# PRIMA-1Met induces myeloma and mantle cell lymphoma cell death, by impairing ROS/GSH balance

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## Objectives

*TP53* is the most frequently mutated gene in cancers, inactivation of p53 pathway is associated with resistance to therapy in numerous cancers, including multiple myeloma (MM) and Mantle Cell Lymphoma (MCL). In both pathologies, therapeutic approaches bypassing p53 resistance are needed.

## Methods

We assessed efficacy of molecules described as "p53 reactivating molecules". Indeed, PRIMA-1Met (APR-246) (PRIMA) was isolated according to its ability to restore apoptosis in SAOS2-His-273 cells in a p53-dependent manner. PRIMA was shown to bind to p53 and to induce a functional re-conformation of the mutant protein. Using a wide number of myeloma cell line (hMCL), primary myeloma cells and MCL cell lines, we investigated whether PRIMA could induce MM and MCL cell death and if this was respective to *TP53* status as described.

## Results

**PRIMA activity wasn't associated with p53 status in hMCLs and MM patients' samples:**

- Lethal dose 50 of PRIMA (LD50) for the whole group was 32µM (95%CI, 20-44) and was irrespective of *TP53* status (Wild Type, wt; Mutated, mut; no p53, neg) (**Fig A.**)
- Silencing of *TP53* by shRNA didn't modify response to PRIMA (**Fig B.**).
- PRIMA didn't upregulate target genes of p53 (p21) (**Fig C.**)
- PRIMA-induced cell death was associated with an increased expression in Noxa mRNA level and was inhibited (-89% cell death) upon Noxa silencing in $OPM2^{Mut}$ cell line (**Fig D.**)

**PRIMA activity was associated with an impaired ROS/GSH balance:**

- PRIMA depleted cells with Gluthatione (GSH) by a mean of 75% (+/-10%) (**Fig E.**) and induced Reactive Oxygen Species (ROS) (**Fig F.**)
- GSH synthesis successively involves GSS and γ-GCS. We showed that PRIMA efficacy correlated with expression of GSS (**Fig G'.**) and that BSO, an irreversible inhibitor of γ-GCS, synergized with PRIMA in 25 out of 27 hMCLs (**Fig G.**)
- In primary MM cells (n=25), 10µM PRIMA induced cell death (median value, 55%). In the presence of BSO, median cell death increased from 32% to 87% in 6 matched experiments, p=0.037; 0% of cell death with BSO alone) (**Fig H.**)

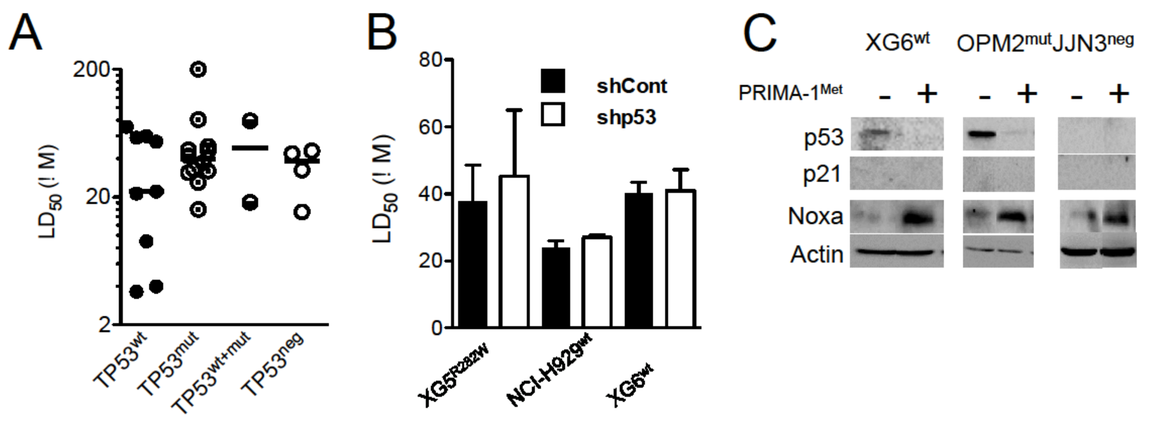
**PRIMA impaired MCL cell lines survival and synergized with BSO**

- LD50 for the whole set of MCL cell lines was 34µM (95%CI, 29-40) (**Fig I.**)
- PRIMA did not induce any significant cell death alone (10µM) but synergized with BSO in both *$TP53^{Wt}$ (Z138, GRANTA-519, JVM2) and* $TP53^{Abn}$ (REC-1, MAVER, MINO, JEKO, UPN1) MCL cell lines (**Fig J.**)

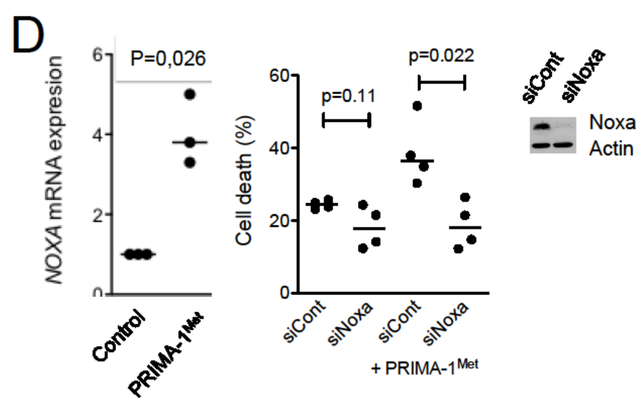
**In Scid-Beige mice bearingJJN3 tumor cells,** PRIMA-1Met (18 mg/kg, intravenous injections, arrows on **Fig H.**), BSO (10 mM, drinking water), or a combination of BSO and PRIMA-1Met were administrated. Body weight and tumor burden were assessed at days 0, 9, 13 and mice were killed at day 16 (due to tumor load)

- Body weight was not significantly impaired upon treatments
- While PRIMA slowed tumor growth ($p<0.05$) when used alone, it fully prevented tumor growth with BSO (and induced tumor regression) (**Fig K.**)

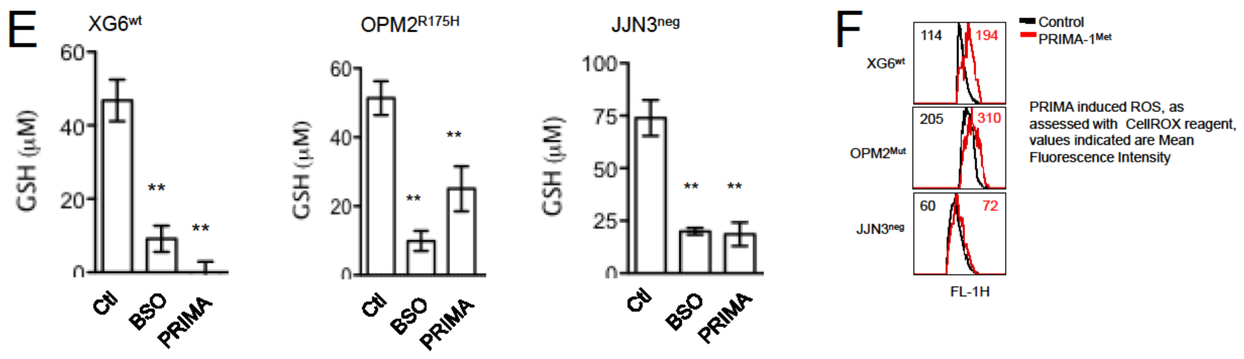
- PRIMA activity was independent of *TP53* status in hMCLs



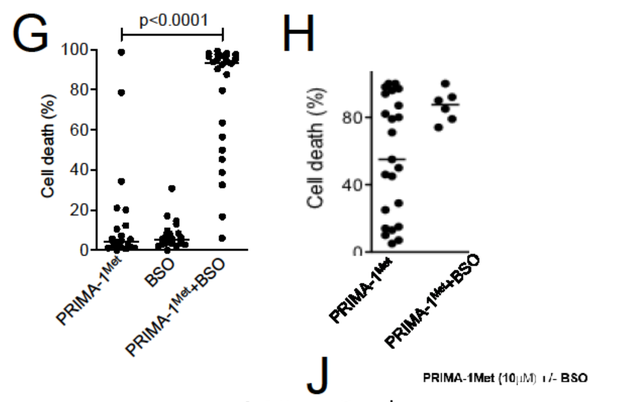

- PRIMA induced death was associated with an increase in Noxa expression



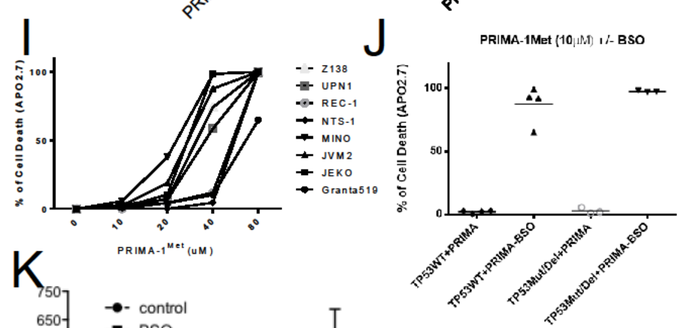

- PRIMA was responsible for an impaired Red/Ox balance in HMCLs



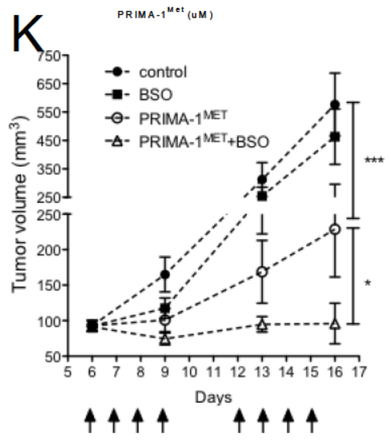

- PRIMA toxicity was enhanced by BSO in both hMCLs and MM patients' samples




- PRIMA toxicity in MCL cell lines




- PRIMA and BSO is effective without overwhelming toxicities in mice




## Conclusion

**PRIMA-$1^{Met}$ activity is independent of *TP53* status in hMCLs and MCL cell lines**

- It strikingly synergizes with BSO, exhibiting high tumor cell toxicity and a major role in impairing Red/Ox balance
- *In vivo* testing has shown very promising results, in order to transfer this data from bench to patients to overcome cell death resistance in patients with MM and MCL, especially for those harboring *TP53* alterations

13icml 168--P
Pathology and biology
Benoit Tessoulin
DOI: 10.3252/pso.eu.13icml.2015
Poster presented at: 13-ICML
Printing supported by: Takeda ONCOLOGY
PosterSessionOnline.com