Captor Therapeutics®

MCL-1: A TARGET WITH IMMENSE POTENTIAL IN ONCOLOGY

Contents

MCL-1 promotes cancer cell growth by inhibiting apoptosis

Apoptosis is a key process for the programmed death of damaged or aged cells and an essential pathway in the elimination of cancerous cells.

Apoptosis can be activated both intracellularly and extracellularly, leading to a series of biochemical changes inside the cell and ultimately cell death. Regardless the factor initiating apoptosis, the process involves activation of a group of proteolytic enzymes from the caspase family, DNA fragmentation, disintegration of the cytoskeleton and the formation of apoptotic bodies. Apoptosis is irreversible after a certain point, so its precise control and regulation is extremely important. In a healthy cell, there is a balance between regulatory proteins that promote (pro-apoptotic) and inhibit (anti-apoptotic) its course. The largest family of these are the Bcl-2 proteins. Pro-apoptotic proteins from the Bcl-2 family include: BID, Bax, Bak, Bad, NOXA and PUMA. Anti-apoptotic proteins include BCL-2, BCL-xL, MCL-1 and survivin.

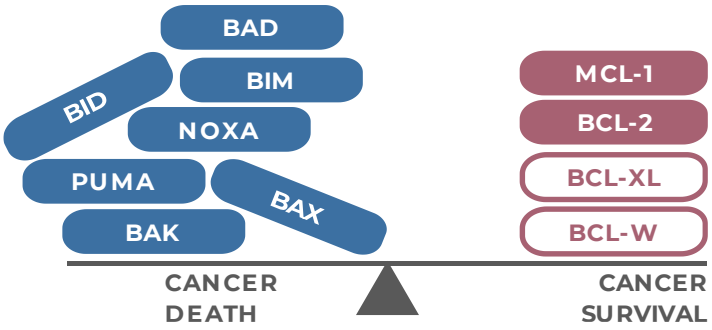

Figure 1: Key apoptotic & anti-apoptotic proteins

The ability to avoid apoptosis is one of the hallmarks of cancer[1], enabling their survival and uninterrupted proliferation. A cell with a dysregulated apoptosis process may not be removed despite the significant DNA damage, as such it can proliferate which allows for clonal selection of the most aggressively proliferating cells. This pattern is replicated in all cancer cells, regardless of the type or cause of cancer[2].

How do cancer cells inhibit apoptosis? They do this by primarily shifting the balance between pro- and anti-apoptotic proteins. Low expression of pro-apoptotic proteins and simultaneous high expression of anti-apoptotic proteins is a characteristic feature of cancer cells[3].

One therapeutic avenue currently being investigated for eliminating cancer cells is to restore their sensitivity to apoptosis using chemotherapeutics, silencing the expression of anti-apoptotic proteins, or epigenetic regulation with substances of natural origin, e.g. alkaloids, terpenoids & quinones[4].

The MCL-1 protein is one of the proteins that is overexpressed in many cancers, e.g.: hematological, breast and lung, where it serves to disrupt the balance between anti- and pro-apoptotic proteins. **It does this by interacting with the pro-apoptotic BAX and BAK proteins, inhibiting their oligomerization and the formation of pores in the mitochondrial membrane. This inhibits the outflow of cytochrome C, the activation of caspases, and consequently the apoptosis process itself, enabling the survival of cancer cells.** This pathway is so fundamental

to cancer cell survival that numerous systemic and targeted therapies, e.g. MEK inhibitors, tamoxifen or paclitaxel, drive the clonal selection of cells towards increased levels of MCL-1, and thus the acquisition of resistance.

Apoptosis is a natural biological process of programmed and controlled death and removal of the body's own cells, and its inhibition is one of the main strategies enabling the survival and multiplication of cancer cells. The MCL-1 protein is overexpressed in many cancer cells, inhibiting the process of apoptosis, and enabling the survival of cancer cells, including when subject to drug therapies.

How do we know that degrading MCL-1 can treat cancer?

MCL-1 is a well-characterized oncogenic protein, whose key role lies in inhibiting apoptosis and **promoting the survival of cancer cells[5].** These features suggest a significant therapeutic potential for drugs that target the degradation of MCL-1.

The important role played by MCL-1 in cancer has been confirmed by numerous studies:

- **Genetic changes** leading to increased levels of MCL-1 protein often occur in cancer cells[6],

- Many **oncogenic signaling pathways are characterized by increased levels of MCL-1**, and greater cell survival[7],

- *In vitro* studies have shown that **the growth of various types of cancer** (including acute myeloid leukemia (AML), B- and T-cell lymphomas, multiple myeloma, some breast cancers, and non-small cell lung cancer) **is dependent on the level of MCL-1 protein**[8],

- AML cells are more sensitive to a decrease in MCL-1 levels compared to normal hematopoietic stem and progenitor cells, suggesting the existence of a safe **therapeutic window**[9],

- A growing problem in AML therapy is acquired resistance to venetoclax, as the survival of patients is on average about 2.4 months. It has been shown that one of the main mechanisms of such resistance is overexpression of MCL-1,

- Overexpression of MCL-1 protein is often associated with **poor prognosis for patients** with oncological diseases[10, 11].

Importantly, a number of *in vivo* studies have confirmed the effectiveness of therapies aimed at inhibiting the activity of the MCL-1 protein, e.g. the treatment of xenograft models of multiple myeloma, lymphoma and acute myeloid leukemia with MCL-1 inhibitors **S63845 and AZD5991** showed inhibition of tumor growth[7, 12]. AZD5991 has also been tested in xenograft models of tumor cells derived from patients with T-cell lymphoma, in which it both induced tumor shrinkage and increased survival of the model organisms[13].

These data provide strong grounds to believe that the use of **drugs that degrade the MCL-1 protein may not only be an effective therapy, but also used to combat MCL-1-induced tumor resistance and sensitize it to existing drugs**.

MCL-1 is a key protein determining the survival of various cancer cells, which impacts their growth and resistance to radiotherapy, chemotherapy, and targeted therapies.

MCL-1 inhibitors and cardiac safety

Despite a strong scientific rationale and years of research by the pharmaceutical industry into the development of MCL-1 inhibitors, **no MCL-1 inhibitor has yet been approved for use.**

Of the MCL-1 inhibitors that have entered clinical trials, (ABBV-467, AMG 176, AMG 397, AZD5991, GS-9716, MIK665 (S64315) & PRT1419), none has yet advanced beyond phase I or I/II clinical trials due to repeated incidences of cardiotoxicity (observed from elevated troponin-I & T levels).

Stage	Compound	Company	Program Status	Comment
1/2	MIK665/ S64315	Novartis/ Servier	Terminated	Elevated troponin-I
1	AMG 176	Amgen/ Beigene	Terminated	Cardiac toxicity observed
1	AMG 397	Amgen/ Beigene	Terminated	Elevated troponin-I
1	AZD5991	AstraZeneca	Terminated	Elevated troponin-I & T
1	ABBV-467	AbbVie	Terminated	Elevated troponin-I & T
1	PRT1419	Prelude Therapeutics	Terminated	No cardiac tox reported
1	GS-9716	Gilead Sciences	On-going	Study recruiting

Table 1: Clinical stage MCL-1 inhibitor programs & current status

Cardiotoxicity seen following treatment with MCL-1 inhibitors is believed to arise from the accumulation of MCL-1 protein within cardiac cells as inhibitors increase MCL-1 stability and prevent its normal degradation by cells[5]. The consequence of this increased stabilization of MCL-1 leads to a cellular rewiring that affects cardiomyocyte viability via necrosis, not apoptosis[14].

Since degraders lower MCL-1 levels, rather than raise them, degradation of MCL-1 is not expected to induce cardiac toxicity. Studies supporting this have shown mice with reduced levels of MCL-1 are viable and do not express signs of cardiac damage[15]. Other studies have also shown that mice deficient in MCL-1 are protected against the development of leukemia & lymphoma[16,17].

The difference between the mechanism of action of inhibitors that **bind and block the interaction of the MCL-1 protein with BAX and BAK proteins,** and **an MCL-1 degrader, which breaks down the protein in the cell preventing its accumulation,** is presented in Figure 2. **Inhibiting the activity of the MCL-1** protein contributes to the accumulation of this protein in the cell, creating a **positive feedback loop** in which more and more drug is necessary to maintain the pharmacological effect. Importantly, classical MCL-1 inhibitors act as protein-protein interaction inhibitors (PPIs), which requires very tight binding with slow times of dissociation.

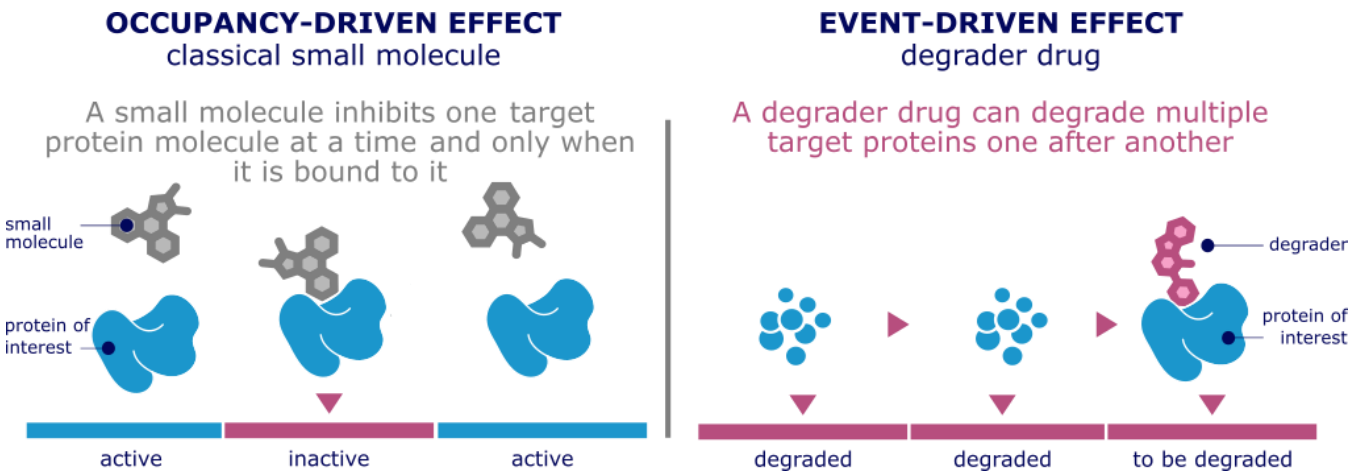

Figure 2: Differentiated pharmacology of degraders vs. inhibitors

No MCL-1 inhibitor has progressed beyond initial clinical studies due to safety concerns arising from cardiac toxicity. This off-target effect is believed to be caused by stabilized MCL-1 inducing necrosis in cardiomyocytes. Since degraders reduce MCL-1 levels, and animal studies demonstrate that lowered levels of MCL-1 do not result in cardiac toxicity, the use of degraders is expected to avoid the class effect seen with inhibitors.

MCL-1 degraders: great potential against various cancers

Degraders are small molecules that form a complex with both the target protein, e.g. MCL-1, and simultaneously an E3 ligase, at which point the target protein has ubiquitin repeatedly added to it. The ubiquinated protein then enters the proteasome where it is degraded.

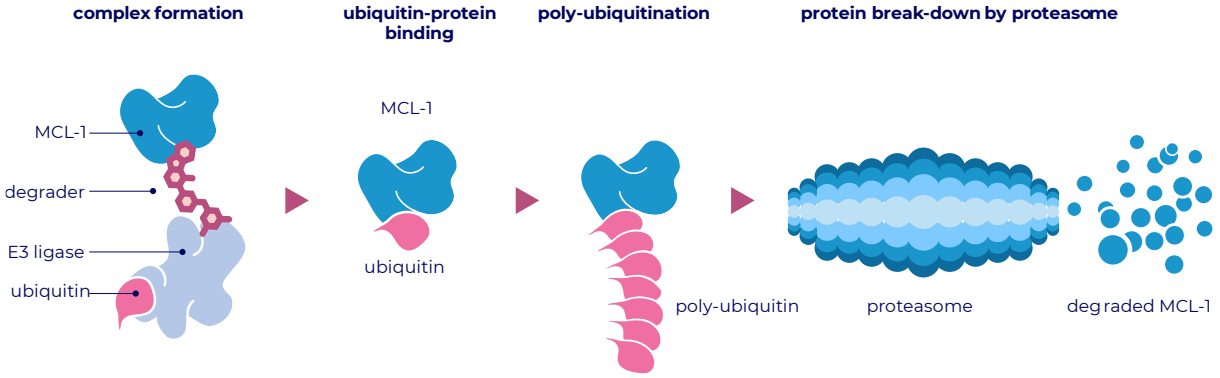

Figure 3: The targeted degradation of MCL-1

MCL-1 is a crucial survival factor for a wide range of hematological cancer cell lines[18], hence most of the initial research has focused on blood cancers. In the case of **multiple myeloma, acute myeloid leukemia and B- and T-cell lymphomas**, a high dependence on MCL-1 has been demonstrated and, in preclinical studies, MCL-1 inhibitors have shown high therapeutic effectiveness in all [8, 11, 13].

Sensitivity to MCL-1 inhibition has also been demonstrated in **solid cancers such as breast and lung cancers**[8]. Analysis of 3,131 cancer samples showed increased levels of MCL-1 expression in 36% of breast cancer cases and 54% of lung cancer cases[19].

Campbell *et al*. showed that high MCL-1 expression correlates with poor prognosis in specific breast cancer subtypes, and the presence of MCL-1 is essential for the development of breast cancer in mice. Campbell *et al* also showed **inhibition of tumor growth after inhibition or silencing of MCL-1 expression** in a mouse model of **triple-negative breast cancer**, an aggressive form of cancer for which there are no effective targeted therapies[7].

Increased MCL-1 expression often accompanies the development of lung adenocarcinoma, the most common **lung cancer**. According to Munkhbaatar *et al*., S63845, an MCL-1 inhibitor, **slowed the development of highly aggressive lung cancer** in mice[20]. Furthermore, Song *et al*. proved that lung cancer cells with a mutation in the EGFR gene increase MCL-1 protein levels in response to treatment with EGFR inhibitors, thereby increasing the resistance of cancer cells. Combining such therapy with a **drug that lowers MCL-1 levels may potentially increase the therapeutic effectiveness of EGFR inhibitors**[21].

Studies on the role of MCL-1 in the biology of cancer and the sensitivity of tumors to reduced levels of MCL-1 suggest a high potential for therapies targeting the degradation of MCL-1 in many cancers, including hematological malignancies and solid tumors.

Development of first-in-class MCL-1-degrading drugs

At Captor Therapeutics, we have used our proprietary **Optigrade™ platform** to identify molecules capable of degrading MCL-1. Through multiple steps based on the design of new chemical molecules and a series of specialized biological tests, we discovered and developed **small molecules capable of eliminating MCL-1 and triggering cancer cell death via apoptosis** not only *in vitro*, but also in mouse xenograft models of human cancer cells. We have made great progress towards bringing *first-in-class* therapeutic MCL-1 degraders to clinical applications.

Since our approach utilizes degraders, which act differently to inhibitors, and don't cause MCL-1 accumulation, we believe there is a reduced risk of cardiotoxicity, something that we have observed in preclinical models.

As already mentioned, it has been shown that acquired resistance to venetoclax is a growing problem in AML therapy, and one of the main mechanisms of such resistance is overexpression of the MCL-1 protein. We have shown that the degradation of MCL-1 in venetoclax-resistant AML cells (cell lines and cells taken from patients) is highly effective in killing them, and that the combination of an MCL-1 degrader with venetoclax is highly synergistic and further enhances the cell death. In a mouse model, we have also shown that the combination of the MCL-1 degrader with venetoclax completely inhibits the growth of leukemic tumors (MV4-11).

Due to the highly aggressive nature of AML with resistance to venetoclax (average survival 2.4 months[22]), we believe an MCL-1 degrader would be a candidate for Fast Track designation with regulatory approval after phase I/II (Accelerated Approval). Should our first-in-class degrader demonstrate MCL-1 degradation in the absence of dose-limiting toxicity, and also show similar benefits in hematological indications such as CLL, Multiple Myeloma & NHL, we believe it would have potential blockbuster status.

Indication	Market Size (Current-Future/ $B)*	Global New Cases[23]	US New Cases[23]	Europe New Cases[23]	5-year Survival[24]
AML†‡	$2.3 - 4.7	336,000	68,400	77,400	32%
CLL†§	$10.9 - 18.3	363,600	51,600	82,400	88%
Multiple Myeloma†	$21.3 - 34.9	538,900	102,500	149,400	60%
NHL	$8.6 - 15.8	1,738,500	270,800	430,500	74%

Figure 4: Market estimates, patient populations & 5-year disease prevalence in key hematological cancers

In the absence of any approved drug targeting MCL-1, our first-in-class degrader offers a unique opportunity to address the significant unmet medical needs in the treatment of numerous cancers.

* Averaged value ranges from market research reports for periods 2021-24 (current) to 2028-32 (future)
† Indication qualifies as an Orphan Disease in the US & EU. (US Orphan Drug Act threshold for a rare disease is <200,000 people affected (https://www.fda.gov/industry/designating-orphan-product-drugs-and-biological-products/orphan-drug-act-relevant-excerpts); EMA threshold for rare disease is 5 per 10,000 (http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:018:0001:0005:EN:PDF), i.e. approx. 224,000)
‡ Assume 23.1% of leukemias are AML (Exp Hematol Oncol. 2020 Jun 19; 9:14
§ CLL accounts for 25% of all leukemias (https://www.cancer.org/cancer/types/chronic-lymphocytic-leukemia/about/key-statistics.html)

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Captor Therapeutics®

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