TT125-802 is a potent and highly selective CBP/p300 bromodomain inhibitor for the treatment of castration resistant prostate cancer and hematological malignancies

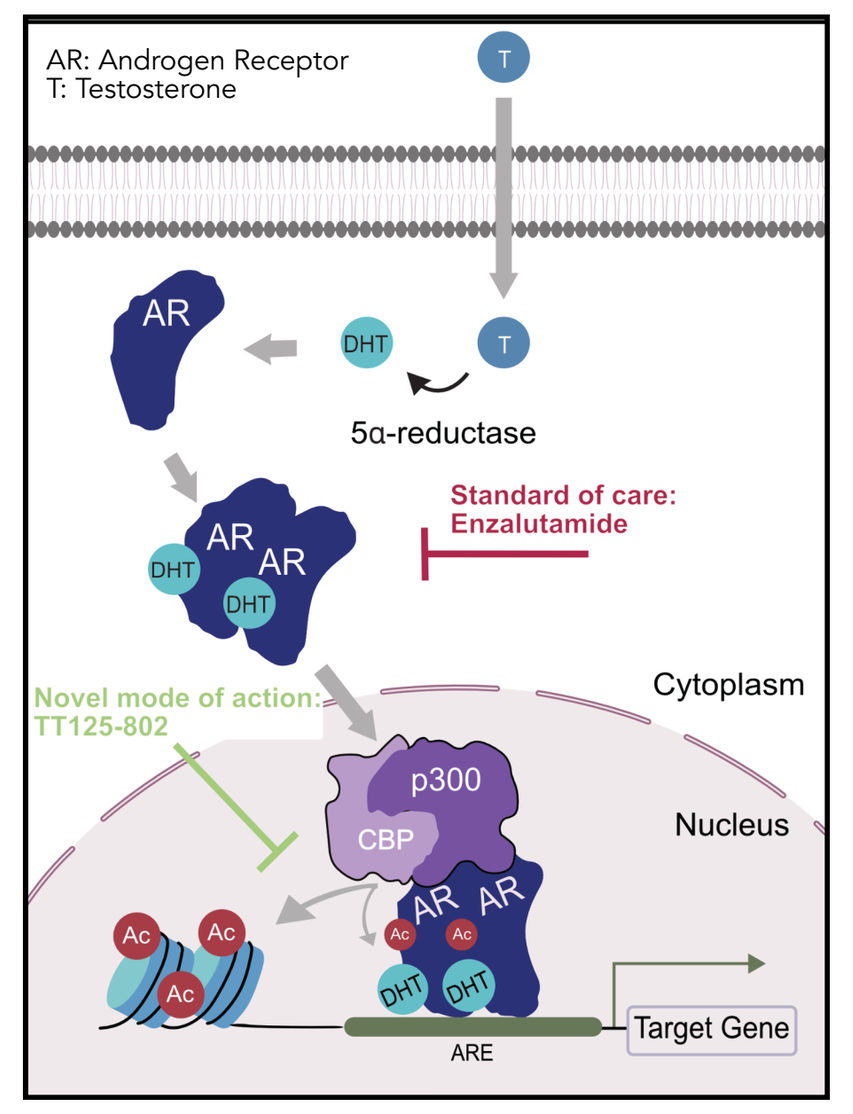
Sara Laudato, Dorothea Gruber, Thomas Bohnacker, Martin Schwill, Charles-Henry Fabritius, Raquel Herrador, Katrin Westritschnig, Thushara Pattupara, Vikram Ayinampudi, Stefanie Flückiger-Mangual
TOLREMO therapeutics AG, Muttenz, Switzerland

TOLREMO therapeutics

Poster #6268

Abstract

The paralogous lysine acetyltransferases CREB-binding protein (CBP) and p300 are key epigenetic regulators involved in diverse signaling pathways in cancer. The bromodomain of CBP/p300 serves as an acetyl-lysine "reader" that allows CBP/p300 to bind chromatin at acetylated histone and non-histone proteins leading to the regulation of gene transcription. CBP/p300 are critical co-activators of nuclear receptors, including the androgen receptor (AR) in castration resistant prostate cancer (CRPC). Thus, inhibition of CBP and p300 is an emerging therapeutic strategy to block the transactivation activity of the AR in CRPC. In addition, inhibition of the bromodomain of CBP/p300 has been described as a therapeutic strategy to treat multiple myeloma (MM) through transcriptional suppression of interferon regulatory factor 4 (IRF4) and concomitant repression of its target genes MYC and MYB.



adapted from Waddell et al 2021

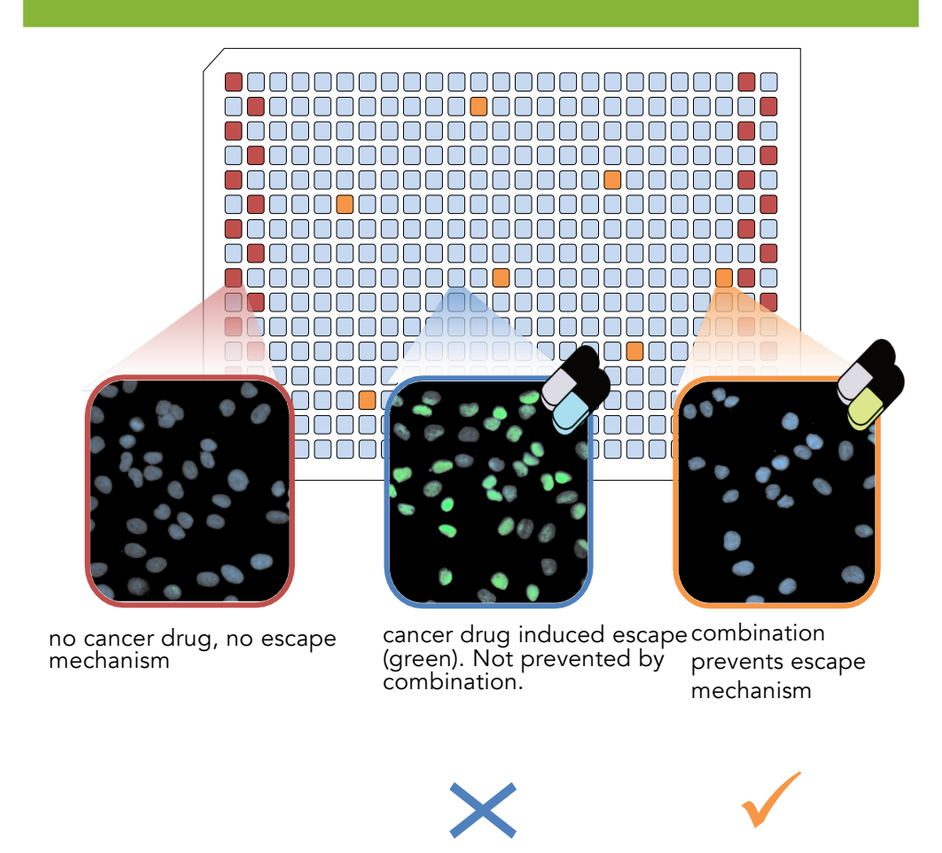
TT125-802 shows selective anti-proliferative activity in AR-dependent prostate cancer cell lines (22Rv-1, C4-2, and LNCaP) and inhibits AR target gene expression (KLK2, KLK3, TMPRSS2, and MYC) in a dose-dependent manner. AR-negative prostate cancer cell lines (DU-145 and PC-3) are insensitive to TT125-802 in vitro, pointing to an AR-selective mode of action. In addition, preclinical studies in CRPC patient-derived xenograft (PDX)-bearing mice showed that the combination treatment of TT125-802 and enzalutamide had a synergistic effect on tumor growth inhibition compared to single agent treatments. TT125-802 reduced mRNA expression of the AR-target genes in tumor samples and decreased plasma PSA levels compared to enzalutamide alone. The combination of both agents reduced levels even further. Moreover, TT125-802 suppressed adaptive drug resistance signatures induced by enzalutamide treatment. In a preclinical model of multiple myeloma (OPM2), TT125-802 had a dose-dependent effect on tumor growth, inducing tumor regressions at the highest dose. Target genes such as MYC, MYB, and IRF4 were potently downregulated in tumors.

We conclude that TT125-802 is a novel, highly selective inhibitor of the bromodomain of CBP/p300. It has therapeutic potential as monotherapy in prostate cancer and multiple myeloma and in combination with next-generation AR inhibitors for patients with lethal prostate cancer.

A FIH study of TT125-802 in cancer patients is on track to start in 2023.

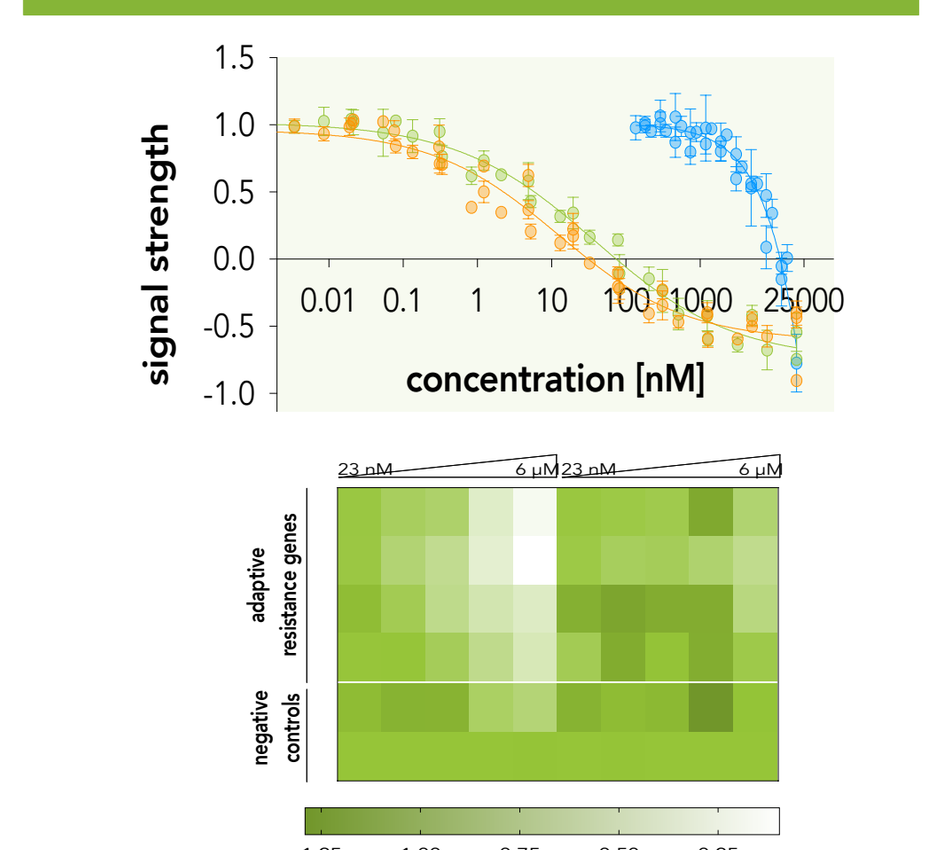
The CBP/P300 inhibitor TT125-802 was developed from a phenotypic screen

Robotic screen focused on adaptive resistance cell state



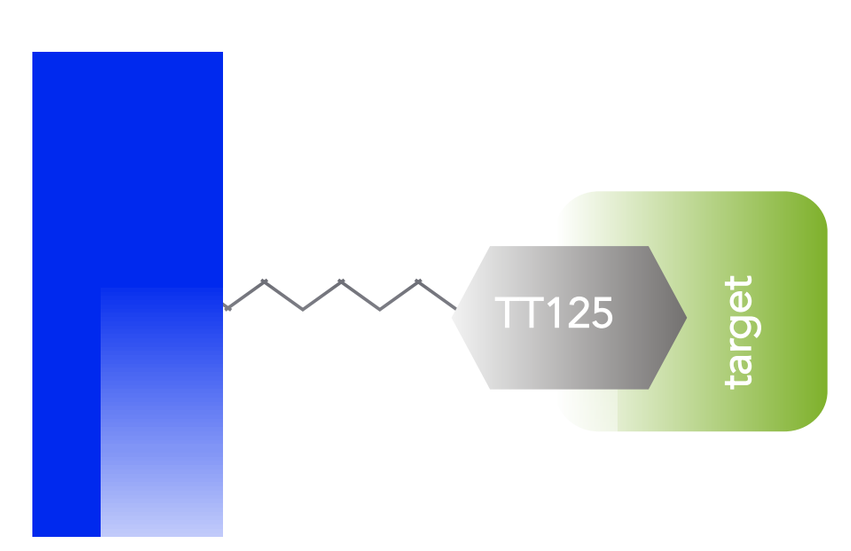
Fully automated, high-throughput phenotypic screen to identify small molecules that prevent non-genetic adaptive drug resistance. TT125 scaffold was identified.

Hit-to-lead using proprietary optimization cascade



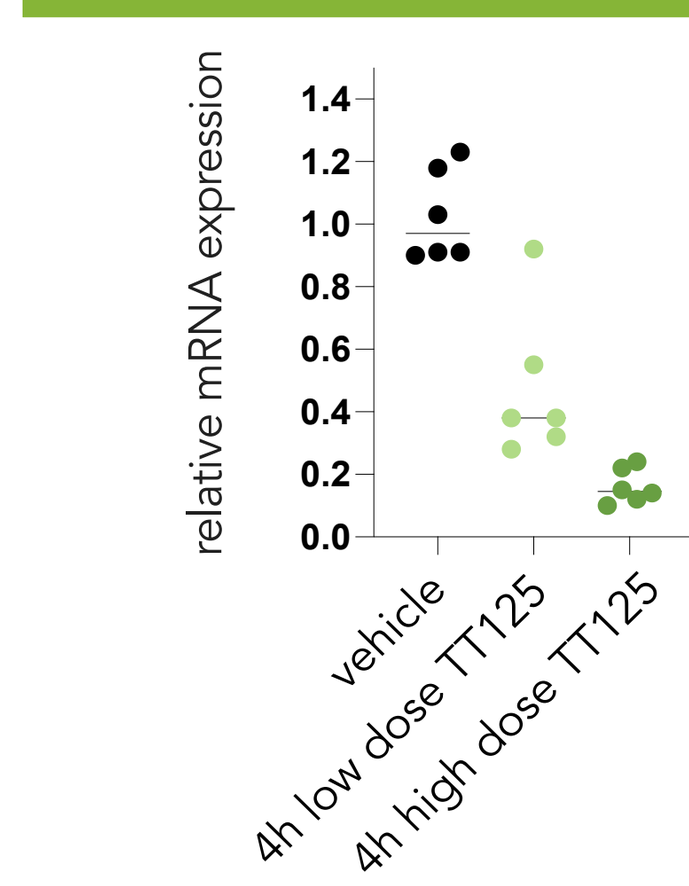
Proprietary, phenotypic compound optimization cascade to drive hit-to-lead phase of TT125 target agnostically.

Identify and validate CBP/P300 as target of TT125



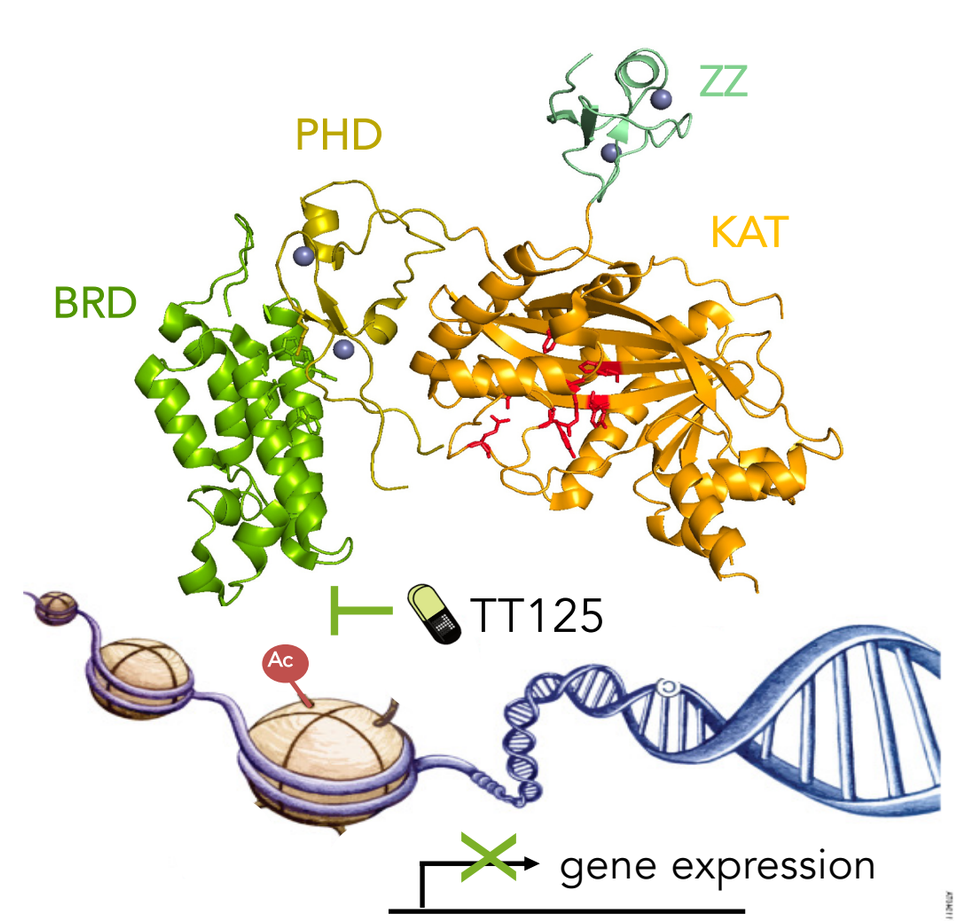
After initial optimization, TT125 scaffold was de-orphaned using chemoproteomics. TT125 binds to the bromodomain of the epigenetic regulators CBP/p300.

Lead optimization driven by rapid in vivo PD model



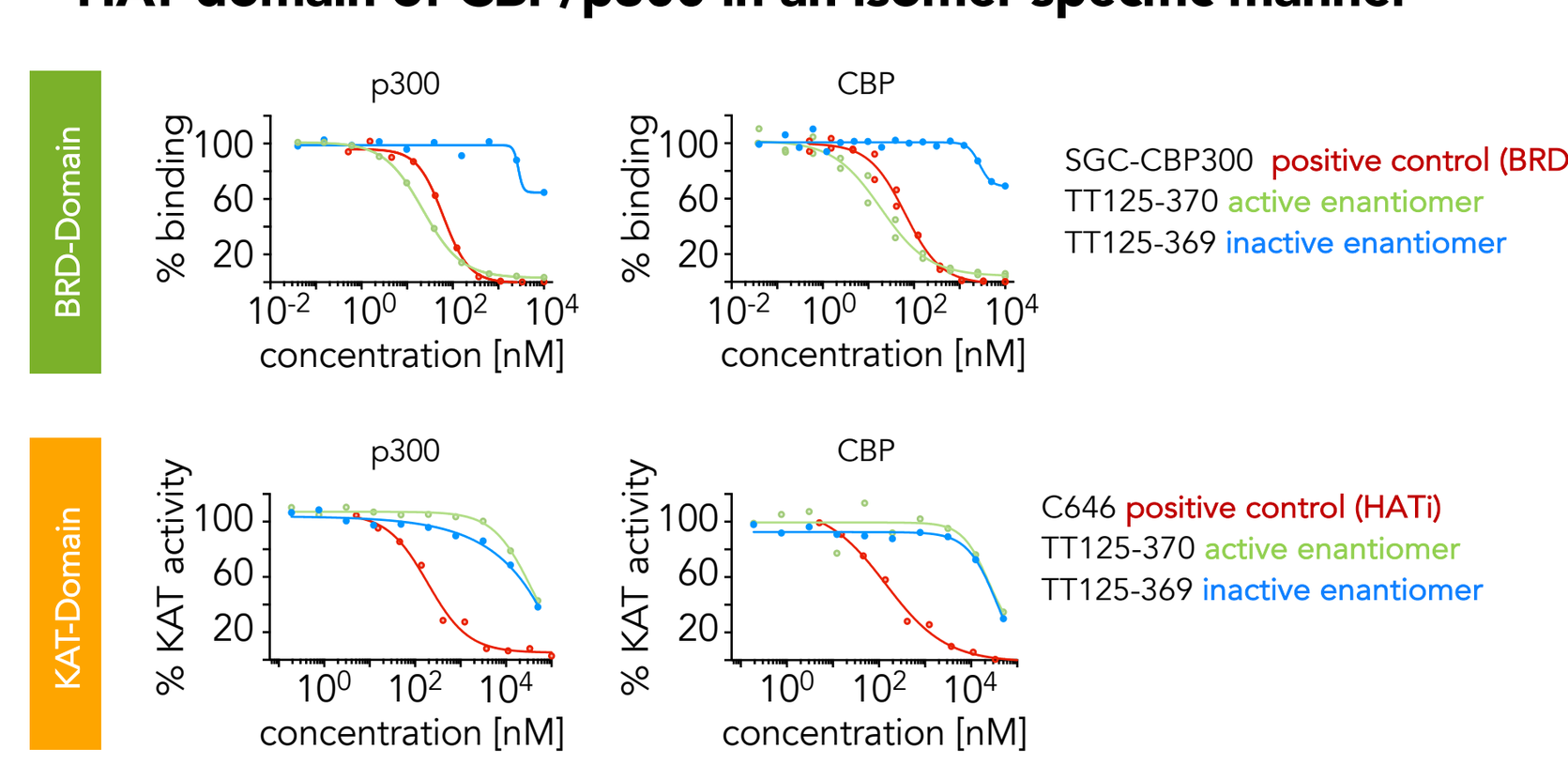
Expression of target genes in xenografts 4h after oral dosing of test compounds was used as rapid in vivo lead optimization model

TT125 binds to the bromodomain of CBP/P300 and it is BET-sparing

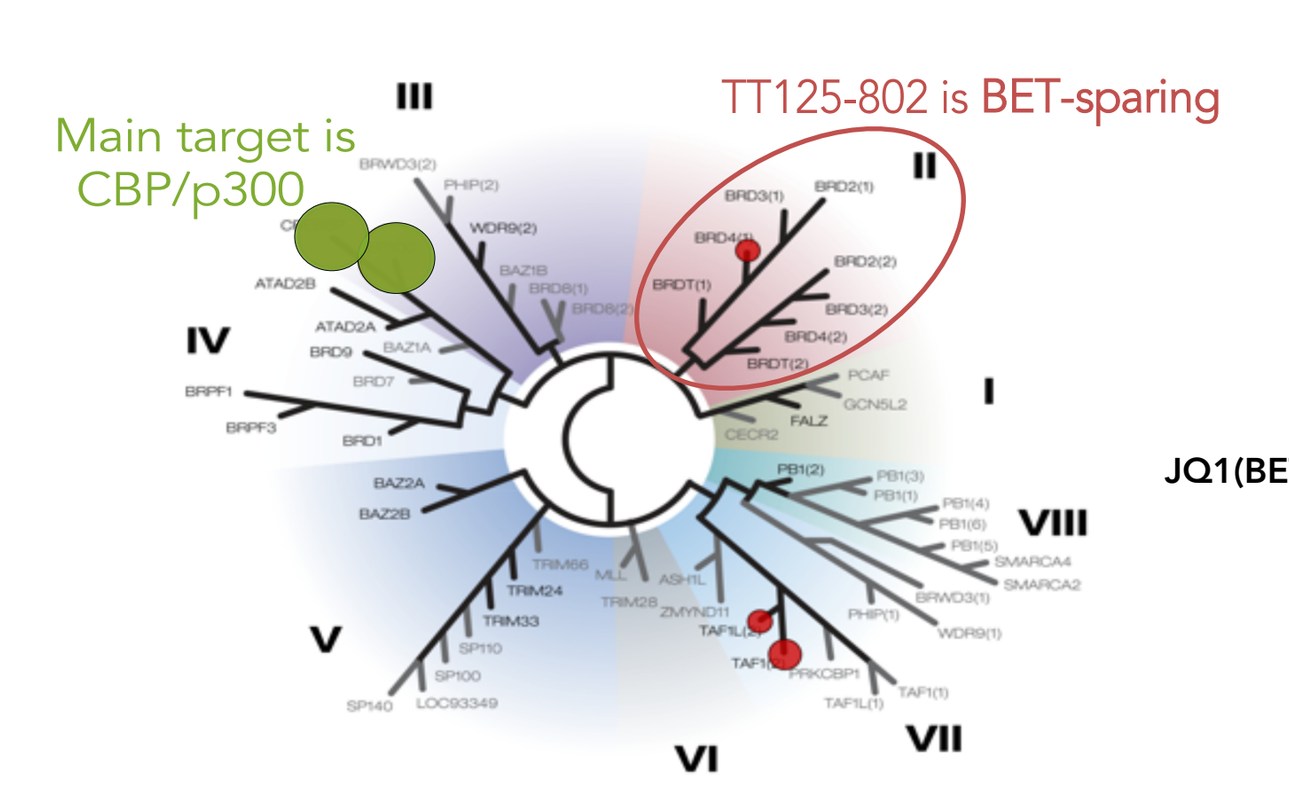


TT125 analogs bind to the bromodomain (BRD) of CBP and p300. They thereby prevent binding to acetylated histones and gene expression of target genes.

TT125 analogs bind the BRD bromodomain but not the catalytic HAT domain of CBP/p300 in an isomer-specific manner



TT125-802 is highly selective to the bromodomain of CBP/p300 and is BET-sparing



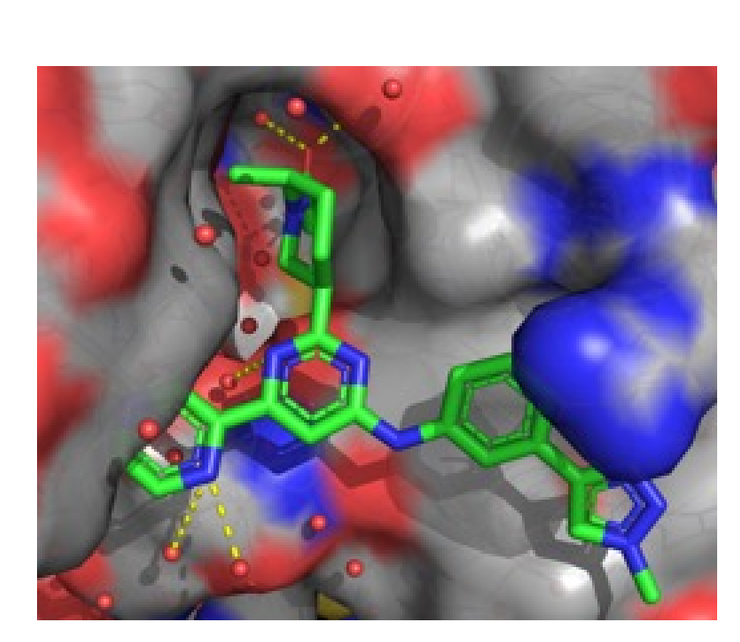
TT125-802 has low affinity for bromodomains of BET proteins

	BRD2 (1,2)	BRD2 (1)	BRD3 (1)	BRD4 (1)
TT125-802	2330	10000	3319	6077
JQ1(BET inhibitor)	16	117	11	18

IC50 (nM)

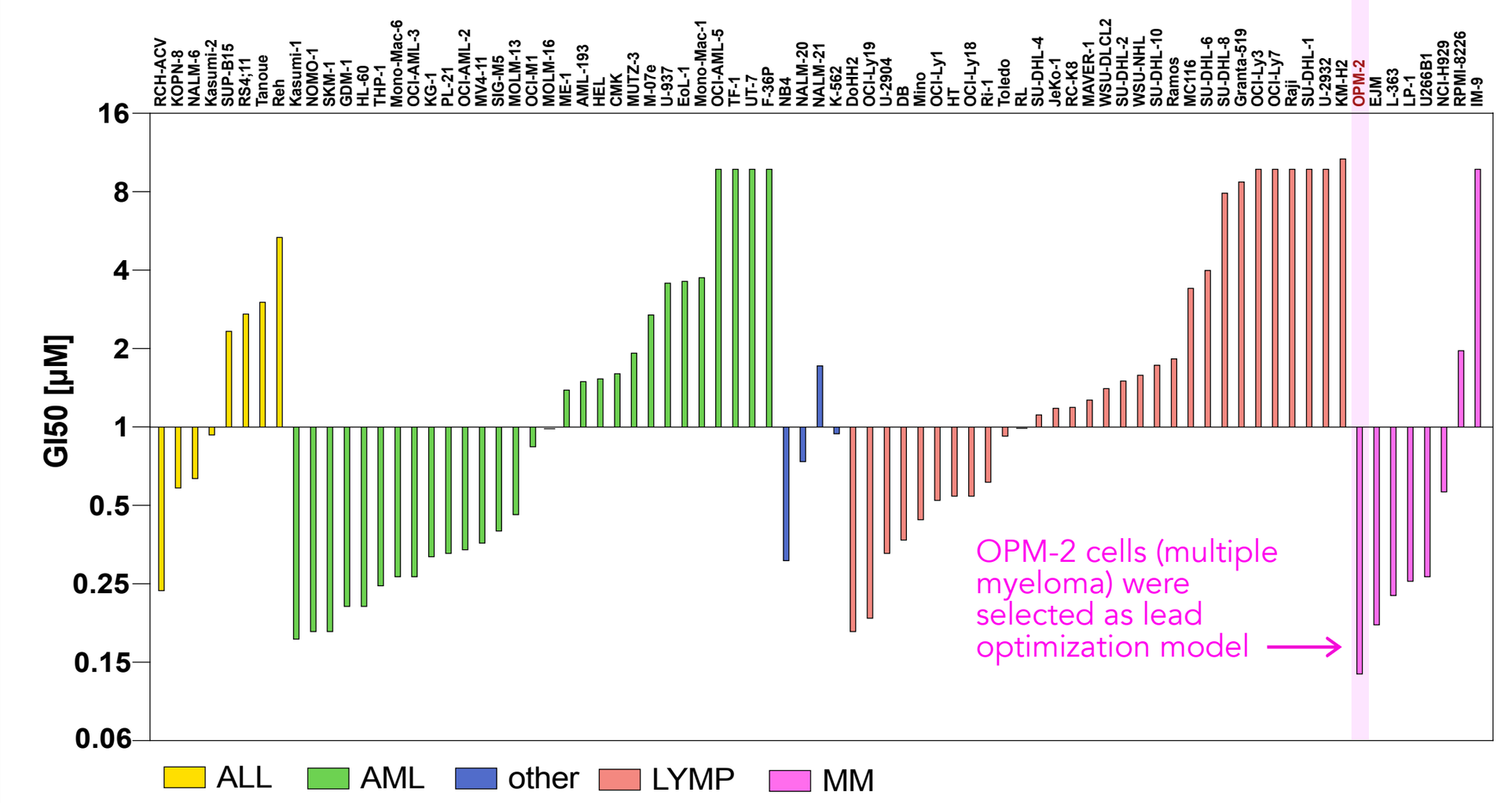
BromoScan K_d: CBP = 2.4nM; p300 =1.5nM; TAF1 = 370nM; all others >> 1000nM

X-ray of CPB bromodomain with TT125 analog

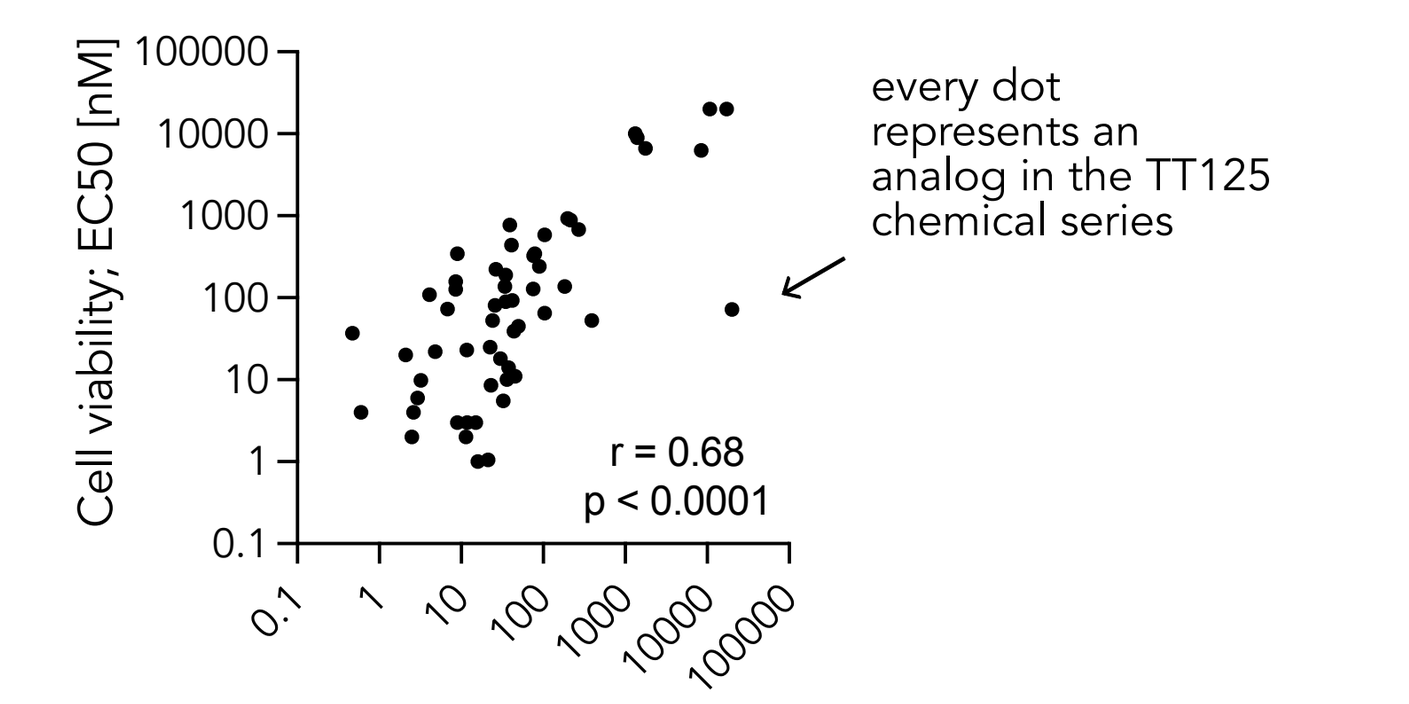


TT125 has anti-proliferative effects in haematological cancer cell lines

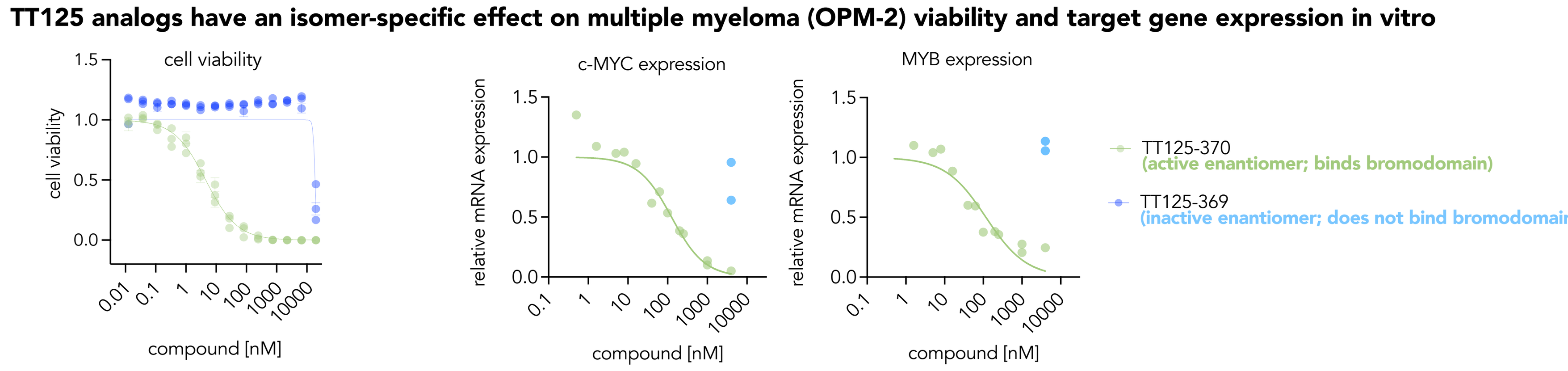
TT125 has single agent efficacy in cell lines of AML, multiple myeloma and lymphoma



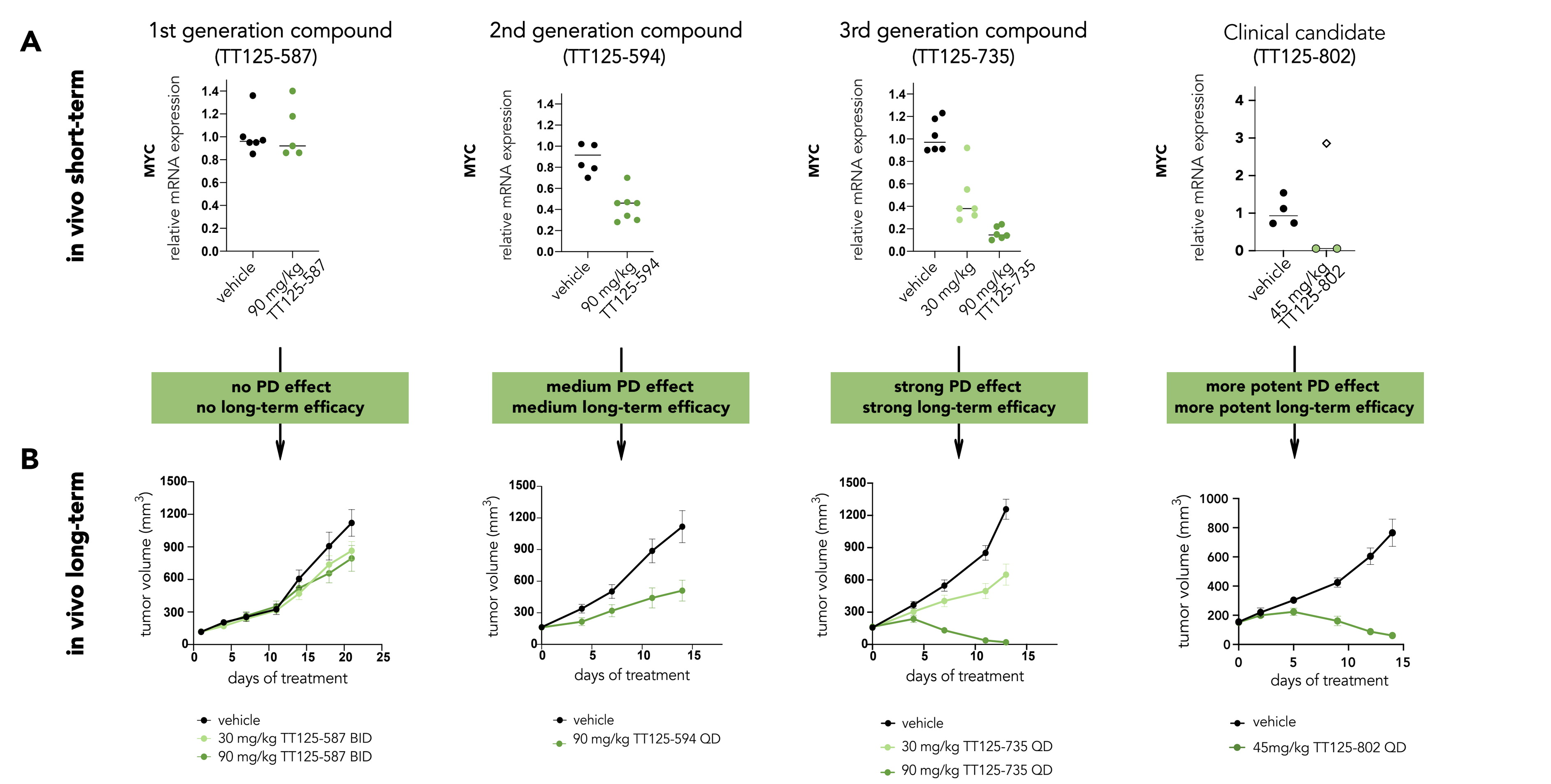
Activity of TT125 analogs in multiple myeloma (OPM-2) viability assay highly correlates with binding to the bromodomain of CBP



TT125 analogs have an isomer-specific effect on multiple myeloma (OPM-2) viability and target gene expression in vitro



Short-term in vivo PD model drove lead optimization of TT125 compound series and translated into long-term efficacy in preclinical multiple myeloma model



A. Short-term PD assay in OPM2 xenograft-bearing mice. Animals were orally dosed with candidate compounds for 4h and mRNA expression was measured. Each dot represents one tumor. **B.** Long-term efficacy of candidate compounds in OPM2 xenografts. Short-term PD readout (higher throughput, short timelines, low animal numbers) translated into long-term tumor control (lower throughput, longer timelines, higher animal numbers).

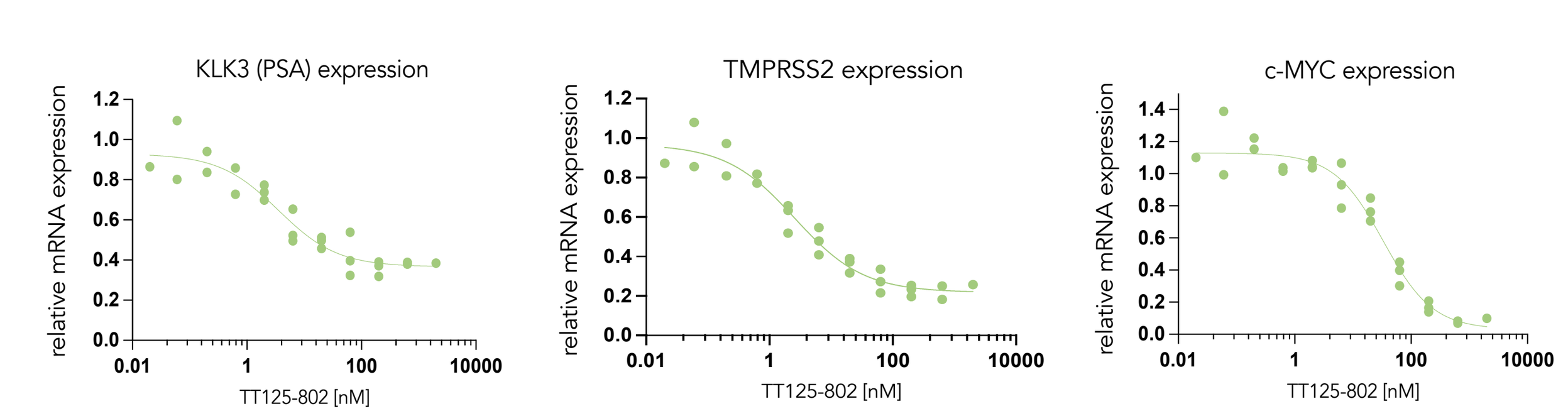
TT125-802 inhibits growth of AR-positive prostate cancer cell lines and dose-dependently inhibits expression of AR target genes and c-MYC

TT125-802 inhibits growth of AR-positive, enzalutamide-resistant prostate cancer cells

Cell line	AR-driven	TT125-802	enzalutamide
22Rv1	+	8	>10000
C4-2	+	31	>10000
LNCaP	+	129	3927
DU145	-	>10000	n/a
PC3	-	10000	n/a

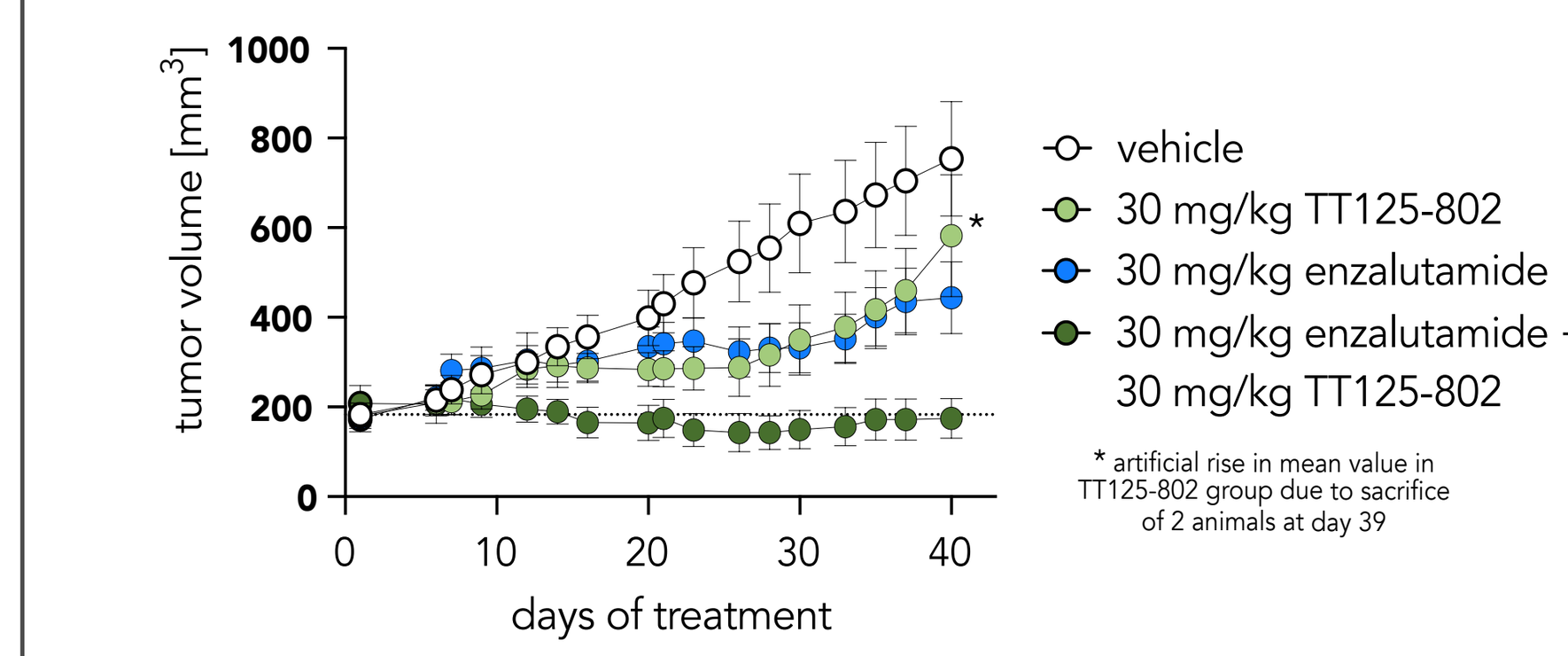
IC50 values (mean of 3 experiments) in nM

TT125-802 inhibits expression of AR target genes and c-Myc in AR-positive LNCaP cells

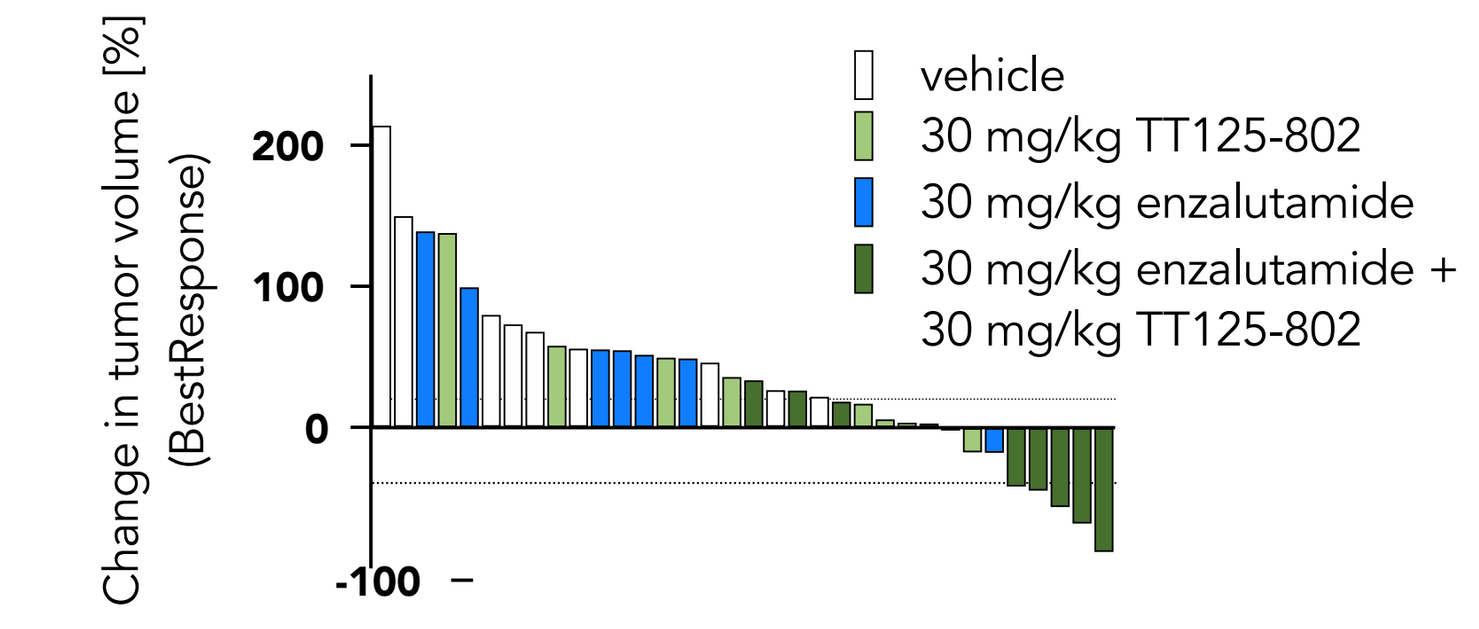


TT125-802 synergizes with enzalutamide in a castration-resistant prostate cancer PDX model

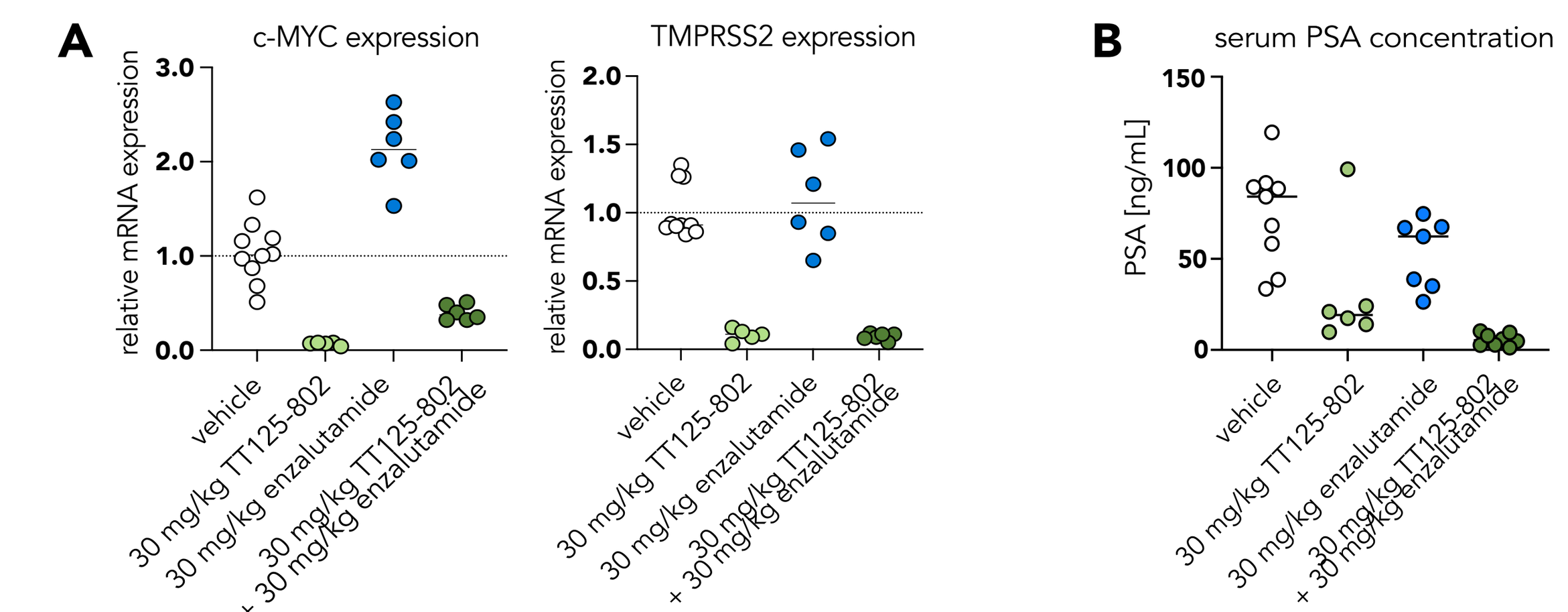
TT125-802 synergizes with enzalutamide and prevents resistance development in prostate cancer PDX model (PR6512)



TT125-802 increases response rates to enzalutamide in prostate cancer PDX model (PR6512)



TT125-802 inhibits expression of c-Myc and AR target genes in prostate cancer PDX model (PR6512)

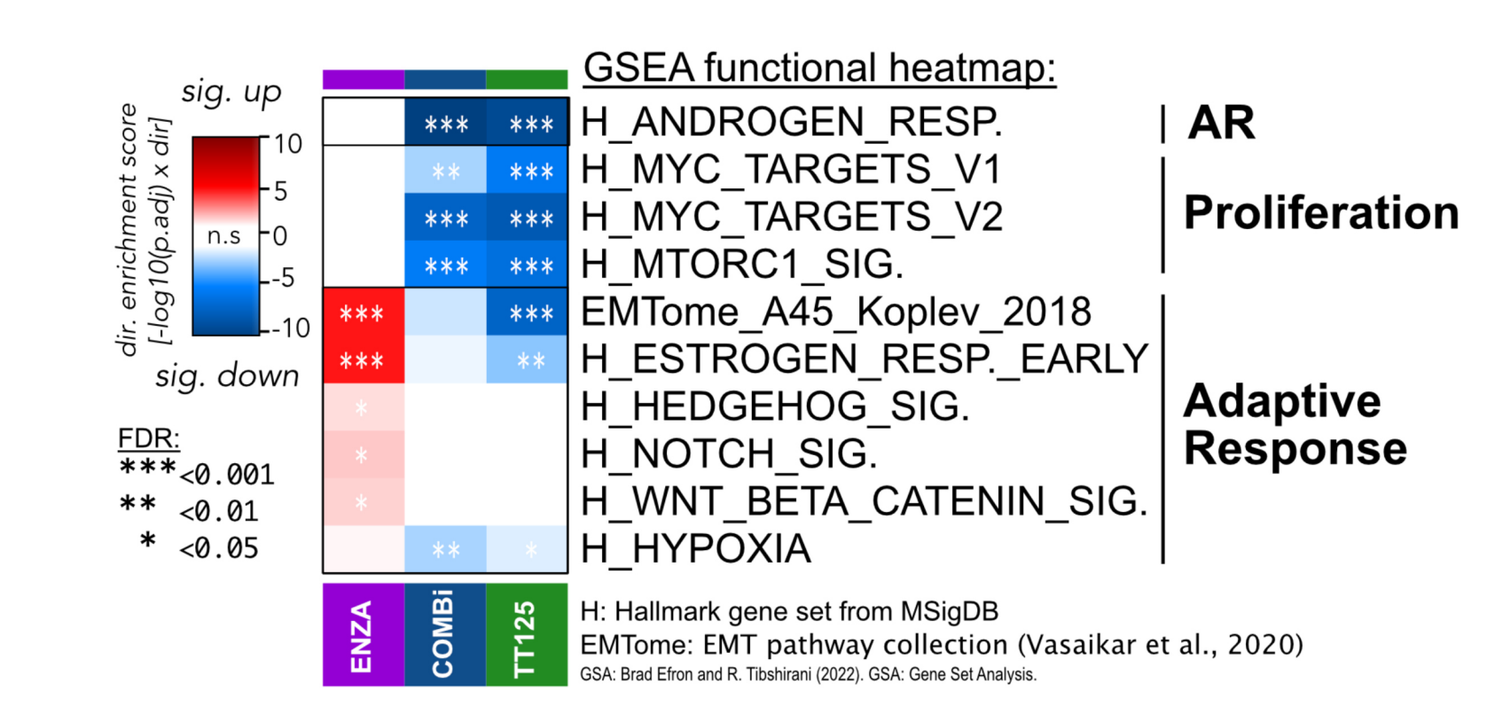


A. TT125-802 inhibits gene expression in tumors harvested 4h after the last dose on Day 41. Each dot represents one animal.

B. TT125-802 lowers serum PSA concentrations in the same PDX model to stronger extent than AR inhibitor enzalutamide.

TT125-802 has dual action in prostate cancer: it downregulates AR and MYC transcriptional programs and it reverts resistance-causing signatures induced by enzalutamide

A TT125-802 counter-regulates resistance-associated transcriptional changes induced by enzalutamide



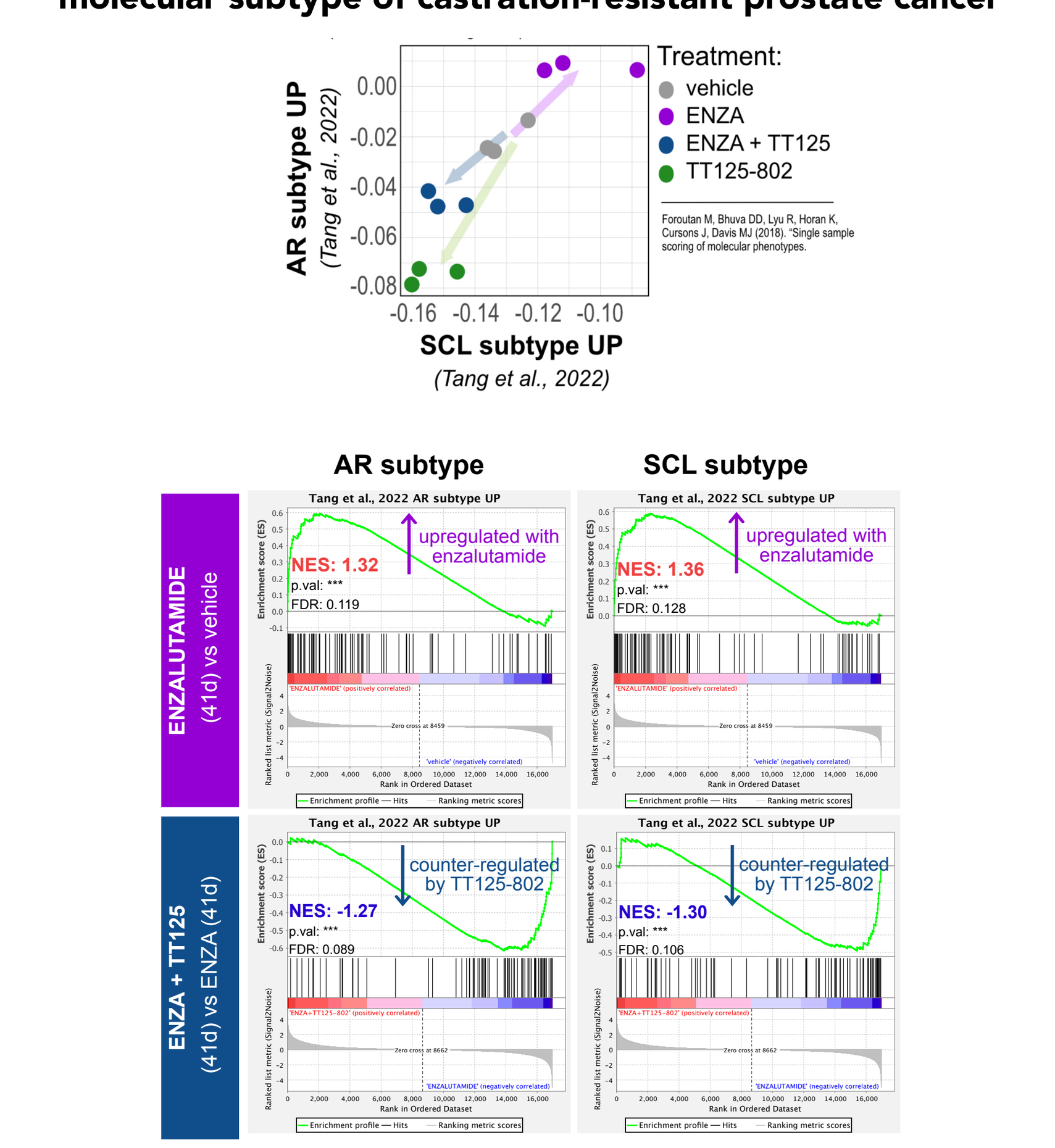
A. RNA-Seq of castration-resistant prostate cancer PDX model (PR6512) treated for 41 days with vehicle, enzalutamide, TT125-802, or a combination of both inhibitors. Enzalutamide inhibits AR and proliferative signatures but also induces resistance-causing gene expression signatures. TT125-802 counter-regulates resistance signatures.

B. Single sample scoring (top) and GSEA enrichment plots (bottom) of clinically relevant molecular phenotypes for AR- and stem cell-like (SCL) subtypes derived from Tang et al. 2022 shows that TT125-802 counter-regulates resistance-associated phenotype switching.

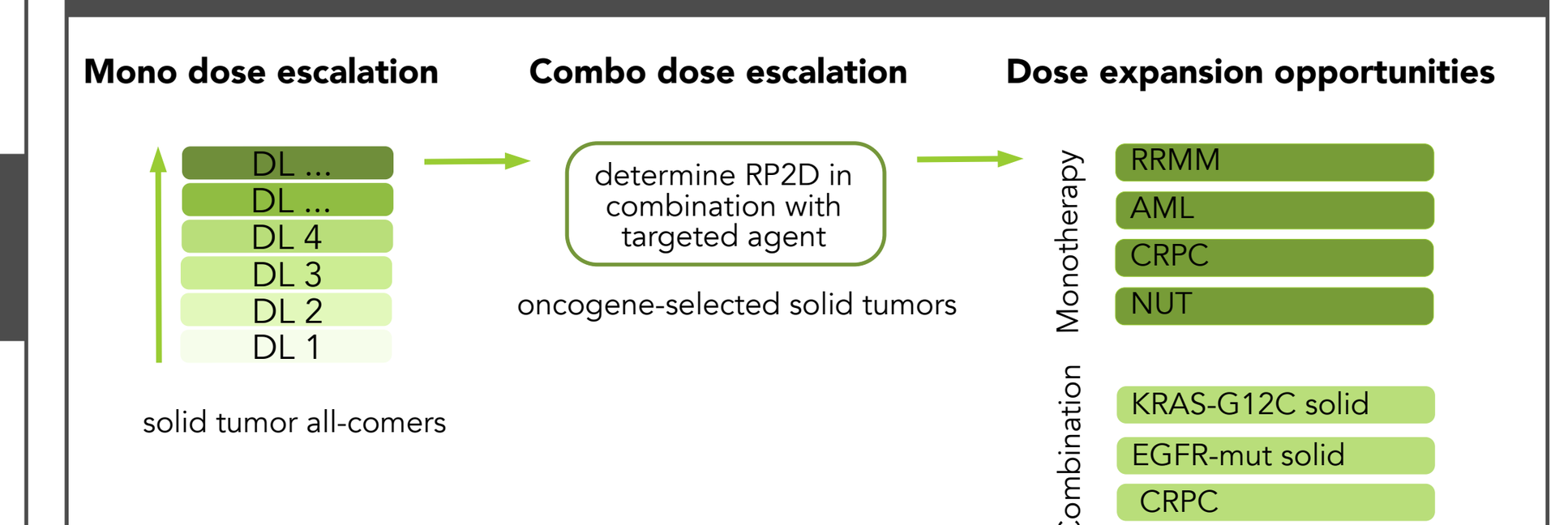
AR inhibitor enzalutamide induces phenotypic plasticity and increases expression of transcriptional signatures of prostate cancer with AR subtype and resistance-associated stem cell-like (SCL) subtype.

When TT125-802 is combined with enzalutamide, resistance-associated phenotype switching is prevented.

B TT125-802 suppresses resistance-causing stem cell-like molecular subtype of castration-resistant prostate cancer



Clinical study design



RP2D recommended phase 2 , DL dose level, RRMM Refractory/relapsed multiple myeloma, AML acute myeloid leukemia, CRPC castration resistant prostate cancer, NUT nuclear testis protein midline carcinoma, NSCLC non-smallcell lung carcinoma, CRC colorectal carcinoma

Poster #3907, T. Bohnacker: Targeting adaptive resistance to EGFR and KRAS G12C inhibitors by TT125-802, a novel and specific CBP/p300 bromodomain inhibitor.

Conclusions

- TT125-802 is a potent, highly selective and orally available small molecule inhibitor that specifically binds to the bromodomain of CBP/p300, thereby preventing the expression of broad transcriptional programs.
- TT125-802 induces tumour regression in preclinical model of multiple myeloma model and suppresses PD markers (MYC, MYB and IRF4) in a dose-dependent manner.
- TT125-802 inhibits the growth of AR-positive prostate cancer cells and downregulates AR target genes KLK3, TMPRSS2, and c-MYC.
- TT125-802 prevents resistance to enzalutamide in AR-positive CRPC PDX model and suppresses Myc and AR target genes.
- TT125-802 has dual action in prostate cancer: it downregulates AR and Myc transriptional programs and suppresses a clinically relevant, resistance-causing stem cell-like molecular subtype of CRPC.
- A Phase 1 clinical study will evaluate TT125-802 alone and in combination with standard-of-care agents.