CARFILZOMIB, NEW DRUG FOR MULTIPLE MYELOMA

ABSTRACT

Revisión de *Carfilzomib*, un inhibidor del sistema *proteosómico* celular, el principal sistema de desguace de las proteínas en las células. El desentrañamiento de la estructura y mecanismo de acción *proteosómico* representó un hito científico merecedor de la concesión del Premio Nobel de Química a sus descubridores, *Aaron Ciechanover*, *Avram Hershiko* e *Irwin Rose*, en el año 2004.

Carfilzomib representa un progreso en relación a *Bortezomib* en varios aspectos: especificidad de acción, irreversibilidad de la inhibición del sistema *proteosómico*, menor incidencia (según los estudios iniciales) de neuropatía periférica; y, principalmente, eficacia clínica en pacientes que, bien son refractarios, o han recaído tras un tratamiento con *Bortezomib*.

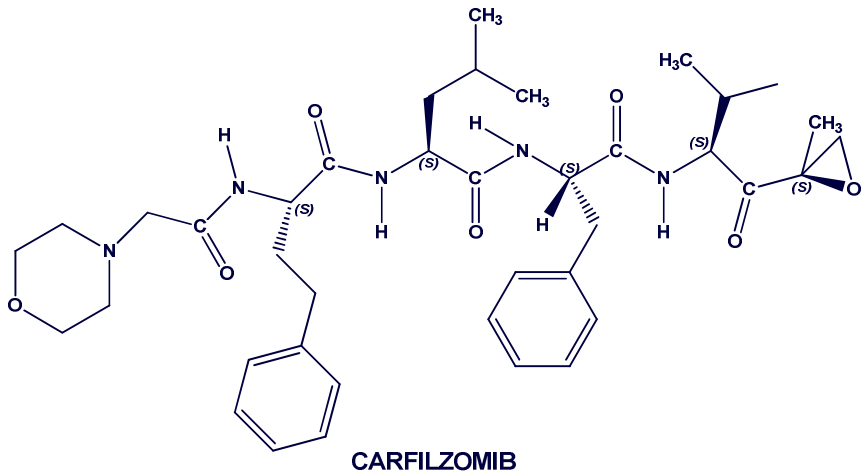
Carfilzomib está indicado bien en régimen de monoterapia, o incluido en protocolos de tratamiento más complejos.

Carfilzomib parece representar un avance para el tratamiento de los estadios más complejos del mieloma múltiple.

INTRODUCTION

Multiple Myeloma is a cancer of antibody producing white blood cells. Other homonyms for this pathology are: Kahler's disease and plasma cell myeloma. In the rest of the article I will use the most common name, multiple myeloma.

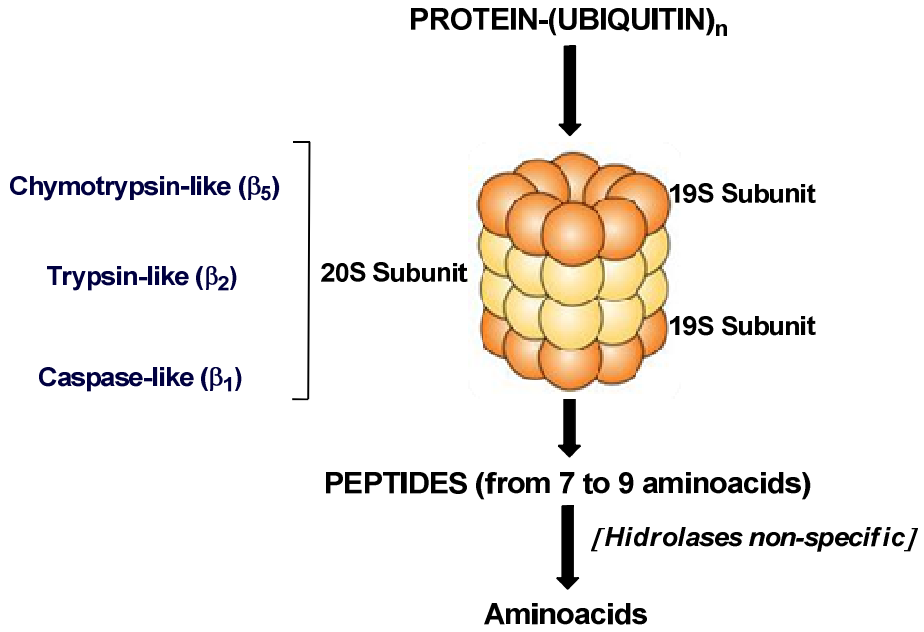
CARFILZOMIB'S MECHANISM OF ACTION



CARFILZOMIB
(*S*)-4-methyl-*N*-((*S*)-1-(((*S*)-3-methyl-1-((*S*)-2-methyloxiran-2-yl)-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-2-((*S*)-2-(2-morpholinoacetamido)-4-phenylbutanamido)pentanamide

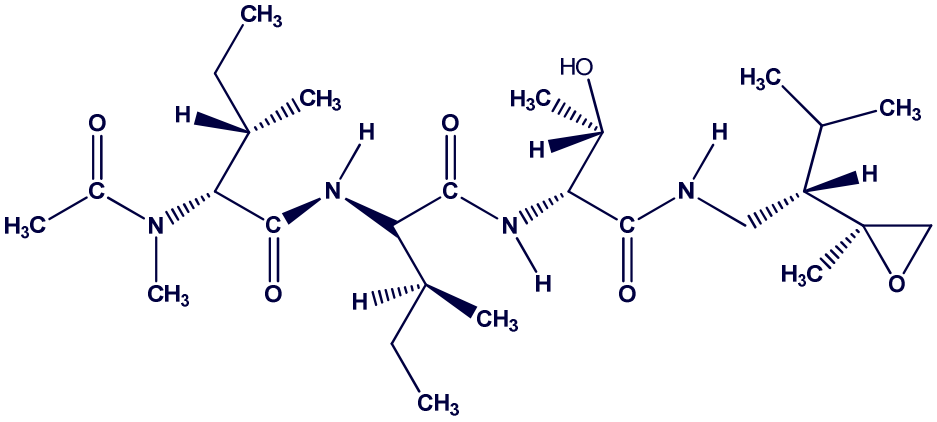
Cells have two ways of degrading proteins: (1) the activity of the lysosomal system; and (2) the proteasomic system [1]. Protein homeostasis is essential for the optimal operation of the gears of complex cellular machinery.
It is easy to infer that the inhibition of any of these protein degradation systems will affect cellular functions, such as signal transduction and cell signaling pathways [2] ending apoptosis (programmed cell death).
The cancer cells show a special susceptibility to the cellular degradation proteasome system [3], [4], [5], [6].

The deciphering of the proteasome protein degradation mechanism was awarded the Nobel Prize in Chemistry, in 2004. Three researchers: Aaron Ciechanover, Avram Hershiko and Irwin Rose were awarded the prize for "the discovery of ubiquitin-mediated protein degradation".



A protein that is recognized by the proteasome (proteasomic protein complex) should be signaled by several monomers of a 76-amino acid polypeptide called ubiquitin (denomination referring to their ubiquity in all cell lines). Before protein can be degraded, no less than 4 ubiquitin monomers must be linking. After that the proteasome system can perform their breaking into smaller peptides, which are hydrolyzed to the free amino acids by nonspecific cytosolic peptidase.
Shaping the proteasome system mimics a scrapping tube (Figure 2). In the first part, [19S proteasome subunit] the protein (labeled by the covalent attachment of

multiple ubiquitin monomers) loses its shape while maintaining its primary structure (amino acid sequence). The denatured protein enters the nucleus of the proteasome system [20S proteasome subunit, consisting of three domains: β5 (chymotrypsin-like), β2 (trypsin-like), and β1 (caspase-like)]. The final part of the proteasome system ("scrapping tunnel") consists of another 19S subunit.
Of all the subunits of the proteasome system, the β5 (chymotrypsin-like) is most susceptible to pharmacological inactivation [7, 8].
The inmunoproteasome [20] is a type of proteasome located in monocytes and lymphocytes. When the TNF (acronym of Tumor Necrosis Factor) or interferon-γ interact with the inmunoproteasome, a series of changes take place: the subunits β5, β2 and β1 are replaced respectively by β5i (also known as LMP7, acronym of Low Molecular Polypeptide 7) β2i (designated the acronym MECL1 of multicatalytic Endopeptidase Complex 1) and β1i (LMP2 of Low Molecular Polypeptide 2).
Inmunoproteosome expression has been observed in multiple myeloma cell lines.
Bortezomib and Carfilzomib inhibit proteasome activity without discriminating between the inmunoproteosome and the proteasome.



Epoxomicin
(2*S*,3*S*)-*N*-((2*R*,3*S*)-3-hydroxy-1-(((*S*)-3-methyl-2-((*S*)-2-methyloxiran-2-yl)butyl)amino)-1-oxobutan-2-yl)-3-methyl-2-((2*R*,3*S*)-3-methyl-2-(*N*-methylacetamido)pentanamido)pentanamide

Carfilzomib is a structural analogue of epoxomicin-3, a product of microbial activity, antitumor activity has been observed following the inhibition of proteasome activity [9, 10].
Carfilzomib therefore selectively inhibits both the activity of the β5 subunit (proteasome) and the β5i activity (or: LMP7) of inmunoproteosome. The inhibition of these subunits is irreversible, so that restoration of proteasome activity requires

the synthesis of new protein subunits. In this it differs from Bortezomib [11], where the inhibition of the proteasome complex subunits is slowly reversible.
The radical of Carfilzomib epoxybutane shows high specificity toward threonine on the amino-terminal end of the catalytic sites, with limited activity of serine-proteases. Instead, Bortezomib preferentially reacts with a variety of serine-proteases, such as the aforementioned "chymotrypsin-like" (β5), but also others such as cathepsin A and G, elastase and chymase [12, 13].

PHARMACOKINETICS OF CARFILZOMIB

Following IV bolus administration, Carfilzomib disappears rapidly from the plasma compartment with a plasma half-life of 15 minutes (as shown by experimental studies on rats) and 7.5 minutes (as shown by experimental studies on monkeys) [14].
Despite its rapid plasma clearance, Carfilzomib leads to prolonged inhibition of 20S proteasome subunit in all tissues except the brain.
The determination of the kinetic of Carfilzomib was established based on one of the two following management protocols:

1. Five days of treatment, followed by nine days of wash-out.
2. Administration on days 1 and 4, followed by nine days of wash-out.

The administration of doses that resulted in ≥ 80% inhibition of proteasome activity was well tolerated by patients [11]. Transient thrombocytopenia was reported, but there was no change in the lymphocyte and neutrophil count.
The recovery of proteasome activity following the administration of single doses was similar in both treatments.
These previous observations were crucial to the design of the first phase I study with escalating doses. In this first clinical trial, O'Connor et al [15], 29 patients were with any one of the following diseases: relapsed or refractory multiple myeloma, lymphomas (Hodgkin and non-Hodgkin) and Waldenstrom's macroglobulinemia. All patients were treated for five consecutive days followed by nine days without treatment, to complete a fourteen-day cycle. The doses of the different arms of the study were as follows:

- 1.2 mg/m2
- 2.4 mg/m2
- 4.6 mg/m2

• 8.4 mg/m2
• 11.0 mg/m2
• 15.0 mg/m2
• 20.0 mg/m2 (maximum dose)

No dose-limiting toxicity was reported in the dose range 1.2mg/m^2 ↔ 15.0mg/m^2. With the highest dose (20.0mg/m^2) grade 3 febrile neutropenia and grade 4-thrombocytopenia (two out of five patients) were shown. Thus, the maximum acceptable dose was set at 15.0 mg/m^2.

Antitumor activity was evident at dose> 11mg/m^2.

The maximum concentration (C_{MAX}) at a dose of 15.0 mg/m^2 was 325.9ng/mL [range: 83.7ng/ml ↔620ng/ml], T_{MAX} (time to reach peak concentrations) was 5.8 minutes [range: 5 ↔ 7], Area Under Curve (AUC) of 9.728ng/ml per hour [range: 1.616ng/ml x hour ↔ 28.426ng/ml hour x hour], $T_{1/2}$ (Half Life) of 28.9 minutes; systemic clearance was 7.054ml/minute [range 950ml/minute↔ 18.511ml/minute], the kidneys and the bladder being the main route of elimination; and the Apparent Volume of Distribution (V_D) was 942L.

Two parameters (C_{MAX} and AUC) are increased according to the dose, but following a complex mathematical function.

Adverse events reported during this trial were: mild to moderate fatigue (14 patients, 48%), nausea (patients, 48%), diarrhea (10 patients, 35%), dyspnea (8 patients, 28%), pyrexia (10 patients, 28%), hypoesthesia (8 patients, 28%), headache (7 patients, 24%), constipation (6 patients, 21%), peripheral edema and systemic edema (7 patients, 24%).

48% (14 patients) experienced grade 3 toxicity, but no patients experienced grade 3 or 4 peripheral neuropathy, although some people included in the study had neuropathy before starting the clinical trial. This information was used to determine a low incidence of neuropathy with Carfilzomib, especially in relation to Bortezomib.

The pharmacodynamic assessment of this study showed that following the first dose of 11mg/m^2 of Carfilzomib, dose-dependent inhibition of chymotrypsin activity (chymotrypsin-like) of the 20S proteasome subunit has been observed, both whole blood and mononuclear cells peripheral blood.

The inhibiting effect accumulates from the first to the fifth day of Carfilzomib treatment. Proteasome activity recovered normal values during the 9 days after

five days of treatment (wash-out period). This recovery was complete in peripheral mononuclear cells, but only partial in whole blood because of the inability to synthesize new erythrocyte proteasomes.

In order to evaluate the differences between the administration of Carfilzomib IV Bolus and intermittent intravenous infusion (30 minutes), the study was extended to Ib/II (PX-171-007) led by Rosen *et al* [16, 17]. Patients with metastatic solid tumors who had not responded to two previous treatments were included. Carfilzomib was injected (IV bolus) on days 1, 2, 8, 9, 15 and 16, in 28-day cycles. During the first cycle, the dose was 20mg/m^2 on day 1; 27mg/m^2, the 2nd day, and 36mg/m^2 on day 8 (maximum dose maintained the rest of the days of treatment). Finally, the dose 20mg/m^2 was selected to start phase II clinical trial.

Fourteen patients were included in the phase I study arm, treated with direct injections (IV bolus) of Carfilzomib; Twenty patients were assigned to receive an intravenous infusion of Carfilzomib, and 65 patients in the phase II study, any of the following diagnoses: lung cancer small cells (12 patients), lung cancer non-small cells (17 patients), ovarian carcinoma (16 women), renal cancer (10 patients), and other malignancies (24 patients, including a subgroup of patients with multiple myeloma). Partial responses were achieved in patients with multiple myeloma, renal cancer or lung cancer non-small cells. The disease stabilised (stopped progressing) for at least 16 weeks in patients with mesothelioma and ovarian, renal, cervical, endometrial cancers; and lung cancers (both small and non-small cells).

The low (almost zero) incidence of neuropathy confirmed the results of previous studies [17], even at the highest dose (36mg/m^2 on day 8 of the treatment cycle). Current studies confirm the efficacy and tolerance of Carfilzomib, regardless of the injection conditions (IV Bolus *versus* Infusion IV).

A second phase II trial (PX-171-002) [18] evaluated the administration on consecutive days (days 1 and 2, 8 and 9; and 15 and 16) every 28 days for three cycles of treatment. Carfilzomib was administered in a variable range of dose (from 1.2 mg/m^2 to 27mg/m^2). This study (PX-171-002) included 37 patients with lymphoma (relapsed or refractory) or multiple myeloma.

The results showed that the minimum effective dose was 15mg/m^2, achieving proteasome inhibition greater than 80%, combined with excellent tolerance.

A favourable response was sustained over intervals 134 days ↔ 392 days, including patients who had relapsed after an initial response to Bortezomib, Thalidomide, Lenalidomide and/or bone marrow transplantation.

IN VITRO STUDIES ON MULTIPLE MYELOMA

In vitro studies conducted on various cell cultures (multiple myeloma, Burkitt's lymphoma, acute lymphocytic leukemia, non-Hodgkin's lymphoma B cells and adenocarcinomas of the colon and rectum, pancreas or lung) have shown that Carfilzomib arrests the cell cycle to leading to apoptosis.

Discussed below four studies on the in vitro activity of Carfilzomib:

a) Study of Demo *et al* [11] on the cytotoxicity of Carfilzomib/ Bortezomib regarding two types of cell cultures: hematologic tumor cells and solid tumor cells.

b) Study of Kuhn *et al* [19] on the potency and specificity with which Carfilzomib inhibits β5 subunit (chymotrypsin-like) proteasome.

c) Study of Suzuki *et al* [20] on the effectiveness of Carfilzomib in cultured colon and rectum adenocarcinoma cells which are resistant to treatment with Bortezomib.

d) Study Trudel *et al* [21] to assess inhibition which Carfilzomib exerts on peripheral blood mononuclear cells as a surrogate for proteasome inhibition.

In the first study cited (a), Carfilzomib shown to be more cytotoxic than Bortezomib, after a brief exposure of hematologic tumor cell cultures to the drug, while cultures of solid tumor and non-tumor cells were insensitive to both Carfilzomib and Bortezomib. When the exposure time is longer, Carfilzomib (from 1 hour to 6 hours) shows, as expected, a higher degree of cytotoxicity and increased time for the recovery of proteasome activity.

In the second study referenced (b), β5 subunit-specific (chymotrypsin-like) of the proteasome complex is inhibited by Carfilzomib. As a result there is an accumulation of substrates "ubiquitinated" that are waiting to be degraded by the proteasome complex. The authors also demonstrated the inhibition of various cell lines by Carfilzomib (ANBL-6, KAS-6, U266 and RPMI-8226), the first two dependent on interleukin-6 (IL6); and U266 and RPMI-8226 independent interleukin-6 (IL6). The inhibition of these types of cell cultures correlated in a linear shape with concentration and duration of exposure to the drug. The no-proliferative effect was greater in cells from multiple myeloma patients not previously treated with Bortezomib (samples from *naïve* patients). Carfilzomib

was active even in cultures of human multiple myeloma (primary or secondary) that were refractory to the addition of Bortezomib.

In the third study (c), cells from human adenocarcinoma of the colon and rectum were exposed to Bortezomib. In cell cultures resistant to Bortezomib, proteasome activity was between 7 times and 11 times higher than that observed in cultures sensitive to Bortezomib. Carfilzomib resulted in prolonged inhibition of proteasome activity in cultures, both sensitive and resistant to Bortezomib. Some multiple myeloma cells refractory to Bortezomib have a mutation located in the β5 proteasome subunit, specifically "Cysteine Arginina24". This mutation is essential for the correct assembly of the proteasome complex, and is the most common mutation in patients with Bortezomib-resistant multiple myeloma [22]. The authors also identified another mutation which, though less frequent, is also responsible for rapid recovery of proteasome activity in patients treated with Bortezomib: the mutation is LMP7 (acronym for Low Molecular Polypeptide 7). Carfilzomib causes the irreversible inhibition of enzymatic activity of the proteasome in cultured cells, with either of the aforementioned two types of mutations.

These preclinical in vitro studies show that Carfilzomib causes apoptosis in both *naïve* patients and patients previously treated with Bortezomib.

In the fourth study (d), the authors employed a dual assay (with substrate for the activity flurogenic β5, and immunoa**d**sorption to quantify the activity β5, LMP7, and MECL1) comparing the vulnerability of the proteasome activity in bone marrow cells and peripheral blood mononuclear cells. The study confirmed that inhibition of proteasome activity in the mononuclear cells obtained from the capillaries is an excellent marker for the inhibition of multiple myeloma cells; cells derived from bone marrow.

IN VITRO STUDIES ON NON-HODGKIN LYMPHOMA IN ASSOCIATION WITH INHIBITORS HISTONE DEACETYLASE

A study led by Dasmahapatra [24] noted that Vorinostat, an inhibitor of the enzyme "histone-deacetylase", increased the activity of Carfilzomib on B cell lymphoma, both sensitive and refractory to treatment with Bortezomib.

This complex mechanism of action involves several mitochondrial injuries: caspase activation, and apoptosis-derived from the activation of mitogen-associated p38 kinase. Also, the abrogation of activation of nuclear factor-B κ (intermediate

inhibition of histone-deacetylase), inactivation of AKT, and the acetylation of Ku70 were observed. These biochemical modifications contribute to synergistic activity. Although these processes have not been studied to date, the possibility of synergy between Carfilzomib and "inhibitor of histone-deacetylase" opens up new therapeutic possibilities.

ESTABLISHMENT OF DOSES FOR PHASE I CLINICAL TRIALS

Demo et al. [11] demonstrated that Carfilzomib causes apoptosis in xenotransplantation models of human B cell lymphoma, colorectal cancer and Burkitt's lymphoma. Different treatment protocols were tried, and when Carfilzomib was administered in cycles of two consecutive days, the best results were achieved. This procedure resulted in the greatest inhibition of proteasome activity (> 80%) in most tissues, and this scheme was selected for Phase I clinical trials.

CLINICAL TRIALS AND EFFECTS

An open (open-label, in statistical jargon) multicentre study showed excellent results when mono-therapy with Carfilzomib was administered in patients with relapsed or refractory multiple myeloma [25]. 46 patients with multiple myeloma, which relapsed after successful treatment, were treated with intravenous doses of Carfilzomib 20mg/m^2 on days 1 and2, 8 and 9, 15 and 16, every 28 days, up to a maximum of 12 cycles. All patients were previously treated with anthracyclines and/or alkylating agents. The average number of cycles of chemotherapy treatment with these drugs was 5 [range: 2↔15]. 83% had undergone bone marrow transplantation. Patients received an average of three cycles of Carfilzomib, according to the protocol indicated previously. Clinical efficacy was defined as a response equal to, or exceeding, the minimum expected. Results: 10 patients (26%) achieved a response equal to, or exceeding, the minimum (clinical efficacy criteria). No patients achieved a complete remission of the neoplasic process. Five patients with Bortezomib refractory disease achieved a partial response. The average time to disease progression was 6.2 months, and the average duration of response was 7.4 months, similar to that observed with Bortezomib in the SUMMIT and APEX trials.

12 cycles of treatment (maximum expected) was completed by 10% of patients.

As regards adverse effects, a first estimate percentage is as follows:

- Peripheral neuropathy (<10%).
- Fatigue (65%).
- Anemia (65%).
- Thrombocytopenia (46%).
- Neutropenia (20%).
- Nausea (37%).
- Upper respiratory tract infections (37%).
- Diarrhea (33%).
- Serum creatinine (33%), not always related to treatment with Carfilzomib.
- Acute renal failure (approximately 9%, cause by tumor lysis and Carfilzomib nephrotoxicity).

In this first phase II study (IIa), effectiveness of Carfilzomib, administered in monotherapy to patients with Bortezomib-resistant multiple myeloma, was shown.

The results of the previous study led to a second phase II trial, also conducted by the Multiple Myeloma Research Consortium. This study, called PX-171-003-AI, included 266 patients with refractory multiple myeloma who had been treated with at least two prior therapies that had included Bortezomib, Thalidomide (or Lenalidomide), and an alkylating drug.

Treatment:

- 1st Cycle of 28 days: Carfilzomib (20mg/m^2) on days 1 and 2, 8 and 9, 15 and 16.
- 2nd Cycle, and all the subsequent cycles (up to 12 cycles): Carfilzomib in escalating dose, up to a maximum dose of 27mg/m^2 (cycle 12), keeping the same schedule of administration at all levels.

Criteria: overall clinical response rate (no tumor progression).

Results: overall response was achieved in 24% of patients, with an average effectiveness of 7.4 months [range: 6.2↔10.3 months]. One patient attained a complete response (0.4%), 12 got responses catalogued as excellent (4.7%), 48 achieved partial responses (19%), and 32 had responses defined as minimal (12%), the latter were not considered in 24% of the overall response. Moreover, 83 patients (32%) slowed the progression of their disease for at least 6 weeks. 11% of patients completed 12 cycles of treatment.

The pattern of adverse effects was: hematologic type [thrombocytopenia (22%), anemia (20%), lymphopenia (10%), neutropenia (8%)], pneumonia (8%), fatigue

(7%), hyponatremia (5%), and hypercalcemia (5%). Although some patients had neuropathy at the beginning of the study, new cases of neuropathy, or worsening neuropathy, were incidental and infrequent (<1%).

The findings of this Phase II-study may lead to durable responses even in patients in whom previous Bortezomib treatment, and immunomodulatory therapy, failed. Even in patients with neuropathy, treatment with Carfilzomib was well tolerated, with minimal risk of exacerbation.

A parallel Phase-II multicentre study conducted by Multiple Myeloma Research Consortium was carried out to estimate the effectiveness of Carfilzomib in patients who did not previously receive Velcade® (Bortezomib-*naïve*). This study included two arms, 54 patients and 19 patients respectively:

1. 1st: Carfilzomib (20mg/m^2) on days 1 and 2, 8 and 9, 15 and 16, for 28 days for 12 cycles (maximum) (arm 54 patients).
2. 2nd: Carfilzomib with increasing dose in each cycle [from 20mg/m^2 to 27mg/m^2], following the same protocol of treatment within each cycle (arm 19 patients).

In the 1st study arm (20mg/m^2 of Carfilzomib during all cycles) the overall response rate was 46% (25 of 54 patients). Patients with a favourable overall response [25] were distributed as follows: 1 global response, 5 very good partial responses, and 19 partial responses.

In the 2nd study arm (dose from 20mg/m^2 to 27mg/m^2 at the 12th cycle), the overall response rate was 53%, with 1 excellent partial response, and 9 partial responses. The average duration of the response was 8.8 months, and the time until disease progression was 7.6 months.

The adverse pattern was similar to the previous studies, including fatigue (59%), nausea (41%), dyspnea (36%), anemia (29%), increased creatinine (31%), and upper respiratory tract infections (31%). Carfilzomib tolerance was considered excellent, because it was not necessary to reduce the dose, even in patients with renal insufficiency.

Patients in this study were stratified into high-risk category scores, following the model established by Eastern Cooperative Oncology Group, the cytogenetic profile and β2 microglobulin. Based on these parameters, the overall response rate ranged from 41% to 54%, with low co-morbidity associated with steroid treatment.

DISCUSSION

Biochemically, Carfilzomib, unlike Bortezomib, leads to an irreversible inhibition of the proteasome, with greater selectivity on chymotrypsin subunit (chymotrypsin-like); and superior inhibition with inmunoproteosome over proteosome. However, as usual, there is no known molecular translation of these findings to clinical outcomes.

Although wide information exists on the pleiotropic effects of Bortezomib (dipeptide boronic acid) on the microscopic environment of the bone marrow and the myeloma cells, there is no knowledge regarding Carfilzomib.

Likewise, the lack of selectivity in the inhibition of the proteasome by Bortezomib is the cause of a relatively common neuropathy during treatment with this drug [(26)].

Another experimental proteasome-inhibitor (NPI-0052) was shown to be more potent and less toxic than Carfilzomib. It is justified theoretically on the basis that NPI-0052 inhibits not only the 20S subunit of the proteasome, but other protein domains cluster with caspase and trypsin activity.

It can be considered, therefore, that the differential inhibition in different proteosome subunits of these substances (drugs like [Bortezomib, Carfilzomib] and other potential drug [NPI-0052)]) determines toxicity and tolerance, without compromising the efficiency.

Several prospective Phase II Bortezomib studies [(27), (28), (29), (30), (31), (32)] in patients with relapsed or refractory lymphoma, achieved a partial response ranging from 29% to 50%, with complete remission rates between 4% and 8%, and a time (average) of response of 10 months. Adverse effects observed in patients with lymphoma were similar to those mentioned previously, during the use of Bortezomib for the treatment of patients with multiple myeloma. At present whether it can achieve a similar response with Carfilzomib, in patients with non-Hodgkin lymphoma, is being studied.

Bortezomib, associated with other drugs, has shown good results in patients with refractory multiple myeloma. So, “Bortezomib + Melphalan”, or “Bortezomib + Dexamethasone”, have shown favourable responses ranging between 63% and 70%, with overall response rates ranging from 15% to 23% [(33), (34), (35)].

When Bortezomib has been included in triple therapy protocols (*vg* "Bortezomib + Dexamethasone + Cyclophosphamide", "Adriamycin + Dexamethasone + Bortezomib" or "Bortezomib + Lenalidomide + Dexamethasone") favourable response rates in a very

wide range [51% ↔ 82%] have been achieved , but with overall response rates in the normal range [15% ↔ 27%] [36], [37], [38], [39], [40], [41], [42].

Bortezomib, together with three common drugs in multiple myeloma (quadruple therapy), has shown response ranges from 67% to 92% (partial response), and 44% ↔ 53% (overall response) [43], [44], [45], [46].

Two studies [47], [48] have shown that Carfilzomib may be associated with Lenalidomide and Dexamethasone with favourable responses [59% ↔ 72%], and an overall response of 21%.

Nowadays, there are several potentially useful substances with a pharmacological activity of the proteasome inhibitor, although this depends on how we define a more concrete indication based on the inhibition pattern of the different protein subunits that make up the proteasome complex. Likewise, their potential utility in other clinical settings, besides myeloma and some types of lymphomas, are being assessed.

Zangari *et al* [49] found that high levels of alkaline phosphatase are associated with a favourable response to treatment with Carfilzomib. These increases almost always happened during the second cycle of treatment. Alkaline phosphatase levels during the second cycle of treatment were 15U/L. No increases in alkaline phosphatase levels were detected in any patient refractory to treatment. This raises the possibility of using this biochemical parameter for evaluating the patient's susceptibility to treatment with Carfilzomib beyond the first treatment cycle.

UNCERTAINTY AND CONCLUSIONS

Three decades have passed since the research team, led by Adams and Julian, carried out the chemical synthesis of Bortezomib, and proved its activity *in vitro* and *in vivo* in several experimental models of human cancer.

During this same time a more detailed knowledge of the system "Ubiquitin-Proteasome" has made it feasible to develop drugs against increasingly specific E3 ligases [50] of the complex structure of ubiquitin.

Progress has been great, but some issues remain unresolved. For example, is it more appropriate to develop drugs for a specific protease of the proteasome complex, or is it better to achieve the simultaneous inhibition of various proteases?. Within the framework of strict pharmacological criteria, is it more important to take into account the maximum concentration (C_{MAX}), or the area under the curve (AUC) [which represents the total amount of drug absorbed]?

Notwithstanding these and other unresolved issues, we could conclude that Carfilzomib will find its place in the *armamentaria* pharmacological treatment of multiple myeloma (and perhaps in other neoplasic processes), solving clinical situations where patients are refractory to, or have relapsed following Bortezomib treatment, which will remain the first choice of treatment. In addition, the specificity of action on concrete protein subunits of the proteasome, seems to circumvent the common peripheral neuropathy with Bortezomib treatment.
Some ongoing clinical trials and clinical experience will enable the Carfilzomib framework in the treatment of relapsed or refractory multiple myeloma, either in mono-therapy, or integrated into more complex treatment protocols.

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Zaragoza (Spain). July 19, 2013

López-Tricas JM MD
Hospital Pharmacist
tricastriszar@telefonica.net (To whom correspondence should be addressed)
Website: www.info-farmacia.com
Zaragoza (Spain)