**INTRODUCTION**

**BRD4 inhibition: JQ1**

Members of the Bromodomain and ExtraTerminal (BET) subfamily (BRD2-4) associate with acetylated chromatin and facilitate transcriptional activity by recruiting transcriptional activators. **BRD4** is a ubiquitously expressed transcriptional co-factor, associated with oncogenes as c-myc, whose transcription is ruled by super-enhancers (1). Super-enhancers need an excess of transcription, rendering the gene very sensitive to BRD4 inhibition. Since the survival of transformed cells depends on oncogenes, their depletion induces death preferentially in cancer cells, while preserving normal ones (2). JQ1 was developed as a BET bromodomain inhibitor: targeting BRD4, it has shown specific anticancer activity towards many types of tumor (3), reducing the transcription of target genes, as c-myc. This causes cell-cycle arrest and senescence (4). Furthermore, JQ1 can modulate FOS-like antigen 1 (FOSL1), also known as Fra-1) (5).

On that basis, JQ1 could be an important agent in the combination chemotherapy of MPM.

**The chemotherapy of Malignant pleural mesothelioma (MPM)**

MPM is an asbestos-associated tumor. MPM histology ranges from epithelioid to sarcomatoid, with any combination of both (mixed or biphasic). The gold-standard frontline chemotherapeutic consists of cisplatin and pemetrexed, but the rates of response are quite modest (7). MPM is a chemoresistant tumor characterized by high antioxidant, DNA-repair, and antiapoptotic armory. Genes encoding pro-survival proteins in the NF-κB pathway are overexpressed, including c-myc (8), whose repression is synergistic with cisplatin activity in MPM (9).

**Cisplatin** chemoresistance is directly linked to c-Myc (10). This transcription factor plays a critical role in an epigenetic manner (11) over a broad range of cellular processes, including cell cycle progression, cell growth, differentiation, transformation, angiogenesis, apoptosis and chemoresistance (12). Furthermore, the expression of c-myc gene is rapidly induced by many mitogenic stimuli, that are deregulated in MPM (13) leading to enhanced DNA repair and anti-apoptotic pathways downstream the transcriptional activity of c-Myc (14).

**RESULTS and DISCUSSION**

**in vitro growth inhibition activity**

The MPM cell panel, a cisplatin-resistant sub-line (MM98R) and a mesothelial counterpart (HMC), were challenged with JQ1 or cisplatin, respectively. Among the different MPM phenotypes, the sarcomatoid one (MM98) and even its cisplatin-resistant sub-line MM98R were the most sensitive to JQ1. We then performed a drug combination study, employing cisplatin and JQ1 at fixed molar ratio, i.e. 10:1 on sarcomatoid cells and 3:1 on epithelial (BR95), mixed (MG06) and mesothelial cells, chosen according to **IC<sub>50</sub>** values previously found. The drug combination resulted synergistic on MG06 and on MM98, and additive on the remaining MPM cells. On the contrary, the combination was slightly antagonistic on HMC. (CI<sub>1</sub> = 1).

**Table** shows the **IC<sub>50</sub>** values (µM) obtained after 72h of treatment by means of the resazurin reduction assay. Data in brackets reports the selectivity index, SI, i.e., the ratio between **IC<sub>50</sub>** (HMC) and the **IC<sub>50</sub>** on the tumor MPM cell line. Data are means ± standard deviation of at least 3 replicates. Combination index (CI) was calculated for non-mutually exclusive drugs, and reported for the concentration of drug giving 50% growth inhibition, **IC<sub>50</sub>** CI below 1 means synergism, around 1 additivity, over 1 antagonism.

**Cell cycle distribution**

HMC cell cycle distribution was not affected by any treatment. JQ1 had negligible effect on BR95 and MG06, but induced G<sub>s</sub> arrest in the most sensitive MM98 and MM98R. Cisplatin increased the S phase in BR95, MG06 and MM98, but not MM98R. G<sub>1</sub> increased in MM98 only. Combination treatment allowed cells to recover the same pattern as the control, except in MG06 and, partially, BR95, where the same pattern caused by cisplatin was observed (p<0.05).

The negligible role of Fra-1

The Fra-1-directed antibody gave an intense band around 40 kDa in the positive control (A, A549 lysate), along with a faint band at lower molecular weight (around 30 kDa). The same weak band was observed in all cell lines and decreased in BR95 and MG06, when challenged with JQ1 or with its combination with cisplatin.

**Apoptosis induction**

The activation of the central apoptosis players caspase 3/7 was revealed in cell lysates by means of the cleavage of the fluorescent substrate, Ac-DEVD- AFC, 0.01 g/L. The activity was followed for 1 h, by means of fluorescence at Ex 390/ Em 520 nm (15). The inhibitor, Ac-DEVD-CHO abrogated any signal. Final fold activity (with respect to control wells) is the mean of at least three independent replicates performed in duplicate for each condition.

**Conclusions**

Repressed c-Myc correlated with increased apoptosis both after treatment with JQ1 (MG06, BR95 and MM98R), and with drug combination (in all MPM cell lines). Furthermore, apoptosis was significantly increased by cisplatin with respect to JQ1 in MM98 and MM98R, where synergism was observed.

Cisplatin as a single agent inhibits MPM cell growth, but it rarely induces apoptosis. JQ1 increases the efficacy of cisplatin-based MPM chemotherapy in terms of selectivity and reduced chemoresistance. This shows that c-Myc can be pharmacologically targeted, by means of BRD4 inhibition, commiting MPM cells to apoptosis.

**References**


**Graph**

Bars represent the relative c-Myc protein expression. Data were quantified from immunoblots using ImageJ and normalized over actin level.

Representative immunoblots for c-Myc and loading control (actin).