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# Targeting the p53/xCT/GSH axis with PRIMA-1$^{Met}$ combined with Sulfasalazine shows therapeutic potential in Chronic Lymphocytic Leukemia


Martina Pasino[1], Andrea Speciale[1], Silvia Ravera[2,3], Giovanna Cutrona[4], Rosanna Massara[4], Nadia Bertola[4], Maurizio Viale[5], Irena Velkova[1], Andrea Nicola Mazzarello[2,3], Franco Fais[2,3], Fabrizio Loiacono[6], Serena Matis[7], Giulia Elda Valenti[2], Nicola Traverso[2], Cinzia Domenicotti[2,3], Barbara Marengo[2,3], Bruno Tasso[8], Adalberto Ibatici[9], Emanuele Angelucci[9], Tiziana Vaisitti[10], Paola Monti[1], Paola Menichini[1]

Presenter: Irena Velkova

[1]Neuro-Oncology and Mutagenesis Unit, IRCCS Ospedale Policlinico San Martino, 16132 Genoa, Italy; [2]Department of Experimental Medicine, University of Genoa, 16132 Genoa, Italy; [3]IRCCS Ospedale Policlinico San Martino, 16132 Genoa, Italy; [4]Molecular Pathology Unit, IRCCS Ospedale Policlinico San Martino, 16132 Genoa, Italy; [5]Biotherapy Unit, IRCCS Ospedale Policlinico San Martino, 16132 Genoa, Italy; [6]Cytometry Facility, IRCCS Ospedale Policlinico San Martino, 16132 Genoa, Italy; [7]Molecular Oncology and Angiogenesis Unit, IRCCS Ospedale Policlinico San Martino, 16132 Genoa, Italy; [8]Department of Pharmacy, University of Genoa, 16132 Genoa, Italy; [9]Hematology and Cellular Therapy Unit, IRCCS Ospedale Policlinico San Martino, 16132 Genoa, Italy; [10]Department of Medical Sciences, University of Torino, 10124 Turin, Italy.


## OBJECTIVES

The primary goal of this work is to study a novel therapeutic strategy for CLL, specifically targeting high-risk patient groups characterized by p53 protein alterations. The objective is to investigate the ability of combined treatment with PRIMA-1$^{Met}$ and sulfasalazine (SAS) to overcome drug resistance by targeting both p53-dependent and GSH pathways.

## CONCLUSIONS

In CLL cells, PRIMA-1$^{Met}$ significantly influences the antioxidant response and the induction of reactive oxygen species (ROS), decreasing cell survival. The combination of PRIMA-1$^{Met}$ and SAS further causes a redox imbalance and increases oxidative stress in both wt and mutant p53 CLL cells, resulting in a synergistic effect on cell survival. However, CLL cells with mutant p53 are more severely compromised in their ability to handle oxidative stress compared to cells expressing wild-type p53.


**CONTACTS**

irena.velkova@hsanmartino.it
martina.pasino01@gmail.com
paola.menichini@hsanmartino.it


OSPEDALE POLICLINICO SAN MARTINO
Sistema Sanitario Regione Liguria
Istituto di Ricovero e Cura a Carattere Scientifico

## INTRODUCTION

Chronic Lymphocytic Leukaemia (CLL) is a highly heterogeneous form of leukaemia characterized by complex karyotype aberrations and gene mutations at different genes. The mutations at the ***TP53*** tumour suppressor gene represent an important hallmark of the disease since they may strongly influence therapeutic success. The deletion at chromosome 17p13.1 (del17p) and/or *TP53* mutations, are considered the most important predictive biomarkers in CLL.

This work aims to investigate the effects of a combination therapy using **PRIMA-1$^{Met}$** and **sulfasalazine** (**SAS**) as a novel and promising therapeutic strategy.

**PRIMA-1$^{Met}$** is a small molecule that promotes the correct refolding of mutant p53 proteins, restoring their pro-apoptotic function. Additionally, it can disrupt intracellular redox balance, increase oxidative stress, and promote cell death.

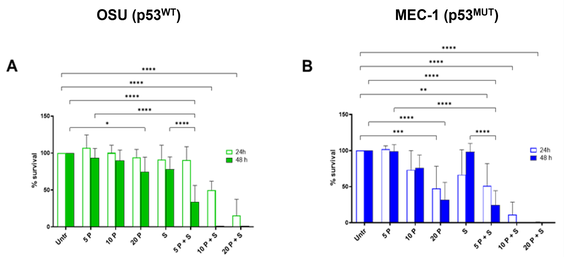
In parallel, **SAS**—an anti-inflammatory drug already approved for chronic inflammatory diseases—inhibits the cystine transporter xCT (SLC7A11), which is essential for glutathione (GSH) synthesis, one of the cell's major antioxidants. SAS impairs the tumor cell's ability to detoxify reactive oxygen species, thereby increasing its susceptibility to oxidative stress-induced cell death.

## RESULTS

In OSU and MEC-1 cells the combined treatment with PRIMA-1$^{Met}$ and SAS induces cell death (**Fig. 1**) resulting in a synergistic effect on cell survival (**Fig.2**). Decreased survival is likely related to GSH depletion operated by both molecules, that in MEC-1 cells was associated to a significant increase of ROS (**Fig.3**).

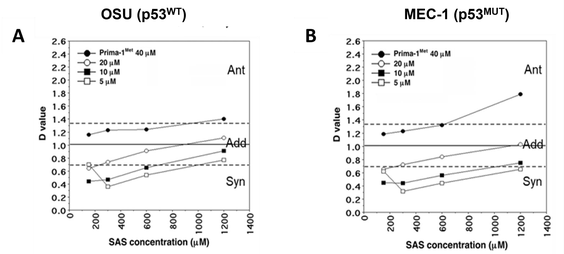
Primary CLL cells expressing both p53$^{wt}$ and p53$^{mut}$ showed a significant cell sensitivity to PRIMA-1$^{Met}$ and SAS treatment (**Fig. 4**). In CLL primary cells the redox balance is significantly impaired (**Fig.5**): a general decline in antioxidant capacity was observed in both wt and mutant p53-expressing cells. This compromised defense further contributed to the accumulation of oxidative damage following treatment with PRIMA-1$^{Met}$ or SAS. Consistent with observations in cell lines, primary CLL cells carrying mutant p53 showed the highest levels of lipid peroxidation, particularly after the double treatment.

### Figure 1. Decreased survival of OSU (A) and MEC-1 (B) cells after treatment with PRIMA-1$^{Met}$ (P) ± SAS (S)



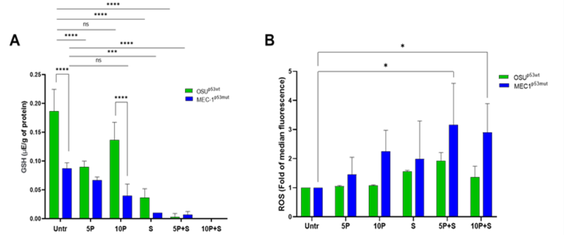

Cell viability was analyzed by MTT assays in cells exposed for 24 h and 48 h to increasing concentrations of PRIMA-1$^{Met}$ (5, 10, 20 μM) alone or in combination with Sulfasalazine (300 μM).

### Figure 2. PRIMA-1$^{Met}$ and SAS can synergistically influence CLL cell survival, irrespective of their p53 status



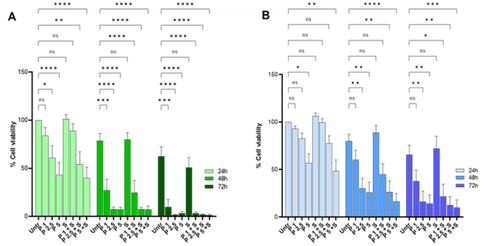

PRIMA-1$^{Met}$ synergizes with SAS at the lowest drug concentrations (5μM and 10 μM PRIMA-1$^{Met}$), up to 1200 μM SAS, giving a D value <1. The concentration of 20 μM PRIMA-1$^{Met}$ can be considered additive (D = 1) only when higher SAS concentrations are used, while at 40 μM PRIMA-1$^{Met}$, the activity of the combination of the two drugs is never synergistic (D >1).

### Figure 3. PRIMA-1$^{Met}$ and SAS deplete the intracellular GSH (A) in both cell lines and augment ROS levels (B) significantly in MEC-1 cells



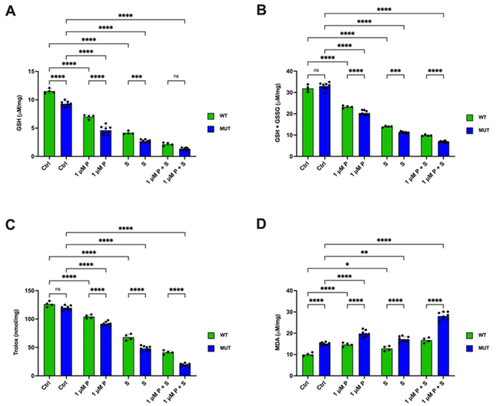

GSH levels were measured by HPLC, ROS levels were detected by cytofluorimetric analysis.

### Figure 4. PRIMA-1$^{Met}$ ± SAS treatment reduce the viability of *TP53*-wild type and *TP53*-mutant primary CLL cells




Viability was calculated as the percentage of double Annexin V and PI negative cells.

### Figure 5. PRIMA-1$^{Met}$ and SAS significantly affect the modulation of metabolic markers in CLL primary cells




Determination of (A) GSH level, (B) GSH + GSSG content, (C) cellular total antioxidant capacity, (D) malondialdehyde (MDA) intracellular level in CLL patients' cells following 1 μM PRIMA-1$^{Met}$ alone and in combination with 300 μM SAS.

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