Golcadomide Demonstrates Potent Antiproliferative Activity in T-Cell Lymphoma via Degradation of IKZF1 and IKZF3

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Introduction

- T-cell lymphomas (TCLs) are a heterogeneous group of aggressive non-Hodgkin lymphomas characterized by poor prognosis and limited treatment options.
- The aggressive biology, molecular diversity, and resistance to conventional treatments underscore a critical unmet need for novel, targeted therapeutic strategies.
- Golcadomide is a potential, first-in-class, oral CELMoD™ agent designed for the treatment of lymphoma, with preferential distribution to lymphoid organs and enhanced activity in lymphoma cell lines.
- Golcadomide drives the closed, active conformation of cereblon to induce rapid and deep degradation of IKZF1 and IKZF3, leading to direct cell killing and immunomodulatory activity (**Fig.1**).
- Previous studies were largely focused on B cell lymphoma. In this study, we test the in vitro activity of golcadomide and evaluate its potential as a therapeutic agent for patients with T-cell lymphoma.

Methods

Cell Culture:

Human T-cell lymphoma were cultured at 37°C with 5% CO_2 in the Iscove's modified Dulbecco's medium supplemented with 2 mM Glutamine and 10% FBS (MJ cell line) or RPMI 1460 supplemented with 2 mM Glutamine and 10% FBS (all other cell lines).

Generation of Mutant Overexpression Cell Lines:

T-cell lymphoma cells were transduced with packaged lentivirus to create stable cell lines overexpressing the nondegradable mutant protein. The virus-containing medium was removed 24 hours post-infection and replaced with medium containing 10 µM Blasticidin for selection.

Immunoblot Analysis:

T-cell lymphoma cell lines were treated with indicated concentration of lenalidomide, golcadomide, or the vehicle, DMSO, and cultured for the specified time prior to collection and storage at -80 °C prior to processing. Frozen pellets were then lysed and processed. Soluble fraction of the samples (20 µg total protein) were loaded onto NuPAGE™ Tris-Glycine 4-12% gels. Immunoblot signal was detected using the LiCor Odyssey® infrared detection system.

Flow Cytometry Based Substrate Degradation Assay:

ATL -3I and MT-2 cells were treated with lenalidomide or golcadomide for 24 hours. Cells were stained with live/dead dye according to manufacturer's instructions, washed, and fixed for 10 minutes at room temperature. Then cells were permeabilized; Fc block, Aiolos and Ikaros antibodies were added sequentially. Cells were washed after 30 minutes and resuspended in stain buffer for acquisition by LSR Fortessa.

Flow Cytometry Based Proliferation and Apoptosis Assay:

effect of compounds on the proliferation and apoptosis of the T-cell lymphoma cell lines was assessed utilizing a 96-well plate flow cytometry assay after incubation with the compounds. The cell apoptosis and viability were analyzed by flow cytometry Attune (Thermo Fischer Scientific Inc, Canoga Park, CA) using Annexin V and DRAQ7 staining. Data analysis was then performed using FlowJo and non-linear curve-fit regression was performed using GraphPad Prism 10.0.011 to calculate the viable or apoptosis AUC based on the dose-response curves.

Proteomic Study:

Cells were seeded into triplicate wells of a 96-well, U-bottom plate for each time point and treated with the indicated dose series of golcadomide or single dose of lenalidomide. After the specified treatment duration, cells were washed twice with cold PBS and pelleted. Cell pellets were lysed in 55 µL LYSE buffer (PreOmics GmbH, Planegg, Germany) and the cell lysate viscosity reduced by sonication. Cell lysates were processed to mass spectrometry-ready peptides using a PreOmics iST 96-well kit. Recovered peptides were reduced to dryness by vacuum centrifugation and resuspended in LC-LOAD buffer (PreOmics GmbH) at a concentration of 1 µg input protein/µL for analysis. Proteomics data acquisition was performed on a TimsTOF HT mass spectrometer coupled with a nanoElute 2 nanoflow liquid chromatography system (Bruker Corporation, Billerica, MA).

Results

Golcadomide Shows Robust Antiproliferation and Apoptosis Induction in T-Cell Lymphoma

- The antiproliferative and pro-apoptotic activity of golcadomide was evaluated in a panel of 10 TCL cell lines, including adult T-cell leukemia/lymphoma (ATLL), cutaneous T-cell lymphoma (CTCL), and anaplastic large cell lymphoma (ALCL). Cells were treated with increasing concentrations of golcadomide or lenalidomide, and cell viability and apoptosis were assessed using flow cytometry.

- All cell lines exhibited greater sensitivity to golcadomide than to lenalidomide, with golcadomide producing enhanced inhibition of proliferation (**Fig.2**) and stronger induction of apoptosis (**Table 1 and Fig.3**). Importantly, all TCL subtypes tested responded to golcadomide, supporting its potential as a broadly active therapeutic agent

Figure 1. The schematic illustration of how golcadomide works in T-cell lymphoma

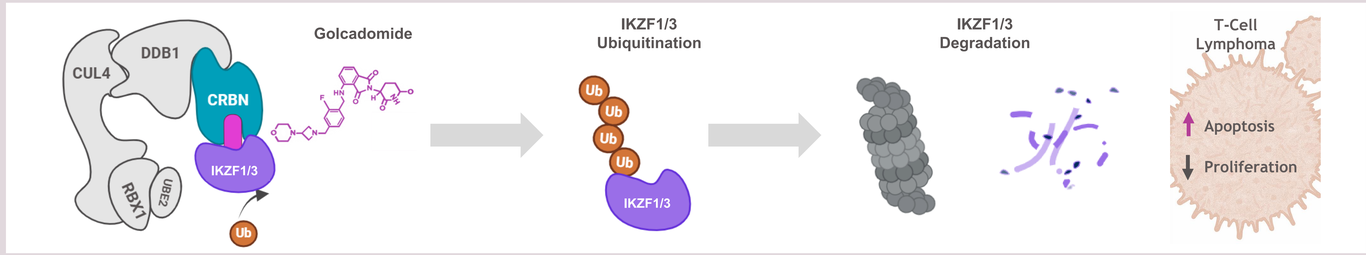
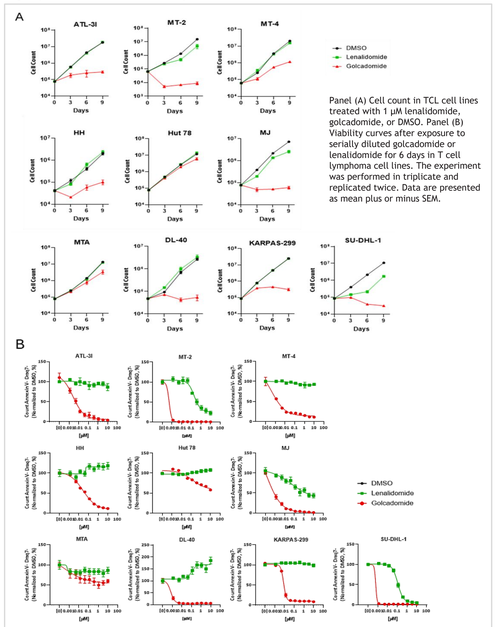


Figure 2. Golcadomide Shows Enhanced Antiproliferation in T-Cell Lymphoma Cells as Compared to Lenalidomide



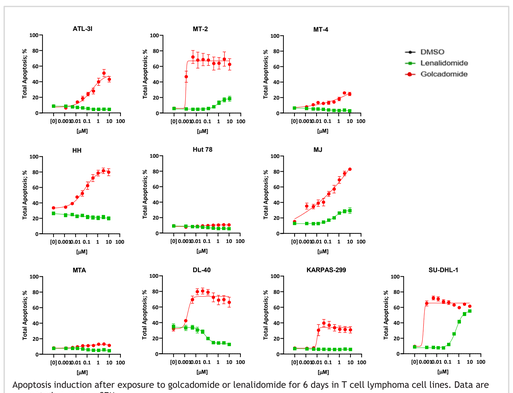
Panel (A) Cell count in TCL cell lines treated with 1 µM lenalidomide, golcadomide, or DMSO. Panel (B) Viability curves after exposure to serially diluted golcadomide or lenalidomide for 6 days in T cell lymphoma cell lines. The experiment was performed in triplicate and replicated twice. Data are presented as mean plus or minus SEM.

Table 1. Apoptosis AUC of T-Cell Lymphoma Cell Lines with Lenalidomide or Golcadomide Treatment.

	Apoptosis; AUC					
	Lenalidomide			Golcadomide		
	Day 3	Day 6	Day 9	Day 3	Day 6	Day 9
ATL-3I	34.75	46.73	54.52	202	448.5	702.8
MT-2	123.8	162.4	327.1	594.2	659.3	727.5
MT-4	78.06	33.78	35.27	181.1	234.3	394.8
HH	164	209.2	193.8	498.4	795	824
Hut78	42.24	59.66	58.12	73.24	106.1	164.2
MJ	76.49	277.9	405.2	290.9	762.5	772
MTA	93.25	53.03	42.27	114.4	124.2	229.1
DL-40	178.1	135.8	300.1	468	685.7	731.5
KARPAS-299	38.33	61.1	22.97	80.65	315.8	581.8
SU-DHL-1	91.07	488.7	281.9	139.3	625.5	784.3

Abbreviation: AUC = area under the curve

Figure 3. Golcadomide Shows Robust Apoptosis Induction in T-Cell Lymphoma Cells as Compared to Lenalidomide

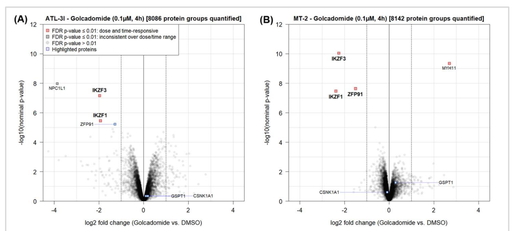


Apoptosis induction after exposure to golcadomide or lenalidomide for 6 days in T cell lymphoma cell lines. Data are presented as mean ± SEM.

IKZF1 and IKZF3 are Key Targets for Golcadomide Activity in T-Cell Lymphoma

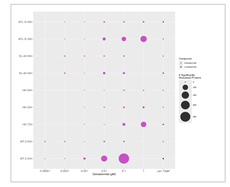
- To characterize golcadomide-induced protein degradation, proteomics mass spectrometry was performed in four TCL cell lines. Golcadomide demonstrated statistically significant and dose-responsive downregulation of the neo-substrates IKZF1 and IKZF3 (**Fig.4**)

Figure 4. Volcano Plots of Proteomics Profile of T-Cell Lymphoma Cell Lines Treated with Golcadomide



Panel (A) Proteome changes upon golcadomide treatment in ATL-3I cells. Panel (B) Proteome changes upon golcadomide treatment in MT-2 cells.

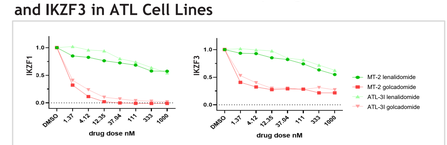
Figure 5. Golcadomide Induced Dose- and Time-Dependent Proteome Changes in T-Cell Lymphoma Cell Line Models



- Extended treatment confirmed that golcadomide elicited both dose- and time-dependent changes in the T-cell lymphoma proteome, while lenalidomide produced minimal effects across these models (**Fig.5**)

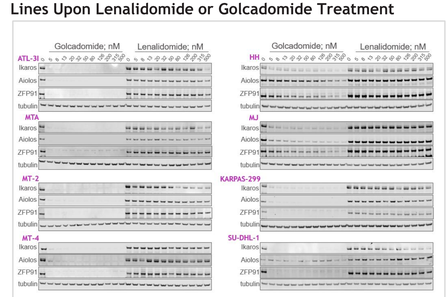
- Flow cytometry analyses (**Fig.6**) and immunoblot (**Fig.7**) confirmed that golcadomide treatment resulted in robust degradation of IKZF1 and IKZF3, which was notably more pronounced than with lenalidomide

Figure 6. Golcadomide Shows Deep Degradation of IKZF1 and IKZF3 in ATL Cell Lines



ATL cell lines were treated for 24 hours with lenalidomide or golcadomide at semi-log dose up to 1 µM. Data is normalized to DMSO control.

Figure 7. Immunoblot Analysis of T-Cell Lymphoma Cell Lines Upon Lenalidomide or Golcadomide Treatment

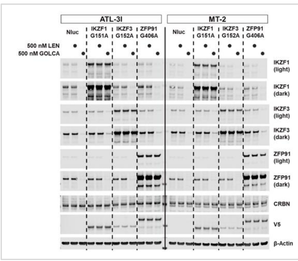


Immunoblot of relevant proteins in TCL upon lenalidomide or golcadomide treatment for 24 hours.

- To determine the substrates necessary for golcadomide's antiproliferative effects, TCL cell lines expressing non-degradable mutant forms of IKZF1 or IKZF3 were generated (**Fig.8**).

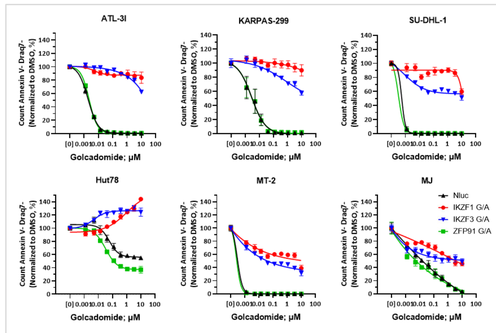
- Overexpression of IKZF1 or IKZF3 mutants effectively protected the cells from golcadomide-induced antiproliferation in all tested models (**Fig.9**).

Figure 8. Overexpression of Nondegradable Mutant in T Cell Lymphoma Cell



Immunoblot confirmation of mutant variant overexpression and protection from golcadomide- or lenalidomide-induced degradation of IKZF1, IKZF3, and ZFP91 in TCL cell lines. Cells were treated for 6 hours with indicated dose of compound or DMSO. Abbreviations: GOLCA, golcadomide; LEN, lenalidomide; Nluc, NanoLuc® luciferase

Figure 9. Nondegradable G/A Mutant IKZF1 and IKZF3 Provided Protection Against Golcadomide-Induced Antiproliferation



Viability curves of T cell lymphoma cells after nine days of golcadomide treatment. The experiment was performed in triplicate and replicated twice. Data are presented as mean ± SEM.

Conclusions

- In summary, these findings support the continued development of golcadomide as a promising therapeutic for T-cell lymphomas.
- Golcadomide's potent efficacy across multiple TCL subtypes and mechanisms involving IKZF1 and IKZF3 degradation highlight a novel strategy to address unmet clinical need in this challenging disease.

References

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- All authors contributed to and approved the presentation.

Declaration of interests

Z. M., E.K., S.W., L.G., A.C., M.T., Y.N., R.G., D.J., M.A., N.B., A.L-G: Bristol Myers Squibb - current employment, current equity holder in publicly-traded company.

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