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Candidozyma auris-infected *Galleria mellonella* larvae: the effect of the humanized monoclonal antibody Dia-T51 and its synergy with amphotericin B

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Fungal infections pose a major worldwide health risk, as newly emerging pathogens and the increasing spread of drug resistance are now severely challenging current therapeutic and diagnostic tools. *Candidozyma auris* embodies both of these aspects as it is an urgent threat that is resistant to several antifungal classes. The lack of effective drugs is driving the development of innovative strategies, among them monoclonal antibodies. This study investigates the in vivo and ex vivo safety and efficacy of the humanized monoclonal antibody Dia-T51, administered alone or in combination with amphotericin B (AMB), in *Galleria mellonella* model. Against *C. auris* infection, Dia-T51 demonstrated good therapeutic efficacy and provided near-complete protection when used prophylactically. The synergy between Dia-T51 and AMB observed in vitro was confirmed in vivo, resulting in enhanced larval survival and a significant reduction in fungal burden. The results strengthen the profile of Dia-T51 as a promising biological agent against fungal infections both for its standalone potential (via direct inhibition and immune-mediated mechanisms) and for its ability to amplify the efficacy of conventional antifungals.

The International Society for Infectious Diseases updated the alarming numbers related to the global burden of fungal infections in September 2024. Public healthcare every year has to face 6.5 million cases of invasive fungal infections with less than half of patients achieving a positive recovery[1]. 2.5 million of the 3.8 million infection-related deaths are attributed to fungal pathogens, even in the absence of underlying comorbidities. Among mycoses candidiasis encompasses cutaneous, mucosal, deep-seated organ, and bloodstream infections (candidemia), with the latter being the most alarming and mortal form with about 1,565,000 cases and 995,000 deaths (63.6%) annually[1,2]. While *Candida albicans*, *Nakoseomyces glabratus*, *C. tropicalis*, *C. parapsilosis*, and *Pichia kudriavzevii* have historically predominated, a new specie, *Candidozyma auris*, has emerged and rapidly spread worldwide. *C. auris* is now classified by the Centers for Disease Control and Prevention (CDC) as the foremost fungal pathogen representing a serious global health threat, and is listed by the World Health Organization (WHO) among its Critical Priority Pathogens[2–4]. *C. auris* is notorious for causing critical nosocomial outbreaks, particularly in intensive care units, by colonizing both abiotic and biotic surfaces. Its lethality is extreme, with reported 30- and 90-day mortality rates following bloodstream infection reaching 30.1% and 44.6%, respectively[5]. The primary concern surrounding this species is its multidrug- and pandrug-resistance affecting all major classes of antifungal drugs[5,6]. Despite recognizing this potential pandemic threat, the progression of fungal infections remains difficult to halt due to several bottlenecks. These include scarce data, unreliable identification, and methodological issues undermining diagnostic procedures. Similarly, therapeutic options are severely limited by the poor antifungal arsenal, the increasing resistance phenomenon, and the difficulties in developing non-toxic novel agents[7]. In the hunt for innovative strategies, immunotherapy targeting conserved fungal structures has emerged as a promising frontier. A pivotal advancement in this field was the characterization of the murine monoclonal antibody (mAb) 2G8, generated against β-1,3-glucans. Since β-1,3-glucan is a ubiquitous and essential component of the fungal cell wall absent in mammalian cells, it represents an ideal selective target[7,8]. Extensive preclinical studies demonstrated that 2G8 possesses intrinsic antifungal activity, inhibiting fungal growth and adherence, and conferring significant protection in murine models of systemic candidiasis, aspergillosis, and cryptococcosis[9–13]. However, the clinical translation of murine antibodies is inherently restricted by their immunogenicity, which risks eliciting Human Anti-Mouse Antibody (HAMA) responses, and by their suboptimal interaction with human effector systems.

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To overcome these barriers and unlock the therapeutic potential of the 2G8 paratope, we developed and characterized its humanized IgG_1 derivative, the humanized monoclonal antibody (hmAb) Dia-T51 (previously designated as H5K1)[14,15]. Dia-T51 surpasses the already high-affinity and specificity of the parental clone for β-1,3-glucans (IC_{50} Dia-T51 0.06 μg/ml vs IC_{50} 2G8 0.12 μg/ml) while possessing a human Fc region to ensure safety and effective recruitment of host immune mechanisms. Previous work demonstrated that Dia-T51 efficiently controls and resolves *C. auris* infections in vitro, both alone and by enhancing phagocytosis in the presence of monocyte-derived macrophages[15]. Furthermore, it showed an additive effect with echinocandins and synergy with amphotericin B (AMB)[15,16]. However, the precise combinatorial parameters required to maximize therapeutic efficacy while minimizing drug exposure remained undefined. Therefore, this work aims to identify the optimal concentration ratio and assess the feasibility and efficacy of Dia-T51 and its combination with AMB in an in vivo setting. We used *Galleria mellonella* larvae as a model to produce candidemia caused by *Candidozyma auris*. Infected insects were treated with the hmAb in single and repeated doses, both therapeutically and prophylactically, and finally, in combination with AMB.

Results

In vitro synergistic activity of Dia-T51 and amphotericin B

To observe the effect of Dia-T51 and amphotericin B (AMB) as monotherapies and in combination and to assess the optimal combinatorial regimen in vitro, a checkerboard microdilution assay was performed against *C. auris* (Fig. 1). This analysis allowed us to pinpoint the specific concentrations where efficacy is maximized. Consistent with its mode of action, Dia-T51 monotherapy displayed low direct inhibitory activity precluding the determination of MIC_{50} or MIC_{90}. Conversely, AMB exhibited potent activity with a MIC_{90} of 0.25 μg/ml. The combinatorial treatment yielded a pronounced synergistic effect: the MIC_{90} of AMB decreased 4-fold to 0.063 μg/ml in the presence of 0.78 μg/ml Dia-T51. The calculated Fractional Inhibitory Concentration Index (FICI) was 0.256, classifying the interaction as synergistic (FICI ≤ 0.5). To evaluate the fungicidal effect, aliquots from optically clear wells were plated on PDA agar. While AMB monotherapy achieved a Minimal Fungicidal Concentration (MFC) of 1 μg/ml, the combination demonstrated complete killing at the significantly reduced AMB concentration of 0.125 μg/ml when combined with 3.3 μg/ml of Dia-T51. Given that antibody efficacy is often dependent on host effector

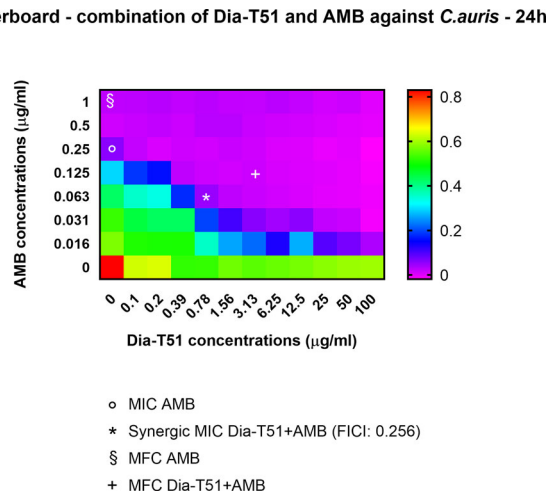


Fig. 1 | Synergistic interaction between Dia-T51 and amphotericin B against *C. auris*. Checkerboard heatmap displaying fungal growth density, ranging from red (maximum growth/control) to violet (complete inhibition/clear wells). Axes represent increasing concentrations of Dia-T51 and amphotericin B. Symbols denote: the minimum inhibitory concentration (MIC_{90}) of AMB (o) and of the combination Dia-T51-AMB (*), and the Minimal Fungicidal Concentration (MFC) of AMB (§) and of the combination Dia-T51-AMB (+).

mechanisms, we utilized the *Galleria mellonella* infection model to validate this synergy in vivo and assess whether the presence of innate immune cells enhances Dia-T51 activity.

Dia-T51 and amphotericin B are not toxic for *Galleria mellonella* larvae

To ensure the viability of the *Galleria mellonella* model, the safety and toxicity of the compounds (Dia-T51 and amphotericin B) and the solvents (PBS and DMSO) were assessed. None of the tested doses of Dia-T51, 1 and 10 mg/kg, demonstrated significant toxicity with a survival rate of 81.8% observed in larvae. The low level of recorded mortality was likely attributable to the advanced observation timepoints (100 and 120 h) and potential injection-related stress, as controls injected with Phosphate-Buffered Saline (PBS) in other studies exhibited full survival (Fig. 2a). Similarly, AMB displayed a favorable safety profile across the three tested concentrations, 50, 5, and 0.5 mg/kg. While the highest dose (50 mg/kg) resulted in an 81.9% survival rate, the two lower concentrations yielded survival rates exceeding 90%. Finally, DMSO tested at the same percentage used for AMB dilution, was non-toxic, with a survival rate of 90.9% (Fig. 2b). These results confirm the safety of the administered doses for use in subsequent in vivo efficacy experiments.

Dia-T51 increases the survival of infected larvae up to 50%

The two non-toxic doses of Dia-T51 previously evaluated in the safety assessment (1 and 10 mg/kg) were used to determine the hmAb efficacy against a lethal *C. auris* infection burden and in the presence of the larval immune system. While all infected and untreated larvae died within 78 h (Median Survival Time, MST: 39 h), treatment with Dia-T51 significantly increased the survival rate and prolonged the MST (Fig. 3a, b). Larvae treated with the hmAb at both doses survived for up to six days. 10 mg/kg resulted in 50.5% survival, with an MST of 138 h. 1 mg/kg yielded a survival rate of 41.7% and an MST of 123 h. Statistical analysis confirmed a significant difference in the overall survival rate and in the MST ANOVA results, Kruskal–Wallis statistic: 14.42, *P* value 0.0007) between both treated groups (1 and 10 mg/kg Dia-T51) and the infected, untreated control group (*C. auris*).

Dia-T51 prophylaxis almost completely recovered the survival of *C. auris* infected larvae

Since the difference in survival between the 10 mg/kg and 1 mg/kg doses of Dia-T51 was marginal, the 1 mg/kg dose was selected for subsequent experiments to maximize its efficiency. We compared the effect of single and double dose regimens in both therapeutic and prophylactic settings.

The comparison between one and two doses applied as post-infection treatments (Fig. 4a) confirmed previous findings: a single 1 mg/kg dose was effective, resulting in 57.1% survival (MST: >150 h, Fig. 4c). The double dose regimen resulted in a slightly decreased survival rate of 42.9% at 144 h (MST: 132 h). Statistical analysis determined that the double dose regimen was not statistically significant in increasing either the survival rate or the MST, both when compared to the single dose treatment and the infected, untreated control group.

The efficacy of Dia-T51 dramatically increased when administered prophylactically. Both the single prophylactic dose and the double regimen (prophylaxis followed by treatment) provided almost complete protection against *C. auris* infection. The single prophylactic dose achieved 92.3% survival (MST: >150 h), and the double regimen resulted in 84.6% (MST: >150 h) survival at 144 h. Significantly, both the single and double prophylactic regimens demonstrated a statistically significant increase in both MST ANOVA results, Kruskal–Wallis statistic: 12.22, *P* value 0.0022) and overall survival rate when compared to the infected, untreated control group (Fig. 4b, d). Prophylactic administration also protected the larvae from hyperinflammation, as evidenced by their light color compared to the brownish color of simultaneously infected controls (Fig. 4e).

In both therapeutic and prophylactic trials, the double-dose regimen resulted in a slightly lower overall survival rate compared to the respective

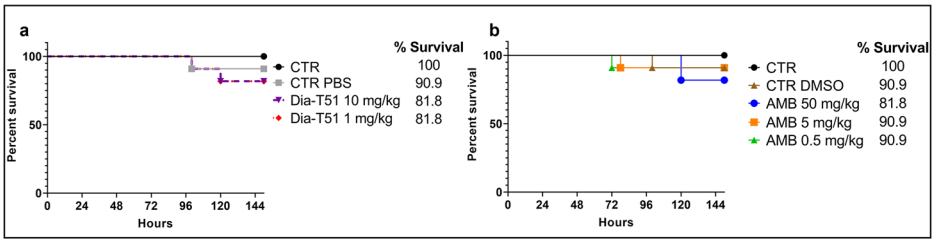


Fig. 2 | In vivo acute toxicity assessment of Dia-T51 and amphotericin B in *Galleria mellonella* larvae. Survival curves demonstrate the safety profile of **(a)** Dia-T51 at two concentrations and **b** Amphotericin B (AMB) at three concentrations over time. CTR represents uninjected control larvae, used to evaluate the overall fitness and natural mortality of the *G. mellonella* model. CTR PBS and CTR DMSO indicate control groups injected solely with the respective solvents (phosphate-buffered saline and dimethyl sulfoxide) DMSO seved to dissolve and initially dilute AMB. It was used at 2.5% (v/v), the same percentage of all the AMB groups. PBS was used for the solubilization and dilution of Dia-T51 and for the final dilution of AMB. Experiments were repeated three times with $n = 5–8$ larvae per group based on supplier availability. Each independent experiment included the full panel of injected groups. The only exception is represented by the uninjected control group which is common to both panels as experiments were performed concurrently. Statistical analysis revealed no significant differences.

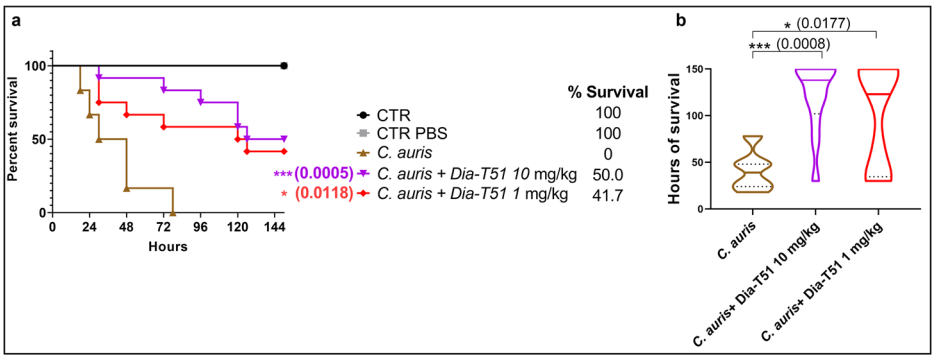


Fig. 3 | Therapeutic effect of Dia-T51 in *Galleria mellonella* larvae infected with a lethal dose of *C. auris*. $3*10^6$ ($\pm 2.83*10^6$ SD) CFU-infected larvae were treated with a single injection of two different doses of Dia-T51. Two aspects were considered: the survival rate (**a**) and the survival time (**b**). CTR and CTR PBS are uninfected control groups with the former being uninjected to assess larval fitness and the latter injected with PBS used for the antibody dilution and inoculum suspension. In (**a**), Kaplan–Meier curves are shown, while in b, violin graphs illustrate the distribution of larval survival time (in hours), showing the median (continuous line) and first and third quartiles (dotted lines). The presented data are derived from four independent experiments ($n = 6$ larvae per group) and were statistically checked to exclude batch effects. All treatment and control groups were assessed simultaneously in every replicate. One-Way ANOVA – non-parametric Kruskal–Wallis test was used. Post-hoc Dunn's multiple comparisons test was performed comparing the mean rank of the groups with the mean rank of the infected, untreated group (*C. auris*). AMA *P* value style was chosen: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$. *P* values are reported in brackets in (**a**) and (**b**).

single-dose regimen. We hypothesize that the mechanical trauma associated with the second injection negatively impacts larval viability. Despite this, from a visual inspection there was no difference among uninfected groups (Supplementary Fig. S1 in Supplementary Information) and the MSTs for the single and double prophylactic regimens were not significantly different (Fig. 4c, d), confirming the sustained and potent protective effect of Dia-T51.

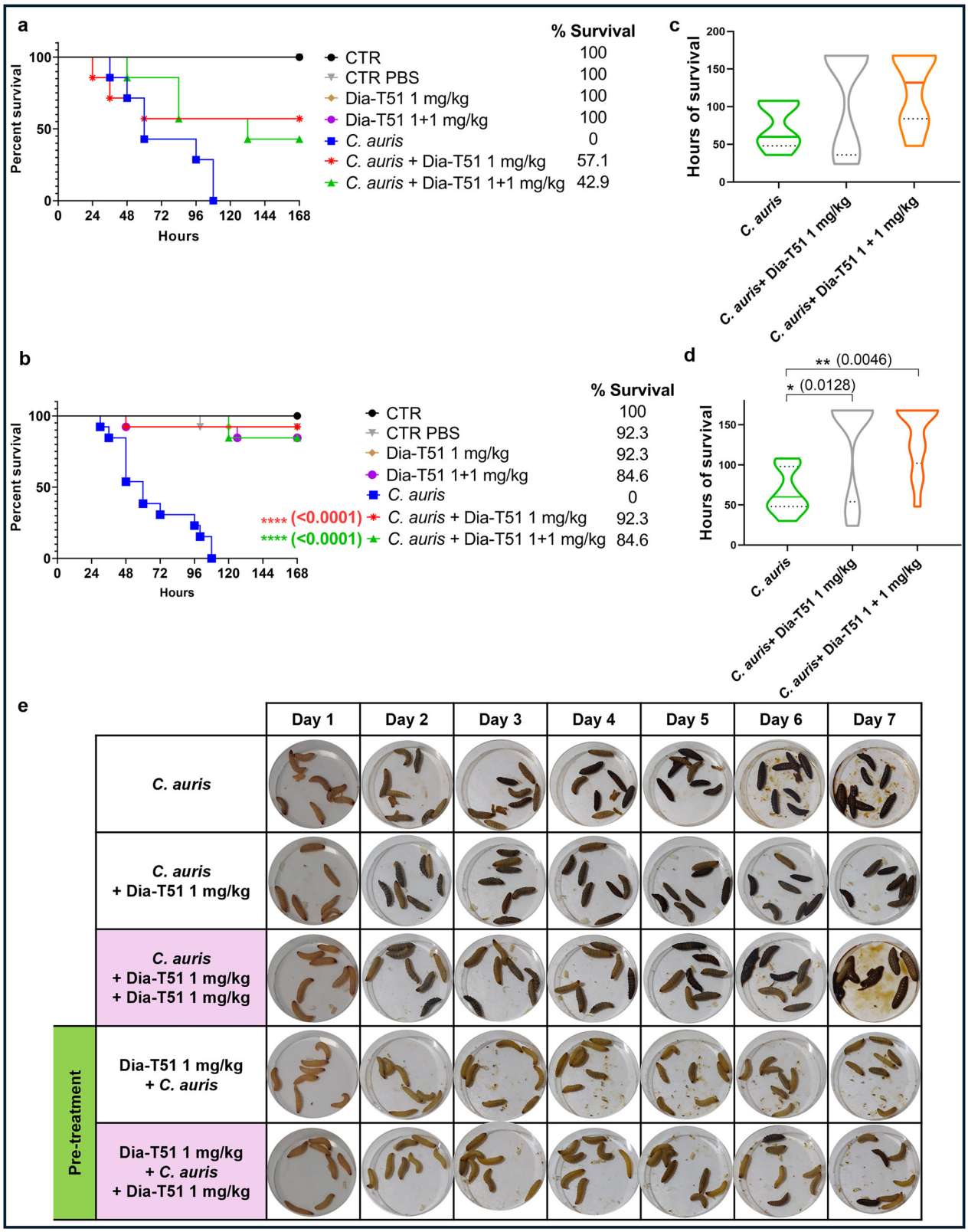
Dia-T51 potentiates the efficacy of sub-therapeutic amphotericin B dose in vivo

The activity of amphotericin B monotherapy was initially assessed against *C. auris* infection. AMB exhibited a clear dose-dependent efficacy, yet its protective effect rapidly diminished with lower concentrations. While the highest tested concentration (50 mg/kg) resulted in a statistically significant 42.8% larval survival rate at 180 h, this activity decreased substantially with 5 mg/kg (28.6% survival) and was completely absent at the lowest dose of 0.5 mg/kg (0% survival) (Fig. 5a). In parallel, Dia-T51 monotherapy at both 10 mg/kg and 1 mg/kg concentrations yielded a 180-h survival rate of 14.3%. Combining Dia-T51 with the ineffective AMB dose of 0.5 mg/kg a remarkable potentiation of antifungal activity was demonstrated (Fig. 5b, c). When AMB was combined with Dia-T51 at 10 mg/kg and 1 mg/kg, the 180-h survival rates reached 71.4% and 28.6%, respectively. Notably, the AMB/Dia-T51 0.5/10 mg/kg combination achieved a survival rate that significantly surpassed the efficacy of the highest AMB concentration used as a single agent. Statistically, this combination demonstrated a significant increase in survival rate when compared to both the infected, untreated group (*C. auris*) and the ineffective concentration of AMB administered alone (0.5 mg/kg). Of note, also the effect of AMB/Dia-T51 0.5/1 mg/kg, which is comparable to that of 5 mg/kg of AMB.

Analysis of the full-time course confirmed the superior protective effect of the combination (Fig. 5e). At 96 h, the AMB/Dia-T51 0.5/10 mg/kg combination maintained 100% survival, dramatically outperforming Dia-T51 alone at 10 mg/kg (57.1%) and AMB alone at 0.5 mg/kg (14.3%). Concurrently, AMB/Dia-T51 0.5/1 mg/kg survival rate was 42.9%, significantly higher than the antibody monotherapy at 1 mg/kg (28.6%).

This sustained effect was maintained at 120 h for the combinations (85.7% and 42.9% survival when hmAb was 10 and 1 mg/kg, respectively), whereas the protective effect of AMB monotherapy at 0.5 mg/kg was null and at its highest dose (50 mg/kg) decreased rapidly, dropping from 85.7% at 96 h to 57.1% at 120 h.

Analysis of the mean survival time confirms the synergistic efficacy of Dia-T51. The combination of the sub-therapeutic AMB dose (0.5 mg/kg) with Dia-T51 (10 and 1 mg/kg) resulted in a maximal MST > 168 h and of 78 h, respectively. Both values surpass those of the single agents (AMB 0.5 mg/kg: 54, Dia-T51 10 mg/kg: 120 and Dia-T51 1 mg/kg: 48). Although a statistically significant difference was calculated only in comparison with the infected, untreated group ANOVA results, Kruskal–Wallis statistic: 22.11, *P* value 0.0024), MST of the combination AMB/Dia-T51 0.5/10 mg/kg is even higher than that of the highest-dose AMB monotherapy (150 h), directly demonstrating the potentiation of AMB's efficacy by Dia-T51.



Ex vivo fungal burden analysis confirms potentiation by Dia-T51

The synergistic effect of Dia-T51 towards amphotericin B was further confirmed ex vivo by quantifying the fungal burden in the *Galleria mellonella* hemolymph via plating on PDA plates (Fig. 6). Compared to the initial injected fungal load (0 h), all tested monotherapy and combination regimens resulted in a statistically significant reduction in *C. auris* colony-forming units (CFUs) at both 3- and 24-h post-treatment. Monotherapy trends presented a general decrease in effectiveness, starting earlier for Dia-T51 and later for AMB.

The combinations demonstrated a massive and superior initial fungicidal effect within 3 h[17–19], consistently exceeding the CFU reduction achieved by either Dia-T51 and AMB monotherapies at all observed

Fig. 4 | Therapeutic and prophylactic efficacy of Dia-T51 against *C. auris*. Comparative analysis of single and double dose regimens (1 mg/kg and 1 + 1 mg/kg, respectively) of Dia-T51 against $3*10^6$ ($\pm 2.83*10^6$ SD) CFU-infected *Galleria mellonella* larvae. In the therapeutic regimens (**a**, experiment repeated in triplicate, $n = 5–7$ larvae per group based of the supplier availability) Kaplan–Meyer survival curves are reported to compare the single and double dose administered after fungal inoculation. In the prophylactic settings (**b**, experiment repeated four times, $n = 5–7$ larvae per group) Kaplan–Meyer curves indicates the survival of insects when administered 24 h before infection (single dose, 1 mg/kg) and administered 24 h before infection and 3 h post-infection (double dose, 1 + 1 mg/kg). In survival time analysis (**c, d**) violin plots illustrates the distribution of larval survival time (in hours) for the therapeutic regimen (**c**) and the prophylactic regimen (**d**). Plots show the median (continuous line) and first and third quartiles (dotted lines). The results are cumulative of the independent replicates, which were statistically verified for consistency to exclude batch effects. All groups were included in every independent experimental run. Larval phenotype (**e**) is visible in images of a representative experimental replicate demonstrating the course of infection and inflammation. CTR is the uninjected control group, CTR PBS is the group injected once with PBS, solvent used for antibody dilution and inoculum suspension. One-Way ANOVA – non-parametric Kruskal–Wallis test was used. Post-hoc Dunn's multiple comparisons test was performed comparing the mean rank of the groups with the mean rank of the infected, untreated group (*C. auris*). AMA *P* value style was chosen: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$. *P* values are reported in brackets in (**b**) and (**d**).

timepoints and statistically significative at 3 h. While no statistically significant difference in CFU numbers was observed among all treated groups at 48 h, analysis of the Log variation highlighted a particular phenomenon: the well-known fungicidal effect of AMB transitioned into a prolonged fungistatic effect at that concentration. This was evidenced by microbial growth at 48 h that surpassed the growth rate observed in the untreated control, potentially suggesting a hormesis effect (Supplementary Tables S1, S2 and S3 in Supplementary Information).

Macroscopic monitoring (Supplementary Fig. S2 in Supplementary Information) provided visual evidence correlating fungal clearance with the host's inflammatory response, where black spots indicate intense immune activation based on the production and deposition of melanin onto the pathogen. AMB-treated and untreated larvae showed hyperinflammation at 3 h, which temporarily decreased at 24 h but rebounded at 48 h, coinciding with the observed loss of AMB's protective in vivo efficacy. Conversely, Dia-T51 appeared to positively influence the inflammatory burden or promote alternative clearance mechanisms. By 48 h, larvae treated with the combinations showed rapid recovery, returning to a near-healthy state with few or no black spots. In contrast, melanization persisted longer in larvae treated with Dia-T51 monotherapy. This differential recovery suggests that the combination regimen facilitates a more effective and rapid resolution of systemic infection and inflammation.

Discussion

The *Galleria mellonella* larvae model provides a robust and rapid in vivo system widely utilized for simulating microbial infections, assessing compound toxicity, and determining antimicrobial efficacy. Its advantages, including the absence of ethical issues, low cost, and relatively brief experimental window, establish it as a convenient and reliable tool. This system has demonstrated a strong correlation with infection outcomes observed in mammalian models, validating its use as a scientifically acceptable alternative to higher animals, especially when screening novel therapeutic agents[20]. A unique advantage of the *G. mellonella* model for this specific study lies in the absence of an adaptive immune system. This characteristic simplifies the complexity of in vivo testing, ensuring that the humanized monoclonal antibody (hmAb) Dia-T51 acts as the sole source of immunoglobulin within the animals. This isolation allows us to provide unambiguous evidence of the hmAb's efficacy based purely on its interaction with the larval innate immune system without interference from endogenous host antibodies. The innate immune system of *G. mellonella* exhibits several functional parallels with that of humans. Larval defense mechanisms rely on hemocytes (analogous to leukocytes), including plasmatocytes and granular cells that mediate phagocytosis and nodule formation and oenocytes, which contain mediators of the phenoloxidase cascade leading to melanization[21,22]. The humoral response rather involves melanin, complement-like proteins, and antimicrobial peptides (AMPs). Antifungal immunity is mainly triggered by Pattern Recognition Receptors (PRRs) that recognize fungal cell wall components, such as β-1,3 glucans. Activation of these pathways leads to the production of pro-inflammatory mediators (e.g., cytokines, eicosanoids, AMPs) that promote hemocyte migration, phagocytosis, and the key fungicidal process of melanization[23]. Phagocytic granulocytes and lipid-metabolizing oenocytes encircle the fungal pathogen restricting dissemination and replication. Nodules become centers of inflammation and cytotoxic mediators. There, the secretion of melanin acts as a physical barrier depriving fungal cells of gas and nutrients[24]. Melanization, driven by the phenoloxidase cascade, is not only a fundamental defense mechanism but also serves as a vital macroscopic indicator for monitoring the host's inflammatory state and the infection course[25].

Even if in literature *Galleria mellonella* larvae were infected with a wide range of microbes, yet few works have used *Candidozyma auris* as etiological agent[26]. For this reason, before safety and efficacy testing, the lethality of *C. auris* infection was defined in order to ensure total mortality within a few days but sufficient time for treatment evaluation.

Dia-T51 is the humanized derivative of the murine mAb 2G8. The decision to humanize the existing clone rather than generating de novo antibodies relied on its well-characterized activity and specific epitope recognition, a strategy that reduced development costs and timelines. Initiating a new discovery pipeline would have delayed clinical translation without guaranteeing the identification of a target epitope with equivalent biological relevance. Thus, this approach mitigates the immunogenicity risk and the incompatibility with human recycling and effector systems, paving the way for future clinical advancement. The resulting hmAb Dia-T51, demonstrated superior binding affinity compared to the parental antibody (IC_{50}: 0.06 μg/ml vs 0.12 μg/ml) and proved effective against *C. auris* in vitro[14–16]. These promising results prompted the assessment of its feasibility and efficacy in vivo.

As the first study describing the use of a humanized monoclonal antibody in *Galleria mellonella* larvae, we established that Dia-T51 is well-tolerated at doses of 10 mg/kg and 1 mg/kg, with observed larval deaths attributed solely to natural longevity.

In therapeutic settings, Dia-T51 achieved substantial survival rates (50.5% at 10 mg/kg and 41.7% at 1 mg/kg at 144 h). However, the similarity of these outcomes suggests the absence of a linear dose-dependent survival rate above a critical concentration. This leads to the hypothesis of a saturation effect, likely related not to the antibody's antigen (the fungal β-1,3 glucans) but rather to the limited number of larval hemocytes available for activation and clearance. Despite similar end-point survival rates, the Median Survival Time (MST) was significantly longer for the 10 mg/kg dose in both pathogenic burdens. This difference suggests that while saturation may occur, the higher dose provides a prolonged reservoir of active hmAb, counteracting the rapid consumption of the antibody during pathogen eradication and ultimately extending the time of survival. To further explore dose dynamics, the lower, yet highly effective, dose of 1 mg/kg was selected to compare single and double-dose regimens. The administration of a second dose post-infection did not positively impact on the 144-h survival rate and, in fact, led to a slight decrease. We hypothesize that the mechanical trauma associated with a second injection imposed additional stress, negatively affecting the already compromised health of the larvae. In stark contrast, when administered prophylactically (24 h pre-infection), Dia-T51 achieved an exquisite survival rate of over 90%, confirming its potent protective capacity. The administration of a second dose in the prophylactic regimen (3 h post-infection) also resulted in a slightly reduced survival percentage, further supporting the stress/trauma

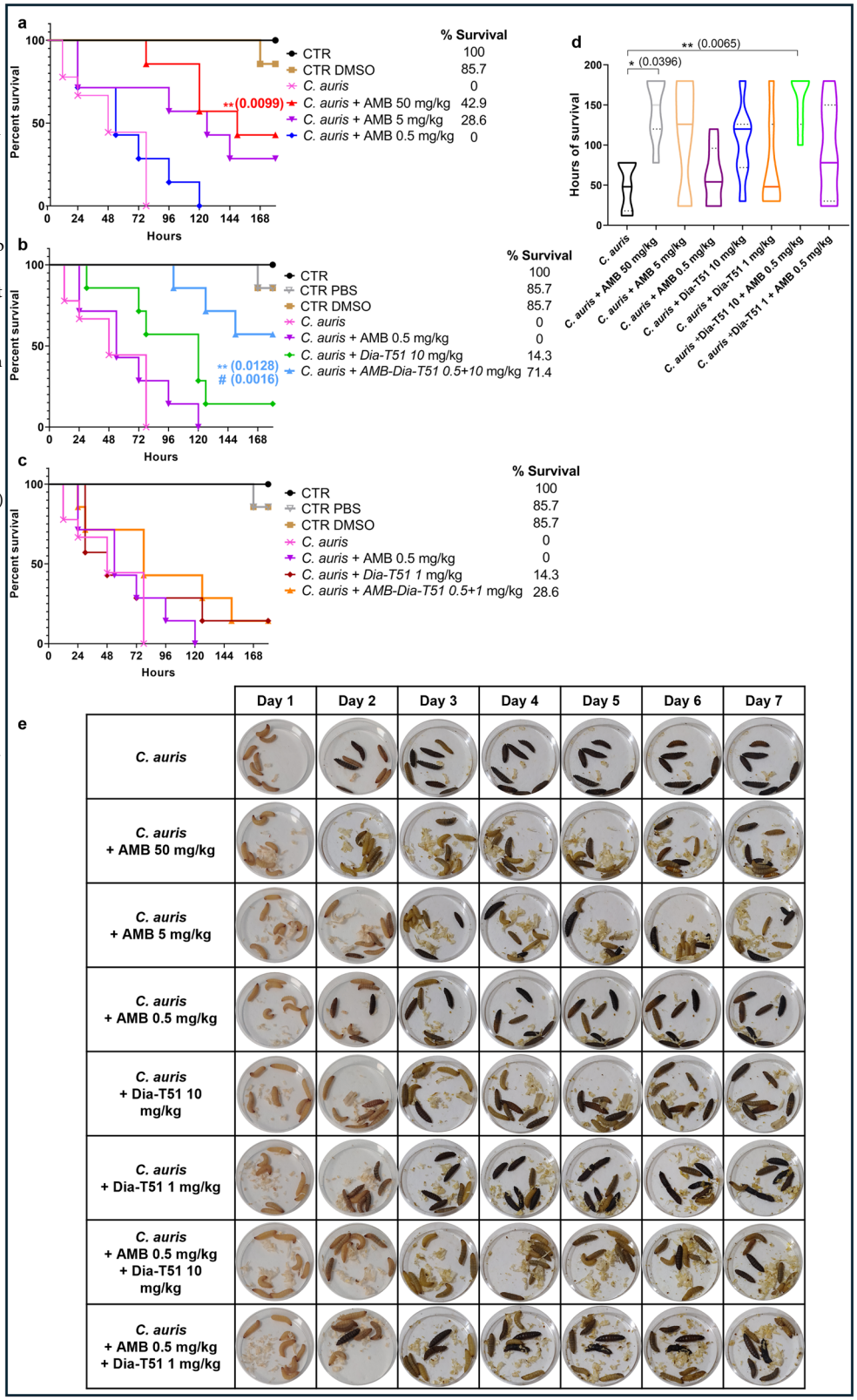


Fig. 5 | Synergistic efficacy of Dia-T51 and sub-therapeutic amphotericin B (AMB) against *C. auris* infection. Comparative analysis of monotherapy and combination therapy regimens against $6.5*10^6$ ($\pm 2.12*10^6$ SD) CFU/mL-infected *Galleria mellonella* larvae (experiment repeated in triplicate $n = 5–7$ larvae per group based on supplier availability). **a–c** Kaplan–Meyer survival curves comparing AMB monotherapy (50, 5, and 0.5 mg/kg), Dia-T51 monotherapy (10 and 1 mg/kg), and the synergistic combination of the sub therapeutic AMB dose (0.5 mg/kg) with both Dia-T51 concentrations. Survival curves represent results from the same experimental triplicates. Data are stratified across panels (**a–c**) to enhance the readability of synergistic interactions. **d** Violin plots illustrate the density of mortality events (in hours) for the infected groups. Results represent pooled data from independent experiments; no significant batch effects were detected. All groups were included in every independent experimental replicate. Plots show the median (continuous line) and first and third quartiles (dotted lines). One-Way ANOVA – non-parametric Kruskal–Wallis test was used, followed by post-hoc Dunn's multiple comparisons test. "*" indicates statistically significant difference compared to infected untreated group (*C. auris*) whereas "#" refers to AMB at 0.5 mg/kg. AMA *P* value style was chosen: $*p < 0.05$ and $**p < 0.01$. *P* values are reported in brackets in panels (**a**), (**b**) and (**d**). **e** Macroscopic monitoring of the course of infection in key groups, allowing for visual assessment of immune system response and inflammation. *C. auris* group received only the injection used to establish the infection. CTR is the uninjected control group; CTR PBS and CTR DMSO are the control groups injected respectively with PBS and DMSO diluted in PBS at the same percentage used for AMB doses preparation.

hypothesis. These prophylactic results, coupled with the minimal melanization observed, suggest two potential mechanisms: (i) Dia-T51 actively controls immune system overstimulation, preventing inflammatory overburden that could damage host tissues; or (ii) Dia-T51 promotes or accelerates alternative defense mechanisms that reduce the overall contribution of the melanization cascade. Given previous in vitro data demonstrating a protective effect on immune cells[15], we favor the hypothesis of the hmAb modulating or enhancing innate immune cell function, but further dedicated studies are required to confirm this mechanism firmly. In both regimens the administration of a second dose prolonged the MST supporting the rationale of a rapid clearance of the antibody.

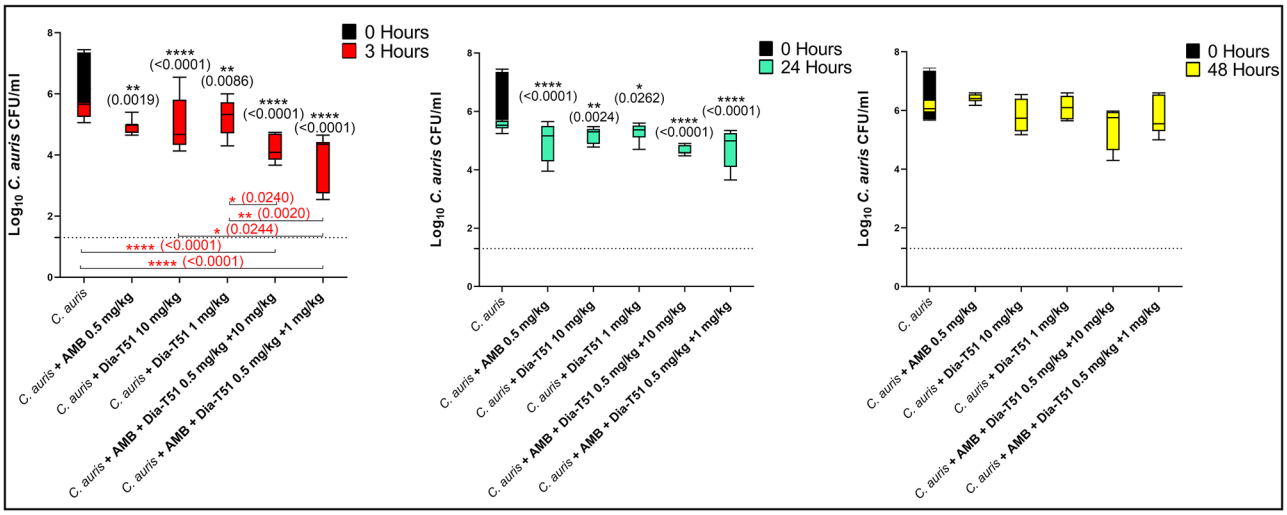


Fig. 6 | Kinetics of fungal burden reduction following monotherapy and combination therapy. Quantification in Logarithmic scale of *C. auris* colony-forming units (CFUs) in the hemolymph extracted from *G. mellonella* larvae at 0-, 3-, 24-, and 48-h post-infection (injected inoculum: $9.4*10^6$ ($\pm 1.17*10^7$ SD CFU/ml) and plated on PDA plates and eye-counted after incubation at 30 °C. Statistical analysis was performed using Two-Way ANOVA – post-hoc Sidak's Multiple Comparisons test. Significance levels are based on AMA *P* value style: $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$ and are reported in brackets. Black asterisks refer to infected, untreated group (*C. auris*) at T0, red asterisks to infected, untreated groups (*C. auris*) of the respective timepoint. *P* values are reported in brackets. An uninfected control group was present in the experimental setting, but results are not reported in figure as no CFU were present in plates. The T0 infected, untreated group was reported at each timepoint as growth reference. The experiment was repeated four times ($n = 3$–4 larvae per group). Each independent experiment included the full panel of treatment and control groups to account for biological variations across different larval batches. Dotted lines represent the Limit of Detection (LOD).

This study also investigated the relationship between Dia-T51 and amphotericin B (AMB), following previous reports of synergy[15,16]. The in vitro data confirmed a potent synergistic effect (FICI: 0.256) and defined the optimal therapeutic ratio. Unlike previous preliminary studies, the concentrations where Dia-T51 maximizes AMB potency have been identified in vitro. This combination strategy resulted in a 4-fold reduction in the AMB MIC_{90} and an 8-fold reduction in the AMB concentration required for a fungicidal effect. This result is clinically pivotal: it suggests that combining low concentrations of Dia-T51 with AMB could allow for the use of subtoxic dosages of the polyene while maintaining – or even enhancing – fungicidal efficacy. Given the well-known dose-limiting nephrotoxicity of AMB, the ability of Dia-T51 to act as a "potentiator" represents a valuable strategy to widen the therapeutic window of this drug. Driven by these compelling findings, we sought to validate the translational potential of this combinatorial approach in the in vivo setting.

In vivo, AMB monotherapy showed clear dose-dependent efficacy but was completely ineffective at the 0.5 mg/kg concentration in *C. auris*-infected larvae. Therefore, AMB at 0.5 mg/kg was combined with Dia-T51 at the two doses of 10 and 1 mg/kg.

The remarkable results of the combinatorial test represent, to our knowledge, the first attempt at testing a small-molecule drug and an antibody in combination within the *G. mellonella* model. As expected, the 0.5 mg/kg AMB dose alone was ineffective. However, the combination with Dia-T51 resulted in a significant synergistic boost: the AMB/Dia-T51 0.5/10 mg/kg combination yielded 71.4% survival at 180 h with an undefined MST, surpassing both the survival rate (42.9%) and the MST (150 h) achieved by the highest AMB dose (50 mg/kg). Importantly, the contribution of Dia-T51 to the combination demonstrated a clear dose-dependent behavior in survival rate (28.6%) and mean survival time (78 h for the lower dose combo). Further evidence was provided by the ex vivo CFU analysis. The combinations exhibited a statistically significant initial fungicidal effect within 3 h, followed by an improved fungistatic long-term effect compared to monotherapies.

Our reliance on the *C. auris* type strain DSM 21092 (Clade II) ensures taxonomic standardization and establishes a baseline for intrinsic susceptibility, unconfounded by the variable resistance mechanisms typical of clinical outbreaks. Given the well-characterized pathogenicity of this strain in the *G. mellonella* model[26], these findings provide a necessary in vivo proof-of-concept. Validation against a broader panel of strains (including multidrug-resistant isolates) and the assessment of potential resistance development remain essential and mandatory prerequisites for future clinical translation.

Despite these promising outcomes, we acknowledge specific limitations inherent to the invertebrate model. *G. mellonella* is a powerful screening tool but does not fully replicate the complexity of mammalian patho/physiology. The lack of an adaptive immune system prevents the assessment of immunological memory, and the open circulatory system does not perfectly mimic mammalian organ perfusion or tissue-specific pharmacokinetics. Furthermore, this model represents an acute infection in an immunocompetent host, which may not fully capture the dynamics of *C. auris* infection in neutropenic or immunosuppressed patients. Finally, host-pathogen interactions and fungal dissemination patterns differ from those in higher vertebrates; the larva's simplified anatomical architecture - lacking complex organ systems found in mammals (e.g., lungs, kidneys, blood-brain barrier) - inevitably restricts the study of tissue-specific tropism and multi-organ failure typically observed in clinical cases[27].

However, regarding the evaluation of a humanized monoclonal antibody, the simplicity of the larval immune system offers distinct experimental advantages. First, the inability to generate host anti-Dia-T51-antibodies, eliminates a major confounding factor often observed in other immunocompetent animal models (neutralization or clearance). Second, while larval hemocytes share functional similarities with mammalian phagocytes, they lack the specific mammalian Fc receptors required for classic antibody recognition and signaling. This biological context suggests that the observed protective efficacy is primarily driven by the specific binding of the antibody to the fungal target, rather than by non-specific immunomodulation mediated by the Fc portion.

Mindful of the strengths and limitations of this study, it is crucial to note that the current landscape of antifungal therapy is severely challenged by the emergence of drug-resistant fungal species. The use of Dia-T51, either as monotherapy or in combination, offers a crucial, innovative strategy to prevent the uncontrolled spread of fungal infections and overcome conventional resistance pathways. By combining an antifungal drug with a novel immune-modulating agent, we offer a solution that can potentiate or

restore the efficacy of ineffective existing drugs against difficult-to-treat and multidrug-resistant fungal species like *C. auris*.

Our findings hold profound clinical implications. Given AMB's known nephrotoxicity, the ability of Dia-T51 to enhance AMB's fungicidal activity and extend its protective effect also in vivo – enabling the use of a sub-therapeutic AMB dose to achieve results superior to high, toxic doses – represents a major clinical breakthrough. This approach offers a viable solution to reduce side effects while enhancing therapeutic outcomes.

Methods

Material

The humanized monoclonal antibody Dia-T51 was kindly provided by Diatheva S.R.L. Amphotericin B was purchased from Sigma Aldrich. The reference strain of *Candidozyma auris* DSM 21092 was purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH and corresponds to the designated type strain of the species originally described by Satoh et al.[28]. Although belonging to the East Asian clade (Clade II) – hence characterized by a low antifungal resistance profile and not primarily associated with systemic infection – this strain was specifically selected to ensure taxonomic standardization and reproducibility. Its pathogenicity and suitability for the *Galleria mellonella* infection model have been previously validated[26], establishing it as a robust reference strain for in vivo efficacy screening. *Galleria mellonella* larvae were purchased from Big Game Fishing, Cattolica (RN), Italy ensuring direct control over life stage, feeding and exclusion of hormone and antibiotic exposure.

Checkerboard microdilution method

To assess the effect of Dia-T51, amphotericin B and their combination in vitro against *C. auris*, a checkerboard microdilution assay was performed[29]. Briefly, fungal suspensions (~10^5 CFU/mL in RPMI-MOPS, pH 7.4) were treated with two-fold serial dilutions of the antibody, AMB, and the combination of both agents in 96-well plates. The starting concentration of the antifungal drug was set at 2× MIC (Minimum Inhibitory Concentration), while the starting concentration of Dia-T51 was established based on previous studies. Plates were incubated at 37 °C for 24 h. Absorbance was measured at 405 nm. The Fractional Inhibitory Concentration Index (FICI) was calculated and interpreted as follows: FICI ≤ 0.5, synergistic interaction; 0.5 < FICI ≤ 1, additive interaction; 1 < FICI < 4, indifferent (no interactions); FICI ≥ 4.0, antagonistic interaction[30]. All experiments were performed in duplicate.

Determination of minimal fungicidal concentration (MFC)

Aliquots harvested from the checkerboard assay wells and exhibiting no visible growth were plated onto Potato Dextrose Agar (PDA). After incubation at 37 °C for 24 h, Colony Forming Units (CFUs) were counted. The MFC was defined as the lowest concentration resulting in a ≥99% reduction in fungal growth compared to the growth control[31].

Experimental design

The sample size for larval studies was determined using the "resource equation" method proposed by Charan and Kantharia because at the beginning it was not possible to assume about effect size[32]. According to this method:

E = Total number of animals − Total number of groups

Where "E", the degree of freedom of the analysis of variance (ANOVA), should lie between 10 and 20. Control groups, including an uninjected control and vehicle controls, were always included. To satisfy the equation while maintaining the minimum number of groups (4), the minimum number of *Galleria mellonella* larvae should be 4, but since the use of larvae does not breach ethical guidelines, based on the supplier availability, the experiments accommodated a larger number of animals (from 5 to 8 larvae per group) and were repeated independently at least three times. In each replicate, larvae from a single batch were randomized into all study groups to ensure that treatment arms were tested simultaneously against their respective controls. Due to the inherent variability in the size of commercially available larval batches, we adopted a flexible allocation strategy to maximize ethical use and statistical robustness: any surplus larvae were systematically allocated to the control groups. Each independent experiment included the full panel of treatment and control groups to account for biological variations across different larval batches.

Larval handling and maintenance

The protocol described by Fuchs et al.[25], was followed. *G. mellonella* larvae were selected based on weight (0.3–0.5 g range) and visually inspected for health. Fresh larvae were immediately used or stored at 4 °C for at most one day. When removed from storage larvae were left adapted to room temperature (20 °C) for at least 2 h. During storage and experiments larvae weren't provided with food or water. During the experiments the larvae were maintained at 36 °C in Petri dishes and monitored by visual observation and touching with laboratory tweezers as after injections larvae can become less motile. Both infections and treatments were performed injecting 20 µl through an insulin syringe (BD Micro-Fine 0.3 ml, 8 mm - 30 G) directly into the hemocoel via the last prolegs. For repeated injections prolegs were alternated. To minimize the confounding impact of injection-related physical trauma in already debilitated larvae, the number of vehicle administrations in untreated and control groups was not matched to the multi-dose treatment regimens. This strategy ensured that baseline mortality reflected the natural course of infection rather than procedural stress.

Dia-T51 and amphotericin B toxicity

The humanized monoclonal antibody Dia-T51 was tested at two doses: 1 and 10 mg/kg. This selection aligns with common therapeutic monoclonal antibody dosing (typically <10 mg/kg) aiming to optimize pharmacokinetics by minimizing effects on serum composition and avoiding overly rapid clearance[33–35]. Amphotericin B was tested at three doses: 0.5, 5, and 50 mg/kg. This choice relies on two different considerations: the first concerns the highest dose which had to be effective without affecting insect health. Previous studies in *G. mellonella* showed toxicity at doses ≥500 mg/kg and melanization and loss of motility at doses ≥150[36]. The second consideration regards the lowest dose which had to be ineffective to assess possible synergy in combination with Dia-T51 (other studies were done with lower concentrations but 0.5 mg/kg was already not effective (data not shown)). Regarding compound preparation, Dia-T51 was dissolved and diluted in Phosphate Buffered Saline (PBS), pH 7.4. AMB was initially dissolved in 100% dimethyl sulfoxide (DMSO). To maintain a constant solvent concentration across all groups, distinct dilution protocols were employed: the 50 mg/kg dose was diluted using PBS, whereas the 5 and 0.5 mg/kg doses were prepared by an initial dilution in 100% DMSO followed by a final dilution step in PBS. This procedure ensured a fixed final DMSO concentration of 2.5% (v/v) across all treatment and vehicle control groups. Vehicle control groups were included in all experiments.

Galleria mellonella larvae infection

Galleria mellonella larvae were injected with 10^3, 10^4, 10^5 and 10^6 colony forming units (CFU) of *Candidozyma auris* cells/larva to assess the proper infection dose causing the death of all the animals in a time frame useful to observe the course of the therapy. Briefly, the counts were adjusted by diluting into PBS pH 7.4 an overnight inoculum grown in RPMI-MOPS medium at 30 °C in agitation. In line with literature, 10^6 CFU/larva caused total lethality within the necessary time frame (data not shown), hence an infection burden of 1–8 10^6 CFU/larva was established for subsequent experiments. Uninfected/uninjected control and uninfected/PBS-injected control groups were included in every experimental set to assess respectively the larval healthy state and the attrition of the experiment due to the injection procedure.

Dia-T51 therapeutic and prophylactic regimens

First regimen – Therapeutic monotreatment: *G. mellonella* larvae were injected simultaneously with *C. auris* suspension and Dia-T51 at the doses of 10 or 1 mg/kg.

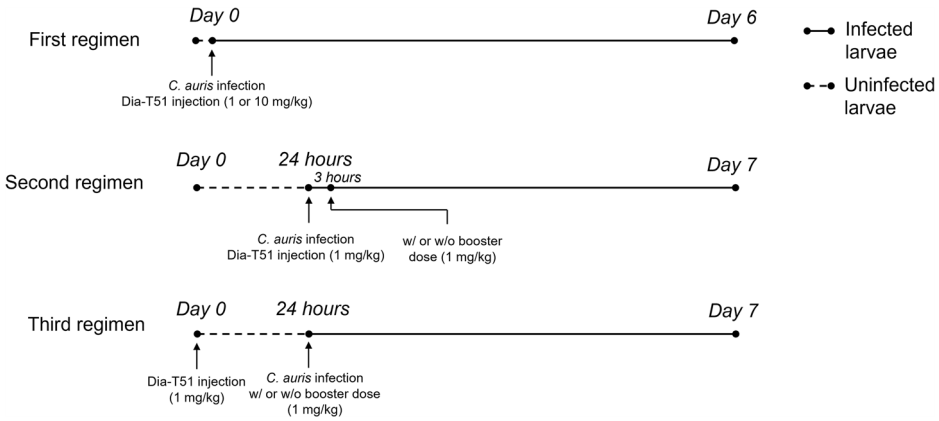


Fig. 7 | Schematic representation of the regimens used for Dia-T51 activity evaluation.

Second regimen – Therapeutic mono- and double-treatment: two groups received the dose of antibody (1 mg/kg) simultaneously with *C. auris* suspension. Of them one group received a booster dose of the antibody (1 mg/kg) after 3 h.

Third regimen – Prophylactic mono- and double-treatment: two groups were injected with Dia-T51 (1 mg/kg) 24 h prior to *C. auris* infection. Of them, one group received a booster dose (1 mg/kg) simultaneously with fungal suspension.

The schematic representation of the regimens is summarized in Fig. 7. In every experimental regimen, uninfected/uninjected, uninfected/PBS-injected, uninfected/Dia-T51-single dose-injected, uninfected/Dia-T51-double dose-injected and infected/untreated control groups were included.

Amphotericin B efficacy and combinatorial regimens

Larvae were injected with *C. auris* suspension and AMB at the concentrations tested for toxicity. The ineffective concentration of 0.5 mg/kg was further used in combination with 10 and 1 mg/kg of hmAb Dia-T51. Insect survival was monitored for seven days after treatment to assess possible long term side effects due to the combinations. As reported for a previous experimental method, stock solutions of Dia-T51 were prepared in Phosphate Buffered Saline (PBS, pH 7.4), whereas amphotericin B (AMB) was solubilized in 100% dimethyl sulfoxide (DMSO). To ensure a consistent vehicle composition across all groups, AMB dilutions were performed using two distinct protocols: the 50 mg/kg dose was diluted exclusively in PBS, while the lower doses (5 and 0.5 mg/kg) underwent an intermediate dilution in DMSO followed by a final step in PBS. This strategy allowed for a fixed final DMSO concentration of 2.5% (v/v) in all AMB-treated and control larvae.

Extraction and plating of hemolymph

From 3 to 4 infected larvae untreated and treated with Dia-T51, AMB and their combinations were killed by freezing at −20 °C for at least 30 min. With a UV-sterilized surgical blade a punctuation was performed at the last abdominal segments. Larvae were gently squeezed and the hemolymph collected in Eppendorf tubes kept on ice during the whole process to avoid melanization. Attention was paid to avoid the incorporation of internal structure such as the fat body and the digestive tube, the latter seat of the host microbiota[37]. The hemolymph was serially diluted in PBS and plated on Potato Dextrose Agar (PDA) plates. Plates were incubated at 30 °C and CFUs were counted after two days. The time points analyzed were 3, 24 and 48 h after infection.

Galleria mellonella larvae dissection

A frozen larva from each group of the previous experiment was dissected. With a UV-sterilized scalpel, a sagittal cut was performed from the thorax to the abdomen. After observation, images were taken with an Inskam316 2MP 1000X Wifi Microscope to document internal features.

Statistical analysis

Data was analyzed using GraphPad Prism. Kaplan–Meier survival analysis with the Log-rank Mantel–Cox test was used to compare survival curves; larvae surviving until the end of the experiment were right-censored at the final timepoint). The One-Way ANOVA non-parametric Kruskal–Wallis test followed by post-hoc Dunn's multiple comparisons test was adopted for analyzing the survival rate and the time of survival. Global significance was assessed prior to post-hoc comparisons. In these statistical analyses, comparisons were restricted to infected, treated groups and infected, untreated groups; uninfected control groups were excluded. Two-way ANOVA with Sidak's multiple comparisons test was chosen for the ex vivo evaluation. A P value < 0.05 was considered statistically significant.

Data availability

All data obtained and analyzed during this study are included in this published article.

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References

1. Nguyen, I. K. Fungal infections: a rising global concern by ISID emerging leader, Afreenish Amir - ISID. https://isid.org/fungal-infections-a-rising-global-concern-by-isid-emerging-leader-afreenish-amir/, https://isid.org/fungal-infections-a-rising-global-concern-by-isid-emerging-leader-afreenish-amir/ (2024).
2. Denning, D. W. Global incidence and mortality of severe fungal disease. *Lancet Infect. Dis.* **24**, e428–e438 (2024).
3. CDC. Antibiotic-resistant Germs: New Threats. *Centers for Disease Control and Prevention* https://www.cdc.gov/drugresistance/biggest-threats.html (2021).
4. *WHO Fungal Priority Pathogens List to Guide Research, Development and Public Health Action* (World Health Organization, Geneva, 2022).
5. Mishra, S. K., Yasir, M. & Willcox, M. *Candida auris*: an emerging antimicrobial-resistant organism with the highest level of concern. *Lancet Microbe* **4**, e482–e483 (2023).
6. C. D. C. Antifungal Susceptibility Testing for *C. auris*. *Candida auris* (*C. auris*), https://www.cdc.gov/candida-auris/hcp/laboratories/antifungal-susceptibility-testing.html (2024).
7. Vanzolini, T. & Magnani, M. Old and new strategies in therapy and diagnosis against fungal infections. *Appl. Microbiol. Biotechnol.* **108**, 147 (2024).
8. Vanzolini, T., Bruschi, M., Rinaldi, A. C., Magnani, M. & Fraternale, A. Multitalented synthetic antimicrobial peptides and their antibacterial, antifungal and antiviral mechanisms. *Int. J. Mol. Sci.* **23**, 545 (2022).
9. Torosantucci, A. et al. A novel glyco-conjugate vaccine against fungal pathogens. *J. Exp. Med.* **202**, 597–606 (2005).

10. Torosantucci, A. et al. Protection by anti-beta-glucan antibodies is associated with restricted beta-1,3 glucan binding specificity and inhibition of fungal growth and adherence. *PloS One* **4**, e5392 (2009).
11. Rachini, A. et al. An anti-beta-glucan monoclonal antibody inhibits growth and capsule formation of Cryptococcus neoformans in vitro and exerts therapeutic, anticryptococcal activity in vivo. *Infect. Immun.* **75**, 5085–5094 (2007).
12. Catellani, M. et al. Optimised production of an anti-fungal antibody in *Solanaceae* hairy roots to develop new formulations against *Candida albicans*. *BMC Biotechnol* **20**, 15 (2020).
13. Bryan, R. A. et al. Toward developing a universal treatment for fungal disease using radioimmunotherapy targeting common fungal antigens. *Mycopathologia* **173**, 463–471 (2012).
14. Di Mambro, T., Vanzolini, T. & Magnani, M. Humanised antibodies against pathogenic fungi. Patent WO2022096455A1 (2022).
15. Di Mambro, T. et al. A new humanized antibody is effective against pathogenic fungi in vitro. *Sci. Rep.* **11**, 19500 (2021).
16. Vanzolini, T., Mambro, T. D., Magnani, M. & Menotta, M. AFM evaluation of a humanized recombinant antibody affecting *C. auris* cell wall and stability. *RSC Adv.* **13**, 6130–6142 (2023).
17. Meletiadis, J., Verweij, P. E., TeDorsthorst, D. T. A., Meis, J. F. G. M. & Mouton, J. W. Assessing in vitro combinations of antifungal drugs against yeasts and filamentous fungi: comparison of different drug interaction models. *Med. Mycol.* **43**, 133–152 (2005).
18. Lepak, A. J. & Andes, D. R. Antifungal pharmacokinetics and pharmacodynamics. *Cold Spring Harb. Perspect. Med.* **5**, a019653 (2014).
19. Andes, D. In vivo pharmacodynamics of antifungal drugs in treatment of candidiasis. *Antimicrob. Agents Chemother.* **47**, 1179–1186 (2003).
20. Gallorini, M. et al. Immunophenotyping of hemocytes from infected *Galleria mellonella* larvae as an innovative tool for immune profiling, infection studies and drug screening. *Sci. Rep.* **14**, 759 (2024).
21. Arteaga Blanco, L. A. et al. Differential cellular immune response of *Galleria mellonella* to *Actinobacillus pleuropneumoniae*. *Cell Tissue Res.* **370**, 153–168 (2017).
22. Pereira, T. C. et al. Recent advances in the use of *Galleria mellonella* model to study immune responses against human pathogens. *J. Fungi Basel Switz.* **4**, 128 (2018).
23. Smith, D. F. Q. & Casadevall, A. Fungal immunity and pathogenesis in mammals versus the invertebrate model organism *Galleria mellonella*. *Pathog. Dis.* **79**, ftab013 (2021).
24. Smith, D. F. Q., Dragotakes, Q., Kulkarni, M., Hardwick, J. M. & Casadevall, A. *Galleria mellonella* immune melanization is fungicidal during infection. *Commun. Biol.* **5**, 1364 (2022).
25. Fuchs, B. B., O'Brien, E., Khoury, J. B. E. & Mylonakis, E. Methods for using *Galleria mellonella* as a model host to study fungal pathogenesis. *Virulence* **1**, 475–482 (2010).
26. Romera, D. et al. The *Galleria mellonella* infection model as a system to investigate the virulence of *Candida auris* strains. *Pathog. Dis.* **78**, ftaa067 (2020).
27. Pereira, T. C. et al. Recent advances in the use of *Galleria mellonella* model to study immune responses against human pathogens. *J. Fungi* **4**, 128 (2018).
28. Satoh, K. et al. *Candida auris* sp. nov., a novel *ascomycetous* yeast isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiol. Immunol.* **53**, 41–44 (2009).
29. Arendrup, M. C., Cuenca-Estrella, M., Lass-Flörl, C. & Hope, W. EUCAST-AFST. EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). *Clin. Microbiol. Infect.* **18**, E246–E247 (2012).
30. Feng, W. & Yang, J. Interpretation of fractional inhibitory concentration index (FICI). *Bio-Protoc. Prepr. Repos.* https://bio-protocol.org/exchange/preprintdetail?id=2404&type=3 (2023).
31. Cantón, E. et al. Minimum fungicidal concentrations of amphotericin B for bloodstream *Candida species*. *Diagn. Microbiol. Infect. Dis.* **45**, 203–206 (2003).
32. Charan, J. & Kantharia, N. D. How to calculate sample size in animal studies? *J. Pharmacol. Pharmacother.* **4**, 303–306 (2013).
33. Dirks, N. L. & Meibohm, B. Population pharmacokinetics of therapeutic monoclonal antibodies. *Clin. Pharmacokinet.* **49**, 633–659 (2010).
34. Ovacik, M. & Lin, K. Tutorial on monoclonal antibody pharmacokinetics and its considerations in early development. *Clin. Transl. Sci.* **11**, 540–552 (2018).
35. An, G. Concept of pharmacologic target-mediated drug disposition (TMDD) in large-molecule and small-molecule compounds. *J. Clin. Pharmacol.* **60**, 149–163 (2020).
36. Marena, G. D., Ramos, M. A. D. S., Lima, L. C., Chorilli, M. & Bauab, T. M. *Galleria mellonella* for systemic assessment of anti-*Candida auris* using amphotericin B loaded in nanoemulsion. *Sci. Total Environ.* **807**, 151023 (2022).
37. Ruiz Barrionuevo, J. M. et al. The bacterial and fungal gut microbiota of the greater wax moth, *Galleria mellonella* L. consuming polyethylene and polystyrene. *Front. Microbiol.* **13**, 918861 (2022).

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Author contributions

T.V. managed conceptualization, methodology, investigation, validation, data curation, formal analysis and project administration. In addition, T.V. handled the visualization and the writing of the original draft. M.M. was responsible for funding acquisition and supervision. All authors provided resources and reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Competing interests

Diatheva S.R.L. supported the study, providing the humanized antibody Dia-T51, but was not involved in carrying out or managing the investigation, nor was it involved in the analysis and interpretation of data and in the preparation of the manuscript. M.M. holds shares in Diatheva S.R.L. V.F. is an employee of Diatheva S.R.L., T.V. and M.M. are inventors of Dia-T51 antibody as declared in patent WO2022096455A1 belonging to Diatheva S.R.L. This does not alter our adherence to the journal policies on sharing data and materials.

Additional information

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