Abstract #17 | Poster

Senolytic capacity of obinutuzumab in t(14;18)-positive GCB DLBCL

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Introduction: Diffuse large B-cell lymphoma (DLBCL) is a heterogenous disease that can be cured in about two thirds of the patients by Rituximab (R)-CHOP. The large phase III GOYA trial failed to show superiority of the second generation glycoengineered CD20 antibody Obinutuzumab (O). However, subgroup analyses revealed that a patient subset with a particularly strong germinal center B-cell (GCB) gene expression profile, according to the lowest quartile of the RNA-based cell-of-origin linear predictor score, achieved a significantly better PFS and OS in the O arm (Oestergraad et al, ASH 2017). Given our previous work on the high senescence susceptibility of Bcl2-overexpressing GCB lymphomas in response to CHOP (Jing-H et al, Genes Dev 2010), we hypothesized here that O may exert superior efficacy in *bcl2*-translocated t(14;18)-positive GCB-DLBCL-cell lines *via* its particular lysosomal killing activity. Long-term persistence of senescent cells, characterized by their enhanced lysosomal compartment, might be detrimental (Milanovic-M et al., Nature 2018).

Methods: Human DLBCL cell lines were treated with Adriamycin (ADR), followed by either R or O, *in vitro*. Further analyses comprised RNA-seq, flow cytometry, immunoblot, fluorescence microscopy using LysoSensor, and RQ-PCR.

Results: T(14;18) (t+) cell lines were enriched for a strong GCB phenotype (Fig 1), thus using the t+ *vs.* t- status as a stratifier in subsequent experiments. Upon ADR, more cells of the t+ *vs* the t- DLBCL entered senescence (Fig 2a). Subsequent treatment with O in the t+ group but not in the t- group produced increased killing which included elimination of senescent cells (Fig 2b). Mechanistically, no R/O differences were noted regarding enhanced caspase 3 cleavage as an indicator of classic apoptosis, but a virtually O-exclusive rise in the pH of viable cells upon elimination of acidic LysoSensor-positive cells (Fig 3), indicative of O-mediated killing *via* the caspase-independent, cathepsin-mediated lysosomal pathway.

Conclusions: O exerts, at least in part, its superior “strong-GCB” efficacy through its senolytic activity in t+ chemo-senescent DLBCL *via* their enhanced susceptibility to lysosomal cell death.

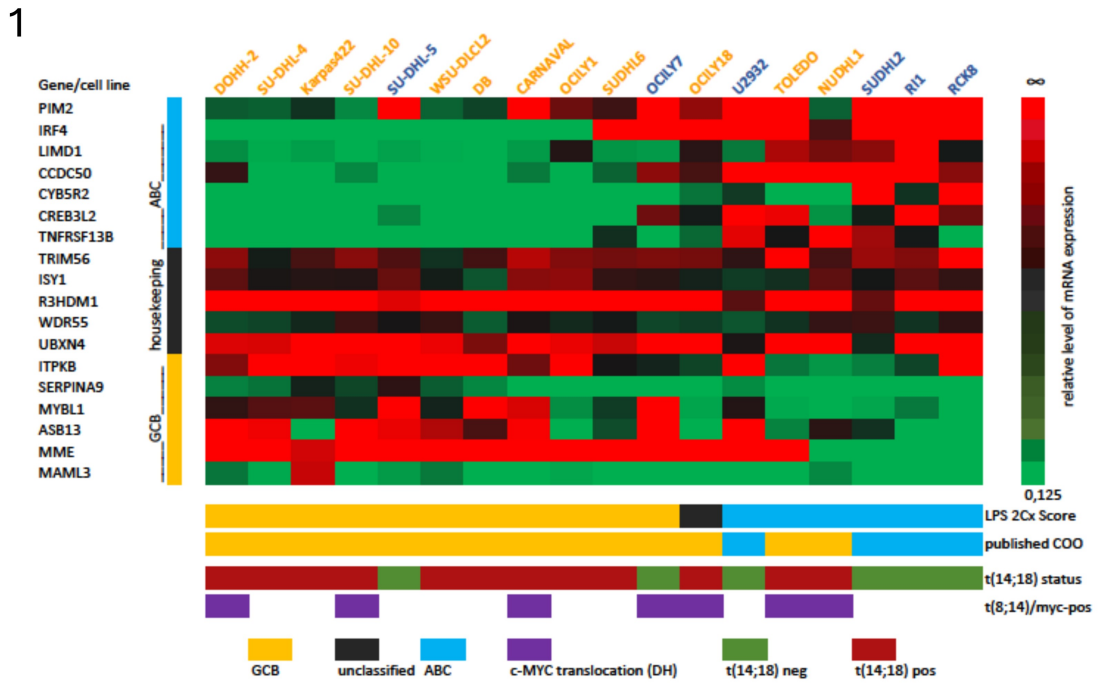

Fig 1: t+ DLBCL is associated with the GCB-subtype
RNA-Seq analysis and calculation of the LPS-Score (Scott et al., JCO, 2015) of our 18 human DLBCL-cell lines was performed. T+ DLBCL cell lines are enriched in the GCB DLBCL subgroup (double hit lymphoma excluded).

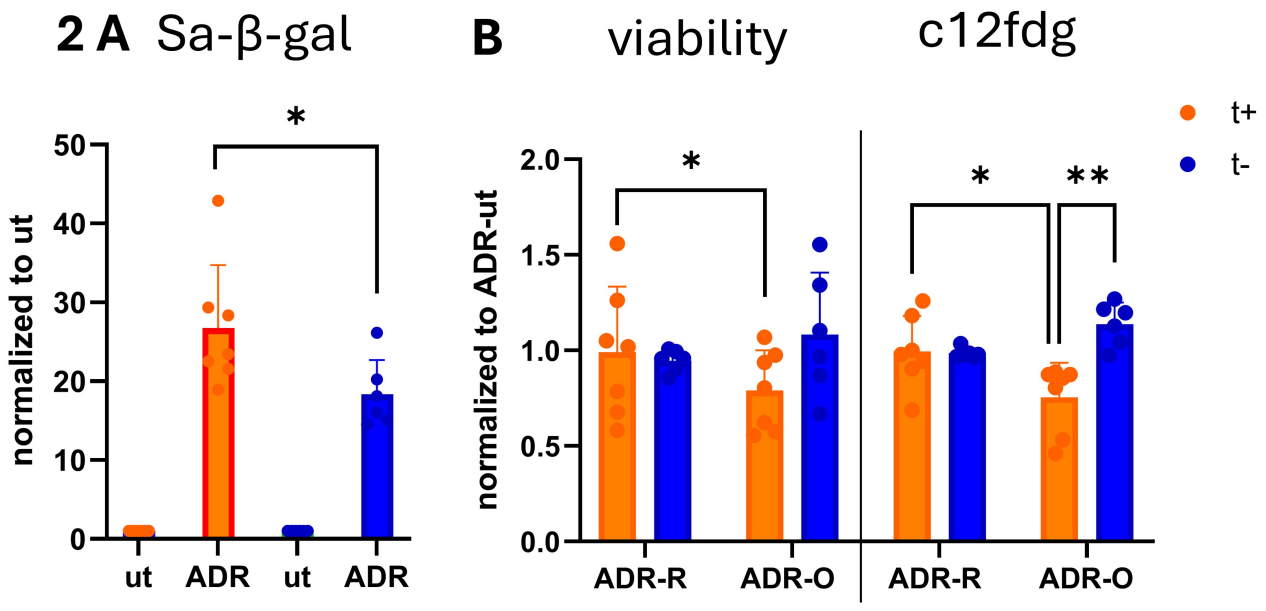

Fig 2: O acts as a senolytic agent against t+ DLBCL cell lines

A, t+ GCB showed a higher percentage of senescence associated β-galactosidase (Sa -β-gal) positive cells upon ADR treatment.

B, O, but not R, produced increase killing of ADR pretreated t+ GCB and eliminated senescent cells, detected via c12fdg, a flow-based staining of Sa-β-gal.

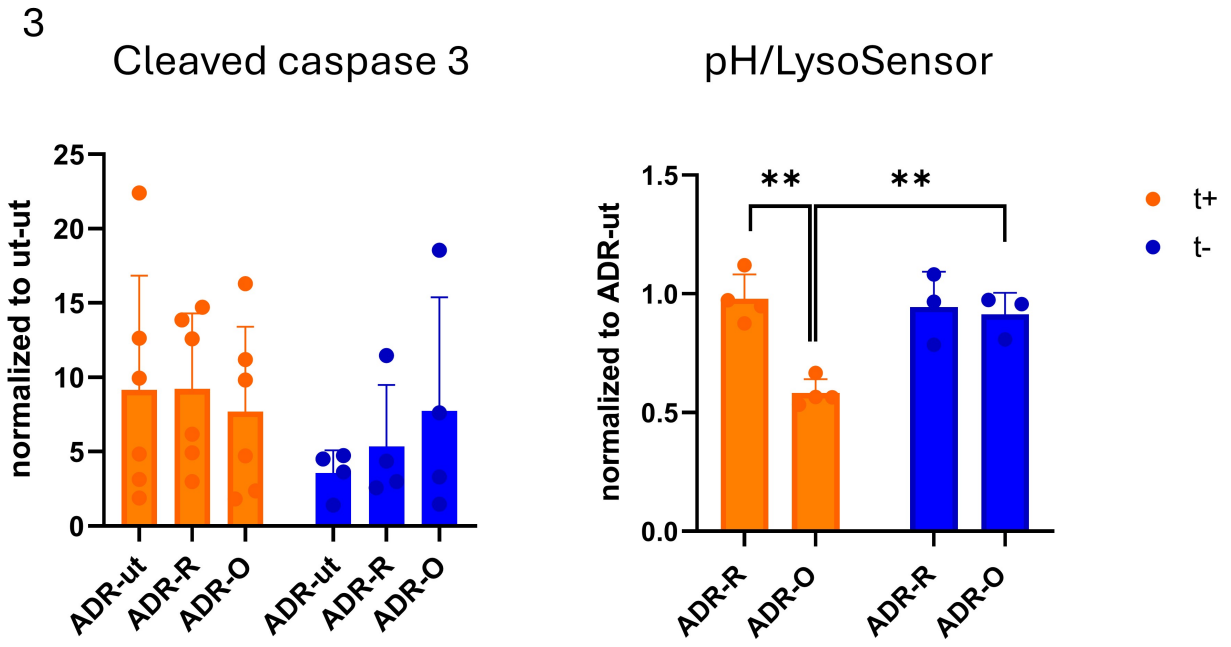

Fig 3: Senolytic activity of O is via the caspase-independent, cathepsin-mediated lysosomal pathway
No R/O differences were noted regarding enhanced caspase 3 cleavage, but a virtually O-exclusive elimination of acidic LysoSensor-positive cells was observed, indicating an O-mediated killing *via* the caspase-independent, cathepsin-mediated lysosomal pathway.