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SHORT REPORT

Haematological Malignancy - Biology

UMG1/CD3ε-bispecific T-cell engager redirects T-cell cytotoxicity against diffuse large B-cell lymphoma

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Summary

UMG1 is a unique epitope of CD43, not expressed by normal cells and tissues of haematopoietic and non-haematopoietic origin, except thymocytes and a minority (<5%) of peripheral blood T lymphocytes. By immunohistochemistry analysis of tissue microarray and pathology slides, we found high UMG1 expression in 20%–24% of diffuse large B-cell lymphomas (DLBCLs), including highly aggressive BCL2^{high} and CD20^{low} cases. UMG1 membrane expression was also found in DLBCL bone marrow-infiltrating cells and established cell lines. Targeting UMG1 with a novel asymmetric UMG1/CD3ε-bispecific T-cell engager (BTCE) induced redirected cytotoxicity against DLBCL cells and was synergistic with lenalidomide. We conclude that UMG1/CD3ε-BTCE is a promising therapeutic for DLBCLs.

KEYWORDS
bispecific T-cell engager, BTCE, CD43, DLBCL, immunotherapy, UMG1

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a highly aggressive malignancy that accounts for 35%–40% of all B-cell lymphomas.[1] Currently, standard first-line therapy cures about 60% of patients while effective treatment of chemo-refractory/resistant (r/r) disease is still a challenging unmet need. Even if a variety of immune therapeutics have been developed, the anti-CD20 Rituximab still provides a cornerstone option.[2] Recently, the treatment of DLBCLs has been revolutionized by novel immunotherapeutic approaches including the use of chimeric antigen receptor-T cells.[3,4] However, these potent engineered cells are not effective in all cases mostly due to the wide heterogeneity of target expression, with consequent failure of response or relapse.[5]

An alternative strategy is based on an off-the-shelf approach to promote immunologic anti-tumour synapsis by bispecific T-cell engagers (BTCEs). Blinatumomab was the first BTCE

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directed against CD19 and the CD3ε subunit of the T-cell receptor. It has prompted the development of similar constructs against CD20, such as mosunetuzumab, odronextamab, glofitamab and epcoritamab.[6] However, many patients still do not respond due to target downmodulation or MYC signalling deregulation, with the consequent progression of disease.[7]

The identification of novel suitable targets represents, therefore, a crucial opportunity for developing new agents for r/r DLCBLs to expand the therapeutic scenario for these aggressive malignancies.

With this objective, we propose the targeting of UMG1, a unique epitope of CD43, as a valuable strategy for the treatment of selected DLBCLs. We recently reported that targeting UMG1[8] with a novel BTCE is safe and highly selective against T-cell acute lymphoblastic leukaemia (T-ALL).[9]

We investigated the UMG1 expression on DLBCLs and the preclinical anti-tumour activity of the UMG1/CD3ε-BTCE as proof of concept for a personalized approach to UMG1-expressing DLBCLs.

MATERIALS AND METHODS

For a more detailed description of the methods used, see Supporting Information (Data S1).

UMG1 mAb and UMG1/CD3E-BTCE

The humanized UMG1 was produced as previously described.[9] The monovalent UMG1/CD3ε-BTCE was generated by 'Knobs-into-holes' technology.

DLBCL cell lines and primary cells

OCI-Ly1, DoHH2 and SU-DHL-4 cells were purchased by DSMZ (Germany). Toledo cells were purchased from ATCC (USA). Cells were grown in standard conditions. Peripheral blood mononuclear cells (PBMCs) from healthy donors and cells from diagnostic bone marrow aspirates from DLBCL patients were collected at our Clinical Units, AOU Renato Dulbecco, teaching Hospital of the Magna Graecia University of Catanzaro (Italy), in accordance with Bioethical Institutional standards.

UMG1 expression was evaluated by flow cytometry (Attune NxT Flow cytometer; Thermo Fisher Scientific, USA); samples were stained with 1 μg/mL of anti-human UMG1-PE (anti-huUMG1; Immunostep) or IgG1-PE (Beckton Dickinson) for 20 min at room temperature.

Immunohistochemistry

DLBCL tissue microarrays (TMAs) (LY2084) were purchased from TissueArray.Com LLC. TMA and pathology slides were incubated with customized immunohistochemistry (IHC) protocols from Dako Omnis with the primary anti-UMG1 antibody (dilution 1:300) at 4°C.

Redirected T-cell cytotoxicity and functional assays

Target lymphoma cells were co-cultured with healthy donor-derived PBMCs labelled with CellTrace Violet viable marker (Thermo Fisher Scientific) in the presence of UMG1/CD3ε-BTCE, BTCE negative control (Anti-βGal-hCD3; InvivoGen) or vehicle (1× PBS).

T-cell cytotoxicity was evaluated by Attune NxT Flow cytometer (Thermo Fisher Scientific) as 7-AAD+/target cells (%). Functional assays on effector cells were performed by flow cytometry analysis of T-cell activation markers.

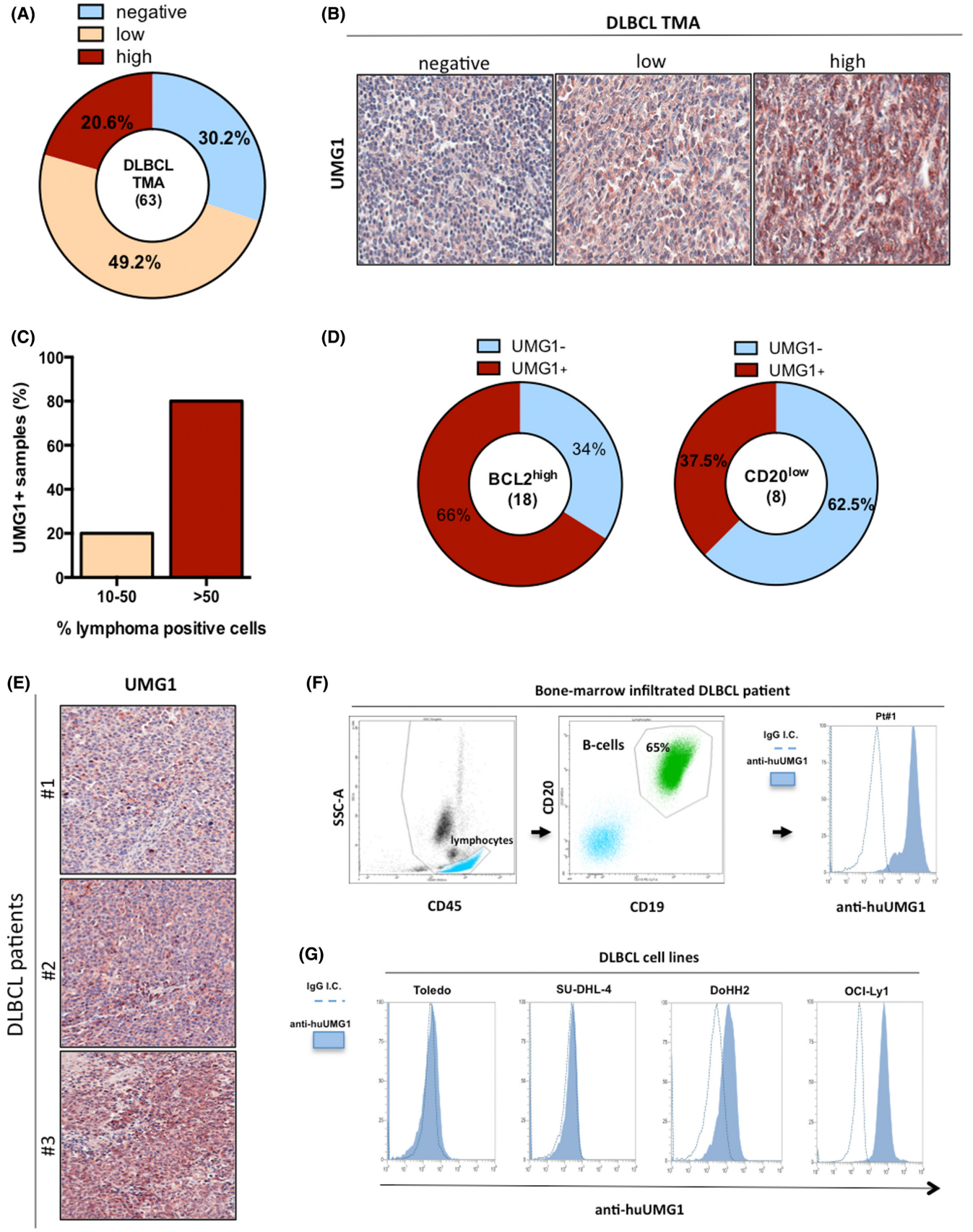
Statistical analysis

Each experiment was performed at least three times and values were reported as means ± standard deviation. Comparisons between groups were analysed with Student's *t*-test, a *p*-value equal to or less than 0.05 was accepted as statistically significant.

RESULTS

UMG1 expression was investigated through IHC on an extensive panel of lymphoma TMAs. Among 63 evaluable DLBCL samples, 13 (20.6%) exhibited high intensity of UMG1 staining, while low staining was observed in an additional 31 cases (49.2%) (Figure 1A,B). Notably, in 80% of high stained samples, we found more than 50% of UMG1-expressing malignant cells (Figure 1C). Furthermore, two relevant findings emerged: UMG1 expression was found in (i) 66% (12 of 18) of $BCL2^{high}$ DLBCL cases, and (ii) in a small cohort (3/8, 37.5%) of $CD20^{low}$ cases (Figure 1D). Moreover, we assessed the UMG1 membrane expression on samples derived from DLBCL patients. We found high UMG1 expression in 5 of 21 (23.8%) pathology slides, through IHC (Figure 1E; Figure S1A,B), and in one of three cases with bone-marrow infiltration, by flow cytometry (Figure 1F; Figure S1C).

FIGURE 1 Expression of UMG1 in DLBCLs. (A) Intensity of UMG1 membrane expression observed on DLBCL samples included in TMA. Relative percentage is shown. (B) Representative IHC images (20×) of DLBCL TMA samples with increasing UMG1 expression. (C) Percentage of UMG1-expressing lymphoma cells among high-stained TMA cases. (D) Percentage of UMG1-expressing DLBCL samples, among $BCL2^{high}$ (*left*) and $CD20^{low}$ (*right*) cases included in TMA. (E) Representative IHC images (20×) of DLBCL patients with different UMG1 expression. (F) Flow cytometry analysis performed on lymphoma cells from bone marrow-infiltrated DLBCL patient. (G) Flow cytometry analysis of UMG1 expression on DLBCL cell lines. DLBCL, diffuse large B-cell lymphoma; IHC, immunohistochemistry; TMA, tissue microarray.



UMG1 expression was also investigated on established lymphoma cell lines. By flow cytometry, we found UMG1 expression on two of four DLBCL cell lines. Specifically, OCI-Ly1 cells exhibited strong expression of the epitope, while DoHH2 cells showed lower expression. In contrast, SU-DHL-4 and Toledo cells did not express UMG1 (Figure 1G).

To validate the translational relevance of our findings, we co-cultured UMG1-expressing and -non-expressing cell lines with PBMCs from healthy donors at various effector:target (E:T) ratios and subsequently exposed co-cultured cells at increasing concentrations of UMG1/CD3ε-BTCE.

The treatment with UMG1/CD3ε-BTCE induced immunologic synapses between T cells and UMG1-expressing DLBCL cells (Figure S1D,E), which translated into significant cytotoxicity in a dose, E:T ratio- and UMG1 expression-dependent fashion. Indeed, significant redirected cytotoxicity was observed in UMG1+ OCI-Ly1 (Figure 2A, *left*) and DoHH2 (Figure S1F) cell lines after UMG1/CD3ε-BTCE treatment, as compared to vehicle or BTCE-negative control (BTCE NC) which was instead directed against

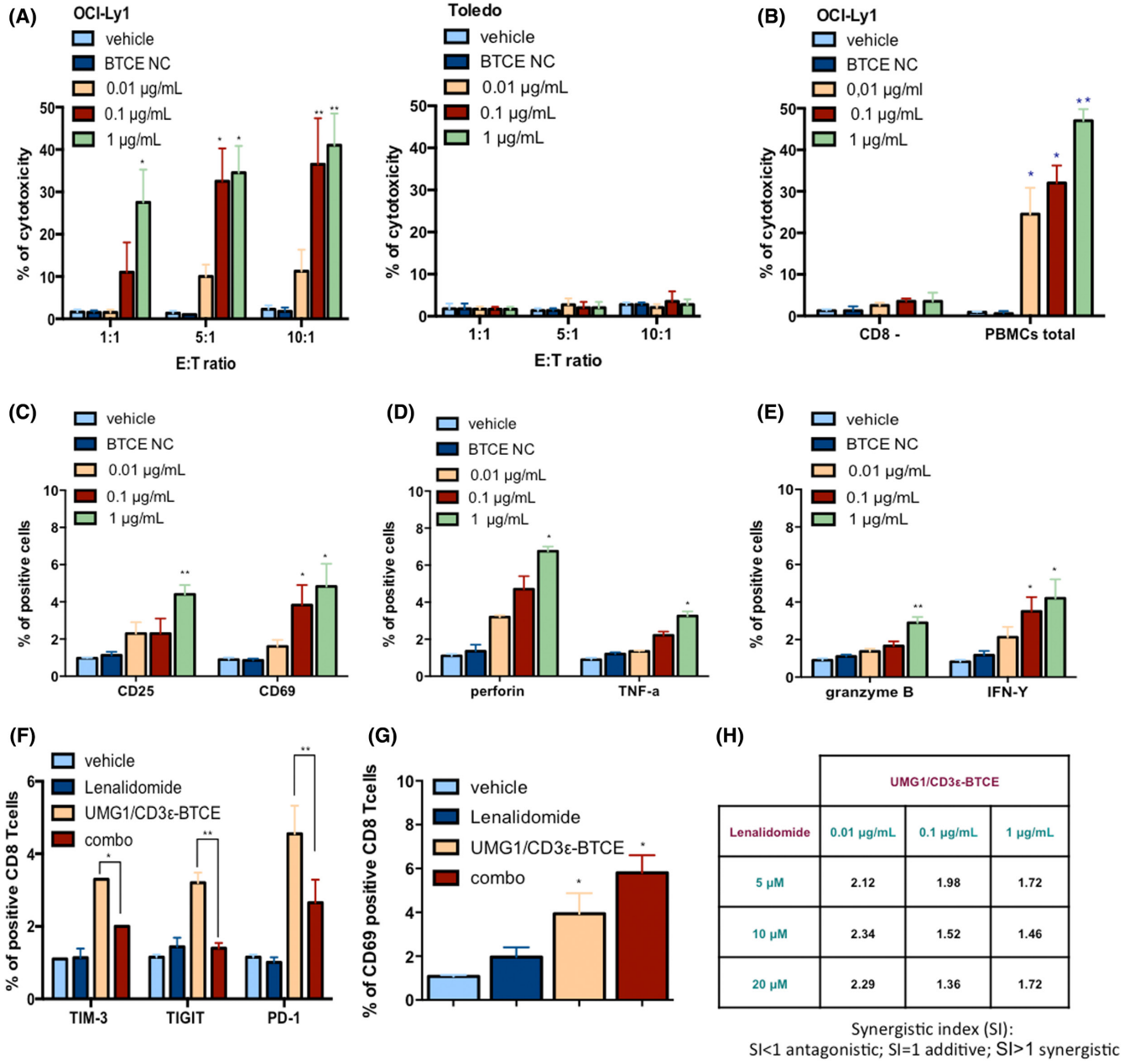


FIGURE 2 UMG1/CD3ε-BTCE redirects and activates T lymphocytes against DLBCL cells. (A) Redirected T-cell-mediated lysis monitored by viable target OCI-Ly1 (UMG1+, *left*) and Toledo (UMG1−, *right*) cells co-cultured with CellTrace Violet-labelled PBMCs at different E:T ratio and treated for 48 h with increasing UMG1/CD3ε-BTCE concentrations. (B) OCI-Ly1 cells incubated with full or CD8 cell-depleted PBMCs as effector cells and treated with increasing UMG1/CD3ε-BTCE concentrations at 10:1 E:T ratio. (C–E) OCI-Ly1 cells were co-cultured for 48 h with PBMCs (E:T = 10:1) in the presence of UMG1/CD3ε-BTCE. Percentages of CD69– and CD25-positive (C), granzyme B and IFN-γ (D) and perforin and TNF-α (E) positive PBMCs are shown. (F, G) OCI-Ly1 cells were co-cultured for 72 h with PBMCs (E:T = 10:1) in the presence of UMG1/CD3ε-BTCE or vehicle, with or without lenalidomide. Flow cytometric analysis of exhaustion (F) and activation marker CD69 (G) expression on PBMCs. (H) Table showing synergistic indexes resulting from combinatorial treatments of OCI-Ly1 with UMG1/CD3ε-BTCE and lenalidomide (72 h time point). Student's *t*-test was applied to calculate statistical significance: $*p < 0.05$; $**p < 0.01$. BTCE, bispecific T-cell engager; PBMC, peripheral blood mononuclear cell.

β-galactosidase and human CD3ε. Conversely, as formal proof of target specificity, no cytotoxic effects were detected after BTCE treatment of UMG1- Toledo cells (Figure 2A, *right*) or in the absence of effector cells (Figure S1G). Afterwards, to demonstrate T-cell-mediated UMG1/CD3ε-BTCE cytotoxicity, OCI-Ly1 cells were co-cultured with total human PBMCs or immunomagnetic T-CD8 cell-depleted PBMCs. Consistently with the mechanism of action, no cytotoxic activity was observed when CD8+ cells were depleted (Figure 2B). Finally, functional effects induced by UMG1/CD3ε-BTCE treatment on PBMCs co-cultured with DLBCL cell lines were evaluated. Importantly, UMG1/CD3ε-BTCE led to concentration-dependent T-lymphocyte activation, as evaluated by the upregulation of early and late activation markers (CD69 and CD25) on CD8+ T lymphocytes, as well as by the release of granzyme B and perforin, the production of pro-inflammatory cytokines, such as TNF-α, IFN-γ (Figure 2C–E; Figure S2A) and the increase of T-cell proliferation (Figure S2B). In addition, concentration-dependent CD107a expression increased in T lymphocytes co-cultured at 10:1 E:T ratio with UMG1-expressing OCI-Ly1 cells, indicating the occurrence of BTCE-induced T-cell degranulation (Figure S2C), which finally led to target cell apoptosis (Figure S2D).

As expected, an increase of exhaustion markers PD-1, TIGIT and TIM-3 on T lymphocytes and an increase of immune checkpoint PD-L1 on lymphoma cells after UMG1/CD3ε-BTCE treatment was observed (Figure S2E,F). To overcome this activation brake, a combination of UMG1/CD3ε-BTCE with immune modulatory drug (IMiD), such as lenalidomide, was investigated. Interestingly, sublethal doses of lenalidomide (Figure S2G) significantly reduced the induction of exhaustion markers by the BTCE (Figure 2F), empowered T-cell activation (Figure 2G) and finally synergized with UMG1/CD3ε-BTCE on cytotoxic activity against OCI-Ly1 cells (Figure 2H).

DISCUSSION

The impressive clinical activity of cancer immune targeting has encouraged the search and discovery of novel targets to provide new opportunities for driving immune effectors against malignant cells.

CD43, a heavily glycosylated surface protein, is highly expressed in B-cell malignancies. It is associated with a non-germinal centre B-cell subgroup and with worse outcome in DLBCL patients.[10] However, the canonical pattern of CD43 expression, as recognized by most available mAbs, makes this target not suitable for immunotherapy since this glycoprotein is also widely expressed in the normal haematopoietic cell compartment, suggesting potential relevant on-target/off-tumour toxicities, limiting so far the clinical-grade development of therapeutics against CD43 towards early trials.[11]

In contrast, the expression of UMG1, a unique CD43-epitope, is restricted to cell surface of cortical thymocytes and of a minority (<5%) of peripheral blood T lymphocytes,[9] while other normal cells or tissues of the human body, including vital organs, do not express the epitope, indicating, therefore, a very promising safe profile of UMG1 pattern of expression for immune therapeutic interventions.

We reported here that UMG1 is significantly expressed on cell membrane of 20–24% DLBCLs, consistently with previously reported CD43 positivity in this disease.[10,12] Notably, UMG1 expression was also found in $CD20^{low}$ and in $BCL2^{high}$ DLBCLs, further suggesting its potential value as a relevant target for a subpopulation of highly aggressive DLBCLs. Therefore, we can speculate that, after clinical cut-off assessment and validation of IHC assay, this fraction of DLBCLs may be suitable for therapeutic intervention. Moreover, we can hypothesize that a larger population of DLBCLs with low levels of UMG1 expression may be potentially targetable, since even few cell surface antigens can activate BTCEs. For example, previous *in vitro* data on anti-FcRH5/CD3 BTCE showed that only 50 FcRH5 molecules per cell are sufficient to induce T-cell redirected cytotoxicity against multiple myeloma cell lines.[13] Furthermore, recent findings on the anti-HER2 trastuzumab deruxtecan have demonstrated that even $HER2^{low}$ breast cancer patients can benefit from this innovative immune-targeted agent, as compared to high expressing patients identified by previous conventional predictive biomarkers.[14] In order to avoid inter-institutional heterogeneity in biomarker assessment, a robust UMG1 IHC companion diagnostic should be set and validated in the early phases of clinical investigation to allow an optimal and unambiguous biomarker driven use of UMG1/CD3ε-BTCE.

Although molecular mechanisms driving UMG1 expression are currently unknown, it is possible to hypothesize that alterations in the glycosylation biosynthetic machinery may be involved. Indeed, cancer cells specifically express on their surface truncated glycans that generate neoantigens with the potential to be new immunotherapeutic targets.[15]

With this aim, we evaluated the *in vitro* activity of the asymmetric IgG-like UMG1/CD3ε-BTCE (2 + 1 format), carrying a CD3ε monovalent binding arm able to reduce non-specific T-cell activation, and a Fc portion to confer long plasma half-life. We showed a concentration-dependent T-cell proliferation and activation, translating into redirected cytotoxicity against DLBCL cells.

MYC plays a critical role as master regulator of B-cell lymphomagenesis. Furthermore, recent evidence support a direct role of MYC in the promotion of immune-suppressive microenvironment which counteracts antitumor activity of BTCE.[7] Consistently, we reported that a combined treatment of UMG1/CD3ε-BTCE and lenalidomide overcomes BTCE-resistance induced by MYC, a critical IMiD target, increasing redirected cytotoxicity on DLBCL cells. Therefore, the use of immune-sensitizing agents, like lenalidomide,[16] can add flexibility in biomarker validation in different clinical settings (mono-therapy vs. combination treatments).

On this basis, we propose UMG1 as a relevant target for the treatment of DLBCLs that express this specific epitope.

UMG1/CD3ε-BTCE can open a novel scenario as a first-in-class off-the-shelf agent for an individualized biomarker-driven approach in the evolving treatment landscape of DLBCL.[17,18]

AUTHOR CONTRIBUTIONS

Daniele Caracciolo, Caterina Riillo, Nicoletta Polerà, Stefania Signorelli, Giada Juli, Maria Cirillo and Leonardo Migale performed experiments and analysed the data; Beatrice Belmonte, Francesco Conforti and Alessandro Gulino performed IHC analysis; Nicoletta Staropoli, Franca Maria Tuccillo, Patrizia Bonelli, Andrea Ballerini, Cristina Pelizon, Maria Teresa Di Martino, Katia Grillone, Serena Ascrizzi, Pierosandro Tagliaferri and Pierfrancesco Tassone provided critical evaluation of experimental data and of the manuscript; Daniele Caracciolo, Pierosandro Tagliaferri and Pierfrancesco Tassone conceived the study and wrote the manuscript; Pierosandro Tagliaferri and Pierfrancesco Tassone supervised the study.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

DATA AVAILABILITY STATEMENT

All data relevant to the study are included in the article.

ETHICS STATEMENT

The study has been approved by the local Ethics Committee (no. 81, 21/3/2019).

PATIENT CONSENT STATEMENT

N/A.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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