should show that mAb also works when given afterwards

Thank you, this an important point about the deployment of antibodies in a clinical context, but slightly tangential to the main message of the manuscript – which is that a target agnostic approach can identify antibodies that are functionally active against a highly complex, bacterial pathogen with multiple potential antigens.

In addition, because Acinetobacter is a nosocomial pathogen and affects vulnerable and immunocompromised intubated patients, the prophylactic context of mAb therapy is relevant, as we have discussed in an opinion piece in Lancet Microbe
[https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247\(20\)30126-9/fulltext](https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247(20)30126-9/fulltext)

The data presented supports the conclusions we draw, we state that the antibodies have prophylactic potential, we do not describe them as being therapeutic.

in Figure 6
they only show one animal experiment with 2-8 mice

We apologise for the lack of clarity; we have attached a revised figure. The data presented in panels a-c are a single n=5 study. The data presented in panels d-f represent 2 experiments combined (n=5 per study), there is some variation in the numbers presented because some of the plates for the counts had to be discarded due to quality control issues. Panels g-i represent a single n=5 study. The lung data specifically had issues with the plating of the bacteria. To overcome these issues, we have now included nasal data from the studies, which has greater numbers of reported values and supports the same conclusion.

they use an isotope specific treatment control in Fig 6 b and in Fig 6d the CFU of the isotope specific control is higher than that of PBS the infection control they only compare the mAb to the isotope control not the PBS control that is the only reason they generate statistical significance. The higher CFU relative to PBS is a problem and suggests anon specific binding and uptake into the spleen of the isotope control they either need to find a better isotope control or show at least real significance compared to the PBS control

This is caused by some experimental variation. The PBS and the isotope control are NOT significantly different. We report significance against PBS in panels d, f, i.

in Fig 6E the numbers are not adequate PBS and Isotope should have each 5 mice to judge significance .

See above, we accept the numbers for the lung are low, but the conclusion is supported by the nasal data. Furthermore, the pattern of protection is repeated in the other challenge model used.

For spleen the CFU are very low 10^2 is barely detection level of CFU so again difficult to judge with one experiment also animal experiments need to all have the same numbers and they should explain why some groups have 2 and some 8 what happened to the rest of the mice

See above – there were some issues with plating and recovery.

The CFU in the spleen are lower, because the bacteria are intranasally administered and there are low levels of recovery. The levels reported are above detection level. This is not just one experiment, but 4 across the whole figure.

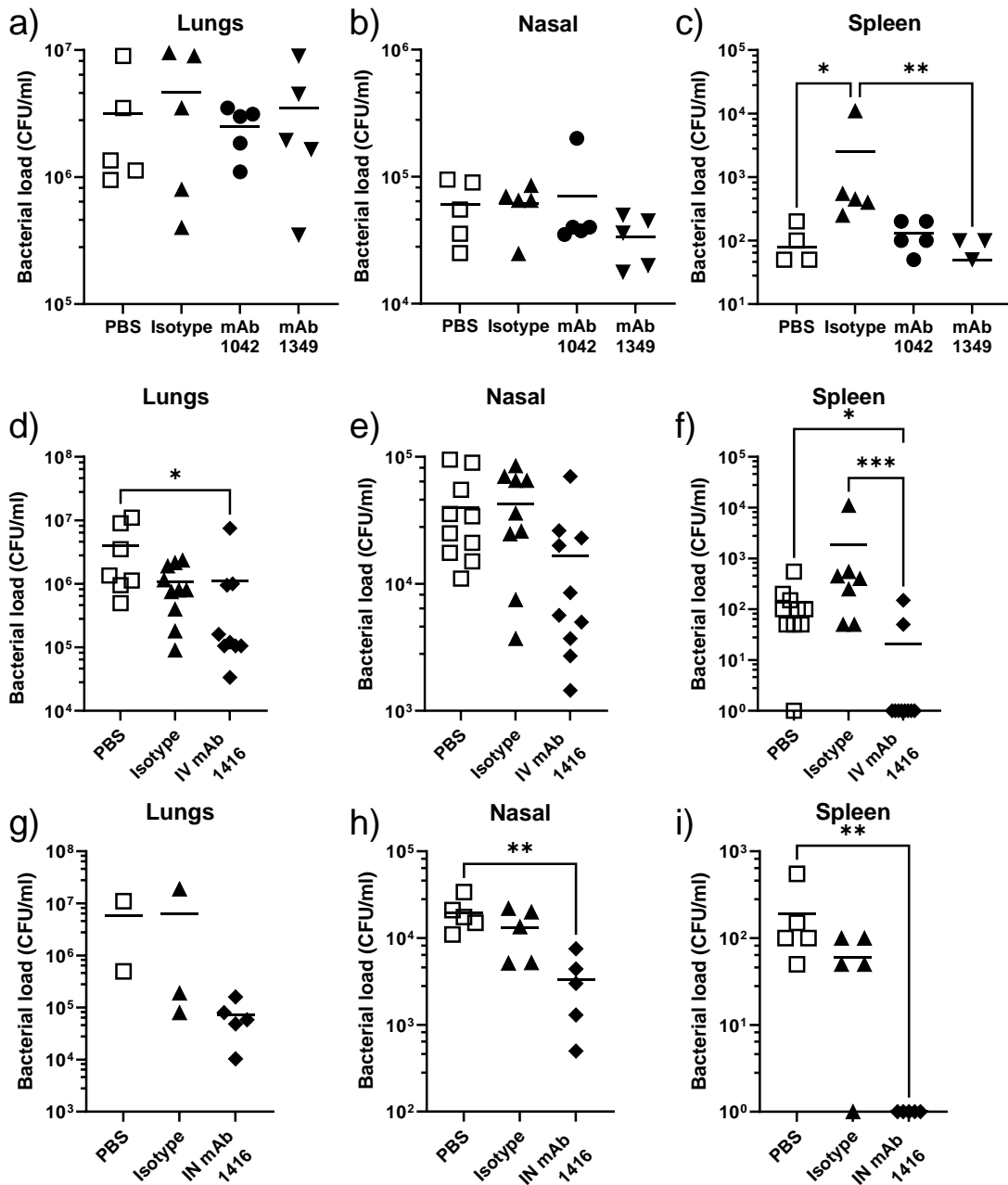


Figure 5. Ability of mAbs to protect in vivo against *A. baumannii* BAL 191. Adult BALB/c mice were intravenously dosed with 10 mg/kg of 1042, 1349, isotype control mAb or left untreated on day -1 and day 0, challenged on day 0 with 5×10^7 CFU *A. baumannii* isolate BAL 191 and culled 24 hours later. Bacterial loads at 24 hours in lungs (a), nasal wash (b) and spleen (c) were enumerated to evaluate protection. In a subsequent study, mice intravenously or intranasally dosed with 10 mg/kg of 1416 or isotype control were similarly evaluated for protection using the KL49 isolate BAL 191. Bacterial loads at 24 hours in lungs and spleen of intravenously dosed (d, e, f) and intranasally dosed mice (g, h, i) were evaluated. Statistical analysis was performed using a Mann-Whitney test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. The data presented in panels a-c are a single $n=5$ study; the data presented in panels d-f represent 2 experiments combined ($n=5$ per study, $n=10$ total); panels g-i represent a single $n=5$ study.

We would like to propose the following edits to the results section to add additional clarity.

Assessment of target engagement for prophylactic protection in vivo

We tested selected mAbs for *in vivo* efficacy in a pre-exposure prophylaxis intranasal mouse challenge model using BAL 191. This challenge model permitted the evaluation of effects of administering mAbs via intravenous (IV) or intranasal (IN) routes on bacterial burden in the upper and lower respiratory tract as well as systemic spread of bacteria to the spleen in mice 24 hours after infection. Compared with untreated mice or mice treated with an isotype control mAb, we did not observe any reduction in BAL 191 bacterial burden in the lungs (Fig 5a) or nasal wash (Fig 5b) of mice treated with 10 mg/kg mAb 1042 targeting OCL1 or mAb 1349 targeting Oxa-23 delivered IV. There was a significant reduction in bacteria recovered from the spleen (Fig 5c). The administration of an IV dose of mAb 1416 of 10 mg/kg reduced bacterial burden in the lung compared to untreated mice (Fig 5d) but not the nasal wash (Fig 5e). There was a significant reduction in the spleen compared to untreated or mAb isotype control mice (Fig 5f). Intranasal administration of 10 mg/kg mAb 1416 mice did not reduce bacterial burden in the lungs compared to untreated mice or isotype control mice (Fig 5g). But it reduced it in the nasal wash (Fig 5h) and provided complete protection from bacterial dissemination to the spleen after challenge (Fig 5i).

Also they still have not shown how many different *Acinetobacter* strains the mAb 1416 would bind, how strain specific is the target and or how common is this KL49 producing *Acinetobacter* strain type.

Figure 3 shows binding of mAb 1416 across carbapenem-resistant *Acinetobacter baumannii* (CRAB) strains isolated in ICUs from three tertiary care hospitals in Vietnam. We show that mAb 1416 clearly binds strains producing KL49 capsule and not strains producing KL2, 3, 6, 10, 30, 31, 32, 40, 52 or 58 capsule subtypes represented in the panel. KL49-producing CRAB is highly represented in this panel (31% of strains) and could be indicative of a recent outbreak (we indicate this in Fig 6a of the manuscript). Literature indicating KL49 associated with CRAB outbreaks and enhanced virulence across multiple continents is accumulating (as discussed in the manuscript) but we appreciate that clinical acknowledgement of KL49-associated virulence is fairly recent as indicated in this recent publication

<https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2024.1351722/full>

It is my understanding that there are 100 distinct capsules. Is this beyond that one ICU a common capsule type? It is my understanding that Of these 100 capsule types, the most common three are KL6, KL10, and KL47, which altogether occur in more than 10% of all nosocomial infections based on Rosesathorn Soontarach et al 2022 does the mAb not bind to any other capsule type?

Based on the clinical strain panel we used, KL6 and KL10 were represented (9% and 7% of all CRAB strains tested). KL47 was not represented in our panel, although the Soontarach et al 2020 (PMID: 32328045) study was based on three tertiary hospitals in Thailand and this may represent geography-specific phylogenetics. Please see above to address the question on whether mAb 1416 binds other capsule types.

In summary, and as defined in *Infect Genet Evol.* 2022 Jan;97:105148, *Infect Drug Resist.* 2020 Nov 12;13:4125-4132 and *Clin Infect Dis.* 2018 Nov 13;67(suppl_2):S179-S188, *Acinetobacter baumannii* producing KL49 are a major cause of mortality in Asia. Whilst we agree that this is not a broadly protective antibody against CRAB strains, it has major potential against a potentially hypervirulent variant. We also want to stress the feasibility of this approach for the identification of such antibodies and would like to highlight that this procedure has identified the major polysaccharide to be a target for inducing protection and we should ultimately aim to identify cross reactive polysaccharide epitopes to prevent infections with these untreatable pathogens.

