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A response to Dr. Pepe and Dr. Fiorentino's letter regarding the original article, "Crosstalk between autophagy and apoptosis in initiating antitumor immune responses in human lymphoma cells"

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Dear Editor,

We sincerely thank Dr. Pepe and Dr. Fiorentino [1] for their thoughtful and constructive Letter to the Editor regarding our study [2] of ganoderic acid DM (GA-DM) in diffuse large B-cell lymphoma (DLBCL). We appreciate their careful consideration of our findings and their discussion of the potential relationships among apoptosis, autophagy, human leukocyte antigen (HLA) class II expression, and cluster of differentiation 4 positive ($CD4^+$) T-cell recognition. We agree that our findings should be interpreted within the limitations of the experimental models used and that additional studies are needed to establish the mechanistic relationships among GA-DM, autophagy, antigen processing, and immune recognition. Our study was intended to provide initial evidence linking GA-DM treatment with regulated cell death, changes in autophagy-associated markers, and enhanced HLA class II-restricted antigen recognition, rather than to establish a causal role for autophagy or demonstrate clinical efficacy.

Autophagic flux and the role of autophagy: We agree that increased microtubule-associated protein 1 light chain 3-I (LC3-I) and LC3-II abundance alone does not establish increased autophagic flux, as LC3 accumulation may reflect either increased autophagosome formation or impaired lysosomal degradation. Likewise, the effects of 3-methyladenine should be interpreted cautiously because of its pharmacological limitations and potential off-target effects. Our findings therefore demonstrate an association between GA-DM exposure and changes in autophagy-related markers rather than establishing that autophagy directly mediates cell death or enhanced antigen presentation. Future studies incorporating lysosomal inhibition, time-dependent LC3-II turnover, p62/SQSTM1 analysis, and tandem fluorescent LC3 reporters will be important for directly assessing autophagic flux and determining whether autophagy is cytoprotective or contributes to GA-DM-induced cell death.

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Viability and interleukin-2 (IL-2) release: We appreciate the important point that target-cell viability and number could influence IL-2 production. Although our interpretation was based on the functional increase in IL-2 following GA-DM treatment, we agree that normalization to viable target-cell numbers would strengthen the conclusion. Future studies will therefore incorporate viability-matched conditions and normalize T-cell responses to viable target-cell numbers to determine whether GA-DM enhances peptide presentation and $CD4^+$ T-cell recognition independently of its effects on lymphoma-cell survival. Evaluation of nonmalignant B cells and other immune-cell populations, together with pharmacologically relevant GA-DM concentrations, will also be important for defining the potential therapeutic window.

HLA-DR4 expression: We agree that the increased proportion of HLA-DR4-positive cells requires careful interpretation in the context of GA-DM-induced changes in viability. Because our experiments did not use viability-dye exclusion and live-cell gating as suggested, selective survival or enrichment of HLA-DR4-high cells cannot be completely excluded. Thus, our data demonstrate an association between GA-DM treatment and increased HLA-DR4 positivity at the population level but do not establish increased HLA-DR4 expression on each viable lymphoma cell. Future viability-controlled flow cytometry and quantitative assessment of HLA-DR4 surface density will help distinguish true upregulation from selective enrichment.

Immunogenic cell death (ICD): We agree that our current findings do not establish ICD. Demonstrating ICD would require assessment of established markers, including calreticulin exposure, extracellular adenosine triphosphate (ATP), and high-mobility group box 1 protein (HMGB1) release, together with functional immune studies. Our study was not intended to characterize GA-DM as an established ICD inducer but rather to raise the possibility that GA-DM-induced cellular stress and regulated cell death may influence antigen presentation and immune recognition.

HLA class II-restricted T-cell assay: The HLA-DR4-engineered lymphoma cells loaded with exogenous IgGκ188-203 peptide and the peptide-specific 2.18a T-cell hybridoma provide a controlled system for evaluating HLA class II-restricted antigen presentation and T-cell recognition. However, we agree that this model does not reproduce endogenous antigen processing in primary DLBCL and does not establish autophagy-dependent generation of endogenous tumor antigens, polyclonal $CD4^+$ T-cell activation, T-cell priming, or cytotoxicity. Future studies using endogenous tumor antigens, primary DLBCL specimens, HLA class II blockade, and polyclonal human $CD4^+$ T cells will be important for establishing the broader biological relevance of our findings.

DLBCL heterogeneity and tissue validation: We agree that DLBCL is highly heterogeneous and that two established cell lines cannot capture the genetic, phenotypic, and microenvironmental complexity of primary tumors. HLA class II deficiency in subsets of DLBCL may also influence the applicability of an HLA class II-dependent therapeutic strategy. Future studies should therefore evaluate primary DLBCL specimens with appropriate molecular and phenotypic characterization, including HLA class II expression, cell-of-origin, and myelocytomatosis (MYC)/B-cell lymphoma 2 (BCL2) status.

We also appreciate the suggestion to perform tissue-based studies combining lymphoma markers such as Paired Box 5 (PAX5), CD79a, or CD20 with HLA-DR, cleaved caspase-3, LC3B, p62/SQSTM1, CD4, and CD68. Such studies could provide valuable information regarding cellular localization and spatial relationships, although static LC3 or p62 staining would not directly establish autophagic flux.

Overall, we thank Dr. Pepe and Dr. Fiorentino for highlighting these important considerations. We agree that future investigations should incorporate viability-controlled experiments, direct measurements of autophagic flux, assessment of ICD-associated signals, quantitative evaluation of HLA class II expression on viable cells, and functional studies using primary DLBCL samples and human $CD4^+$ T cells. Nevertheless, our findings provide a foundation for investigating the intersection of GA-DM-induced cellular stress, apoptosis/autophagy-associated pathways, HLA class II antigen presentation, and $CD4^+$ T-cell recognition in DLBCL. We appreciate the opportunity to clarify the scope and limitations of our study and agree that rigorous mechanistic and translational studies will be necessary to determine its potential therapeutic relevance.

Abbreviations

$CD4^+$: cluster of differentiation 4 positive

DLBCL: diffuse large B-cell lymphoma

GA-DM: ganoderic acid DM

HLA: human leukocyte antigen

ICD: immunogenic cell death

IL-2: interleukin-2

LC3-I: microtubule-associated protein 1 light chain 3-I

Declarations

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AH: Writing—original draft, Writing—review & editing. The author read and approved the submitted version.

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The author declares that there are no conflicts of interest.

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