

# Targeting the Resistance in Multiple Myeloma †

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† Presented at Natural Products and the Hallmarks of Chronic Diseases—COST Action 16112, Luxembourg 25–27 March 2019.

Published: 16 April 2019

**Abstract:** Multiple myeloma is a hematological cancer characterized by the clonal proliferation of malignant plasma cells in the bone marrow. That disease has a rather low incidence but displays a high rate of relapse and resistance to conventional therapies. It is therefore necessary to find new therapeutic strategies to overcome this resistance, which is partly attributed to a subpopulation of cells known as cancer stem cells. Withanolides and HDAC6 selective inhibitors were identified as promising compounds in various resistant multiple myeloma models.

**Keywords:** multiple myeloma; 3D co-culture model; cancer resistance

## 1. Introduction

Multiple myeloma (MM) is a disorder characterized by the clonal proliferation of malignant plasma cells in the bone marrow. One of the main complications in this type of tumor is the lytic lesions in the bone aroused by the activation of osteoclasts and suppression of osteoblasts in a very complex network of interactions between the cancer cells and the bone marrow niche [1]. This malignancy represented 0.8% of all cancers worldwide in 2012, counting for 1% of cancer deaths [2]. Regardless of the improvement in overall survival observed over the past decades, treatment strategies still represent a huge challenge mostly for patients with relapsed and refractory disease [3,4]. It is therefore necessary to find new therapeutic strategies to overcome this resistance.

## 2. Withanolides

Withaferin A (WFA) is a steroidal lactone isolated from the leaves of *Withania somnifera* and has been shown to exert an array of biological activities relevant to various disorders such as cancer [5,6]. In multiple myeloma, WFA induced cell death and was capable of stimulating cell differentiation at relatively low doses as demonstrated by clear morphological changes and alterations in gene expression levels reminiscent of hematopoietic stem cell differentiation [7]. Access to a library of withanolides led to the identification of withanolide D that showed stronger activity than WFA and exerted similar cytostatic effects between MM-sensitive and -resistant cell lines, which were independent of P-glycoprotein efflux [8]. The antiproliferative activity of drugs currently used to treat MM was also evaluated in MM-cancer stem cells (CSCs), RPMI 8226, MM1.S and MM1.R cells. Most of them did not show any activity against the resistant MM-CSCs ( $IC_{50} > 50 \mu M$ ) and displayed a wide range of  $IC_{50}$  values in the other cell lines.

## 3. Histone Deacetylase Inhibitors

Some of the new agents that have displayed great potential in the past years are histone deacetylase (HDAC) inhibitors [9]. HDACs have been reported as dysregulated in MM and the overexpression of HDAC1 and HDAC6 has been associated with poor prognosis [10]. The pan-

HDAC inhibitors presently used in MM treatment, such as panobinostat, display high toxicity despite being very effective in overcoming resistance to bortezomib [11]. Therefore, selective HDAC6 inhibitors could be as active as non-selective inhibitors and at the same time decrease the global toxicity. Ricolinostat is the first HDAC6 inhibitor to reach clinical trials and the first reports showed an improved safety profile when compared with pan-HDAC inhibitors [12]. HDAC inhibitors alone do not display a huge clinical benefit but when combined with other therapies, they showed a great value, with a better outcome in cases of refractory MM [13].

HDAC6 appears to function at various cellular crossroads between two cellular signaling systems, which each involve protein lysine acetylation and ubiquitination [14]. It is a crucial factor in the coordination of the cell response, and it plays an important role in the formation or degradation of cytotoxic protein aggregates in the course of various diseases such as cancer. HDAC6 recruits polyubiquitinated protein aggregates via the ZnF-UBP domain and loads misfolded proteins onto dynein to form the aggresome. The modulation of this pathway represents a strategy to overcome resistance to proteasome inhibitors in MM [15]. The HDAC6 inhibition of several compounds was tested, as well as their antiproliferative activity.

#### 4. 3D Co-Culture Spheroids

Some compounds active in 2D cultures failed during development because of their lack of efficiency in co-culture conditions, due to the supportive function of stromal cells. Therefore, 3D co-culture spheroids, including malignant plasma cells and cells from the microenvironment, were used to evaluate compounds having shown activity in monolayer cultures [16]. MM-CSCs were also introduced in the model in a way that the total amount of CSCs present in the spheroid would correspond to 20% of cancer cells to mimic resistant cancers. By screening compounds in this model not only the individual response of the malignant cells is considered, but also how the spheroid, as an entity, behaves when exposed to the treatment. Therefore, this model better reflects the cellular and molecular complexity found in vivo and was used to study the activity of various compounds with the aim to a more efficient transition between pre-clinical experiments and clinical trials.

**Author Contributions:** Conceptualization, M.F., M.E.I and M.C.; methodology, M.F. and M.E.I.; validation, M.F., M.E.I and M.C.; resources, M.C.; writing—original draft preparation, M.C.; writing—review and editing, M.F., M.E.I and M.C.; supervision, M.C.; project administration, M.C.; funding acquisition, M.C. All authors read and approved the final manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** This work is part of the European COST Action CM1106 “Chemical Approaches to Targeting Drug Resistance in Cancer Stem Cells” and CM1406 “Epigenetic Chemical Biology”. This publication is based upon work from COST Action NutRedOx-CA16112 supported by COST (European Cooperation in Science and Technology).

**Conflicts of Interest:** The authors declare no conflict of interest.

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