

# C-Myc Targeting by Degradation: Novel Dual c-Myc/GSPT1 Degradar GT19715 Exerts Profound Cell Kill *in vitro* and *in vivo* in Acute Myeloid Leukemia and Lymphomas

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## Abstract

**Objective:** The oncoprotein c-Myc governs epigenome and transcriptome and is deregulated in 70% of all human cancers. MYC is highly expressed in TP53 mutant or venetoclax (ven) resistant AML (Sallman, Blood 2021, Nishida, ASH 2021). However, targeting c-Myc or the MYC pathway has not met with success. Targeting oncoproteins utilizing cereblon E3 ligase modulators (CELMoDs) are attractive modalities to specifically target hitherto undruggable oncoproteins.

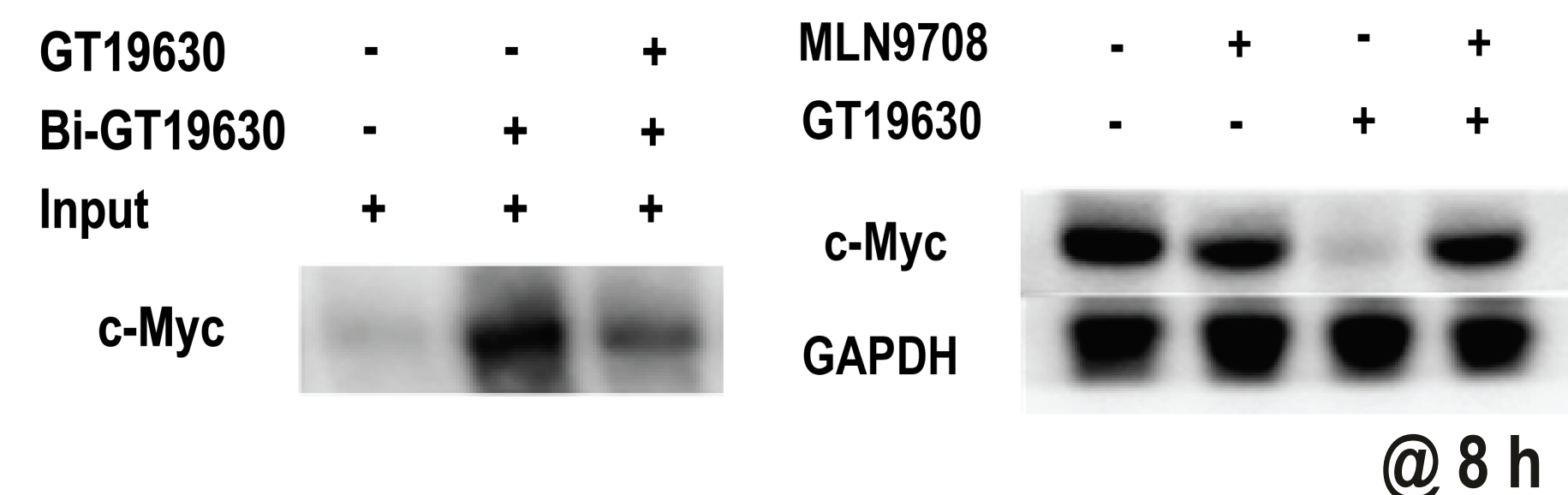
**Design and Setting:** We developed the first c-Myc degrader GT19630 (GT19715, the salt form of GT19630). We tested it in cell-free, cellular assays and in animal studies.

**Results:** GT19630 effectively degraded oncogenic c-Myc protein (IC<sub>50</sub> = 1.5 nM) in HL-60 cells. C-Myc was effectively pulled down by biotinylated GT19630 in a cell-free, in vitro affinity purification assay; and a proteasome inhibitor ixazomib completely blocked c-Myc degradation. IC<sub>50</sub> of GT19715 in HL-60 cells was 1.8 nM, being considerably lower than 40.2 nM, an IC<sub>50</sub> of normal myeloid progenitors in CFU assay, suggesting a therapeutic window. GT19630 shares chemical properties with other CELMoDs and proteomic analyses revealed degradation of translation termination factor G1 to S phase transition proteins 1 (GSPT1), an important factor in LSC survival (Surka et al. Blood 2021). Indeed, GT19630 effectively degrades GSPT1 along with complete degradation of c-Myc in a xenograft model with HL-60 cells, and inhibits tumor growth at a dose as low as 0.3 mg/kg/bid. GT19630 had no effect on normal myeloid lineages in rats at 6 mg/kg. GT19715 eliminates circulating blasts and prolongs survival in the c-Myc-driven systemic Daudi leukemia/lymphoma model. Importantly, GT19715 induces cell killing independent of TP53 status, and baseline c-Myc protein levels significantly correlated with sensitivity to GT19715 in MOLM-13 cells with CRISPR engineered knockout or mutations of TP53 (R<sup>2</sup> = 0.86, P = 0.02). We found that MV4;11 ven resistant (VR) cells demonstrated elevated protein levels of c-Myc, and GSPT1 and exhibited greater sensitivity to GT19715 compared to ven-sensitive parental cells. Finally, GT19715 significantly reduced human CD45+ AML blasts compared to vehicle control in vivo in an AML PDX model.

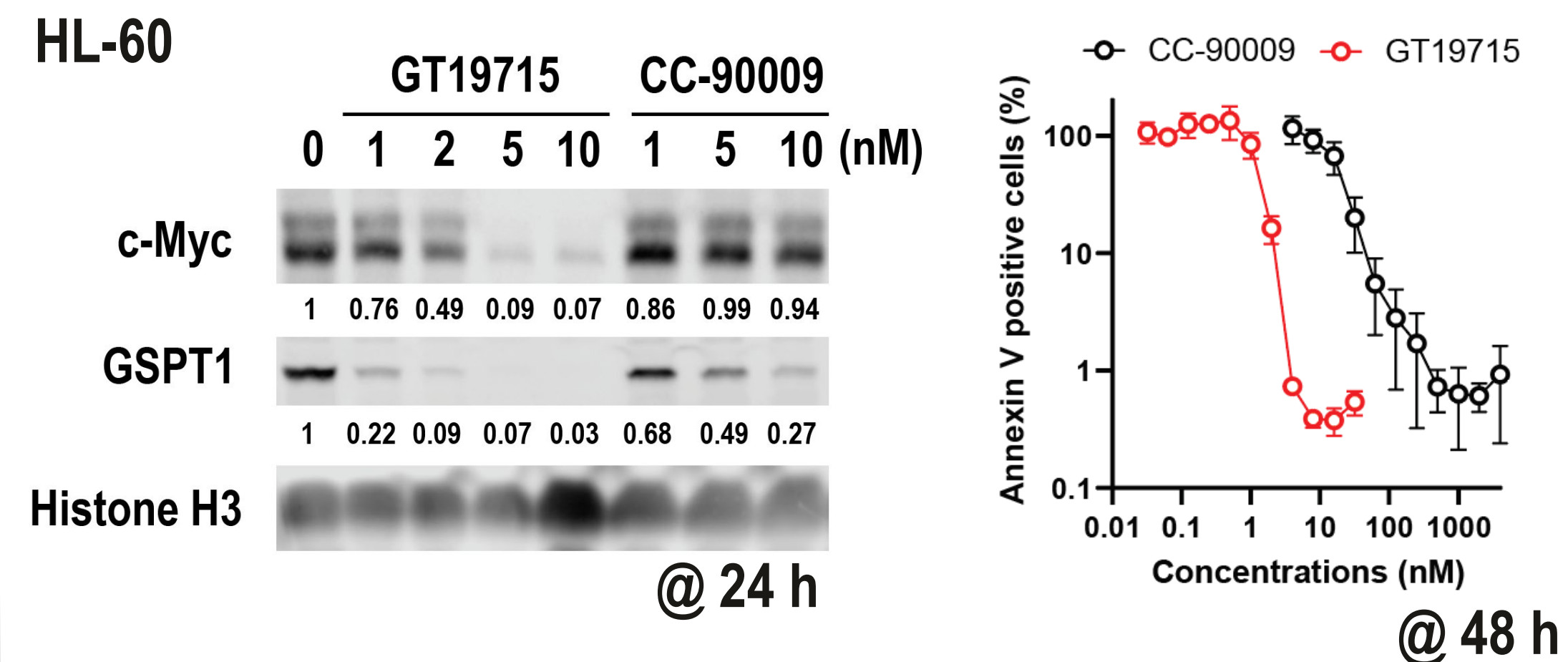
**Conclusion:** First results with the novel dual c-Myc/GSPT1 degrader GT19715 demonstrate promising preclinical anti-lymphoma and -leukemia efficacy, providing rationale for its clinical development.

## Results

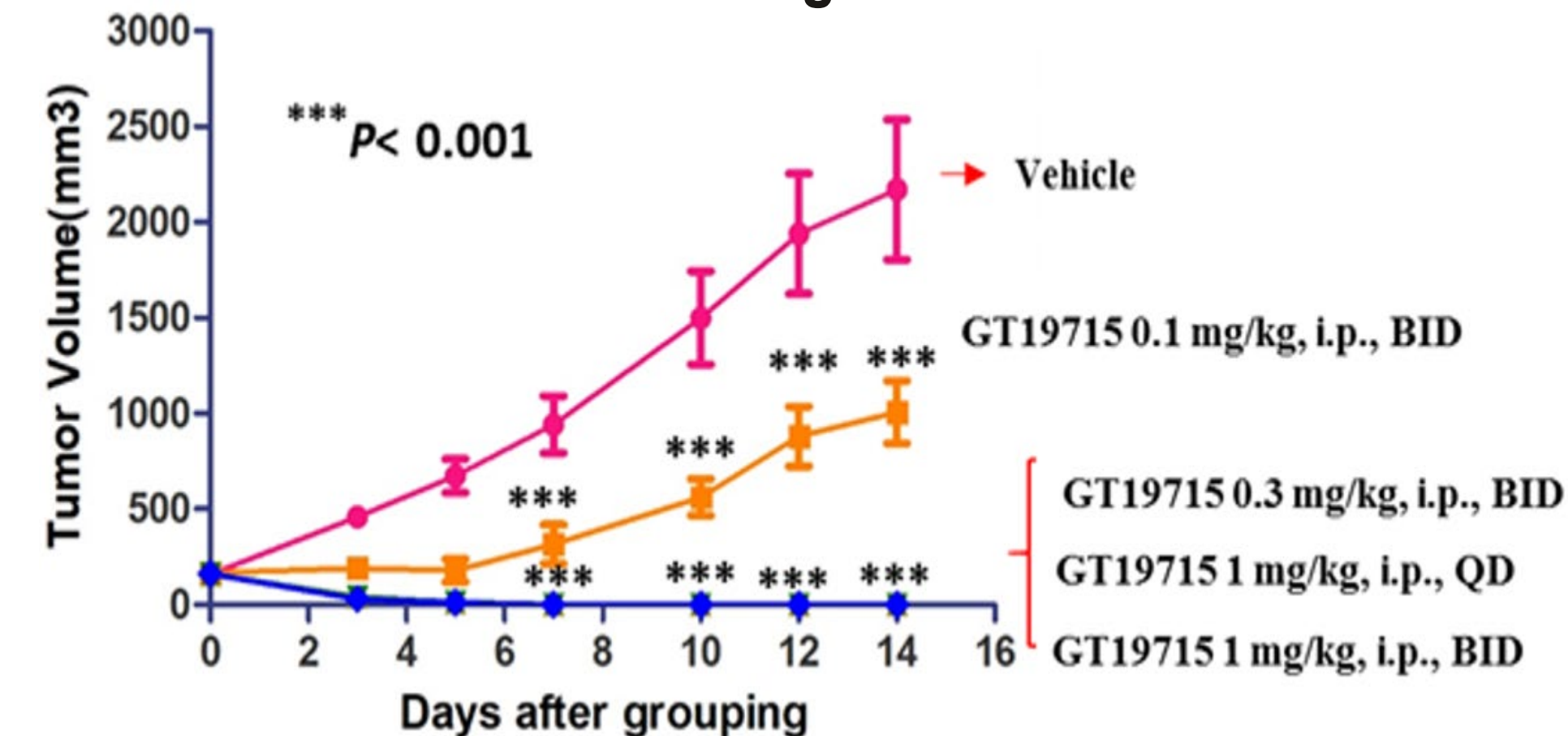
### GT19630 Binds c-Myc Protein and Induce Proteasome-Dependent c-Myc Degradation in MYC-driven HL-60 Cells



### GT19715 Reduces c-Myc and GSPT1 Protein and Induces Cyto reduction in vitro and in vivo

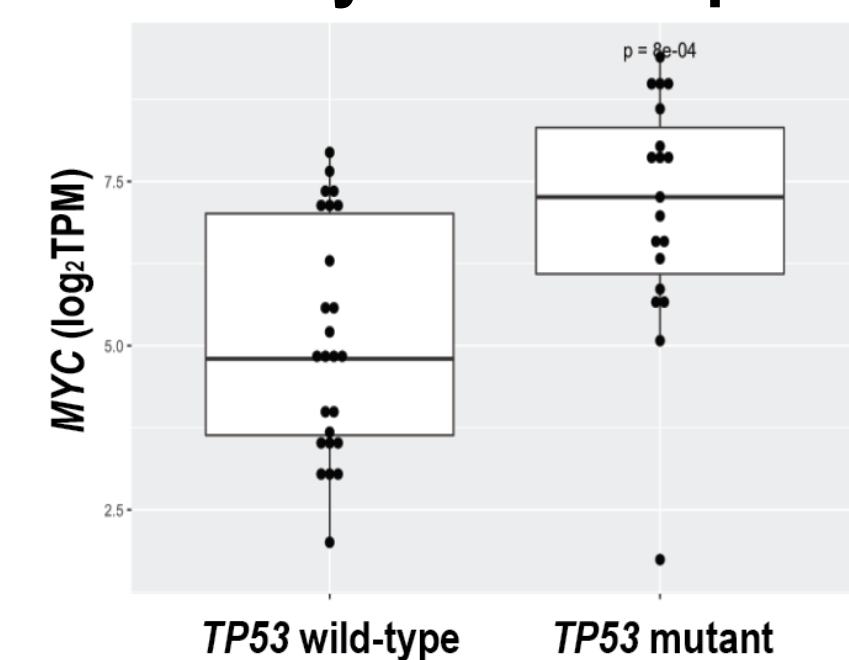


### HL-60 Xenograft Model

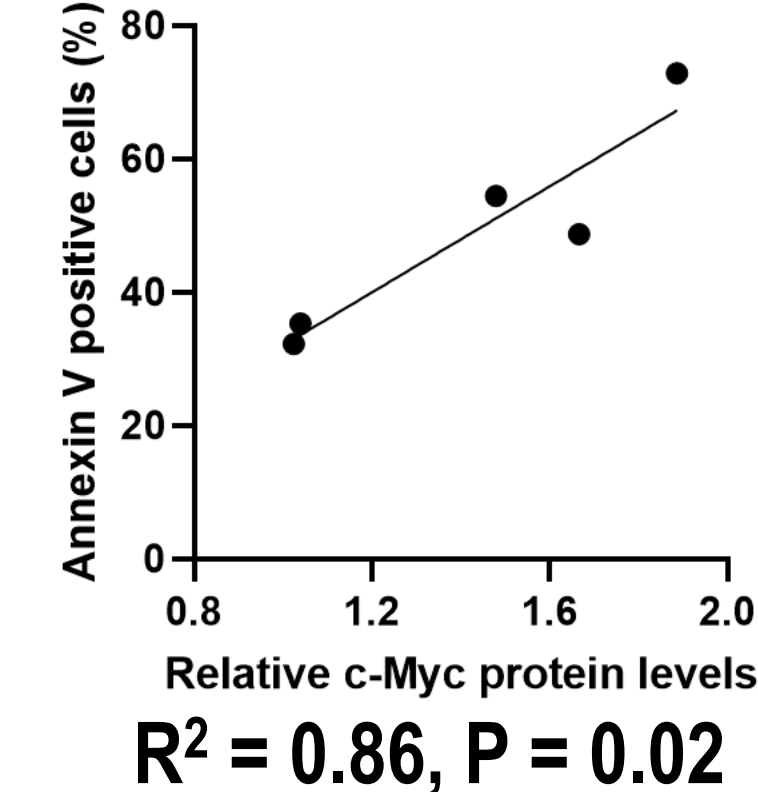


### Higher MYC Expression Levels in TP53 Mutant than in TP53 Wild-type AML - Correlation with Sensitivity to GT19715

#### Primary AML Samples

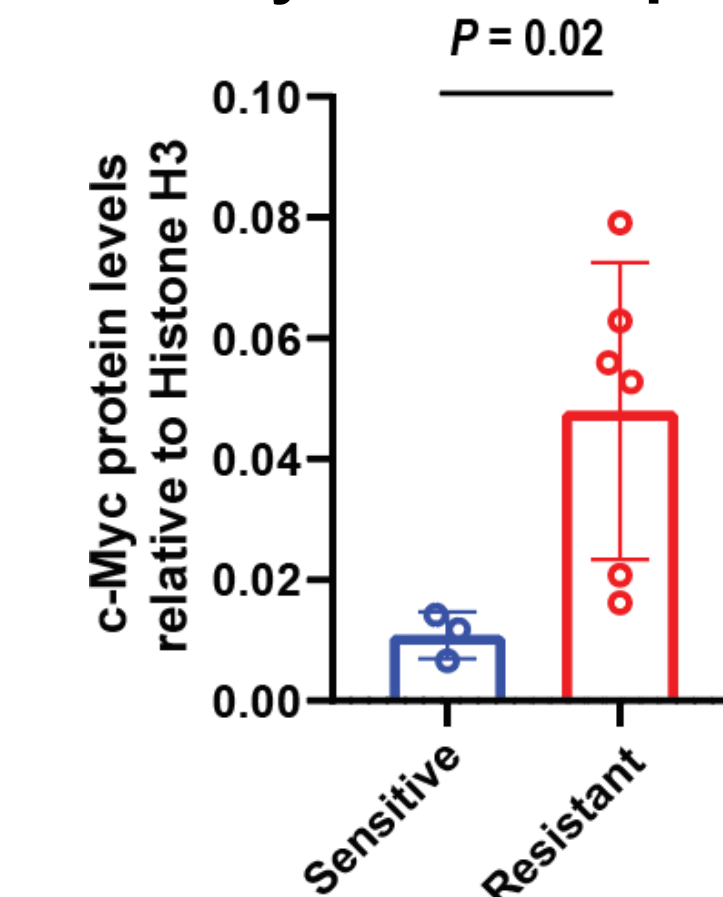


#### MOLM-13 TP53mut/KO

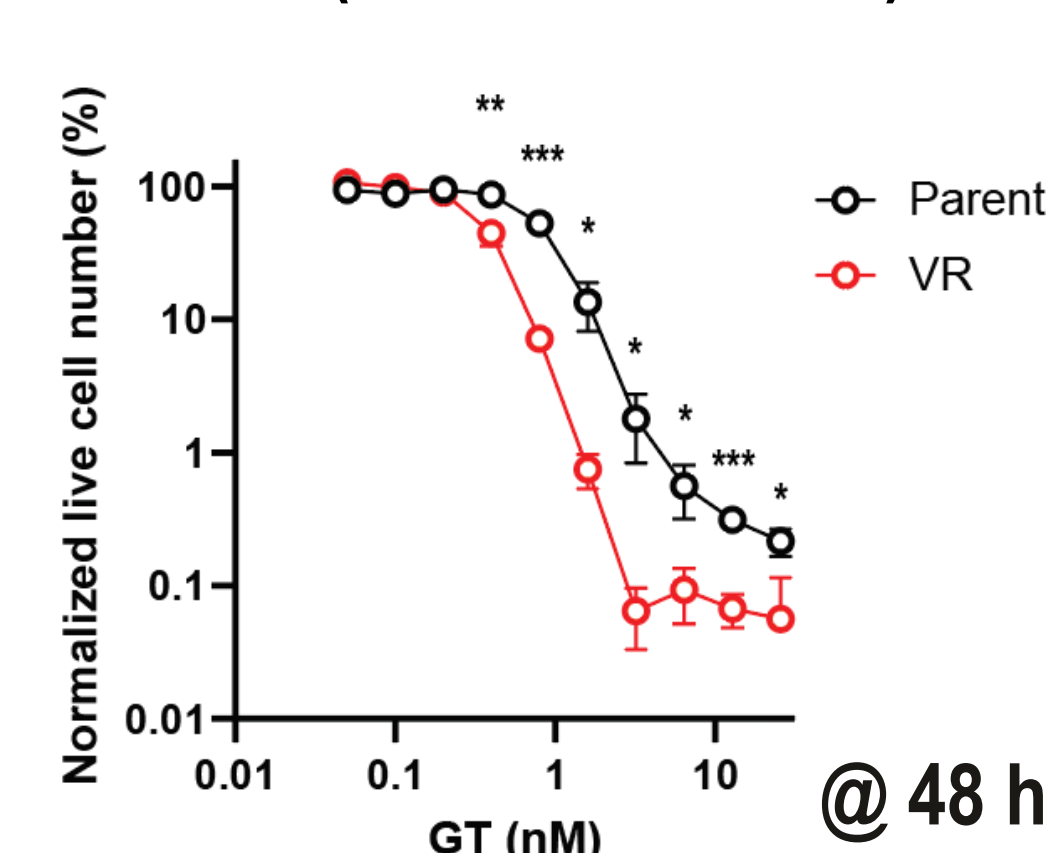


### Venetoclax Resistant AML Samples Exhibit Increased c-Myc Protein Levels and Show Greater Sensitivity to GT19715

#### Primary AML Samples

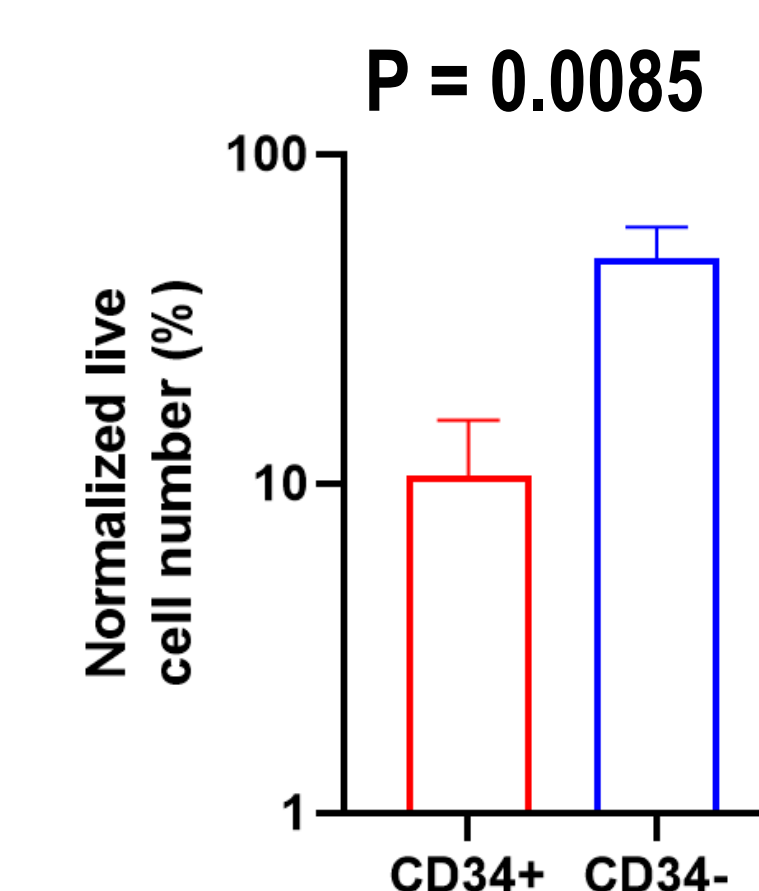


#### MV4;11 (Parent vs Ven-R)

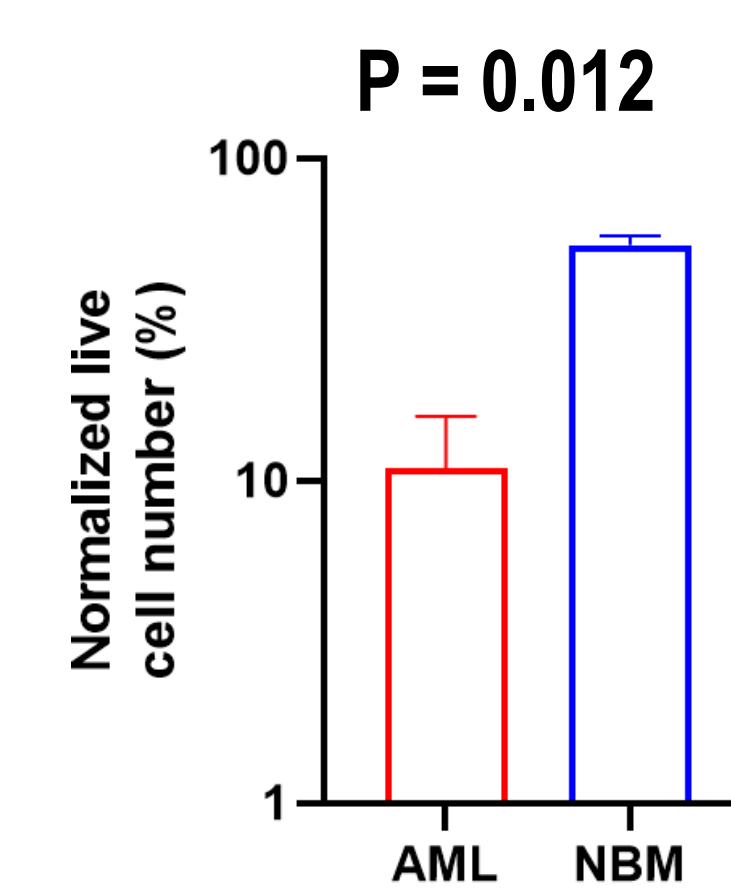


### GT19715 Exerts Greater Sensitivity in Immature CD34+ Primary AML Cells While Sparing CD34+ Normal BM Cells

#### CD34+ vs CD34- AML Cells

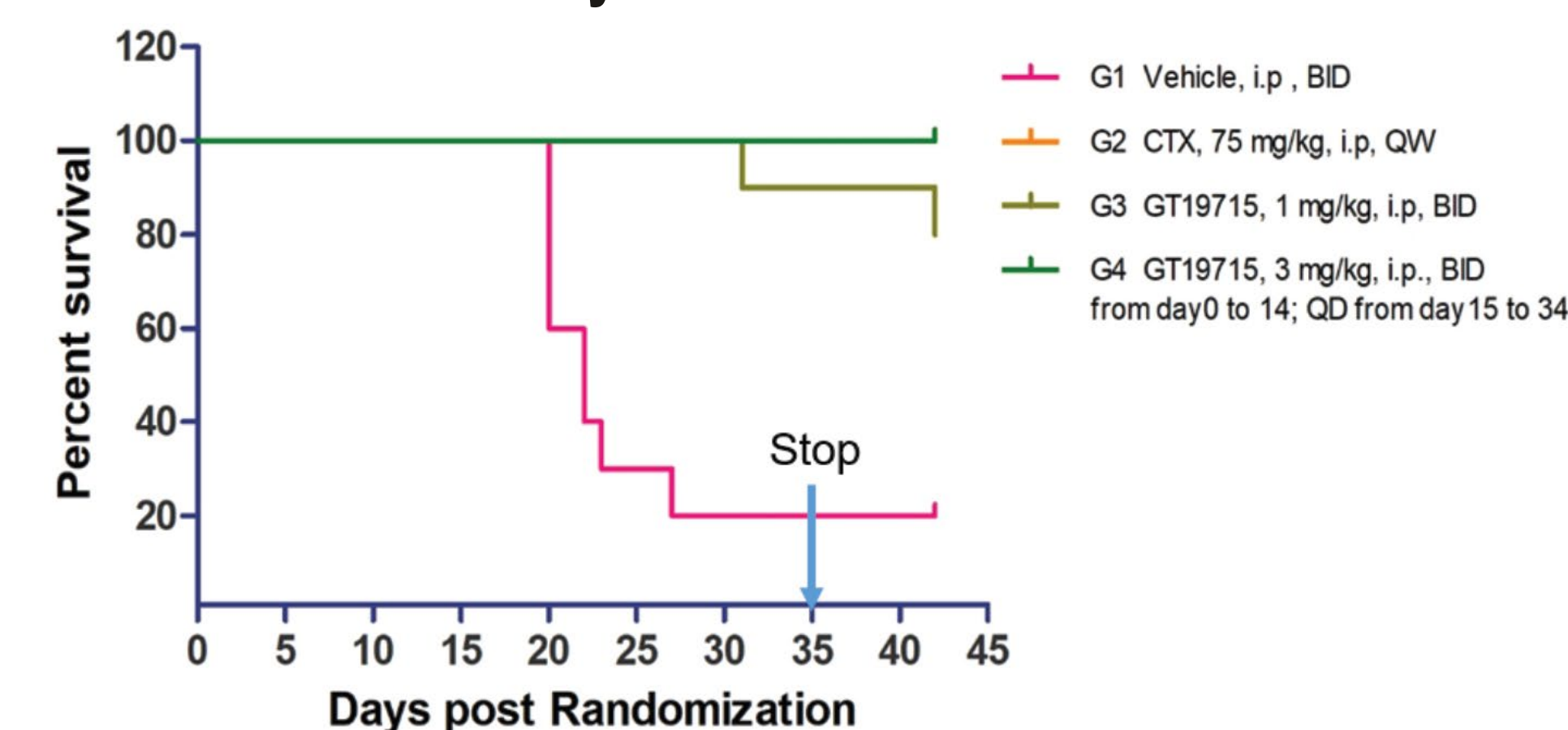


#### AML vs Normal CD34+ Cells

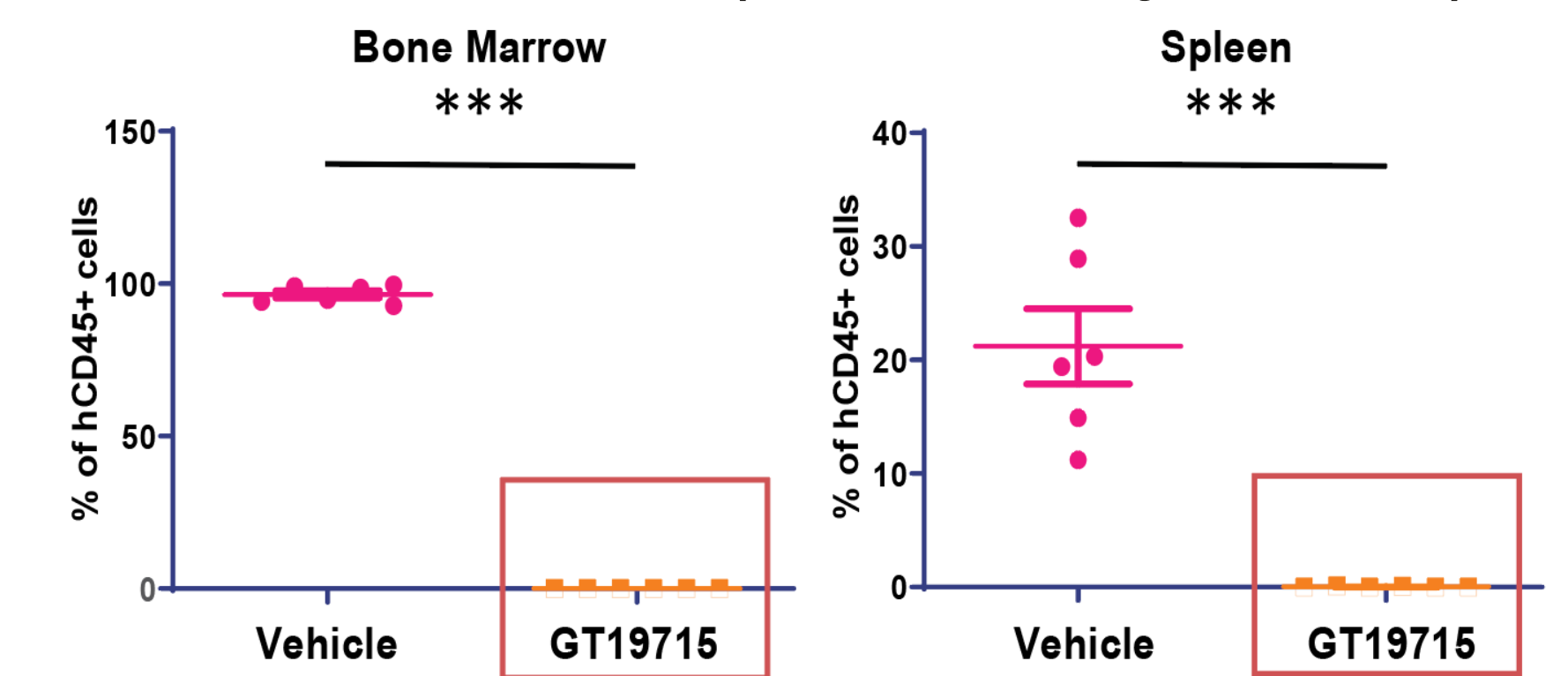


### GT19715 Exerts in vivo Anti-Leukemia/Lymphoma Efficacy

#### Daudi Systemic Leukemia Model



### PDX AML Model (chemotherapy-resistant)



## Summary

- First c-Myc/GSPT1 dual degrader GT19630/GT19715 binds c-Myc and degraded c-Myc and GSPT1 utilizing the ubiquitin proteasome system.
- GT19715 induced tumor reduction in MYC-driven HL-60 xenograft model.
- TP53 mutant AML samples exhibited elevated expression levels of MYC compared to TP53 wild-type ones and c-Myc protein levels correlated with sensitivity to GT19715 in TP53 mutant AML cells.
- Ven-resistant AML cells with elevated c-Myc protein levels responded to GT19715 better than Ven-sensitive AML cells.
- Immature CD34+ AML cells were more sensitive to GT19715 than more mature CD34- AML cells. Notably, normal bone marrow CD34+ cells were less sensitive to GT19715 than CD34+ AML cells, suggesting a therapeutic window.
- GT19715 demonstrated promising anti-leukemia efficacy in MYC-driven Daudi systemic leukemia and PDX AML models.

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