Katedra i Klinika Hematologii i Transfuzjologii

Centrum Medyczne Kształcenia Podyplomowego

Ocena wpływu składowych kompleksu ligazy E3 ubikwityny na przebieg kliniczny i rokowanie u chorych na szpiczaka plazmocytowego poddanych leczeniu immunomodulującemu

lek. Joanna Barankiewicz

Rozprawa doktorska
w dziedzinie nauk medycznych

Promotor: prof. dr hab. n. med. Ewa Lech-Marańda

Warszawa, 2022

Pragnę złożyć serdeczne podziękowania

Pani Prof. dr hab. n. med. Ewie Lech-Marańdzie za wsparcie, zaangażowanie, zaufanie i wyrozumiałość okazaną mi w trakcie realizacji rozprawy doktorskiej oraz całej mojej dotychczasowej pracy.

Współpracownikom z Instytutu Hematologii i Transfuzjologii, zaangażowanym w realizację niniejszego projektu naukowego, w szczególności Aleksandrowi Salomonowi-Perzyńskiemu za motywację i wsparcie w krytycznych etapach realizacji tej pracy oraz Annie Szumerze-Ciećkiewicz za nieoceniony wkład i anielską cierpliwość wobec moich wątpliwości.

Pani Prof. dr hab. n. med. Urszuli Mackiewicz za serce na dłoni w trakcie realizacji Studiów Doktoranckich CMKP.

Pracę dedykuję moim wspaniałym Rodzicom

W skład cyklu publikacji będącego podstawą niniejszej rozprawy doktorskiej wchodzą artykuły wymienione jak niżej:

1. **Barankiewicz J**, Szumera-Ciećkiewicz A, Salomon-Perzyński A, Wieszczy P, Malenda A, Garbicz F, Prochorec-Sobieszek M, Misiewicz-Krzemińska I, Juszczyński P, Lech-Marańda E. *The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients*. Journal of Clinical Medicine. 2021; 10(12):2683. https://doi.org/10.3390/jcm10122683

   Impact factor (IF): 4,964

   Punkty MNiSW: 140

2. **Barankiewicz, J.**; Salomon-Perzyński, A.; Misiewicz-Krzemińska, I.; Lech-Marańda, E. *CRL4CRBN E3 Ligase Complex as a Therapeutic Target in Multiple Myeloma.* Cancers 2022, 14, 4492. https://doi.org/10.3390/cancers14184492

   Impact factor (IF): 6,575

   Punkty MNiSW: 140

Całkowity IF	11,539
Łączna liczba punktów MNiSW	280

NARODOWE CENTRUM NAUKI

Niniejsza rozprawa doktorska powstała w ramach projektu: “Integralność białek kompleksu ligazy E3 ubikwityny jako biomarker odpowiedzi na leczenie immunomodulujące u chorych na szpiczaka plazmocytowego” (2014/3/B/NZ5/03160), finansowanego przez Narodowe Centrum Nauki (ang. *National Science Centre)* w ramach konkursu OPUS.

SPIS TREŚCI

WYKAZ STOSOWANYCH SKRÓTÓW

auto-HSCT –przeszczepienie autologicznych krwiotwórczych komórek macierzystych (ang. *autologous hematopoetic stem-cell transplantation*)

CI – przedział ufności (ang. *confidence interval*)

CR – całkowita remisja (ang. *complete remission*)

CRBN – cereblon (ang. *cereblon)*

CUL4A – białko wchodzące w skład kompleksu ligazy E3 ubitkwityny (ang. *cullin 4A*)

DDB1 – białko wchodzące w skład kompleksu ligazy E3 ubikwityny (ang. *DNA damage-binding protein-1*)

D-VTd – daratumumab, bortezomib, talidomid, deksametazon (ang. *daratumumab, bortezomib, thalidomide, dexamethasone*)

FLC – wolny lekki łańcuch immunoglobulinowy (ang. *free-light chain*)

HR – współczynnik ryzyka (ang. *hazard ratio*)

IKZF1 – czynnik transkrypcyjny Ikaros (ang. *Ikaros family zinc finger 1*)

IKZF3 – czynnik transkrypcyjny Aiolos (ang. *Ikaros family zinc finger protein 3*)

IL-2 – interleukina 2 (ang. *interleukin-2*)

IMiDs – leki immunomodulujące (ang. *immunomodulatory drugs*)

IFN-γ – interferon gamma (ang. *interferon gamma)*

ISS – Międzynarodowy Indeks Prognostyczny (ang. *International Staging System*)

RBX1 – białko wchodzące w skład kompleksu ligazy E3 ubitkwityny (ang. *RING-box protein 1*)

Rd – lenalidomid, deksametazon (ang. *lenalidomide, dexamethasone*)

MM – szpiczak plazmocytowy (ang. *multiple myeloma*)

MRD – mierzalna choroba resztkowa (ang. *measurable residual disease*)

PD – progresja choroby (ang. *progressive disease*)

Pd – pomalidomid, deksametazon (ang. *pomalidomide, dexamethasone*)

PR – częściowa remisja (ang. *partial response*)

PFS – przeżycie wolne od progresji (ang. *progression-free survival)*

rFLC – stosunek stężeń łańcuchów lekkich immunoglobulin w surowicy (ang. *serum free-light chain ratio*)

SD – stabilizacja choroby (ang. *stable disease*)

Td – talidomid, deksametazon (ang. *thalidomide, dexamethasone)*

VGPR – bardzo dobra częściowa remisja (ang. *very good partial response*)

VRd – bortezomib, lenalidomid, deksametazon (ang. *bortezomib, lenalidomide, dexamethasone*)

VTd – bortezomib, talidomid, deksametazon (ang. *bortezomib, thalidomide, dexamethasone*)

ZARYS PROBLEMU

Szpiczak plazmocytowy (*ang. multiple myeloma*, MM) jest chorobą nowotworową układu chłonnego, charakteryzującą się klonalnym rozrostem komórek plazmatycznych, tj. limfocytów B będących na ostatnim etapie dojrzewania i różnicowania. Pomimo faktu, że szpiczak plazmocytowy jest nowotworem rzadkim i stanowi około 1% wszystkich chorób nowotworowych, wśród rozrostów hematoonkologicznych MM obejmuje około 10% i jest jedną z najczęstszych chorób nowotworowych układu chłonnego [1], [2]. Według rejestrów prowadzonych przez Narodowy Instytut Raka (ang. *National Cancer Institute*, NCI), zapadalność roczna na MM w Stanach Zjednoczonych, na podstawie danych epidemiologicznych z lat 2010 – – 2019 , ustabilizowała się i wynosi aktualnie 6,9 przypadków na 100 000 osób [3]. W Polsce obserwuje się stały wzrost zarówno liczby nowych zachorowań na MM, jak i liczby zgonów z powodu MM. Według danych z Krajowego Rejestru Nowotworów, w ciągu ostatnich dwudziestu lat wskaźniki te uległy podwojeniu, **Tabela nr 1.**

Tabela nr 1. Epidemiologia szpiczaka plazmocytowego w Polsce na podstawie danych z Krajowego Rejestru Nowotworów.

Rok	**Liczba zachorowań**	**Współczynnik zachorowalności**	**Liczba zgonów**	**Współczynnik umieralności**
1999	829	2,15	762	1,98
2004	1122	2,94	1090	2,85
2009	1132	2,97	1169	3,06
2014	1498	3,89	1282	3,33
2019	1713	4,46	1410	3,67

Obecnie celem leczenia MM jest uzyskanie stanu całkowitej remisji (ang. *complete remission*, CR) z negatywizacją mierzalnej choroby resztkowej (ang. *measurable residual disease*, MRD), wydłużenie przeżycia wolnego od progresji choroby (ang. *progression-free* survival, PFS) oraz całkowitego przeżycia (ang. *overall survival*, OS). Obserwowany wzrost odsetków 5-letnich przeżyć z 35,5% w roku 2000 do około 54% w roku 2016 wyraża postęp, jaki dokonał się w leczeniu MM w ciągu ostatnich

dwóch dekad [3]. Ta poprawa rokowania związana jest z ugruntowaniem roli konsolidacji leczenia za pomocą wysokodawkowanej chemioterapii wspomaganej transplantacją autologicznych komórek krwiotwórczych (ang. *autologous hematopoietic stem cell transplantation*, auto-HSCT) oraz wprowadzeniem do leczenia nowych klas leków, takich jak: leki immunomodulujące, inhibitory proteasomu oraz przeciwciała monokonalne ukierunkowane na antygeny powierzchniowe komórek MM. Pomimo znaczącej poprawy wyników leczenia, MM pozostaje nowotworem nieuleczalnym, prowadzącym rocznie do 13 000 zgonów w Stanach Zjednoczonych i około 20 000 zgonów w krajach Unii Europejskiej [4].

Szczególną grupą leków, które istotnie przyczyniły się do poprawy wyników leczenia MM są leki immunomodulujące (ang. *immunomodulatory drugs*, IMiDs). Pierwszym przedstawicielem tej grupy był talidomid. Lek ten pierwotnie był stosowany przeciwwymiotnie u kobiet ciężarnych i zyskał złą sławę z powodu poważnych embriotoksycznych działań niepożądanych. Niemniej, jego wprowadzenie w 2006 roku do leczenia chorych na MM zapoczątkowało przełomowe zmiany w terapii MM, które trwają do dziś. Obecnie zastosowanie talidomidu i jego nowszych pochodnych, tj. lenalidomidu i pomalidomidu, stanowi podstawę schematów terapeutycznych, szeroko stosowanych w rutynowej praktyce klinicznej, w terapii nowo rozpoznanego i nawrotowego MM [5], [6]. W leczeniu pierwszej linii u pacjentów kwalifikujących się do auto-HSCT rekomendowane jest stosowanie schematów zawierających talidomid w skojarzeniu z bortezomibem i deksametazonem (VTd) lub z bortezomibem, deksametazonem i daratumumabem (D-VTd), alternatywnie lenalidomid w skojarzeniu z bortezomibem i deksametazonem (VRd). Z kolei u pacjentów, którzy z powodu schorzeń współistniejących lub wieku nie kwalifikują się do konsolidacji leczenia z zastosowaniem auto-HSCT, zaleca się leczenie indukujące remisję lenalidomidem w skojarzeniu z deksametazonem (Rd) lub schematami trójlekowymi z bortezomibem (VRd) bądź daratumumabem (DRd).

Pacjenci z nawrotowym lub opornym na leczenie MM stanową szczególne wyzwanie w codziennej praktyce klinicznej. Znaczącą rolę IMiDs w leczeniu nawrotowego MM podkreśla fakt, że poza determinantami klinicznymi, na dobór terapii drugiej i kolejnych linii znaczący wpływ ma określenie, czy u danego pacjenta rozwinęła się oporność na lenalidomid w toku dotychczasowego leczenia. U pacjentów, którzy nie otrzymywali wcześniej leczenia według schematu Rd, opcję terapeutyczną stanowi schemat Rd [7]

lub jego skojarzenie z inhibitorem proteasomu drugiej generacji (karfilzomibem [8], iksazomibem [9]) lub przeciwciałem monoklonalnym (daratumumabem [10], elotuzumabem [11]). W przypadku pacjentów, u których rozwinęła się oporność na lenalidomid opcję terapeutyczną, obok schematów niezawierających IMiD, stanowią skojarzenia pomalidomidu z deksametazonem (Pd) [12] lub trójlekowe połączenia Pd z przeciwciałem monoklonalnym (daratumumabem [13], izatuksymabem [14], elotuzumabem [15]), inhibitorem proteasomu (bortezomibem [16], karfilzomibem [17], iksazomibem [18]) lub z lekiem alkilującym (cyklofosfamidem [19]). Ostateczna decyzja terapeutyczna dotycząca wyboru odpowiedniego schematu leczenia jest uzależniona od czynników zależnych od pacjenta (wiek, stan sprawności, schorzenia współistniejące, powikłania występujące w trakcie poprzednich linii leczenia) oraz od charakterystyki samej choroby (czas remisji, obecność niekorzystnych czynników rokowniczych, oporność na poprzednie linie leczenia), a w warunkach polskich dodatkowo ta decyzja ograniczona jest kryteriami refundacyjnymi poszczególnych produktów leczniczych.

Szeroki wybór opcji terapeutycznych, jakimi obecnie dysponujemy w leczeniu MM, zobowiązuje nas do pogłębiania wiedzy o mechanizmach działania poszczególnych cząsteczek oraz poszukiwania potencjalnych biomarkerów odpowiedzi na leczenie i czynników prognostycznych, pozwalających na identyfikację pacjentów, którzy mogą odnieść największą korzyść kliniczną z zastosowanej terapii.

Przez wiele lat dokładny mechanizm działania talidomidu i jego pochodnych pozostawał nieznany, a kolejne doniesienia z badań podstawowych wskazywały, że efekt działania IMiDs najlepiej charakteryzuje określenie „plejotropowy". Leki z tej grupy wykazują działanie antyangiogenne, antyproliferacyjne, modulują aktywność osteoklastów oraz komórek układu odpornościowego (tj. limfocytów T oraz komórek NK), a także oddziałują na interakcje komórek MM z mikrośrodowiskiem szpiku kostnego [20], [21]. Pomimo identyfikacji wielu ścieżek aktywności IMiDs, mechanizm molekularny leżący u podstaw ich działania przypisywany jest bezpośredniej interakcji z białkiem CRBN (ang. *cereblon*), które wraz z białkami DDB1 (ang. *DNA damage-binding protein-1*), CUL4A (ang. *cullin 4A*) oraz RBX1 (ang. *RING-box protein 1*) współtworzy kompleks o aktywności ligazy E3 ubikwityny ($CRL4^{CRBN}$) [22]. Interakcja IMiDs z CRBN powoduje zmianę profilu białek, które podlegają ubikwitynacji przez kompleks $CRL4^{CRBN}$, a następnie są kierowane na drogę proteasomalnej degradacji. W terapii MM kluczowymi „neosubstratami" dla kompleksu $CRL4^{CRBN}$ są czynniki transkrypcyjne

IKF1 (ang. *Ikaros family zinc finger 1*) oraz IKZF3 (ang. *Ikaros family zinc finger protein 3*), których ubikwitynacja i wtórna proteasomalna degradacja wywołuje efekt cytotoksyczny wobec komórek MM oraz moduluje aktywność limfocytów T i komórek NK poprzez regulację wydzielania INF-γ oraz IL-2 [23]–[26]. Dotychczas opublikowane wyniki badań podstawowych wskazują, że wyciszenie genu *CRBN* zmniejsza żywotność komórek MM oraz indukuje oporność na antyproliferacyjne działanie lenalidomidu i pomalidomidu [27], [28]. Zaś ekspresja białka CRBN koreluje z wynikami leczenia schematami zawierającymi IMiDs [29]–[31]. Niewiele jednak wiadomo, na temat predykcyjnego i prognostycznego znaczenia pozostałych składowych kompleksu $CRL4^{CRBN}$ u pacjentów z rozpoznaniem MM leczonych za pomocą leków immunomodulujących.

CEL PRACY

W pracy zbadano zagadnienie zależności pomiędzy ekspresją składowych kompleksu ligazy E3 ubikwityny ($CRL4^{CRBN}$) a przebiegiem klinicznym i rokowaniem u chorych na szpiczaka plazmocytowego, poddanych leczeniu z wykorzystaniem IMiDs.

Wyróżniono następujące cele szczegółowe pracy:

1. Ocena ekspresji składowych kompleksu $CRL4^{CRBN}$ w archiwalnych trepanobioptatach pobranych od chorych na MM leczonych IMiDs.
2. Ocena zależności pomiędzy ekspresją składowych kompleksu $CRL4^{CRBN}$ a uznanymi czynnikami prognostycznymi, ocenianymi w rutynowej praktyce klinicznej.
3. Ocena zależności pomiędzy ekspresją składowych $CRL4^{CRBN}$ a uzyskaną odpowiedzią na leczenie IMiDs.
4. Weryfikacja zależności pomiędzy ekspresją składowych $CRL4^{CRBN}$ i odległymi wynikami leczenia z wykorzystaniem IMiDs.
5. Omówienie możliwości wykorzystania kompleksu $CRL4^{CRBN}$ jako celu terapeutycznego u pacjentów z rozpoznaniem MM.

WYNIKI

W ramach pracy doktorskiej przeprowadzono retrospektywne badanie z wykorzystaniem archiwalnych trepanobioptatów pobranych od pacjentów z rozpoznaniem szpiczaka plazmocytowego, poddanych leczeniu immunomodulującemu, w Instytucie Hematologii i Transfuzjologii w latach 2010-2015.

Do badania włączono 130 pacjentów, w tym 81 (62%) pacjentów z nowo rozpoznanym MM, poddanych leczeniu pierwszej linii z wykorzystaniem talidomidu i 49 (38%) pacjentów z nawrotowym MM, którzy otrzymali leczenie oparte na lenalidomidzie. Mediana wieku pacjentów w momencie rozpoczęcia leczenia za pomocą IMiDs wynosiła 62,5 roku (zakres, 32-85 lat), szczegółowa charakterystyka kliniczna oraz laboratoryjna badanej grupy została przedstawiona w **Tabeli nr 2.** Nie obserwowano istotnych statystycznie różnic pomiędzy grupami poddanymi leczeniu z wykorzystaniem IMiDs, z wyjątkiem predominacji płci żeńskiej (66% vs 49%) oraz obecności zmian osteolitycznych (64% vs 28%) w grupie leczonej talidomidem.

Tabela nr 2. Szczegółowa charakterystyka kliniczna i laboratoryjna grupy badanej.

PARAMETR		**n=130**
Wiek, lata		62,5 (32-85)
Płeć	mężczyźni	52 (40%)
	kobiety	78 (60%)
Izotyp białka monoklonalnego	IgG	86 (66%)
	IgA	29 (22%)
	IgE	1 (1%)
	FLC	14 (11%)
ISS	I	23 (18%)
	II	42 (32%)
	III	36 (28%)
	brak danych	29 (22%)
Albumina, g/dl		3,59 (±0.54) n=128
β2-mikroglobulina, mg/l		4,43 (1.56-26.15) n=100
Białko monoklonalne, g/dl		3,6 (±2,01) n=125
rFLC	< 100	43 (33%)
	≥ 100	43 (33%)
	brak danych	44 (33%)
Hemoglobina, g/dl	< 10	69 (53%)
	≥ 10	61 (47%)
Wapń, mmol/l	≤2,55	108 (83%)
	> 2,55	21 (16%)
	brak danych	1 (1%)
Kreatynina, mg/dl	≤ 2	122 (94%)
	> 2	8 (6%)

Zmiany osteolityczne	obecne	66 (50%)
	brak	58 (45%)
	brak danych	6 (5%)
Plazmocyty w szpiku kostnym, %		67,5 (12,5- 95)
Lek immunomodulujący	talidomid	81 (62%)
	lenalidomid	49 (38%)
Liczba cykli leczenia		6 (1-56)

Dane są przedstawione jako: liczby (odsetki) lub mediany (rozstęp międzykwartylowy). Zastosowane skróty: ISS (ang. *International Staging System*), FLC (ang. *free-light* chain), rFLC (ang. *serum free-light chain ratio*).

Ocena ekspresji składowych kompleksu $CRL4^{CRBN}$

W przeprowadzonym badaniu oceniono obecność składowych kompleksu $CRL4^{CRBN}$ z wykorzystaniem technik immunohistochemicznych. Zidentyfikowano obecność białek CRBN, CUL4A, DDB1, IKZF1 i IKZF3 odpowiednio w 54%, 51%, 49%, 71% i 54% analizowanych trepanobioptatach. Wykazano zależność pomiędzy ekspresją białek tworzących kompleks $CRL4^{CRBN}$ dla par: CUL4A-IKZF3 (p=0,023) i DDB1-IKZF1 (p=0,007). Nie obserwowano wzajemnych zależności pomiędzy ekspresją pozostałych składowych $CRL4^{CRBN}$ ani czynników transkrypcyjnymi zależnych od aktywności kompleksu pod wpływem IMiDs (IKZF1, IKZF3).

Zależności pomiędzy ekspresją składowych kompleksu $CRL4^{CRBN}$ a czynnikami klinicznymi MM

W wyniku przeprowadzonych analiz statystycznych wykazano, że w grupa pacjentów z ekspresją CRBN, tj. CRBN (+), charakteryzuje się wyższym stężeniem białka monoklonalnego (mediana 4,1 g/dl vs. 2,7 g/dl, p=0,006), β2-mikroglobuliny (mediana 4,6 mg/l vs 3,9 mg/l, p=0,047) oraz niższym stężeniem hemoglobiny we krwi obwodowej (< 10 g/l, p=0,030) w momencie rozpoczęcia leczenia IMiDs w porównaniu do grupy CRBN(-). Ponadto, w grupie pacjentów z ekspresją DDB1, tj. DDB1(+), obserwowano wyższe stężenie β2-mikroglobuliny (mediana 5,04 g/dl vs 3,94 g/dl, p=0,016) i niższe stężenie hemoglobiny we krwi obwodowej (<10 g/l, p=0,036). Dodatkowo, zidentyfikowano zależność pomiędzy klasą białka monoklonalnego dominującego w przebiegu MM, a ekspresją IKZF1 oraz IKZF3 (odpowiednio p=0,040 i p<0,001).

Zależności pomiędzy ekspresją składowych $CRL4^{CRBN}$ a uzyskaną odpowiedzią na leczenie

W analizie zależności pomiędzy ekspresją składowych $CRL4^{CRBN}$ a odpowiedzią uzyskaną na leczenie oparte na IMiDs, potwierdzono że pacjenci w grupie CRBN(+) charakteryzują się lepszymi wynikami leczenia w porównaniu do grupy CRBN(-). Odpowiednio częściej uzyskiwano co najmniej częściową remisję (≥ PR vs <PR, p=0,012) oraz co najmniej bardzo dobrą częściową remisję (≥ VGPR vs <VGPR, p=0,032) w grupie CRBN(+). Dodatkowo pokazano, że w grupie pacjentów z ekspresją CUL4A, tj. CUL4A(+) również częściej obserwuje się korzystniejsze wyniki leczenia w zakresie uzyskiwanej odpowiedzi na leczenie IMiDs, odpowiednio co najmniej PR (≥ PR vs < PR, p=0,007) oraz przynajmniej VGPR (≥ VGPR vs <VGPR p=0,027), w porównaniu do grupy bez ekspresji CUL4A. Nie obserwowano istotnych statystycznie różnic pomiędzy ekspresją DDB1, IKZF1 i IKZF3, a uzyskaną odpowiedzią na leczenie oparte na IMiDs.

Zależności pomiędzy ekspresją składowych $CRL4^{CRBN}$ i odległymi wynikami leczenia z wykorzystaniem IMiDs

W analizowanej grupie mediana czasu obserwacji w wyniosła 4,75 lat (zakres 0,5-16,9). W ocenianym okresie udokumentowano, że grupa pacjentów CUL4A(+) charakteryzuje się mniejszym ryzykiem progresji MM w porównaniu do grupy bez ekspresji CUL4A (HR=0,66; 95% CI 0,44-0,99; p=0,046). W analizie jednoczynnikowej zidentyfikowano niekorzystne czynniki prognostyczne dla OS, tj. ekspresję DDB1 (HR=3,48; 95% CI 1,75-6,93; p<0,001), obecność zmian osteolitycznych (HR=2,44; 95% CI 1,31-4,53; p=0,005), starszy wiek pacjenta (HR=1,04; 95% CI 1,01-1,08; p=0,008) oraz wyższe wyjściowo stężenie β2-mikroglobuliny we krwi obwodowej (HR=1,10; 95% CI1,04-1,15; p<0,001). W analizie wieloczynnikowej uwzględniono zmienne niezależne, oceniane w analizie jednoczynnikowej, tj. wiek, stężenie białka monoklonalnego, rodzaj białka monoklonalnego, ilościową ocena plazmocytów w szpiku kostnym, stężenie β2-mikroglobuliny, albuminy, kreatyniny, wapnia całkowitego, hemoglobiny, liczbę płytek krwi we krwi obwodowej oraz ekspresję składowych kompleksu $CRL4^{CRBN}$. W toku przeprowadzonej analizy potwierdzono niezależny, niekorzystny wpływ poniższych parametrów, ocenianych bezpośrednio przed leczeniem: stężenie β2-mikroglobuliny (HR=1,06; 95% CI 1,01-1,12; p=0,026), obecność zmian osteolitcznych (HR=2,44; 95% CI 1,19-5,01; p=0.015), oraz ekspresja DDB1 (HR=3,38;

95% CI 1,65-6,75; p<0,001) na prawdopodobieństwo całkowitego przeżycia chorych na MM leczonych IMiDs.

Omówienie możliwości terapeutycznych opartych na wykorzystaniu $CRL4^{CRBN}$ jako punktu uchwytu w leczeniu MM

W pracy poglądowej dokonano przeglądu literatury w zakresie aktualnej wiedzy dotyczącej wykorzystania kompleksu jako celu terapeutycznego w MM. Omówiono aktualne doniesienia w zakresie mechanizmu działania IMiDs oraz wyniki najważniejszych badań klinicznych oceniających terapie opartą na IMiDs. Ponadto scharakteryzowano kolejną generację cząsteczek modulujących aktywność $CRL4^{CRBN}$ (ang. *Cereblon E3 ligase modulators*, CELMoDs), przedstawiając mechanizm ich działania, dotychczas uzyskane wyniki badań podstawowych oraz badań klinicznych wczesnych faz oceniających CELMoDs. W artykule przedstawiono również możliwości wykorzystania kompleksu $CRL4^{CRBN}$ do precyzyjnego kierowania wybranych białek na drogę proteolizy przy użyciu chimerycznych białek adaptorowych, tj. PROTACs (ang. *proteolysis targeting chimeras*) – najnowszej metody inżynierii proteomicznej o wysokim potencjale terapeutycznym.

WNIOSKI

Uzyskane wyniki badań przemawiają za predykcyjnym znaczeniem ekspresji CRBN i CUL4A oraz prognostycznym znaczeniem ekspresji DDB1 u chorych na MM leczonych IMiDs. Realizacja szczegółowych celów niniejszej rozprawy pozwoliła na sformułowanie następujących wniosków szczegółowych:

1. W 130 archiwalnych trepanobioptatach pobranych od pacjentów z rozpoznaniem MM bezpośrednio przed rozpoczęciem leczenia opartego na IMiDs, stwierdzono obecność białek zaangażowanych w tworzenie kompleksu $CRL4^{CRBN}$ – CRBN, CUL4A, DDB1 oraz ekspresję białek zależnych od aktywności $CRL4^{CRBN}$ – IKZF1 oraz IKZ3, wykorzystując techniki immunohistochemiczne rutynowo stosowane w procesie diagnostycznym MM.
2. Istnieje zależność pomiędzy ekspresją CRBN a wyższym stężeniem białka monoklonalnego, β2-mikroglobuliny oraz niższym stężeniem hemoglobiny we krwi obwodowej w momencie rozpoczęcia leczenia immunomodulującego. Podobnie, ekspresja DDB1 związana jest wyższym stężeniem β2-mikroglobuliny oraz niższym stężenie hemoglobiny we krwi obwodowej bezpośrednio przed leczeniem opartym na IMiDs.
3. Pacjenci w grupie z ekspresją CRBN charakteryzują się lepszymi wynikami leczenia w porównaniu do grupy, w której tej ekspresji się nie identyfikuje. Chorzy uzyskują znamiennie częściej PR oraz VGPR w wyniku leczenia opartego na IMiDs. Podobnie, obecność CUL4A również cechuje grupę pacjentów, którzy częściej uzyskują co najmniej PR lub VGPR w wyniku leczenia talidomidem lub lenalidomidem.
4. Do niekorzystnych czynników rokowniczych, mających wpływ na czas całkowitego przeżycia należą: ekspresja DDB1, obecność zmian osteolitycznych, starszy wiek pacjenta oraz wyższe wyjściowo stężenie β2-mikroglobuliny we krwi obwodowej. Uzyskane wyniki analizy wieloczynnikowej potwierdzają prognostyczny, niekorzystny wpływ stężenia β2-mikroglobuliny, obecności zmian osteolitycznych oraz ekspresji DDB1 na prawdopodobieństwo całkowitego przeżycia chorych na MM leczonych IMiDs.
5. Eksploracja znaczenia kompleksu $CRL4^{CRBN}$ w ujęciu terapeutycznym szpiczaka plazmocytowego stanowi szczególną wartość w zakresie identyfikacji

potencjalnych biomarkerów uzyskiwanej odpowiedzi na leczenie i szacowania odległych wyników terapii opartej na IMiDs. W przyszłości może stanowić wartość dodaną w zakresie dynamicznie rozwijających się terapii opartych na aktywności kompleksu ligazy E3 ubikwitny.

Journal of
Clinical Medicine

MDPI

Article

The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients

Joanna Barankiewicz [1,2,*], Anna Szumera-Ciec´kiewicz [3], Aleksander Salomon-Perzyński [1], Paulina Wieszczy [4], Agata Malenda [1], Filip Garbicz [5], Monika Prochorec-Sobieszek [3], Irena Misiewicz-Krzemińska [5], Przemysław Juszczyński [5] and Ewa Lech-Marańda [1,2,*]

1 Department of Hematology, Institute of Hematology and Transfusion Medicine, 02-776 Warsaw, Poland; asalomon@ihit.waw.pl (A.S.-P.); malenda.agata@gmail.com (A.M.)
2 Department of Hematology and Transfusion Medicine, Center of Postgraduate Medical Education, 02-776 Warsaw, Poland
3 Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, 02-776 Warsaw, Poland; szumann@gmail.com (A.S.-C.); monika.prochorec@interia.pl (M.P.-S.)
4 Department of Gastroenterology, Hepatology and Clinical Oncology, Centre of Postgraduate Medical Education, 02-781 Warsaw, Poland; p.wieszczy@gmail.com
5 Department of Experimental Hematology, Institute of Hematology and Transfusion Medicine, 02-776 Warsaw, Poland; filip.garbicz@gmail.com (F.G.); imisiewiczk@ihit.waw.pl (I.M.-K.); pjuszczynski@ihit.waw.pl (P.J.)
* Correspondence: jbarankiewicz@ihit.waw.pl (J.B.); emaranda@ihit.waw.pl (E.L.-M.)

Citation: Barankiewicz, J.; Szumera-Ciec´kiewicz, A.; Salomon-Perzyn´ski, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemin´ska, I.; Juszczyn´ski, P.; Lech-Maran´da, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. *J. Clin. Med.* **2021**, *10*, 2683. https://doi.org/10.3390/jcm10122683

Academic Editors: Ioannis Ntanasis-Stathopoulos and Evangelos Terpos

Received: 30 May 2021
Accepted: 15 June 2021
Published: 18 June 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Immunomodulatory drugs (IMiDs) are effective in the treatment of multiple myeloma (MM), myelodysplastic syndrome with deletion of chromosome 5q and other haematological malignancies. Recent studies showed that IMiDs bind to cereblon (CRBN), a substrate receptor of the CRL4–CRBN complex, to induce the ubiquitination and degradation of IKZF1 and IKZF3 in MM cells, contributing to their anti-myeloma activity. We aimed to determine whether the CRL4– CRBN complex proteins' expression predicts the prognosis of MM patients treated with IMiDs. Here, we evaluated the expression of CRL4–CRBN complex proteins and their downstream targets with immunohistochemistry (IHC) staining in 130 bone marrow samples from MM patients treated with thalidomide or lenalidomide-based regimens. We found that the expression of CRBN and CUL4A was associated with the superior IMiD-based treatment response (p = 0.007 and p = 0.007, respectively). Moreover, the CUL4A expression was associated with improved PFS (HR = 0.66, 95% CI 0.44–0.99; p = 0.046) and DDB1 expression showed a negative impact on OS both in the univariate (HR = 2.75, 95% CI 1.65–4.61; p = 0.001) and the multivariate (HR 3.67; 95% CI 1.79–7.49; p < 0.001) analysis. Overall, our data suggest that the expression of DDB1, CUL4A and CRBN assessed by IHC predicts the clinical course of MM patients and identifies patients with a high probability of responding to IMiD-based therapy.

Keywords: DNA damage-binding protein 1; cereblon; cullin 4a; thalidomide; lenalidomide; immunomodulatory drugs; multiple myeloma

1. Introduction

Multiple myeloma (MM) is the third most common haematologic malignancy in the European Union, with approximately 33,000 new cases and 20,000 deaths annually [1]. Despite the impressive therapeutic progress that has occurred in recent decades, the development of drug resistance is typical during the clinical course of MM, and most patients eventually relapse and require further therapy [2]. The introduction of thalidomide, a first-in-class immunomodulatory drug (IMiD), in 2006 was one of the milestones in MM therapy history. Thalidomide and its newer derivatives such as lenalidomide and pomalidomide,

along with proteasome inhibitors, are the backbone of most combination regimens used in the treatment of MM. In Poland and other European countries, thalidomide-based regimens are the most common therapy for young and fit newly diagnosed MM patients; lenalidomide and next-generation IMiDs are available for relapsed/refractory groups of patients. IMiDs have been shown to have a pleiotropic anti-cancer effect, including anti-angiogenic, anti-proliferative, anti-inflammatory and immune-modulatory effects [3]. The primary molecular target for IMiDs is cereblon (CRBN), which functions as a substrate receptor in the cullin-4 RING E3 ubiquitin ligase (CRL4–CRBN) complex co-formed by other proteins, such as DNA damage-binding protein 1 (DDB1), cullin 4A (CUL4A), and regulator of cullins-1 (ROC1) [4,5]. By binding CRBN, IMiDs modify the substrate specificity of the CLR4–CRBN complex leading to ubiquitination and degradation of the lymphoid transcription factors Ikaros (IKZF1) and Aiolos (IKZF3), and the casein kinase 1α (CK1α) [6–8]. Therefore, the degradation of IKZF1 and IKZF3 decreases the expression of IRF4 and its downstream target MYC, resulting in growth inhibition of multiple myeloma cells [7,9,10]. In T cells, IKZF1 and IKZF3 are transcriptional repressors of the IL-2 gene [11,12]; their degradation therefore releases repression and causes an increased production of IL-2, leading to T and NK cell activation [13].

Recent studies have established a correlation between CRBN expression levels and clinical response to IMiD treatment, however the results are non-conclusive. High expression of CRBN in patients receiving thalidomide maintenance for 2 years was associated with longer PFS in the HOVON-65/GMMG-HD4 trial, while no association was noted in those on bortezomib maintenance [14]. CRBN high expression has also been shown to enhance lenalidomide therapy's effects in terms of treatment response [15]. Conversely, the loss of CRBN protein and CRBN mRNA level led to lenalidomide resistance in myeloma cells and a poor outcome in MM patients [9,16,17].

In this study, we separately evaluated the expression of CRL4–CRBN complex proteins (CRBN, DDB1, CUL4A) and their downstream targets (IKZF1, IKZF3) with IHC staining, using FFPE bone marrow samples from 130 MM patients treated with IMiDs. In the samples from MM patients, we aimed to compare the expression of CRBN-CRL4 complex proteins and their downstream targets in the malignant plasma cells and assess their potential correlation with the MM patients' clinical course, despite the type of IMiD therapy or stage of the disease.

2. Materials and Methods

2.1. Patients and Bone Marrow Samples

The study retrospectively analysed 130 patients diagnosed with MM from 2010 to 2015 at the Institute of Haematology and Transfusion Medicine, Warsaw, Poland. The following data were obtained about the patients: age, sex, disease stage according to the International Staging System (ISS), type of monoclonal protein and its concentration, free light chain (FLC) type and FLC ratio, haemoglobin, calcium and creatinine concentration, the presence of osteolytic lesions, the percentage of plasma cell infiltration in the bone marrow. All patients received therapy with IMiDs: 81 with thalidomide for newly diagnosed MM (NDMM) and 49 with lenalidomide for relapsed/refractory disease (RRMM). Duplicate records of the same patients were rejected from the analysis. IHC evaluation was performed just before the initiation of treatment with thalidomide or lenalidomide-based regimens. The study was conducted according to the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Institute of Haematology and Transfusion Medicine, Warsaw, Poland.

2.2. Treatment Response

The treatment responses, progression-free survival (PFS) and overall survival (OS), were evaluated according to the International Myeloma Working Group panel consensus (19, 20). The study follow-up was defined as the time from the MM diagnosis to death, of any cause, or to the date of last observation with the cut-off date of 6 August 2018.

2.3. Immunohistochemistry Staining

All trephine samples had an established diagnosis according to the histopathological recommendations (WHO and International Myeloma Working Group) for monoclonal plasma cell proliferative disorders. The trephine biopsies were treated with a combined fixative and decalcifier solution (40% formaldehyde, glacial acetic acid, NaCl, H_2O distilled) and then routinely processed staining with haematoxylin and eosin. Immunohistochemistry was performed using an automated immunohistochemical stainer (Dako Denmark A/S, Glostrup, Denmark), and mono- and polyclonal antibodies were applied, including anti-DDB1 (clone: LS-B3138, 1:500 LSBio, Lifespan Biosciences, Seattle, WA, USA), Aiolos/IZKF3 (clone: NBP2-24495, 1:50, NovusBio, Novus Biologicals, Abingdon, UK), CUL4a (clone: NBP1-44439, 1:300, NovusBio, Novus Biologicals, Abingdon, UK), anti-CRBN (cloneCRBN65, 1:2000, Celgene Corporation, New York, NY, USA) and anti-Ikaros/IZK1 (clone: ab26083, 1:50, Abcam, Cambridge, UK). Plasma cells were visualised by the reaction with the CD138 antibody (clone: MI15, RTU, Dako Omnis, Agilent, Santa Clara, CA, USA). All stainings were performed according to the manufacturer's instructions, and the EnVision Detection System (Dako, Denmark A/S, Glostrup, Denmark) was used for signal detection. A positive staining controls were applied for each antibody: anti-Ikaros/IZKF1–tonsil, anti-DDB1–adrenal gland, anti-Aiolos/IKZF3–tonsil, CUL4a–colon, anti-CRBN–liver. Negative (isotype) control stainings were performed using a ready to use FLEX Negative Mouse Control (a cocktail of mouse IgG1, IgG2a, IgG2b, IgG3 and IgM; code nr IR750; Dako Denmark A/S, Glostrup, Denmark). All neoplastic cells were scored independently by two experienced haematopathologists (M.P-S. and A.S-C.) for each target protein's immunoreactivity based on staining intensity and the percentage of cells staining positively. Based on in-house validation, the cut-offs (>30% for anti-DDB1, Aiolos/IKZF3, anti-CRBN, ≥30% for DDB1 and ≥80% for anti-Ikaros/IKZF1) of strongly and/or intermediate positive neoplastic plasma cells were implemented as a final distinction between the positive and negative results of the staining. Two independent pathologists reviewed the samples, and discrepancies were revised to determine the consensus result. All microphotographs were taken by a microscope DP72 Olympus BX63 camera (Olympus, Tokyo, Japan).

2.4. Statistical Analysis

Categorical variables were compared using the chi-squared test or the Fisher test, depending on the number of observations in each 2-by-2 table. Continuous variables were compared using the t-Student test if they followed normal distribution, or the Wilcoxon test if they did not follow normal distribution. The distribution of the variables was checked by plotting histograms. A survival function with 95% confidence intervals was estimated using the Kaplan–Meier method. To estimate the hazard ratios and 95% confidence intervals, the Cox proportional hazard model was used. For the multivariable models, the forward stepwise variable selection was applied at a 0.15 significance level. All tests were two-sided and were performed at a 0.05 significance level. All analyses were performed using Statistica software, ver. 13.1.

3. Results

3.1. Patients' Characteristics

The patients' clinical characteristics are summarised in Table 1. Among the 130 patients included in the analysis, 81 (62%) were treated with thalidomide in the frontline setting and 49 (38%) received lenalidomide for RRMM. The median age of the patients at the initiation of the IMiD-based treatment was 62.5 years (range, 32–85 years), with female predominance (52 men and 78 women). The isotype of monoclonal proteins was as follows: IgG in 86 patients (66%), IgA in 29 patients (22%), light chains in 14 patients (11%) and IgE protein in 1 patient (1%). The distribution according to the ISS was 18%, 32% and 28%, with a score of 1, 2 and 3, respectively (missing data for 22% of patients). A decreased haemoglobin level (<10 g/dL) was observed in 69 patients (53%), hypercalcemia (>2.55 mmol/L) was

noticed in 21 patients (16%) and renal impairment was noted in 8 patients (6%). Frontline treatment with thalidomide was combined with cyclophosphamide and dexamethasone in the majority of patients (n = 62, 77% of thalidomide-based regimens); lenalidomide was applied in combination with dexamethasone for all RRMM cases. A comparison of the patients' baseline characteristics revealed no significant differences between the IMiD-based treatment groups, except for higher rates of female patients (66% vs. 49%) and higher rates of osteolytic lesions in the thalidomide group (64% vs. 28%), Supplement Table S1. The final analyses were performed with data from all of the patients treated with IMiD-based regimens.

Table 1. Overall clinical patients' characteristics. Data are shown as a number (percentage) or median (interquartile range). Abbreviations: ISS—International Staging System; FLC—free light chain; BM—bone marrow; IMiD—immunomodulatory drug.

Parameter		Overall (n = 130)
Age, years		62.5 (32–85)
Sex	male	52 (40%)
	female	78 (60%)
Isotype of M-protein	IgG	86 (66%)
	IgA	29 (22%)
	IgE	1 (1%)
	FLC	14 (11%)
ISS	stage I	23 (18%)
	stage II	42 (32%)
	stage III	36 (28%)
	no data	29 (22%)
Albumin, g/dL		3.59 (±0.54) n = 128
β2-microglobulin, mg/L		4.43 (1.56–26.15) n = 100
Serum M-protein, g/dL		3.6 (±2.01) n = 125
Serum FLC ratio	<100	43 (33%)
	≥100	43 (33%)
	no data	44 (33%)
Haemoglobin, g/dL	<10	69 (53%)
	≥10	61 (47%)
Calcium, mmol/L	≤2.55	108 (83%)
	>2.55	21 (16%)
	no data	1 (1%)
Creatinine, mg/dL	≤2 mg/dL	122 (94%)
	>2 mg/dL	8 (6%)
Osteolytic lesions	yes	66 (50%)
	no	58 (45%)
	no data	6 (5%)
BM plasma cells, %		67.5 (12.5–95)
IMiD	thalidomide	81 (62%)
	lenalidomide	49 (38%)
Cycles of IMiD-based treatment		6 (1–56)

3.2. CRL4–CRBN Complex Proteins IHC Staining

The IHC staining for all evaluated proteins is shown in Supplement Figures S1 and S2. According to the predefined cut-off values, the positivity (+) of CRBN, CUL4A, DDB1, IKZF1 and IKZF3 was observed in 54%, 51%, 49%, 71% and 54% of cases, respectively. We evaluated the internal associations between the proteins' expression involved in the CRL4–CRBN complex, and positive ones were observed for pairs: CUL4A–IKZF3 (p = 0.023) and

DDB1–IKZF1 ($p = 0.007$). There were no other significant associations for the CRBN–CRL4 complex or its activity-dependent transcriptional factors (IKZ1, IKZF3).

3.3. CRL4–CRBN Complex Proteins' Associations with Clinical Features

The group of patients with CRBN$^{(+)}$ had a significantly higher serum concentration of monoclonal protein (median 4.1 g/dL vs. 2.7 g/dL, $p = 0.006$), β2-microglobulin (median 4.6 mg/L vs. 3.9 mg/L, $p = 0.047$) and a haemoglobin concentration lower than 10 g/dL ($p = 0.030$) before treatment initiation. The DDB1$^{(+)}$ was associated with elevated β2-microglobulin (median 5.04 g/dL vs. 3.94 g/dL, $p = 0.016$), a haemoglobin concentration below 10 g/dL ($p = 0.036$) and a shorter IMiD exposure (median number of cycles 5.7 vs. 6.4; $p = 0.022$). A high expression of proteins involved in B-cell maturation and a switch of immunoglobulin classes—IKZF1 and IKZF3—were associated with the isotype of serum monoclonal protein ($p = 0.040$ and $p = 0.0001$, respectively).

3.4. CRBN and CUL4a as Predictive Markers of IMiD-Based Treatment Response

Among those analysed, all patients' data had a median number of cycles, with IMiD-based treatment, of 6 (range, 1–56). The patients with CRBN$^{(+)}$ had a significantly superior response to treatment than those with CRBN$^{(-)}$ (ORR ≥ PR vs. SD/PD, $p = 0.012$; ORR ≥ VGPR vs. PR/SD/PD, $p = 0.032$, Table 2). The CUL4A$^{(+)}$ was also associated with a better response to treatment than the CUL4A$^{(-)}$ group of patients (ORR ≥ PR vs. SD/PD, $p = 0.007$; ORR ≥ VGPR vs. <VGPR, $p = 0.027$, Table 2). There were no significant differences observed in the expression of DDB1, IKZF1 and IKZF3 in terms of treatment response. After a median follow-up of 4.75 years (range, 0.5–16.9), only CUL4A$^{(+)}$ impacts progression-free survival (HR = 0.66, 95% CI 0.44–0.99; $p = 0.046$), Figure 1A.

Table 2. Quality of IMiD-based treatment response stratified by CRBN and CUL4A expression. Abbreviations: CR—complete response; VGPR—very good partial response; PR—partial response; SD—stable disease; PD—progressive disease; ORR—overall response rate; *p*-Value < 0.5 is bolded.

	CRBN$^{(-)}$ $n = 60$	**CRBN$^{(+)}$ $n = 70$**	***p*-Value**	**CUL4A$^{(-)}$ $n = 64$**	**CUL4A$^{(+)}$ $n = 66$**	***p*-Value**
	n (%)	*n* (%)		*n* (%)	*n* (%)	
CR	4 (7)	7 (10)		1 (1)	10 (15)	
VGPR	8 (13)	19 (27)	$p = 0.105$	12 (19)	15 (23)	**$p = 0.011$**
PR	19 (32)	25 (36)		20 (31)	24 (36)	
SD	20 (33)	12 (17)		19 (30)	13 (20)	
PD	9 (15)	7 (10)		12 (19)	4 (6)	
ORR (≥PR)	31 (52)	51 (73)	**$p = 0.0126$**	33 (52)	49 (74)	**$p = 0.007$**
ORR (<PR)	29 (48)	19 (27)		31 (48)	17 (26)	
ORR (≥VGPR)	12 (20)	26 (37)	**$p = 0.0321$**	13 (20)	25 (38)	**$p = 0.027$**
ORR (<VGPR)	48 (80)	44 (63)		51 (80)	41 (62)	

3.5. DDB1 as a Prognostic Marker of MM Patients' Survival upon IMiD-Based Treatment

The median overall survival rate of the analysed group was 4.25 years (range, 0.5–16.9). The univariate analyses revealed that a poor overall survival rate was associated with DDB1$^{(+)}$ (HR = 3.48, 95% CI 1.75–6.93; $p < 0.001$, Figure 1B), the presence of osteolytic lesions (HR 2.44; 95% CI 1.31–4.53, $p = 0.005$), an older age (HR 1.04, 95% CI 1.01–1.08, $p = 0.008$) and a higher β2-microglobulin concentration (HR 1.10, 95% CI 1.04–1.15, $p < 0.001$) at the initiation of IMiD-based treatment (Figure 2). Multivariate Cox analysis using the forward stepwise elimination method and including all variables assessed in univariate analysis (age, serum M-protein, the isotype of immunoglobulin, bone marrow plasma cells, β2-microglobulin, albumin, creatinine, calcium, haemoglobin, platelets and expression

of CRL4–CRBN complex proteins) confirmed that a poor prognosis was independently predicted by DDB1$^{(+)}$ (HR 3.38; 95% CI 1.65–6.75; $p < 0.001$), presence of osteolytic lesions (HR 2.44; 95% CI 1.19–5.01, $p = 0.015$) and β2-microglobulin concentration (HR 1.06; 95% CI 1.01–1.12, $p = 0.026$), Figure 2. As a result, DDB1 expression was confirmed as the significant prognostic marker in MM patients.

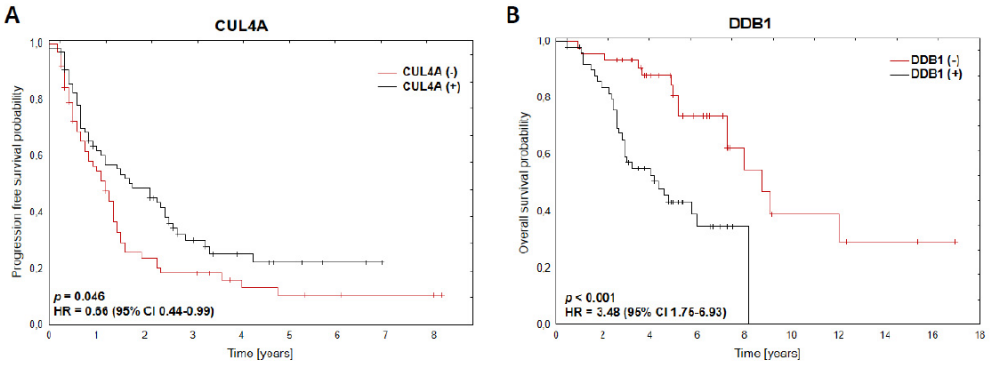


Figure 1. (**A**) Kaplan–Meier survival curves for PFS according to CUL4A expression (classified as positive and negative results of IHC staining), $n = 130$. (**B**) Kaplan–Meier survival curves for OS according to DDB1 expression (classified as positive and negative results of IHC staining), $n = 97$.

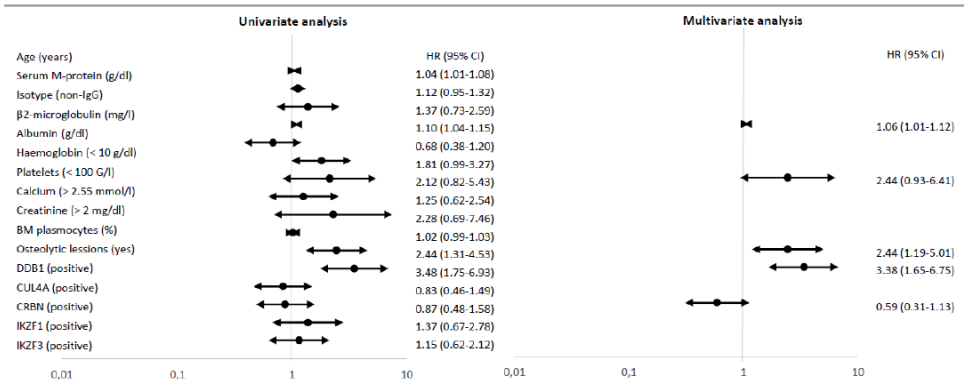


Figure 2. Univariate and multivariate Cox regression analyses for OS ($n = 97$). If the hazard ratio (HR) is greater than 1, then the predictor is associated with an increased risk of death.

4. Discussion

To the best of our knowledge, this is the first study showing that the expression of DDB1 and CUL4A assessed by routine, diagnostic IHC evaluation of bone marrow samples is associated with the outcome of multiple myeloma patients treated with IMiDs. Our analyses also revealed that CRBN expression impacts the superior response to thalidomide or lenalidomide-based treatment in line with previously published data.

It was shown that positive CRBN IHC staining is associated with a superior response rate in patients with newly diagnosed and RRMM treated with thalidomide–dexamethasone (TD) and lenalidomide–dexamethasone (LD) [18]. However, Dimopoulos et al. used CRBN IHC staining in FFPE bone marrow samples of 23 MM patients and found

no correlation between the CRBN protein level and the sensitivity or intrinsic resistance to lenalidomide-based therapy [19]. These conflicting results should be considered in the context of the limited ability of commercially available assays to measure the CRBN protein level in MM reliably. Gandhi et al. have developed a novel CRBN monoclonal antibody CRBN65 and have shown its superiority over other commercially available antibodies in IHC staining [16]. Using the CRBN65 antibody, we found that MM patients with $CRBN^{(+)}$ have a superior response rate to thalidomide- or lenalidomide-based therapy compared to those with $CRBN^{(-)}$ (ORR ≥PR vs. SD/PD, $p = 0.012$; ORR ≥ VGPR vs. PR/PD/SD, $p = 0.032$). In line with our findings, several studies using different approaches to assessing CRBN gene expression, such as real-time PCR [15,20] or gene expression profiling [21], have demonstrated the predictive value of CRBN gene expression in MM patients treated with TD, LD or pomalidomide with dexamethasone. In general, across all of these studies, higher CRBN gene expression was associated with a superior response rate to treatment with IMiD monotherapy [21], or in combination with dexamethasone [15,20,21]. Conversely, low CRBN gene expression determined unresponsiveness to the abovementioned therapy [21]. This recent study also showed that MM patients with hyperdiploid karyotype have a better response rate with IMiD-based therapy and achieve a longer time to next treatment when IMiD-based therapy is applied, compared to their non-hyperdiploid counterparts [22]. It is postulated that it may be associated with the higher expression of CRBN which characterises hyperdiploid-myeloma patients [21].

Here, we show that the CUL4A protein level, a component of the CRL4–CRBN complex, has predictive value in MM patients treated with thalidomide- or lenalidomide-based therapy with higher response rates (ORR ≥ PR vs. SD/PD, $p = 0.007$; ORR ≥ VGPR vs. <VGPR, $p = 0.027$), translated into a favourable PFS (HR = 0.66, 95% CI 0.44–0.99; $p = 0.046$). CUL4A has been shown to play an oncogenic role in various cancer types [23–25]. In MM, CUL4A promotes proliferation, invasion and migration of plasma cells [26]. The positive correlation between the high expression of CUL4A and thalidomide sensitivity was also demonstrated in prostate cancer cell lines [27].

We also show here that DDB1 expression is independently associated with poor OS in both univariate and multivariate analyses (HR 3.38; 95% CI 1.65–6.75; $p < 0.001$). To the best of our knowledge, this is the first report showing the prognostic impact of the DDB1 protein level on the survival of MM patients. DDB1 is essential for DNA repair and plays an important role in many signaling pathways related to carcinogenesis. Moreover, DDB1 overexpression in malignant cells may lead to resistance to anti-cancer therapy [28]. Recently, a high expression of DDB1 was identified as a poor prognostic factor in pancreatic cancer [29]. In our study, $CRBN^{(+)}$ and $DDB1^{(+)}$ were associated with clinical features corresponding to the high burden of MM disease (a higher serum concentration of monoclonal protein or β2-microglobulin, as well as a haemoglobin concentration lower than 10 g/dL). However, there were no significant associations between the expression of those two proteins. The superior response rate in the $CRBN^{(+)}$ group of patients confirms the established mechanism of IMiD action via direct interaction of IMiDs and CRBN. The DDB1 negative impact on the OS and the lack of associations with response rates or PFS may indicate that this component of CRL4–CRBN is more involved in other signaling pathways related to MM cell survival. Therefore, further studies are needed to gain more insight into the DDB1 mechanism of action in MM cells.

The relationship between the expression of CRL4–CRBN downstream targets and IMiD activity remains unclear and inconclusive. Lu et al. [8] found that some MM cell lines with a higher expression of IKZF1 or IKZF3 showed resistance to the lenalidomide. In contrast, Zhu et al. [30] showed that low IKZF1 transcript levels were correlated with a poor response to IMiDs. They also found that higher IKZF1, but not IKZF3, gene expression was associated with better OS. In contrast, Pourabdollah et al. [31] showed that especially IKZF3 expression is correlated with a better outcome in refractory MM patients treated with lenalidomide. In our study, we did not observe any associations of IKZF1/IKZF3 expression in the response rate or MM patients' survival. Therefore, the significant associations

of IKZF1$^{(+)}$ and IZKF3$^{(+)}$ with the isotype of serum monoclonal protein (p = 0.040 and p = 0.0001, respectively) confirm their contribution to switch immunoglobulin classes during B-cell maturation [32,33] and verify the reliability of applied assays.

There is an increasing amount of evidence indicating that IMiD-resistance arises, at least in part, from the acquisition and selection of mutations in genes coding protein downstream or components of the CLR4–CRBN complex, especially *CRBN* and *IKZF1* [34,35]. Consistently, a large-scale genomic and transcriptomic analysis including patients with treatment-naïve-, lenalidomide-refractory and pomalidomide-refractory MM recently showed an increase in the frequency of CRBN aberrations (namely, point mutations, copy number variants, structural variations and exon 10 spliced transcript) with progressive IMiD exposure. Eventually, alterations in CRBN were found in one third of the pomalidomide-resistant patients [36]. It needs to be emphasised here that lenalidomide-refractory patients who harbored CRBN aberrations had a significantly shorter PFS when pomalidomide-based therapy was applied.

However, one should be aware of several limitations of this study. First, there is controversy regarding the most appropriate IHC antibodies, testing method and scoring system for evaluating protein expression using IHC in bone marrow samples. Thus, it would be important to establish replicable methods for the quantitative evaluation ofCRL4–CRBN complex protein expression in FFPE bone marrow samples from MM patients. Second, to provide a sufficient sample size, this study was performed in a retrospective manner, and clinical data were collected from different treatment approaches (both thalidomide- and lenalidomide-based regimens) applied for newly diagnosed or relapsed/refractory MM patients. Third, cytogenetic and molecular data were not available for an analysed group of MM patients. That additional information might produce more powered results in terms of response to IMiD treatment or survival estimations.

In the era of multiple treatment options for patients with MM, having reliable predictive tools is crucial in clinical practice. Our results add some novelty to the understanding of the prognostic and predictive role of some protein components of the CLR4–CRBN complex in MM patients treated with IMiDs. Nevertheless, given the complexity of the molecular mechanisms involved in sensitivity or resistance to IMiD-based therapy, the predictive and prognostic role of the CLR4–CRBN complex cannot be reliably established without a comprehensive analysis and understanding of genetic, transcriptomic and proteomic data obtained from treatment-naïve MM patients, as well as those who have become resistant to thalidomide, lenalidomide and pomalidomide.

In conclusion, this study revealed that the expression of CUL4A and CRBN assessed by routine IHC in FFPE bone marrow samples needs further evaluation as a potential predictive factor for MM patients treated with IMiDs. Moreover, DDB1 expression was found as an independent prognostic factor for the overall survival of patients with MM. Therefore, assessment of the CRL4–CRBN expression in bone marrow samples may improve identifying the MM patients who most benefit from IMiD-based therapies, despite the type of immunomodulatory drug or stage of the disease.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10122683/s1. Table S1. Overall clinical patients' characteristics with detailed division into thalidomide and lenalidomide-based treatment; Figure S1. The immunohistochemical characterization of malignant plasma cells; Figure S2. Positive and negative DDB1 IHC profiles in multiple myeloma

Author Contributions: E.L.-M., P.J. and J.B. designed the research study, J.B., A.S.-C., A.S.-P., M.P.-S., F.G. and A.M. performed the research, J.B., P.W. and I.M.-K. analysed the data, J.B. wrote the paper, A.S.-P., E.L.-M., I.M.-K. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from the National Science Centre in Poland (2014/3/B/NZ5/03160).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Institute of Hematology and Transfusion Medicine.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We would like to thank Celgene Corporation (New York, United States) for sharing with us the CRBN65 antibody for the detection of CRBN in FFPE bone marrow samples.

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

References

1. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2019. *CA Cancer J. Clin.* **2019**, *69*, 7–34. [CrossRef]
2. Kumar, S.K.; Rajkumar, V.; Kyle, R.A.; Van Duin, M.; Sonneveld, P.; Mateos, M.-V.; Gay, F.; Anderson, K.C. Multiple myeloma. *Nat. Rev. Dis. Prim.* **2017**, *3*, 17046. [CrossRef] [PubMed]
3. Raza, S.; Safyan, R.; Suzanne, L. Immunomodulatory Drugs (IMiDs) in Multiple Myeloma. *Curr. Cancer Drug Targets* **2017**, *17*, 846–857. [CrossRef]
4. Ito, T.; Ando, H.; Suzuki, T.; Ogura, T.; Hotta, K.; Imamura, Y.; Yamaguchi, Y.; Handa, H. Identification of a Primary Target of Thalidomide Teratogenicity. *Science* **2010**, *327*, 1345–1350. [CrossRef] [PubMed]
5. Fischer, E.S.; Scrima, A.; Böhm, K.; Matsumoto, S.; Lingaraju, G.M.; Faty, M.; Yasuda, T.; Cavadini, S.; Wakasugi, M.; Hanaoka, F.; et al. The Molecular Basis of CRL4DDB2/CSA Ubiquitin Ligase Architecture, Targeting, and Activation. *Cell* **2011**, *147*, 1024–1039. [CrossRef]
6. Petzold, G.; Fischer, E.S.; Thomä, N.H. Structural basis of lenalidomide-induced CK1α degradation by the CRL4CRBN ubiquitin ligase. *Nat. Cell Biol.* **2016**, *532*, 127–130. [CrossRef] [PubMed]
7. Krönke, J.; Udeshi, N.D.; Narla, A.; Grauman, P.; Hurst, S.N.; McConkey, M.; Svinkina, T.; Heckl, D.; Comer, E.; Li, X.; et al. Lenalidomide Causes Selective Degradation of IKZF1 and IKZF3 in Multiple Myeloma Cells. *Science* **2014**, *343*, 301–305. [CrossRef]
8. Lu, G.; Middleton, R.E.; Sun, H.; Naniong, M.; Ott, C.J.; Mitsiades, C.S.; Wong, K.-K.; Bradner, J.E.; Kaelin, W.G., Jr. The Myeloma Drug Lenalidomide Promotes the Cereblon-Dependent Destruction of Ikaros Proteins. *Science* **2014**, *343*, 305–309. [CrossRef]
9. Zhu, Y.X.; Braggio, E.; Shi, C.-X.; Bruins, L.A.; Schmidt, J.E.; Van Wier, S.; Chang, X.-B.; Bjorklund, C.C.; Fonseca, R.; Bergsagel, P.L.; et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. *Blood* **2011**, *118*, 4771–4779. [CrossRef]
10. Bjorklund, C.C.; Lu, L.; Kang, J.; Hagner, P.; Havens, C.G.; Amatangelo, M.; Wang, M.; Ren, Y.; Couto, S.S.; Breider, M.; et al. Rate of CRL4CRBN substrate Ikaros and Aiolos degradation underlies differential activity of lenalidomide and pomalidomide in multiple myeloma cells by regulation of c-Myc and IRF4. *Blood Cancer J.* **2015**, *5*, e354. [CrossRef]
11. Georgopoulos, K.; Moore, D.D.; Derfler, B. Ikaros, an early lymphoid-specific transcription factor and a putative mediator for T cell commitment. *Science* **1992**, *258*, 808–812. [CrossRef]
12. Gandhi, R.; Kumar, D.; Burns, E.J.; Nadeau, M.; Dake, B.; Laroni, A.; Kozoriz, D.; Weiner, H.L.; Quintana, F.J. Activation of the aryl hydrocarbon receptor induces human type 1 regulatory T cell–like and Foxp3+ regulatory T cells. *Nat. Immunol.* **2010**, *11*, 846–853. [CrossRef]
13. Gandhi, A.K.; Kang, J.; Havens, C.G.; Conklin, T.; Ning, Y.; Wu, L.; Ito, T.; Ando, H.; Waldman, M.F.; Thakurta, A.; et al. Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4 CRBN. *Br. J. Haematol.* **2014**, *164*, 811–821. [CrossRef]
14. Broyl, A.; Kuiper, R.; Van Duin, M.; Van Der Holt, B.; El Jarari, L.; Bertsch, U.; Zweegman, S.; Buijs, A.; Hose, D.; Lokhorst, H.M.; et al. High cereblon expression is associated with better survival in patients with newly diagnosed multiple myeloma treated with thalidomide maintenance. *Blood* **2013**, *121*, 624–627. [CrossRef]
15. Heintel, D.; Rocci, A.; Ludwig, H.; Bolomsky, A.; Caltagirone, S.; Schreder, M.; Geiselhart, S.; Gisslinger, H.; Zojer, N.; Jäger, U.; et al. High expression of cereblon (CRBN) is associated with improved clinical response in patients with multiple myeloma treated with lenalidomide and dexamethasone. *Br. J. Haematol.* **2013**, *161*, 695–700. [CrossRef]
16. Gandhi, A.K.; Mendy, D.; Waldman, M.; Chen, G.; Rychak, E.; Miller, K.; Gaidarova, S.; Ren, Y.; Wang, M.; Breider, M.; et al. Measuring cereblon as a biomarker of response or resistance to lenalidomide and pomalidomide requires use of standardized reagents and understanding of gene complexity. *Br. J. Haematol.* **2013**, *164*, 233–244. [CrossRef] [PubMed]
17. Franssen, L.E.; Nijhof, I.S.; Couto, S.; Levin, M.-D.; Bos, G.M.; Broijl, A.; Klein, S.K.; Ren, Y.; Wang, M.; Koene, H.R.; et al. Cereblon loss and up-regulation of c-Myc are associated with lenalidomide resistance in multiple myeloma patients. *Haematologica* **2018**, *103*, e368–e371. [CrossRef]
18. Huang, S.-Y.; Lin, C.-W.; Lin, H.-H.; Yao, M.; Tang, J.-L.; Wu, S.-J.; Chen, Y.-C.; Lu, H.-Y.; Hou, H.-A.; Chen, C.-Y.; et al. Expression of cereblon protein assessed by immunohistochemicalstaining in myeloma cells is associated with superior response of thalidomide- and lenalidomide-based treatment, but not bortezomib-based treatment, in patients with multiple myeloma. *Ann. Hematol.* **2014**, *93*, 1371–1380. [CrossRef] [PubMed]

19. Dimopoulos, K.; Munch-Petersen, H.F.; Eskelund, C.W.; Sjö, L.D.; Ralfkiaer, E.; Gimsing, P.; Grønbaek, K. Expression of CRBN, IKZF1, and IKZF3 does not predict lenalidomide sensitivity and mutations in the cereblon pathway are infrequent in multiple myeloma. *Leuk. Lymphoma* **2018**, *60*, 180–188. [CrossRef] [PubMed]
20. Bedewy, A.M.L.; El-Maghraby, S.M. Do baseline Cereblon gene expression and IL-6 receptor expression determine the response to thalidomide-dexamethasone treatment in Multiple myeloma patients? *Eur. J. Haematol.* **2014**, *92*, 13–18. [CrossRef] [PubMed]
21. Schuster, S.R.; Kortuem, K.M.; Zhu, Y.X.; Braggio, E.; Shi, C.-X.; Bruins, L.A.; Schmidt, J.E.; Ahmann, G.; Kumar, S.; Rajkumar, S.V.; et al. The clinical significance of cereblon expression in multiple myeloma. *Leuk. Res.* **2014**, *38*, 23–28. [CrossRef]
22. Abdallah, N.; Rajkumar, S.V.; Greipp, P.; Kapoor, P.; Gertz, M.A.; Dispenzieri, A.; Baughn, L.B.; Lacy, M.Q.; Hayman, S.R.; Buadi, F.K.; et al. Cytogenetic abnormalities in multiple myeloma: Association with disease characteristics and treatment response. *Blood Cancer J.* **2020**, *10*, 82. [CrossRef]
23. Li, T.; Wu, S.; Jia, E.A.L.; Cao, W.; Yao, Y.; Zhao, G.; Li, H. CUL4 E3 ligase regulates the proliferation and apoptosis of lung squamous cell carcinoma and small cell lung carcinoma. *Cancer Biol. Med.* **2020**, *17*, 357–370. [CrossRef] [PubMed]
24. Yi, L.-J.; Yi, L.-J.; Ding, N.; Ren, J. CUL4A promotes proliferation and inhibits apoptosis of colon cancer cells via regulating Hippo pathway. *Eur. Rev. Med. Pharmacol. Sci.* **2020**, *24*, 10518–10525. [CrossRef] [PubMed]
25. Wang, X.; Chen, T. CUL4A regulates endometrial cancer cell proliferation, invasion and migration by interacting with CSN6. *Mol. Med. Rep.* **2020**, *23*. [CrossRef] [PubMed]
26. Yang, Y.; Wang, S.; Li, J.; Qi, S.; Zhang, D. CUL4A as a marker and potential therapeutic target in multiple myeloma. *Tumor Biol.* **2017**, *39*, 1–7. [CrossRef]
27. Ren, S.; Xu, C.; Cui, Z.; Yu, Y.; Xu, W.; Wang, F.; Lu, J.; Wei, M.; Lu, X.; Gao, X.; et al. Oncogenic CUL4A determines the response to thalidomide treatment in prostate cancer. *J. Mol. Med.* **2012**, *90*, 1121–1132. [CrossRef]
28. Lee, K.J.; Mann, E.; Wright, G.; Piett, C.G.; Nagel, Z.D.; Gassman, N.R. Exploiting DNA repair defects in triple negative breast cancer to improve cell killing. *Ther. Adv. Med. Oncol.* **2020**, *12*. [CrossRef]
29. Zhang, Y.; Lei, Y.; Xu, J.; Hua, J.; Zhang, B.; Liu, J.; Liang, C.; Meng, Q.; Yu, X.; Shi, S. Role of Damage DNA-Binding Protein 1 in Pancreatic Cancer Progression and Chemoresistance. *Cancers* **2019**, *11*, 1998. [CrossRef]
30. Zhu, Y.X.; Braggio, E.; Shi, C.-X.; Kortuem, K.M.; Bruins, L.A.; Schmidt, J.E.; Chang, X.-B.; Langlais, P.; Luo, M.; Jedlowski, P.; et al. Identification of cereblon-binding proteins and relationship with response and survival after IMiDs in multiple myeloma. *Blood* **2014**, *124*, 536–545. [CrossRef]
31. Pourabdollah, M.; Bahmanyar, M.; Atenafu, E.G.; Reece, D.; Hou, J.; Chang, H. High IKZF1/3 protein expression is a favorable prognostic factor for survival of relapsed/refractory multiple myeloma patients treated with lenalidomide. *J. Hematol. Oncol.* **2016**, *9*, 123. [CrossRef] [PubMed]
32. Corteés, M.; Georgopoulos, K. Aiolos Is Required for the Generation of High Affinity Bone Marrow Plasma Cells Responsible for Long-Term Immunity. *J. Exp. Med.* **2004**, *199*, 209–219. [CrossRef]
33. Sellars, M.; Reina-San-Martin, B.; Kastner, P.; Chan, S. Ikaros controls isotype selection during immunoglobulin class switch recombination. *J. Exp. Med.* **2009**, *206*, 1073–1087. [CrossRef]
34. Barrio, S.; Munawar, U.; Zhu, Y.X.; Giesen, N.; Shi, C.-X.; Da Viá, M.; Sanchez, R.; Bruins, L.; Demler, T.; Müller, N.; et al. IKZF1/3 and CRL4CRBN E3 ubiquitin ligase mutations and resistance to immunomodulatory drugs in multiple myeloma. *Haematologica* **2020**, *105*, E237–E241. [CrossRef] [PubMed]
35. Kortüm, K.M.; Mai, E.K.; Hanafiah, N.H.; Shi, C.-X.; Zhu, Y.-X.; Bruins, L.; Barrio, S.; Jedlowski, P.; Merz, M.; Xu, J.; et al. Targeted sequencing of refractory myeloma reveals a high incidence of mutations in CRBN and Ras pathway genes. *Blood* **2016**, *128*, 1226–1233. [CrossRef] [PubMed]
36. Gooding, S.; Ansari-Pour, N.; Towfic, F.; Estévez, M.O.; Chamberlain, P.P.; Tsai, K.-T.; Flynt, E.; Hirst, M.; Rozelle, D.; Dhiman, P.; et al. Multiple cereblon genetic changes are associated with acquired resistance to lenalidomide or pomalidomide in multiple myeloma. *Blood* **2021**, *137*, 232–237. [CrossRef] [PubMed]

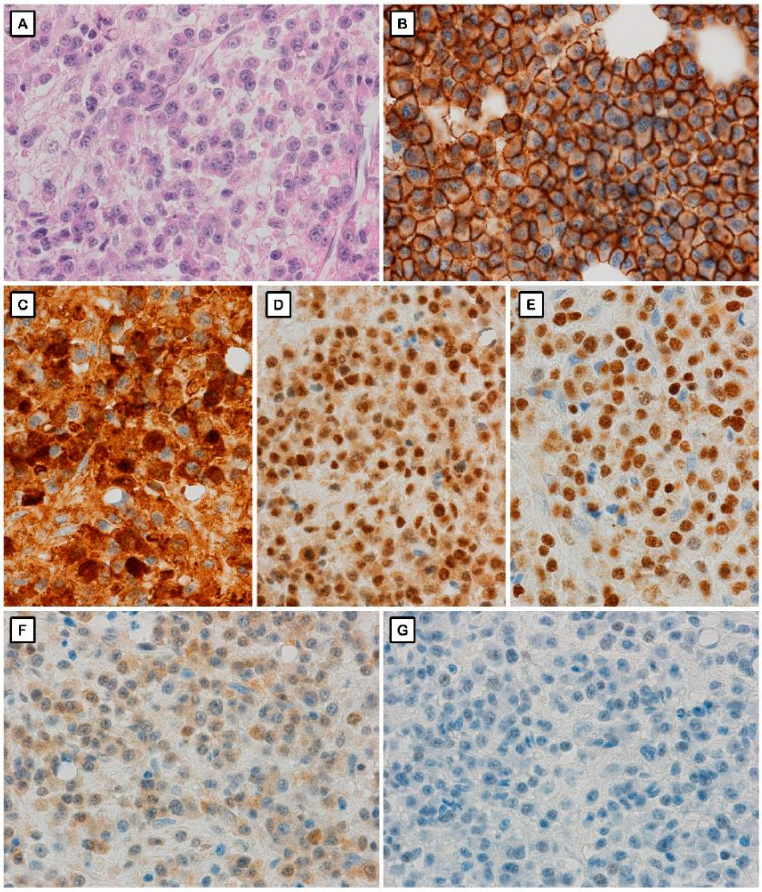
Supplementary materials

Supplementary Table S1. Overall clinical patients' characteristics with detailed division into thalidomide and lenalidomide-based treatment.
Abbreviations: ISS - International Staging System; FLC – free light chain; BM – bone marrow; IMiD – immunomodulatory drug; LEN – lenalidomide; THAL – thalidomide; LD – lenalidomide, dexamethasone; TD – thalidomide, dexamethasone; CTD – cyclophosphamide, thalidomide, dexamethasone; VTD – bortezomib, thalidomide, dexamethasone; MPT – melphalan, prednisone, thalidomide

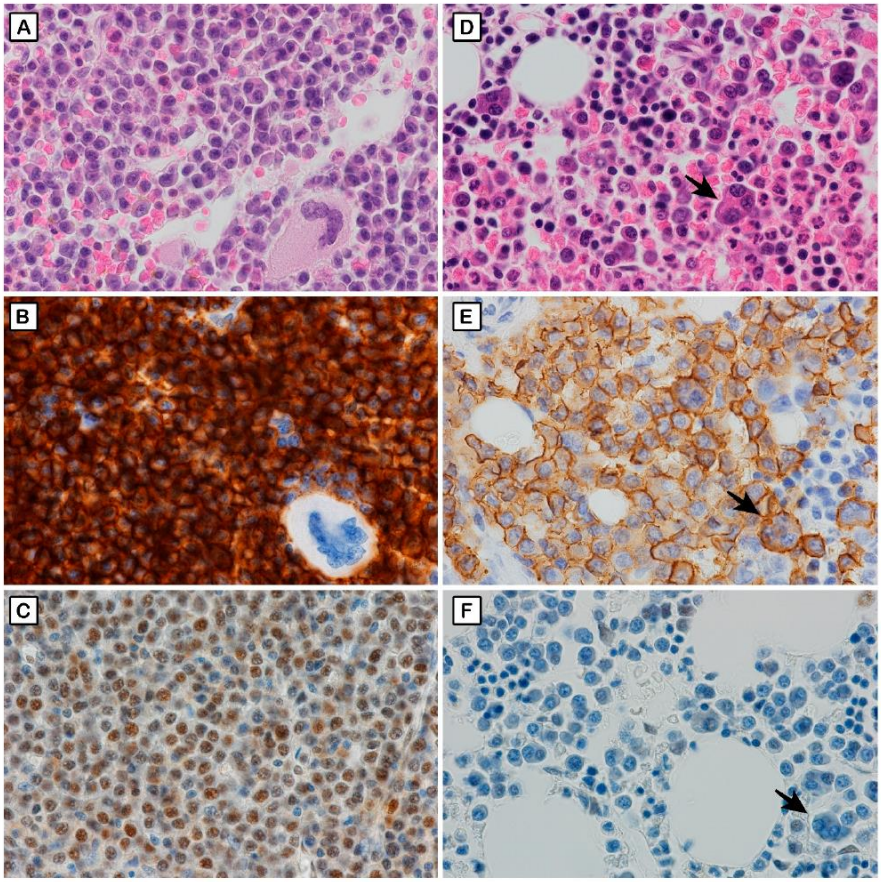
Parameter		LEN-based treatment (n=49)	THAL-based treatment (n=81)	P-value
Age, years		61.4 (32-80)	63 (47-85)	0.226
Sex	male	25 (51%)	27 (34%)	**0.046**
	female	24 (49%)	54 (66%)	
Isotype of M-protein	IgG	33 (68%)	53 (65%)	0.279
	IgA	8 (16%)	21 (26%)	
	IgE	1 (2%)	0	
	FLC	7 (14%)	7 (9%)	
ISS	stage I	12 (24%)	11 (12%)	0.052
	stage II	12 (24%)	30 (37%)	
	stage III	8 (16%)	28 (36%)	
	no data	17 (36%)	12 (15%)	
Albumin, g/dl		3.67 (±0.54)	3.56 (±0.54) n=79	0.363
β2-microglobulin, mg/l		3.7 (1.56, 18.64) n=31	4.6 (1.75, 26.15) n=69	0.073
Serum M-protein, g/dl		3.53 (±1,80) n=45	3.63 (±2,12)	0.723
Serum FLC ratio	<100	14 (29%)	29 (36%)	0.267
	>=100	19 (39%)	24 (30%)	
	no data	16 (32%)	28 (34%)	
Haemoglobin, g/dl	< 10	18 (37%)	43 (53%)	0.071
	≥ 10	31 (63%)	38 (47%)	
Calcium, mmol/l	≤ 2,55	42 (86%)	66 (82%)	0.631
	> 2,55	7 (14%)	14 (17%)	
	no data	0	1 (1%)	
Creatinine, mg/dl	≤ 2 mg/dl	47 (96%)	75 (93%)	0.444
	> 2 mg/dl	2 (4%)	6 (7%)	
Osteolytic lessions	yes	14 (28%)	52 (64%)	**0.001**
	no	29 (59%)	29 (36%)	
	no data	6 (13%)	0	
BM plasma cells, %		65 (17.5-95) n=48	70 (12.5-95) n=80	
Cycles of IMiD-based treatment (median, range)	6 (1-56)	9.5 (3-56)	6 (1-24)	

Prior lines of treatment	n/a	4 (1-8)	n/a	
Treatment regimen	LD	49 (100%)		
	TD		2 (2%)	
	CTD		62 (77%)	
	VTD		2 (2%)	
	MPT		15 (19%)	

Supplementary Figure S1. The immunohistochemical characterization of malignant plasma cells (#5 case): A – HE; B – CD138; C – positive IZKF3; D – positive CUL4A; E – positive IKZF1; F – positive CRBN; G – negative DDB1; (all photographs in 600x magnification).



Supplementary Figure S2. Positive and negative DDB1 IHC profiles in multiple myeloma; case #1: A – HE; B – CD138; C – DDB1 positive (>30% of strongly positive cells); case #2: D - HE; E – CD138; F – DDB1 negative (≤30% of strongly positive cells); (arrow – atypical plasma cell with triple nuclei; all photographs in 600x magnification).



cancers

MDPI

Review

CRL4^{CRBN} E3 Ligase Complex as a Therapeutic Target in Multiple Myeloma

Joanna Barankiewicz [1,2,*], Aleksander Salomon-Perzyński [1], Irena Misiewicz-Krzemińska [3] and Ewa Lech-Marańda [1,2]

1 Department of Hematology, Institute of Hematology and Transfusion Medicine, 02-776 Warsaw, Poland
2 Department of Hematology and Transfusion Medicine, Center of Postgraduate Medical Education, 02-776 Warsaw, Poland
3 Department of Experimental Hematology, Institute of Hematology and Transfusion Medicine, 02-776 Warsaw, Poland
* Correspondence: jbarankiewicz@ihit.waw.pl

Simple Summary: Immunomodulatory drugs (IMiDs) are effective in the treatment of multiple myeloma (MM) and other hematological malignancies. Cereblon (CRBN), a target of IMiDs, forms the CRL4 E3 ubiquitin ligase complex (CRL4^{CRBN}) with DDB1, CUL4A and RBX1. The insight into the molecular mechanism of IMiDs action has advanced dramatically since the identification of cereblon (CRBN) as their direct target. Targeting CRBN by IMiDs modifies CRL4^{CRBN} substrate specificity towards non-physiological protein targets which are subsequently ubiquitinated and degraded by the proteasome. To date, IMiDs are the only known group of protein degraders used in clinical practice. This review provides the current state of knowledge about thalidomide and its derivatives' mechanisms of action, and highlights the future perspectives for targeted protein degraders.

Abstract: Multiple myeloma (MM) is the second most common hematological malignancy with a recurrent clinical course. The introduction of immunomodulatory drugs (IMiDs) was one of the milestones in MM therapy leading to a significant improvement in patients' prognosis. Currently, IMiDs are the backbone of MM therapy in newly diagnosed and relapsed/refractory settings. It is now known that IMiDs exert their anti-myeloma activity mainly by binding cereblon (CRBN), the substrate receptor protein of the CRL4 E3 ubiquitin ligase (CRL4^{CRBN}) complex. By binding CRBN, IMiDs alter its substrate specificity, leading to ubiquitination and proteasomal degradation of proteins essential for MM cell survival. Following the success of IMiDs, it is not surprising that the possibility of using the CRL4^{CRBN} complex's activity to treat MM is being further explored. In this review, we summarize the current state of knowledge about novel players in the MM therapeutic landscape, namely the CRBN E3 ligase modulators (CELMoDs), the next generation of IMiDs with broader biological activity. In addition, we discuss a new strategy of tailored proteolysis called proteolysis targeting chimeras (PROTACs) using the CRL4^{CRBN} to degrade typically undruggable proteins, which may have relevance for the treatment of MM and other malignancies in the future.

Keywords: multiple myeloma; cereblon; immunomodulatory drugs; cereblon E3 ligase modulators; proteolysis targeting chimeras

check for updates

Citation: Barankiewicz, J.; Salomon-Perzyński, A.; Misiewicz-Krzemińska, I.; Lech-Marańda, E. CRL4^{CRBN} E3 Ligase Complex as a Therapeutic Target in Multiple Myeloma. *Cancers* **2022**, *14*, 4492. https://doi.org/10.3390/cancers14184492

Academic Editors: Sabina Ševčíková and Jiří Minařík

Received: 30 August 2022
Accepted: 14 September 2022
Published: 16 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

The ubiquitin-proteasome pathway plays an essential role in the proteins' degradation. This process is mediated by a cascade of enzymatic reactions engaging a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3), which are recycled and activated by ATP during the whole ubiquitination pathway [1]. The role of E3 is the determination of the substrate specificity for ubiquitination and subsequent degradation in the proteasome. The human genome encodes more than 600 E3 ubiquitin

ligases, and the cullin-RING ubiquitin ligases (CRLs) represent the largest E3 ligase family, which take part in numerous cellular homeostatic processes, e.g., signal transduction, cell cycle regulation, DNA damage response, regulation of transcription and embryonic development [2,3]. CRL4 E3 ubiquitin ligase is a complex of RING finger domain protein (Roc1, also named RBX1), cullin4 (CUL4) scaffold protein, and DDB1–CUL4 associated proteins, which determine the substrate specificity for the CRL4 E3 activity. Cereblon (CRBN) is one of the CRL4 E3 substrate receptors, and this protein is crucial for the action of small molecules, such as immunomodulatory drugs (IMiDs). Targeting CRBN by IMiDs modifies its substrate specificity towards non-physiological proteins which are subsequently ubiquitinated and degraded by the proteasome [4–10], Figure 1A. This mechanism of action has shown particular relevance in the treatment of multiple myeloma (MM), the second most common hematological malignancy with a recurrent clinical course leading to 20,000 deaths per year in the European Union [11]. The introduction of IMiDs-based treatment has been a game changer for patients with MM, significantly improving their prognosis.

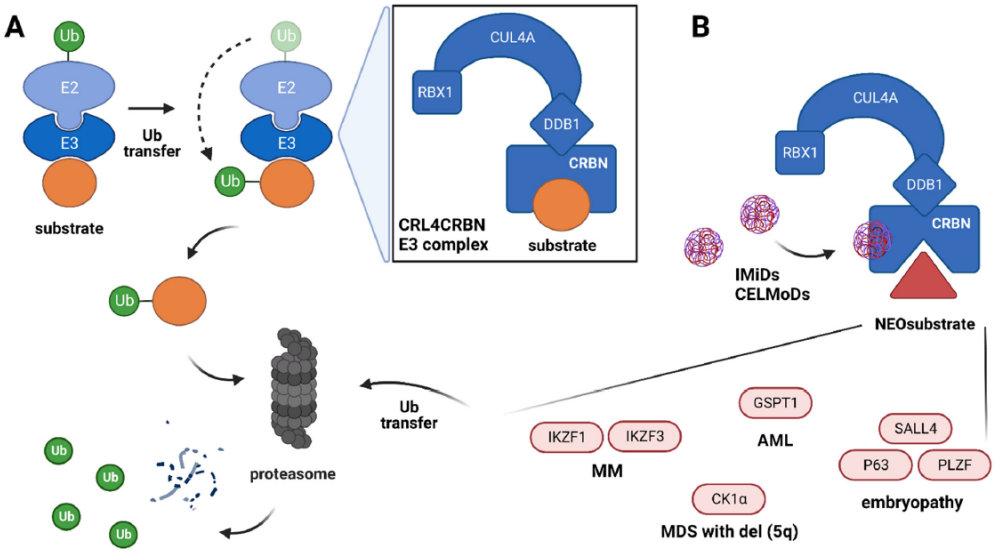


Figure 1. (**A**) Overview of the ubiquitination process via $CRL4^{CRBN}$ E3 ligase complex. The E3 ligase recognizes the E2-Ub complex and target substrate with subsequent transfer of Ub from E2 to the substrate. This process results in Ub-substrate transfer to the proteasome and proteolytic degradation with Ub recycle. The $CRL4^{CRBN}$ E3 ligase complex (enlarged) is formed by cereblon (CRBN)—a substrate recruiter, and other proteins such as DNA damage binding protein 1 (DDB1), cullin 4A (CUL4A), and regulator of cullins-1 (RBX1). (**B**) Mechanism of CRBN-mediated effects upon exposure to thalidomide and its derivatives. Binding IMiDs/CELMoDs to the CRBN leads to the recognition of different substrates (neosubstrates) for ubiquitination and successive protein degradation.

Although acting on the E3 ligase-related function of CRBN appears to be the main mechanism for the anti-myeloma activity of IMiDs [12,13], recent reports indicate that IMiDs also act by modulating other properties of CRBN, such as chaperone function [14,15]. Therefore, to emphasize the broader biological activity, the next generation of IMiD is called 'CELMoDs' (Cereblon E3 ligase modulators). In recent years, the $CRL4^{CRBN}$ complex, along with other E3 ligases, is widely explored as the target of degradation typically "undruggable" proteins by heterobifunctional small molecules, known as proteolysis targeting chimeras (PROTACs).

Here, we review the ways of modulating $CRL4^{CRBN}$ E3 ligase activity in a CRBN-dependent manner in established and upcoming therapeutic approaches in multiple myeloma.

2. Immunomodulatory Drugs (IMiDs)

2.1. Mechanism of IMiDs' Action

The introduction of thalidomide in 2006, a first-in-class IMiD, was one of the milestones in MM therapy. Together with its new generation derivatives, such as lenalidomide and pomalidomide, along with proteasome inhibitors and monoclonal antibodies, these drugs are placed as a standard of care for MM patients at all disease stages. Before the clarification of the molecular mechanism of action, thalidomide and its analogues were characterized by modulation of T cells, NK and NK-T cells functions by inducing the production of cytokines, including IL-2 (interleukin-2) and interferon-γ [16–18]. Thus, thalidomide and its analogs are called immunomodulatory drugs in addition to their anti-angiogenic activity, disruption of the myeloma cell-bone marrow stromal interaction, and downregulation of osteoclastogenesis [19,20].

The game-changer in the exploration of IMiDs molecular mechanism of action was information that thalidomide interacts with CRBN, and this interplay led to the teratogenic side effects and limb malformations of newborns [21]. Then, it was shown that CRBN expression was required for the anti-myeloma activity of IMiDs as CRBN knockdown leads to resistance to lenalidomide and pomalidomide in MM cell lines [12]. Our group and others showed that CRBN expression is associated with a response to thalidomide and lenalidomide-based treatment in MM patients [22–25]. Recent molecular studies with lenalidomide- and pomalidomide-resistant MM patients revealed some CRBN molecular alterations (e.g., point mutation, structural variation, copy loss, or exon 10 spliced transcript of CRBN) associated with IMiDs' exposure [26]. Nevertheless, the low frequency and clonal fraction of identified CRBN mutations cannot be responsible for IMIDs resistance in the majority of patients [27,28]. As IMiDs resistance is one of the main challenges in MM treatment, its mechanism of resistance needs to be explored in future studies.

The subsequent key findings in IMIDs mechanism of action were presented in 2014. Two papers demonstrated that lenalidomide's interaction with CRBN changes its substrate specificity to induce the proteasomal-dependent degradation of transcriptional factors IZKF1 and IKZF3 (named also Ikaros and Aiolos, respectively) [5,6]. IKZF1 and IKZF3 were defined as CRBN 'neosubstrates' because they only become CRBN targets in the presence of IMiDs. Degradation of IKZF1/3 regulates the expression of other genes, such as *IRF4* and *MYC*, and is essential for the proliferation and survival of MM cells [29,30]. Disruption of the IKZF1/3-IRF4-MYC transcriptional axis is of special importance in MM cells survival [31], in contrast to studies with primary effusion lymphoma cell lines, where IMiDs triggered downregulation of IRF4 expression independently of both IKZF1 and IKZF3 [32]. The investigation of the lenalidomide mechanism of action in other hematological malignancies, such as myelodysplastic syndrome with del5q, identified the next neosubstrate of CRBN; a casein kinase 1 α (CK1α; encoded on chromosome 5q by CSNK1A1) [9]. The deletion of the 5q region leads to reduced baseline expression of CK1α and sensitizes MDS cells to lenalidomide, which causes a unique opportunity to exert its apoptotic effect. In MM cells, inactivation of CK1α induces cell cycle arrest and overcomes the bone marrow stromal protection, indicating that lenalidomide-dependent degradation of CK1α may complement its anti-myeloma activity [33–35]. Moreover, the

group of thalidomide neosubstrates includes also PLZF, SALL4 and P63 proteins, which were identified as its teratogenicity mediators [36–39]. Recent screening studies conducted by mass-spectrometry and high-throughput sequencing of engineered cell lines revealed the multiple potential IMiDs neosubstrates [40,41], which need to be validated under physiological conditions and translated to the clinical effects of IMiDs. The established neosubstrates for $CRL4^{CRBN}$ E3 ligase under IMiDs impact are shown in Figure 1B.

The differences in neosubstrates repertoire degraded under IMiDs activity may reflect the various adverse events observed during MM therapy. The most common side effect of thalidomide is chronic axonal neuropathy [42], in contrast to other IMIDs characterized by myelosuppression as the most frequent toxicity. Myelosuppressive effect of lenalidomide and pomalidomide refers to the IKZF1 degradation and subsequent downregulation of the transcription factor PU.1 [43] and GATA1 [42], resulting in neutropenia and thrombocytopenia, respectively. As thalidomide is a much less potent IKZF1 degrader relative to lenalidomide and pomalidomide, it may not induce this toxicity as strongly as its newer derivatives.

IMiDs can modulate the $CRL4^{CRBN}$ E3 ligase activity toward the degradation of various proteins with different affinity to the specific neosubstrates. The unique patterns of substrate specificity may translate the diversity in clinical efficacy and toxicity profile of these medicines.

2.2. *Clinical Efficacy of IMiDs*

Although the role of thalidomide in MM treatment has been steadily declining since the introduction of lenalidomide, in many countries where access to lenalidomide is limited, the combination of thalidomide, dexamethasone and bortezomib (VTD) is still the key approach in patients with newly diagnosed MM who are eligible for high-dose chemotherapy followed by autologous stem-cell transplantation (auto-HSCT). Recently, VTD induction prior to auto-HSCT has been shown to achieve an objective response (at least partial response [PR]) in almost 95% of patients, confirming previously reported results [44]. As shown in the recent phase 3 CASSIOPEIA trial, the clinical benefits of VTD in terms of depth of response, rate of measurable residual disease (MRD) negativity and progression-free survival (PFS) can be further enhanced by the addition of daratumumab, a first-in-class monoclonal antibody targeting CD38, i.e., an antigen commonly expressed on the surface of MM cells [45].

The high efficacy and favorable toxicity profile of lenalidomide have made this drug the cornerstone of most regimens currently used in MM therapy, both as initial treatment and in relapsed/refractory settings. The phase 3 PETHEMA/GEM2012 trial of 458 MM patients eligible for auto-HSCT showed significant activity of lenalidomide, dexamethasone and bortezomib (VRD) combination in pre-transplant induction (6 cycles) and post-transplant consolidation (additional 2 cycles) with high rates of both deep responses (≥very good partial response [VGPR], 75%; complete response [CR], 50%) and MRD negativity (45%) assessed after consolidation [46]. The phase 2 randomized GRIFFIN study recently showed that the addition of daratumumab to VRD induction (D-VRD) (given for 4 cycles) and post-transplant consolidation (given for additional 2 cycles) significantly improved depth of response (≥VGPR, 91% vs. 73%; ≥CR, 52% vs. 42%; MRD negativity rate, 51% vs. 20%) compared to VRD alone [47]. The efficacy and safety of D-VRD as a frontline treatment for transplant-eligible MM patients will be further evaluated in the phase 3 PERSEUS trial (NCT03710603).

The superiority of VRD over RD alone was demonstrated in the phase 3 SWOG S0777 trial in treatment-naive MM patients not intended for immediate auto-HSCT. Longer progression-free survival (PFS) (median, 43 vs. 30 months) and overall survival (OS) (median, 75 vs. 64 months) were observed in VRD compared to the RD arm [48]. The ENDURANCE trial showed that in a group of patients with no intention for immediate auto-HSCT, treatment with a combination of the second-generation proteasome inhibitor carfilzomib with RD (KRD) did not provide clinical benefit in terms of PFS over VRD [49].

More recently, the MAIA study including patients ineligible for auto-HSCT due to age or comorbidities showed that adding daratumumab to RD (DRD) led to a 47% and 32% reduction in the risk of progression and death, respectively, compared to RD alone [50]. Given these results, both VRD and DRD have been established as the preferred therapeutic options for patients with newly diagnosed MM who are not eligible for auto-HSCT [51].

The treatment of relapsed/refractory MM is a major challenge in clinical practice. For patients who have not previously been exposed to lenalidomide, RD alone (especially in frail patients) [52], or combined with carfilzomib (the ASPIRE trial) [53,54] ixazomib (the TOURMALINE trial) [55], daratumumab (the POLLUX trial) [56] or elotuzumab (the ELOQUENT-2 trial) [57,58] are highly relevant therapeutic options. In turn, for lenalidomide-refractory patients, in addition to IMID-free regimens (e.g., DKD [the CANDOR trial] [59], DVD [the CASTOR trial] [60] and KD alone [the ENDEAVOR trial] [61], pomalidomide-based approaches are of great clinical value. Depending on previous therapies, performance status and comorbidities, patients with relapsed/refractory MM may benefit from pomalidomide-dexamethasone given alone or in combination with anti-CD38 antibodies (i.e., daratumumab (the APOLLO trial) [62] or isatuximab [the ICARIA-MM trial] [63], elotuzumab (the ELOQUENT-3 trial) [64], proteasome inhibitors (i.e., bortezomib [the OPTIMISMM trial] [65], carfilzomib [66] and ixazomib [67]) along with cytotoxic agents (e.g., cyclophosphamide [68]). The results of the randomized clinical trials with IMiDs are summarized in Table 1.

Lenalidomide is also placed as the standard of care in maintenance therapy of MM after auto-HSCT or in nontransplant settings for newly diagnosed patients. In four phase 3 randomized trials, prolonged PFS was observed with hazard ratios (HRs) ranging from 0.47 to 0.57 in favor of the lenalidomide arm vs observation/placebo post auto-HSCT [69–72]. Moreover, three clinical trials' meta-analysis documented longer overall survival (OS) of patients with lenalidomide maintenance [73]. On the other hand, one is aware of the risk of secondary malignancies during long-term exposure to lenalidomide [74], especially the several recent reports that emerged about acute B-cell leukemia with diverse clinical courses and treatment outcomes [75–78].

There is room for pomalidomide and new cereblon E3 ligase modulators (CELMoDs, described below) in the maintenance therapy of MM because of their higher efficacy and more favorable toxicity profile, which is of special interest during long-term therapy.

Table 1. Summarize the results of randomized clinical trials with IMiDs.

Trial	Phase	Regimen	Outcome
Newly-Diagnosed MM with Transplant Intent			
CASSIOPEIA [45]	3	Dara-VTD VTD	mPFS: NR vs. NR (HR = 0.47; $p < 0.0001$) MRD (-): 64% vs. 44% ($p < 0.0001$)
PETHEMA/GEM2012 [46]	3	VRD	mPFS: NR; MRD (-): 29% (post induction), 42% (post auto-HSCT) and 45% (post consolidation)
GRIFFIN [47]	3	Dara-VRD VRD	2y-PFS: 96% vs. 90% MRD (-): 51% vs. 20% ($p < 0.0001$)
Newly-Diagnosed MM with Non-Transplant Intent			
SWOG S0777 [48]	3	VRD RD	mPFS: 43 vs. 30 mo (HR = 0.71; $p = 0.0018$) mOS: 75 vs. 64 mo (HR = 0.71; $p = 0.025$)
ENDURANCE [49]	3	KRD VRD	mPFS: 34.6 vs. 34.4 months ($p = 0.74$)
MAIA [50]	3	Dara-RD VRD	mPFS: NR vs. 34.4 mo (HR = 0.53; $p < 0.0001$)

Table 1. *Cont.*

Trial	Phase	Regimen	Outcome
Relapsed/Refractory MM			
Dimopoulos et al. [52]	3	RD placebo-D	mTTP, 11.3 vs. 4.7 months ($p < 0.001$)
ASPIRE [54]	3	KRD RD	mPFS: 26 vs. 18 mo (HR 0.69; $p = 0.0001$) mOS: 48 vs. 40 mo (HR = 0.79; $p = 0.0045$)
TOURMALINE [55]	3	IRD placebo-RD	mPFS: 20.6 vs. 14.7 mo (HR = 0.74; $p = 0.01$)
POLLUX [56]	3	Dara-RD RD	mPFS: 44.5 vs. 17.5 mo (HR = 0.44; $p < 0.0001$)
ELOQUENT-2 [57,58]	3	Elo-RD RD	mPFS: 19.4 vs. 14.9 mo (HR = 0.70; $p < 0.001$) mOS: 48.3 vs. 39.6 mo (HR = 0.82; $p = 0.04$)
CANDOR [59]	3	Dara-KD KD	mPFS: 28.6 vs. 15.2 mo (HR = 0.59; p <0.0001)
CASTOR [60]	3	Dara-VD VD	mPFS: 16.7 vs. 7.1 mo (HR = 0.31; $p < 0.0001$)
ENDEAVOR [61]	3	KD VD	mPFS: 18.7 vs. 9.4 mo (HR = 0.53; $p < 0.0001$) mOS, 47.6 vs. 40 mo (HR = 0.79; $p = 0.01$)
APOLLO [62]	3	Dara-PD PD	mPFS: 12.4 vs. 6.9 mo (HR = 0.63; $p = 0.0018$)
ICARIA-MM [63]	3	Isa-PD PD	mPFS: 11.5 vs. 6.5 mo (HR = 0.596; $p = 0.001$) mOS: 24.6 vs. 17.7 mo (HR = 0.76; $p = 0.028$)
ELOQUENT-3 [64]	2	Elo-PD PD	mPFS: 10.3 vs. 4.7 mo (HR = 0.54; $p = 0.008$) mOS: 29.8 vs. 17.4 mo (HR = 0.59; $p = 0.0217$)
OPTIMISMM [65]	3	PVD VD	mPFS: 11.2 vs. 7.1 mo (HR = 0.61; $p < 0.0001$)

Abbreviations: D: dexamethasone; Dara: daratumumab; Elo: elotuzumab; HR: hazard ratio; IRD: ixazomib, lenalidomide and dexamethasone; Isa: isatuximab; KD: carfilzomib and dexamethasone; KPD: carfilzomib, pomalidomide and dexamethasone; KRD: carfilzomib, lenalidomide and dexamethasone; MM: multiple myeloma; MRD: measurable residual disease; mo: months; mOS: median OS; mPFS: median PFS; NR: not reached; ORR: objective response rate; OS: overall survival; PD: pomalidomide and dexamethasone, PFS: progression-free survival; PVD: bortezomib, pomalidomide and dexamethasone; RD: lenalidomide and dexamethasone; TTP: time to progression; VD: bortezomib and dexamethasone; VRD: bortezomib, lenalidomide and dexamethasone; VTD: bortezomib, thalidomide and dexamethasone; y, years.

3. Cereblon E3 Ligase Modulators (CELMoDs)

3.1. Mechanism of CELMoDs' Action

Even though the enigma of IMiDs' different ways of action is still not fully deciphered, we have to make room for the novel, intentionally designed, class of $CRL4^{CRBN}$ players, referred to as CRBN E3 ligase modulation drugs (CELMoDs). This group of "next-generation" IMiDs is represented by CC-92480 (mezigdomide), CC-220 (iberdomide), CC-122 (avadomide) and CC-885. Chemically, CELMoDs share with IMiDs the conserved glutarimide rings for interaction with CRBN. The second, extended region of their structures (corresponding to the phthalimide ring in thalidomide) varies between each CELMoD and determines the interaction with CRBN and new $CRL4^{CRBN}$ E3 substrates, as shown in Figure 2A,B.

One of the key features that differentiate CELMoDs from IMiDs is the enhanced affinity to the CRBN. The raw data varies between the published results depending on the used assays. Still, most publications document the approximately 10–20-fold higher CRBN-affinity of CELMoDs compared to lenalidomide or pomalidomide [79,80]. Consistent with increased affinity, the greater CELMoDs' potency in degradation of IKZF1 and IKZF3 is observed compared to classical IMiDs [81], Figure 2C.

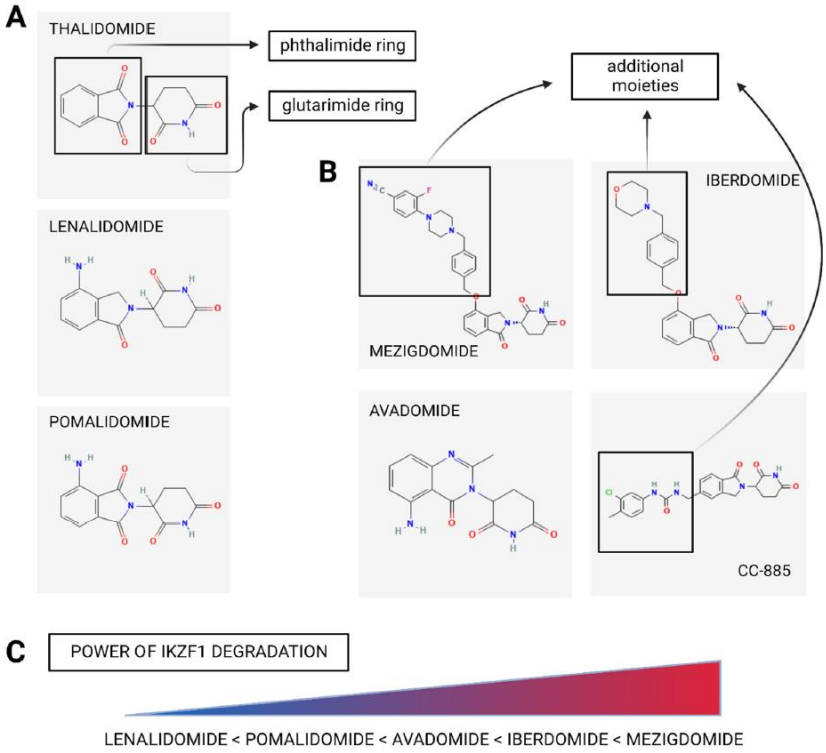


Figure 2. IMIDs/CELMoDs differences in chemical structure and power of IKZF1 degradation. Chemically, IMiDs (**A**) and CELMoDs (**B**) share glutarimide ring for binding to the tri-tryptophan pocket of CRBN, but the second structural region varies between each drug and determines the interaction with CRBN and neoubstrates. (**C**) The comparison of the IMiDs and CELMoDs potency in IKZF1 degradation.

In contrast to known IMIDs' neosubstrates, CC-885 was found to exert the antitumor activity by CRBN-dependent ubiquitination and degradation of the translational terminal factor, GSPT1. Degradation of GSPT1 is detrimental in acute myeloid leukemia (AML) cell lines and patient-derived AML samples [82]. Furthermore, a recent analysis based on mass-spectroscopy proteomics also identified dose- and time-dependent degradation of BNIP3L in $CRBN^{+/+}$, but not $CRBN^{-/-}$ cells exposed to CC-885 compound. That data uncover a novel role of CC-885 in regulating degradation of mitochondria (mitophagy) by targeting BNIP3L for $CRL4^{CRBN}$ E3 ligase-dependent ubiquitination [83]. In MM cell lines, CC-885 selectively induced the ubiquitination and degradation of CDK4 in a CRBN-dependent manner, suggesting that CDK4 destruction contributed to its cytotoxicity in MM pre-clinical model [84].

The CC-220 has a higher than IMiDs affinity to CRBN and potency for IKZF1/3 degradation, but does not degrade CK1a or GSPT1. It is worth mentioning that iberdomide and other CELMoDs (CC-122, CC-92480) have the activity in lenalidomide- or pomalidomide-resistant cell lines with decreased CRBN expression [85,86].

Unlike other IMiDs or CELMoDs, recent basic studies with avadomide revealed the CRBN-dependent degradation of ZMYM2 (ZNF198), a transcriptional factor involved in

rearrangements with FGFR1 and FLT3. This makes CC-122 a potential drug for patients with aggressive hematological malignances harboring translocations resulting in fusion oncoproteins ZMYM2–FGFR1 and ZMYM2–FLT3 [87].

3.2. Clinical Efficacy of CELMoDs

3.2.1. CC-92480 (Mezigdomide)

Recently, the preliminary results of the phase 1/2 CC-92480-MM-002 study have been reported. A total of 19 patients with relapsed/refractory MM after a median of 3 (range, 2–4) lines of prior therapy had received a combination of CC-92480 (mezigdomide), bortezomib and dexamethasone [88]. All patients were previously exposed to lenalidomide and half of them received pomalidomide in addition. The mezigdomide-bortezomib-dexamethasone combination has shown promising clinical activity with an objective response (≥PR) achieved in almost 75% of cases and a median duration of response of 10 months. The toxicity profile was predictable and acceptable, with cytopenias being the most commonly reported grade 3 or 4 treatment-emergent adverse event. In this study, evaluation of other mezigdomide-dexamethasone combinations containing a next-generation PI (carfilzomib or ixazomib) or anti-CD38 antibody (daratumumab or isatuximab) or anti-SLAMF7 antibody (elotuzumab) is planned (NCT03989414). Another phase 1 study (NCT03374085) has recently demonstrated the mezigdomide-dexamethasone doublet to be an effective approach in a group of 66 heavily pre-treated (a median of 6 previous therapies) patients with prior exposure to lenalidomide (89%), pomalidomide (83%) and anti-CD38 antibodies (78%) [89]. The objective response rate at the therapeutic dose was almost 50%, and responses were achieved independently of resistance to IMIDs. The most common adverse events were myelosuppression. The study is ongoing, and further findings are highly anticipated.

3.2.2. Iberdomide (CC-220)

Triplet combinations with iberdomide (CC-220) have shown a favorable safety profile and promising clinical activity in heavily pretreated MM patients, according to the preliminary results of the phase 1/2 CC-220-MM-001 study (NCT02773030) [90]. The iberdomid-daratumumab-dexamethasone (IberDd) cohort included 63% and 58% of daratumumab-resistant and quadruple-refractory (defined as refractory to ≥1 IMIDs, 1 PI, 1 anti-CD38 monoclonal antibodies and 1 steroid) patients, respectively. Similarly, a high representation of refractory patients (PI-refractory, 76%; quadruple-refractory, 48%) was included in the iberdomide-bortezomib-dexamethasone (IberVD) cohort. Nevertheless, the objective response rate was 35% in the IberDd and 50% in the IberVD cohort. Importantly, responses to IberDd and IberVd were achieved irrespective of daratumumab- and bortezomibe-refractoriness. It is worth highlighting that a significant proportion of patients derived clinical benefit from iberdomide-based therapy due to achieving a minimal response or stable disease. The clinical benefit rate and the disease control rate were 47% and 88% (for the IberDd cohort) and 65% and 85% (for IberVD cohort), respectively [90]. Cytopenias were the most common complication of the combination therapy. The IberDd combination for the treatment of relapsed/refractory MM is planned to be compared with DRd in the phase 3 EXCALIBER-RRMM trial (NCT04975997). Additionally, IberVD as a frontline approach for MM patients ineligible for HDT-auto-HSCT will be evaluated in the phase 2 BOREALIS trial (NCT05272826).

In the phase 1/2 CC-220-MM-001 study (NCT02773030), iberdomide in combination with dexamethasone was evaluated [91]. Almost all of the 107 enrolled patients were triple refractory (refractory to IMID, PI and anti-CD38 monoclonal antibody), 25% had an extramedullary disease, and 30% had high-risk cytogenetics. Treatment with iberdomide and dexamethasone led to a response in 26% of patients. Median PFS and OS were 3 and 11 months, respectively. Interestingly, patients who had previously received anti-BCMA therapy had similar response rates (ORR of 25%). There were no new concerns about the toxicity of the combination therapy. The efficacy and safety of iberdomid-dexamethasone

combined with other anti-myeloma agents, i.e., carfilzomib (NCT05199311, NCT02773030), ixazomib (NCT04998786), cyclophosphamide (NCT04392037) and idecabtagene vicleucel (the KarMMa-7 trial, NCT04855136) are currently being investigated in several early phase studies.

3.2.3. Avadomide (CC-122)

The results of the first-in-human study of avadomide monotherapy in the treatment of various advanced hematological malignancies, including two cases of heavily pretreated MM have recently been published [92]. Although no objective responses were observed, in one MM case avadomide led to long-term disease stabilization. In two other early phase studies, avadomide both in monotherapy and in combination with the anti-CD20 antibodies showed promising efficacy in the treatment of relapsed/refractory non-Hodgkin's lymphoma [93,94].

3.2.4. CC-885

Another CELMoD, CC-885 has shown anti-cancer activity in several preclinical studies [83,84,95]. However, to the best of our knowledge, CC-885 is not yet evaluated in clinical trials.

4. Proteolysis Targeting Chimeras (PROTACs)

As described previously, selective protein degradation is a treatment strategy of high clinical value, and this therapeutic approach is desirable not only for MM patients. An interesting method for novel drug design is to hijack the activity of E3 ubiquitin ligases for ubiquitination and degradation of the proteins of "our" interest (POIs). The extensive studies in IMiDs mechanism of action led to the development of "degronimids"—bifunctional compounds in which a thalidomide-like element is paired with one of many different small molecules to cause ubiquitination of proteins binding to these latter molecules [96]. This engineered technique for protein degradation is more commonly known as proteolysis targeting chimeras (PROTACs). The PROTAC molecules consist of three elements: (1) a small molecule compound that binds specifically to the target protein, (2) a compound that binds specifically to the E3 ubiquitin ligase, often called "molecular glue" and (3) a "linker" that connects the two above elements and also affects its tertiary structure, water solubility, and stability, Figure 3.

The PROTAC technique does not require binding to the target protein's active site, so this approach has a great advantage in overcoming the potential limitations of classical small-molecule protein inhibitors (transient targeting of non-covalent inhibitors; resistance caused by protein overexpression or point mutations). This novel strategy brings us closer to degrading "undruggable" proteins, such as crucial oncogenic proteins.

Currently, most PROTACs use the $CRL4^{CRBN}$ and von Hippel-Lindau (VHL) E3 ubiquitin ligase as a recruiting ligase. Thus, IMiDs are often considered pioneers in respect to the "molecular glue" part of PROTAC since they promote the interaction of CRBN with a multitude of therapeutically relevant neosubstrates.

The first CRBN-based PROTAC was developed in 2015, with the structure of thalidomide capturing CRBN and bromodomains as protein of interest (by BET inhibitor—JQ1). The resulting compound dBET1 has been shown to induce highly selective CRBN-dependent BET protein degradation in MM and AML cell lines [96]. The next-generation PROTACs based on $CRL4^{CRBN}$—pomalidomide interaction also targets BET proteins (ARV 825), which showed promising activity against MM cells, including in vivo activity in a mice model [97,98]. Effective PROTACs targeting other MM promising oncoproteins such as CDK4 and CDK6 [99,100] and MCL-1 [101] have also been described.

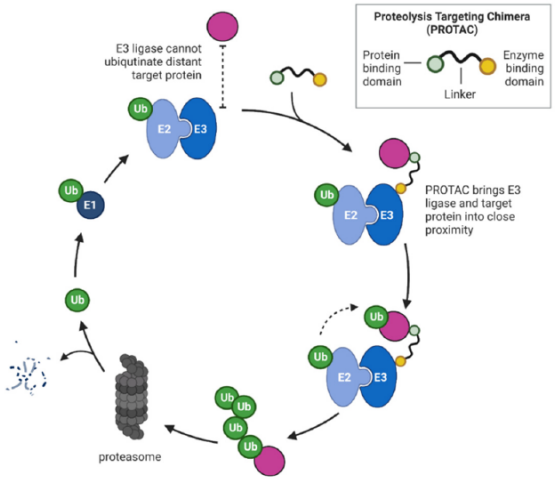


Figure 3. The mechanism of PROTAC-mediated targeted protein degradation. The PROTAC works as "molecular glue" and brings the target protein closer to E3 ligase to form a multi-protein complex. This result in Ub transfer from E2 to the target and subsequent degradation in the proteasome. Excluding the proteolyzed target, the classic component of the ubiquitination pathway and PROTAC recycle during the whole process.

It should be noted that in the case of MM, this strategy may be limited due to the resistance of MM cells that arises during treatment with IMiDs or CELMoDs. This may affect the efficacy of PROTACs based on the $CRL4^{CRBN}$ E3 ubiquitin ligase by changes in the CRBN expression and mutation in the gene encoding CRBN. Fortunately, the human genome encodes more than 600 E3 ubiquitin ligases [3,102], so far only a few have been used for PROTAC's generation: VHL, MDM2 (Murine double minute 2), IAPs (inhibitor of apoptosis proteins) and CRBN. The latest comprehensive investigation of PROTACs targeting different proteins but running via the same E3 ligase showed cross-resistance. In turn, the sequential exposure to other E3 ligases (CRBN of VHL) for the same target overcame this effect [103].

To date, degronimids and other PROTACs are being studied extensively in a broad spectrum of hematologic malignancies and other cancers in preclinical studies [104–106]. In 2019, the first potential PROTAC-based drugs entered the first-in-human clinical trial in metastatic and castration-resistant prostate cancer (ARV-110; NCT03888612) and advanced breast cancer (ARV-471; NCT04072952), resulting in acceptable toxicity profile and the first evidence of the PROTACs' clinical activity [107,108]. In August 2022, clinical trials with ARV-110 (ADRENT, NCT0388861) and ARV-471 (VERITAC, NCT04072952) are running phase 2 trials. In the hematology field, a first-in-human phase 1 trial of a first-in-class oral BTK degrader with IMiD-like activity (NX-2127), is currently enrolling the patients with relapsed/refractory B-cell malignancies (NCT04830137). Similarly, another BTK degrader with IMID backbone (NX-5948) has entered the 1 phase trial in adults with relapsed/refractory B-cell malignancies, including also primary central nervous system lymphoma (NCT05131022). Recently, the STAT3 degrader (KT-333) also on the IMiD backbone was approved to enter the phase 1 trial in adults with refractory B-cell non-Hodgkin lymphoma, T-cell lymphomas and solid tumors [109].

5. Conclusions and Future Directions

The introduction of IMiDs has changed the therapeutic landscape of multiple myeloma once and for all, and together with other advances, has led to significant improvement in MM treatment outcomes. Currently, these drugs are the standard of care for induction therapy for newly-diagnosed MM patients, maintenance therapy after auto-HSCT, and treatment of relapsed/refractory MM.

The lifetime of thalidomide from a teratogenic "dark remedy" to the first-in-class IMiD, along with an extensive investigation of its mechanism of action, gave us a unique lesson about the possibility of precise and re-directed protein ubiquitination. The identification of CRBN as a thalidomide binding protein was followed by the discovery that IMiDs modulate the ubiquitin ligase activity of $CRL4^{CRBN}$ towards non-physiological targets for proteasome degradation. For now, plenty of new $CRL4^{CRBN}$ interactors have been discovered as a result of broad IMiDs/CELMoDs activity investigations. The design and development of selective protein degraders based on $CRL4^{CRBN}$ and other E3 ligases may represent the quintessence of personalized medicine, as targeted protein degraders apparently can induce degradation of any cancer vulnerability.

CELMoDs seem to be an attractive therapeutic option for MM refractory to IMiDs, but further deep proteomic investigations of resistant MM cells (especially at the stage of MRD) can reveal resistance mediating "undruggable" proteins that can become targets for PROTACs utility.

Even though IMiDs are one of the most important drugs used in MM therapy, the landscape of their therapeutic area is enlarging to other hematologic malignancies. The efficacy of lenalidomide was proven in the treatment of relapsed mantle cell lymphoma, follicular lymphoma and marginal zone lymphomas. Furthermore, CELMoDs recruitment of new $CRL4^{CRBN}$ substrates (e.g., GSTP1) makes them attractive for the treatment of AML. The pluripotent mechanism of IMiDs/CELMoDs action makes them attractive to complement other therapies, especially immunotherapy. The potential to enhance anti-tumor immune responses by overcoming an immunosuppressive effect of the tumor microenvironment brings them promising candidates for combined therapies with immune-engagers, such as monoclonal or bispecific antibodies and chimeric antigen receptor (CAR)-T cell therapies.

Author Contributions: Conceptualization: J.B. and E.L.-M.; methodology: J.B. and E.L.-M.; investigation: J.B., A.S.-P. and I.M.-K.; resources: J.B., A.S.-P. and I.M.-K.; writing—original draft preparation: J.B., A.S-P. and I.M.-K.; writing-review and editing: J.B., A.S.-P., I.M.-K. and E.L.-M.; visualization: J.B.; supervision: E.L.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by a grant from National Science Centre in Poland (2014/3/B/NZ5/03160).

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

References

1. Finley, D.; Ciechanover, A.; Varshavsky, A. Ubiquitin as a central cellular regulator. *Cell* **2004**, *116*, S29–S34. [CrossRef]
2. Nguyen, H.C.; Wang, W.; Xiong, Y. Cullin-RING E3 Ubiquitin Ligases: Bridges to Destruction. *Macromol. Protein Complexes* **2017**, *83*, 323–347. [CrossRef]
3. Nguyen, V.-N.; Huang, K.-Y.; Weng, J.T.-Y.; Lai, K.R.; Lee, T.-Y. UbiNet: An online resource for exploring the functional associations and regulatory networks of protein ubiquitylation. *Database* **2016**, *2016*. [CrossRef]
4. Zhu, Y.X.; Braggio, E.; Shi, C.-X.; Kortuem, K.M.; Bruins, L.A.; Schmidt, J.E.; Chang, X.-B.; Langlais, P.; Luo, M.; Jedlowski, P.; et al. Identification of cereblon-binding proteins and relationship with response and survival after IMiDs in multiple myeloma. *Blood* **2014**, *124*, 536–545. [CrossRef]
5. Lu, G.; Middleton, R.E.; Sun, H.; Naniong, M.; Ott, C.J.; Mitsiades, C.S.; Wong, K.-K.; Bradner, J.E.; Kaelin, W.G., Jr. The Myeloma Drug Lenalidomide Promotes the Cereblon-Dependent Destruction of Ikaros Proteins. *Science* **2014**, *343*, 305–309. [CrossRef]
6. Krönke, J.; Udeshi, N.D.; Narla, A.; Grauman, P.; Hurst, S.N.; McConkey, M.; Svinkina, T.; Heckl, D.; Comer, E.; Li, X.; et al. Lenalidomide Causes Selective Degradation of IKZF1 and IKZF3 in Multiple Myeloma Cells. *Science* **2014**, *343*, 301–305. [CrossRef]

7. Gandhi, A.K.; Kang, J.; Havens, C.G.; Conklin, T.; Ning, Y.; Wu, L.; Ito, T.; Ando, H.; Waldman, M.F.; Thakurta, A.; et al. Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex $CRL4^{CRBN}$. *Br. J. Haematol.* **2013**, *164*, 811–821. [CrossRef]
8. Fischer, E.S.; Böhm, K.; Lydeard, J.R.; Yang, H.; Stadler, M.B.; Cavadini, S.; Nagel, J.; Serluca, F.; Acker, V.; Lingaraju, G.M.; et al. Structure of the DDB1–CRBN E3 ubiquitin ligase in complex with thalidomide. *Nature* **2014**, *512*, 49–53. [CrossRef]
9. Krönke, J.; Fink, E.C.; Hollenbach, P.W.; Macbeth, K.J.; Hurst, S.N.; Udeshi, N.D.; Chamberlain, P.P.; Mani, D.R.; Man, H.W.; Gandhi, A.K.; et al. Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. *Nature* **2015**, *523*, 183–188. [CrossRef]
10. Petzold, G.; Fischer, E.S.; Thomä, G.P.E.S.F.N.H. Structural basis of lenalidomide-induced CK1α degradation by the CRL4CRBN ubiquitin ligase. *Nature* **2016**, *532*, 127–130. [CrossRef]
11. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2019. *CA Cancer J. Clin.* **2019**, *69*, 7–34. [CrossRef]
12. Zhu, Y.X.; Braggio, E.; Shi, C.-X.; Bruins, L.A.; Schmidt, J.E.; Van Wier, S.; Chang, X.-B.; Bjorklund, C.C.; Fonseca, R.; Bergsagel, P.L.; et al. Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. *Blood* **2011**, *118*, 4771–4779. [CrossRef]
13. Franssen, L.E.; Nijhof, I.S.; Couto, S.; Levin, M.-D.; Bos, G.M.; Broijl, A.; Klein, S.K.; Ren, Y.; Wang, M.; Koene, H.R.; et al. Cereblon loss and up-regulation of c-Myc are associated with lenalidomide resistance in multiple myeloma patients. *Haematologica* **2018**, *103*, e368–e371. [CrossRef]
14. Heider, M.; Eichner, R.; Stroh, J.; Morath, V.; Kuisl, A.; Zecha, J.; Lawatscheck, J.; Baek, K.; Garz, A.-K.; Rudelius, M.; et al. The IMiD target CRBN determines HSP90 activity toward transmembrane proteins essential in multiple myeloma. *Mol. Cell* **2021**, *81*, 1170–1186.e10. [CrossRef]
15. Costacurta, M.; He, J.; Thompson, P.E.; Shortt, J. Molecular Mechanisms of Cereblon-Interacting Small Molecules in Multiple Myeloma Therapy. *J. Pers. Med.* **2021**, *11*, 1185. [CrossRef]
16. Haslett, P.A.; Corral, L.G.; Albert, M.; Kaplan, G. Thalidomide Costimulates Primary Human T Lymphocytes, Preferentially Inducing Proliferation, Cytokine Production, and Cytotoxic Responses in the CD8+ Subset. *J. Exp. Med.* **1998**, *187*, 1885–1892. [CrossRef]
17. Davies, F.; Raje, N.; Hideshima, T.; Lentzsch, S.; Young, G.; Tai, Y.-T.; Lin, B.; Podar, K.; Gupta, D.; Chauhan, D.; et al. Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma. *Blood* **2001**, *98*, 210–216. [CrossRef]
18. Hayashi, T.; Hideshima, T.; Akiyama, M.; Podar, K.; Yasui, H.; Raje, N.; Kumar, S.; Chauhan, D.; Treon, S.P.; Richardson, P.; et al. Molecular mechanisms whereby immunomodulatory drugs activate natural killer cells: Clinical application. *Br. J. Haematol.* **2004**, *128*, 192–203. [CrossRef]
19. D'Amato, R.J.; Loughnan, M.S.; Flynn, E.; Folkman, J. Thalidomide is an inhibitor of angiogenesis. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4082–4085. [CrossRef]
20. Charliński, G.; Vesole, D.H.; Jurczyszyn, A. Rapid Progress in the Use of Immunomodulatory Drugs and Cereblon E3 Ligase Modulators in the Treatment of Multiple Myeloma. *Cancers* **2021**, *13*, 4666. [CrossRef]
21. Ito, T.; Ando, H.; Suzuki, T.; Ogura, T.; Hotta, K.; Imamura, Y.; Yamaguchi, Y.; Handa, H. Identification of a Primary Target of Thalidomide Teratogenicity. *Science* **2010**, *327*, 1345–1350. [CrossRef]
22. Broyl, A.; Kuiper, R.; Van Duin, M.; Van Der Holt, B.; El Jarari, L.; Bertsch, U.; Zweegman, S.; Buijs, A.; Hose, D.; Lokhorst, H.M.; et al. High cereblon expression is associated with better survival in patients with newly diagnosed multiple myeloma treated with thalidomide maintenance. *Blood* **2013**, *121*, 624–627. [CrossRef]
23. Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. *J. Clin. Med.* **2021**, *10*, 2683. [CrossRef]
24. Heintel, D.; Rocci, A.; Ludwig, H.; Bolomsky, A.; Caltagirone, S.; Schreder, M.; Pfeifer, S.; Gisslinger, H.; Zojer, N.; Jäger, U.; et al. High expression of cereblon (*CRBN*) is associated with improved clinical response in patients with multiple myeloma treated with lenalidomide and dexamethasone. *Br. J. Haematol.* **2013**, *161*, 695–700. [CrossRef]
25. Misiewicz-Krzeminska, I.; De Ramón, C.; Corchete, L.A.; Krzeminski, P.; Rojas, E.A.; Isidro, I.; García-Sanz, R.; Martínez-López, J.; Oriol, A.; Bladé, J.; et al. Quantitative expression of Ikaros, IRF4, and PSMD10 proteins predicts survival in VRD-treated patients with multiple myeloma. *Blood Adv.* **2020**, *4*, 6023–6033. [CrossRef]
26. Gooding, S.; Ansari-Pour, N.; Towfic, F.; Estévez, M.O.; Chamberlain, P.P.; Tsai, K.-T.; Flynt, E.; Hirst, M.; Rozelle, D.; Dhiman, P.; et al. Multiple cereblon genetic changes are associated with acquired resistance to lenalidomide or pomalidomide in multiple myeloma. *Blood* **2021**, *137*, 232–237. [CrossRef]
27. Jones, J.R.; Barber, A.; Le Bihan, Y.-V.; Weinhold, N.; Ashby, C.; Walker, B.A.; Wardell, C.P.; Wang, H.; Kaiser, M.F.; Jackson, G.H.; et al. Mutations in CRBN and other cereblon pathway genes are infrequently associated with acquired resistance to immunomodulatory drugs. *Leukemia* **2021**, *35*, 3017–3020. [CrossRef]
28. Salomon-Perzyński, A.; Barankiewicz, J.; Machnicki, M.; Misiewicz-Krzemińska, I.; Pawlak, M.; Radomska, S.; Krzywdzińska, A.; Bluszcz, A.; Stawiński, P.; Rydzanicz, M.; et al. Tracking Clonal Evolution of Multiple Myeloma Using Targeted Next-Generation DNA Sequencing. *Biomedicines* **2022**, *10*, 1674. [CrossRef]

29. Shaffer, A.L.; Emre, N.C.T.; Lamy, L.; Ngo, V.N.; Wright, G.; Xiao, W.; Powell, J.; Dave, S.; Yu, X.; Zhao, H.; et al. IRF4 addiction in multiple myeloma. *Nature* **2008**, *454*, 226–231. [CrossRef]
30. Lopez-Girona, A.; Heintel, D.; Zhang, L.-H.; Mendy, D.; Gaidarova, S.; Brady, H.; Bartlett, J.B.; Schafer, P.H.; Schreder, M.; Bolomsky, A.; et al. Lenalidomide downregulates the cell survival factor, interferon regulatory factor-4, providing a potential mechanistic link for predicting response. *Br. J. Haematol.* **2011**, *154*, 325–336. [CrossRef]
31. Bjorklund, C.C.; Lu, L.; Kang, J.; Hagner, P.; Havens, C.G.; Amatangelo, M.; Wang, M.; Ren, Y.; Couto, S.S.; Breider, M.; et al. Rate of CRL4CRBN substrate Ikaros and Aiolos degradation underlies differential activity of lenalidomide and pomalidomide in multiple myeloma cells by regulation of c-Myc and IRF4. *Blood Cancer J.* **2015**, *5*, e354. [CrossRef]
32. Patil, A.; Manzano, M.; Gottwein, E. CK1α and IRF4 are essential and independent effectors of immunomodulatory drugs in primary effusion lymphoma. *Blood* **2018**, *132*, 577–586. [CrossRef]
33. Manni, S.; Carrino, M.; Manzoni, M.; Gianesin, K.; Nunes, S.C.; Costacurta, M.; Tubi, L.Q.; Macaccaro, P.; Taiana, E.; Cabrelle, A.; et al. Inactivation of CK1α in multiple myeloma empowers drug cytotoxicity by affecting AKT and β-catenin survival signaling pathways. *Oncotarget* **2017**, *8*, 14604–14619. [CrossRef]
34. Carrino, M.; Tubi, L.Q.; Fregnani, A.; Nunes, S.C.; Barilà, G.; Trentin, L.; Zambello, R.; Semenzato, G.; Manni, S.; Piazza, F. Prosurvival autophagy is regulated by protein kinase CK1 alpha in multiple myeloma. *Cell Death Discov.* **2019**, *5*, 98. [CrossRef]
35. Hu, Y.; Song, W.; Cirstea, D.; Lu, D.; Munshi, N.C.; Anderson, K.C. CSNK1α1 mediates malignant plasma cell survival. *Leukemia* **2014**, *29*, 474–482. [CrossRef]
36. Matyskiela, M.E.; Couto, S.; Zheng, X.; Lu, G.; Hui, J.; Stamp, K.; Drew, C.; Ren, Y.; Wang, M.; Carpenter, A.; et al. SALL4 mediates teratogenicity as a thalidomide-dependent cereblon substrate. *Nat. Chem. Biol.* **2018**, *14*, 981–987. [CrossRef]
37. A Donovan, K.; An, J.; Nowak, R.P.; Yuan, J.C.; Fink, E.C.; Berry, B.C.; Ebert, B.L.; Fischer, E.S. Thalidomide promotes degradation of SALL4, a transcription factor implicated in Duane Radial Ray syndrome. *eLife* **2018**, *7*. [CrossRef]
38. Asatsuma-Okumura, T.; Ando, H.; De Simone, M.; Yamamoto, J.; Sato, T.; Shimizu, N.; Asakawa, K.; Yamaguchi, Y.; Ito, T.; Guerrini, L.; et al. p63 is a cereblon substrate involved in thalidomide teratogenicity. *Nat. Chem. Biol.* **2019**, *15*, 1077–1084. [CrossRef]
39. Yamanaka, S.; Murai, H.; Saito, D.; Abe, G.; Tokunaga, E.; Iwasaki, T.; Takahashi, H.; Takeda, H.; Suzuki, T.; Shibata, N.; et al. Thalidomide and its metabolite 5-hydroxythalidomide induce teratogenicity via the cereblon neosubstrate PLZF. *EMBO J.* **2021**, *40*, e105375. [CrossRef]
40. An, J.; Ponthier, C.M.; Sack, R.; Seebacher, J.; Stadler, M.B.; Donovan, K.; Fischer, E.S. pSILAC mass spectrometry reveals ZFP91 as IMiD-dependent substrate of the CRL4CRBN ubiquitin ligase. *Nat. Commun.* **2017**, *8*, 15398. [CrossRef]
41. Sievers, Q.L.; Petzold, G.; Bunker, R.D.; Renneville, A.; Słabicki, M.; Liddicoat, B.J.; Abdulrahman, W.; Mikkelsen, T.; Ebert, B.L.; Thomä, N.H. Defining the human C2H2 zinc finger degrome targeted by thalidomide analogs through CRBN. *Science* **2018**, *362*, eaat0572. [CrossRef] [PubMed]
42. Liu, A.; Li, S.; Donnenberg, V.; Fu, J.; Gollin, S.M.; Ma, H.; Lu, C.; Stolz, N.B.; Mapara, M.Y.; Monaghan, S.A.; et al. Immunomodulatory drugs downregulate IKZF1 leading to expansion of hematopoietic progenitors with concomitant block of megakaryocytic maturation. *Haematologica* **2018**, *103*, 1688–1697. [CrossRef] [PubMed]
43. Pal, R.; Monaghan, S.A.; Hassett, A.C.; Mapara, M.Y.; Schafer, P.; Roodman, G.D.; Ragni, M.V.; Moscinski, L.; List, A.; Lentzsch, S. Immunomodulatory derivatives induce PU.1 down-regulation, myeloid maturation arrest, and neutropenia. *Blood* **2010**, *115*, 605–614. [CrossRef] [PubMed]
44. Rosiñol, L.; Oriol, A.; Teruel, A.I.; Hernández, D.; López-Jiménez, J.; de la Rubia, J.; Granell, M.; Besalduch, J.; Palomera, L.; González, Y.; et al. Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: A randomized phase 3 PETHEMA/GEM study. *Blood* **2012**, *120*, 1589–1596. [CrossRef]
45. Moreau, P.; Attal, M.; Hulin, C.; Arnulf, B.; Belhadj, K.; Benboubker, L.; Béné, M.C.; Broijl, A.; Caillon, H.; Caillot, D.; et al. Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): A randomised, open-label, phase 3 study. *Lancet* **2019**, *394*, 29–38. [CrossRef]
46. Rosiñol, L.; Oriol, A.; Rios, R.; Sureda, A.; Blanchard, M.J.; Hernández, M.T.; Martínez-Martínez, R.; Moraleda, J.M.; Jarque, I.; Bargay, J.; et al. Bortezomib, lenalidomide, and dexamethasone as induction therapy prior to autologous transplant in multiple myeloma. *Blood* **2019**, *134*, 1337–1345. [CrossRef]
47. Voorhees, P.M.; Kaufman, J.L.; Laubach, J.P.; Sborov, D.W.; Reeves, B.; Rodriguez, C.; Chari, A.; Silbermann, R.; Costa, L.J.; Anderson, L.D., Jr.; et al. Daratumumab, lenalidomide, bortezomib, and dexamethasone for transplant-eligible newly diagnosed multiple myeloma: The GRIFFIN trial. *Blood* **2020**, *136*, 936–945. [CrossRef]
48. Durie, B.G.M.; Hoering, A.; Abidi, M.H.; Rajkumar, S.V.; Epstein, J.; Kahanic, S.P.; Thakuri, M.; Reu, F.; Reynolds, C.M.; Sexton, R.; et al. Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): A randomised, open-label, phase 3 trial. *Lancet* **2017**, *389*, 519–527. [CrossRef]
49. Kumar, S.K.; Jacobus, S.J.; Cohen, A.D.; Weiss, M.; Callander, N.; Singh, A.K.; Parker, T.L.; Menter, A.; Yang, X.; Parsons, B.; et al. Carfilzomib or bortezomib in combination with lenalidomide and dexamethasone for patients with newly diagnosed multiple myeloma without intention for immediate autologous stem-cell transplantation (ENDURANCE): A multicentre, open-label, phase 3, randomised, controlled trial. *Lancet Oncol.* **2020**, *21*, 1317–1330. [CrossRef]

50. Facon, T.; Kumar, S.K.; Plesner, T.; Orlowski, R.Z.; Moreau, P.; Bahlis, N.; Basu, S.; Nahi, H.; Hulin, C.; Quach, H.; et al. Daratumumab, lenalidomide, and dexamethasone versus lenalidomide and dexamethasone alone in newly diagnosed multiple myeloma (MAIA): Overall survival results from a randomised, open-label, phase 3 trial. *Lancet Oncol.* **2021**, *22*, 1582–1596. [CrossRef]
51. Callander, N.S.; Baljevic, M.; Adekola, K.; Anderson, L.D.; Campagnaro, E.; Castillo, J.J.; Costello, C.; Devarakonda, S.; Elsedawy, N.; Faiman, M.; et al. NCCN Guidelines®Insights: Multiple Myeloma, Version 3.2022. *J. Natl. Compr. Cancer Netw.* **2022**, *20*, 8–19. [CrossRef]
52. Dimopoulos, M.; Spencer, A.; Attal, M.; Prince, H.M.; Harousseau, J.-L.; Dmoszynska, A.; Miguel, J.S.; Hellmann, A.; Facon, T.; Foà, R.; et al. Lenalidomide plus Dexamethasone for Relapsed or Refractory Multiple Myeloma. *N. Engl. J. Med.* **2007**, *357*, 2123–2132. [CrossRef] [PubMed]
53. Stewart, A.K.; Rajkumar, S.V.; Dimopoulos, M.A.; Masszi, T.; Špička, I.; Oriol, A.; Hájek, R.; Rosiñol, L.; Siegel, D.S.; Mihaylov, G.G.; et al. Carfilzomib, Lenalidomide, and Dexamethasone for Relapsed Multiple Myeloma. *N. Engl. J. Med.* **2015**, *372*, 142–152. [CrossRef] [PubMed]
54. Siegel, D.S.; Dimopoulos, M.A.; Ludwig, H.; Facon, T.; Goldschmidt, H.; Jakubowiak, A.; Miguel, J.S.; Obreja, M.; Blaedel, J.; Stewart, A.K. Improvement in Overall Survival with Carfilzomib, Lenalidomide, and Dexamethasone in Patients with Relapsed or Refractory Multiple Myeloma. *J. Clin. Oncol.* **2018**, *36*, 728–734. [CrossRef]
55. Moreau, P.; Masszi, T.; Grzasko, N.; Bahlis, N.J.; Hansson, M.; Pour, L.; Sandhu, I.; Ganly, P.; Baker, B.W.; Jackson, S.R.; et al. Oral Ixazomib, Lenalidomide, and Dexamethasone for Multiple Myeloma. *N. Engl. J. Med.* **2016**, *374*, 1621–1634. [CrossRef] [PubMed]
56. Bahlis, N.J.; Dimopoulos, M.A.; White, D.J.; Benboubker, L.; Cook, G.; Leiba, M.; Ho, P.J.; Kim, K.; Takezako, N.; Moreau, P.; et al. Daratumumab plus lenalidomide and dexamethasone in relapsed/refractory multiple myeloma: Extended follow-up of POLLUX, a randomized, open-label, phase 3 study. *Leukemia* **2020**, *34*, 1875–1884. [CrossRef]
57. Lonial, S.; Dimopoulos, M.; Palumbo, A.; White, D.; Grosicki, S.; Spicka, I.; Walter-Croneck, A.; Moreau, P.; Mateos, M.V.; Magen, H.; et al. Elotuzumab Therapy for Relapsed or Refractory Multiple Myeloma. *N. Engl. J. Med.* **2015**, *373*, 621–631. [CrossRef]
58. Dimopoulos, M.A.; Lonial, S.; White, D.; Moreau, P.; Weisel, K.; San-Miguel, J.; Shpilberg, O.; Grosicki, S.; Špička, I.; Walter-Croneck, A.; et al. Elotuzumab, lenalidomide, and dexamethasone in RRMM: Final overall survival results from the phase 3 randomized ELOQUENT-2 study. *Blood Cancer J.* **2020**, *10*, 1–10. [CrossRef]
59. Dimopoulos, M.; Quach, H.; Mateos, M.-V.; Landgren, O.; Leleu, X.; Siegel, D.; Weisel, K.; Yang, H.; Klippel, Z.; Zahlten-Kumeli, A.; et al. Carfilzomib, dexamethasone, and daratumumab versus carfilzomib and dexamethasone for patients with relapsed or refractory multiple myeloma (CANDOR): Results from a randomised, multicentre, open-label, phase 3 study. *Lancet* **2020**, *396*, 186–197. [CrossRef]
60. Mateos, M.-V.; Sonneveld, P.; Hungria, V.; Nooka, A.K.; Estell, J.A.; Barreto, W.; Corradini, P.; Min, C.-K.; Medvedova, E.; Weisel, K.; et al. Daratumumab, Bortezomib, and Dexamethasone Versus Bortezomib and Dexamethasone in Patients with Previously Treated Multiple Myeloma: Three-year Follow-up of CASTOR. *Clin. Lymphoma Myeloma Leuk.* **2019**, *20*, 509–518. [CrossRef]
61. Dimopoulos, M.A.; Goldschmidt, H.; Niesvizky, R.; Joshua, D.; Chng, W.-J.; Oriol, A.; Orlowski, R.Z.; Ludwig, H.; Facon, T.; Hajek, R.; et al. Carfilzomib or bortezomib in relapsed or refractory multiple myeloma (ENDEAVOR): An interim overall survival analysis of an open-label, randomised, phase 3 trial. *Lancet Oncol.* **2017**, *18*, 1327–1337. [CrossRef]
62. Dimopoulos, M.A.; Terpos, E.; Boccadoro, M.; Delimpasi, S.; Beksac, M.; Katodritou, E.; Moreau, P.; Baldini, L.; Symeonidis, A.; Bila, J.; et al. Daratumumab plus pomalidomide and dexamethasone versus pomalidomide and dexamethasone alone in previously treated multiple myeloma (APOLLO): An open-label, randomised, phase 3 trial. *Lancet Oncol.* **2021**, *22*, 801–812. [CrossRef]
63. Richardson, P.G.; Perrot, A.; San-Miguel, J.; Beksac, M.; Spicka, I.; Leleu, X.; Schjesvold, F.; Moreau, P.; Dimopoulos, M.A.; Huang, J.S.-Y.; et al. Isatuximab plus pomalidomide and low-dose dexamethasone versus pomalidomide and low-dose dexamethasone in patients with relapsed and refractory multiple myeloma (ICARIA-MM): Follow-up analysis of a randomised, phase 3 study. *Lancet Oncol.* **2022**, *23*, 416–427. [CrossRef]
64. Dimopoulos, M.A.; Dytfeld, D.; Grosicki, S.; Moreau, P.; Takezako, N.; Hori, M.; Leleu, X.; Leblanc, R.; Suzuki, K.; Raab, M.S.; et al. Elotuzumab plus Pomalidomide and Dexamethasone for Multiple Myeloma. *N. Engl. J. Med.* **2018**, *379*, 1811–1822. [CrossRef]
65. Richardson, P.G.; Oriol, A.; Beksac, M.; Liberati, A.M.; Galli, M.; Schjesvold, F.; Lindsay, J.; Weisel, K.; White, D.; Facon, T.; et al. Pomalidomide, bortezomib, and dexamethasone for patients with relapsed or refractory multiple myeloma previously treated with lenalidomide (OPTIMISMM): A randomised, open-label, phase 3 trial. *Lancet Oncol.* **2019**, *20*, 781–794. [CrossRef]
66. Shah, J.J.; Stadtmauer, E.A.; Abonour, R.; Cohen, A.D.; Bensinger, W.I.; Gasparetto, C.; Kaufman, J.L.; Lentzsch, S.; Vogl, D.T.; Gomes, C.L.; et al. Carfilzomib, pomalidomide, and dexamethasone for relapsed or refractory myeloma. *Blood* **2015**, *126*, 2284–2290. [CrossRef]
67. Krishnan, A.; Kapoor, P.; Palmer, J.M.; Tsai, N.-C.; Kumar, S.; Lonial, S.; Htut, M.; Karanes, C.; Nathwani, N.; Rosenzweig, M.; et al. Phase I/II trial of the oral regimen ixazomib, pomalidomide, and dexamethasone in relapsed/refractory multiple myeloma. *Leukemia* **2018**, *32*, 1567–1574. [CrossRef]
68. Garderet, L.; Kuhnowski, F.; Berge, B.; Roussel, M.; Escoffre-Barbe, M.; Lafon, I.; Facon, T.; Leleu, X.; Karlin, L.; Perrot, A.; et al. Pomalidomide, cyclophosphamide, and dexamethasone for relapsed multiple myeloma. *Blood* **2018**, *132*, 2555–2563. [CrossRef]

69. Attal, M.; Lauwers-Cances, V.; Marit, G.; Caillot, D.; Moreau, P.; Facon, T.; Stoppa, A.M.; Hulin, C.; Benboubker, L.; Garderet, L.; et al. Lenalidomide Maintenance after Stem-Cell Transplantation for Multiple Myeloma. *N. Engl. J. Med.* **2012**, *366*, 1782–1791. [CrossRef]
70. Palumbo, A.; Cavallo, F.; Gay, F.; Di Raimondo, F.; Ben Yehuda, D.; Petrucci, M.T.; Pezzatti, S.; Caravita, T.; Cerrato, C.; Ribakovsky, E.; et al. Autologous Transplantation and Maintenance Therapy in Multiple Myeloma. *N. Engl. J. Med.* **2014**, *371*, 895–905. [CrossRef]
71. Holstein, S.A.; Jung, S.-H.; Richardson, P.G.; Hofmeister, C.C.; Hurd, D.D.; Hassoun, H.; Giralt, S.; Stadtmauer, E.A.; Weisdorf, D.J.; Vij, R.; et al. Updated analysis of CALGB (Alliance) 100104 assessing lenalidomide versus placebo maintenance after single autologous stem-cell transplantation for multiple myeloma: A randomised, double-blind, phase 3 trial. *Lancet Haematol.* **2017**, *4*, e431–e442. [CrossRef]
72. Jackson, G.H.; Davies, F.E.; Pawlyn, C.; Cairns, D.A.; Striha, A.; Collett, C.; Hockaday, A.; Jones, J.R.; Kishore, B.; Garg, M.; et al. Lenalidomide maintenance versus observation for patients with newly diagnosed multiple myeloma (Myeloma XI): A multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol.* **2019**, *20*, 57–73. [CrossRef]
73. McCarthy, P.L.; Holstein, S.A.; Petrucci, M.T.; Richardson, P.G.; Hulin, C.; Tosi, P.; Bringhen, S.; Musto, P.; Anderson, K.C.; Caillot, D.; et al. Lenalidomide Maintenance After Autologous Stem-Cell Transplantation in Newly Diagnosed Multiple Myeloma: A Meta-Analysis. *J. Clin. Oncol.* **2017**, *35*, 3279–3289. [CrossRef] [PubMed]
74. Jones, J.R.; Csg, O.B.O.T.N.H.-O.; Cairns, D.A.; Gregory, W.M.; Collett, C.; Pawlyn, C.; Sigsworth, R.; Striha, A.; Henderson, R.; Kaiser, M.F.; et al. Second malignancies in the context of lenalidomide treatment: An analysis of 2732 myeloma patients enrolled to the Myeloma XI trial. *Blood Cancer J.* **2016**, *6*, e506. [CrossRef]
75. Chavez, B.M.; Barnell, B.E.; Griffith, M.; Skidmore, Z.; Griffith, O.; Tian, L.; Wartman, L.D. B-Cell Acute Lymphoblastic Leukemia Arising in Patients with a Preexisting Diagnosis of Multiple Myeloma Is a Novel Cancer with High Incidence of TP53 Mutations. *Blood* **2020**, *136*, 20. [CrossRef]
76. Germans, S.K.; Kulak, O.; Koduru, P.; Oliver, D.; Gagan, J.; Patel, P.; Anderson, L.D.; Fuda, F.S.; Chen, W.; Jaso, J.M. Lenalidomide-Associated Secondary B-Lymphoblastic Leukemia/Lymphoma—A Unique Entity. *Am. J. Clin. Pathol.* **2020**, *154*, 816–827. [CrossRef]
77. Khan, S.R.; Tariq, M.; Fayyaz, S.M.; Soomar, S.M.; Moosajee, M. Lenalidomide induced secondary Acute Lymphoblastic Leukemia in a Multiple Myeloma patient: A case-report. *Leuk. Res. Rep.* **2022**, *17*. [CrossRef]
78. Sinit, R.; Hwang, D.G.; Vishnu, P.; Peterson, J.F.; Aboulafia, D.M. B-cell acute lymphoblastic leukemia in an elderly man with plasma cell myeloma and long-term exposure to thalidomide and lenalidomide: A case report and literature review. *BMC Cancer* **2019**, *19*, 1–9. [CrossRef]
79. Matyskiela, M.E.; Zhang, W.; Man, H.-W.; Muller, G.; Khambatta, G.; Baculi, F.; Hickman, M.; Lebrun, L.; Pagarigan, B.; Carmel, G.; et al. A Cereblon Modulator (CC-220) with Improved Degradation of Ikaros and Aiolos. *J. Med. Chem.* **2017**, *61*, 535–542. [CrossRef]
80. Thakurta, A.; Pierceall, W.E.; Amatangelo, M.D.; Flynt, E.; Agarwal, A. Developing next generation immunomodulatory drugs and their combinations in multiple myeloma. *Oncotarget* **2021**, *12*, 1555–1563. [CrossRef]
81. Hansen, J.D.; Correa, M.; Nagy, M.A.; Alexander, M.; Plantevin, V.; Grant, V.; Whitefield, B.; Huang, D.; Kercher, T.; Harris, R.; et al. Discovery of CRBN E3 Ligase Modulator CC-92480 for the Treatment of Relapsed and Refractory Multiple Myeloma. *J. Med. Chem.* **2020**, *63*, 6648–6676. [CrossRef] [PubMed]
82. Matyskiela, M.E.; Lu, G.; Ito, T.; Pagarigan, B.; Lu, C.-C.; Miller, K.; Fang, W.; Wang, N.-Y.; Nguyen, D.; Houston, J.; et al. A novel cereblon modulator recruits GSPT1 to the CRL4CRBN ubiquitin ligase. *Nature* **2016**, *535*, 252–257. [CrossRef]
83. Hao, B.-B.; Li, X.-J.; Jia, X.-L.; Wang, Y.-X.; Zhai, L.-H.; Li, D.-Z.; Liu, J.; Zhang, D.; Chen, Y.-L.; Xu, Y.-H.; et al. The novel cereblon modulator CC-885 inhibits mitophagy via selective degradation of BNIP3L. *Acta Pharmacol. Sin.* **2020**, *41*, 1246–1254. [CrossRef] [PubMed]
84. Zhao, M.; Hu, M.; Chen, Y.; Liu, H.; Chen, Y.; Liu, B.; Fang, B. Cereblon modulator CC-885 induces CRBN-dependent ubiquitination and degradation of CDK4 in multiple myeloma. *Biochem. Biophys. Res. Commun.* **2021**, *549*, 150–156. [CrossRef] [PubMed]
85. Bjorklund, C.C.; Kang, J.; Lu, L.; Amatangelo, M.; Chiu, H.; Hagner, P.; Gandhi, A.K.; Pourdehnad, M.; Klippel, A.; Thakurta, A. CC-122 Is a Cereblon Modulating Agent That Is Active in Lenalidomide-Resistant and Lenalidomide/Dexamethasone-Double-Resistant Multiple Myeloma Pre-Clinical Models. *Blood* **2016**, *128*, 1592. [CrossRef]
86. Bjorklund, C.C.; Kang, J.; Amatangelo, M.; Polonskaia, A.; Katz, M.; Chiu, H.; Couto, S.; Wang, M.; Ren, Y.; Ortiz, M.; et al. Iberdomide (CC-220) is a potent cereblon E3 ligase modulator with antitumor and immunostimulatory activities in lenalidomide- and pomalidomide-resistant multiple myeloma cells with dysregulated CRBN. *Leukemia* **2019**, *34*, 1197–1201. [CrossRef]
87. Renneville, A.; Gasser, J.A.; Grinshpun, D.E.; Beltran, P.M.J.; Udeshi, N.D.; Matyskiela, M.E.; Clayton, T.; McConkey, M.; Viswanathan, K.; Tepper, A.; et al. Avadomide Induces Degradation of ZMYM2 Fusion Oncoproteins in Hematologic Malignancies. *Blood Cancer Discov.* **2021**, *2*, 250–265. [CrossRef]
88. Richardson, P.G.; Ocio, E.; Raje, N.S.; Gregory, T.; White, D.; Oriol, A.; Sandhu, I.; Raab, M.-S.; LeBlanc, R.; Rodriguez, C.; et al. CC-92480, a Potent, Novel Cereblon E3 Ligase Modulator (CELMoD) Agent, in Combination with Dexamethasone (DEX) and Bortezomib (BORT) in Patients (pts) with Relapsed/Refractory Multiple Myeloma (RRMM): Preliminary Results from the Phase 1/2 Study CC-92480-MM-002. *Blood* **2021**, *138*, 2731. [CrossRef]

89. Richardson, P.G.; Vangsted, A.J.; Ramasamy, K.; Trudel, S.; Martínez, J.; Mateos, M.-V.; Rodríguez Otero, P.; Lonial, S.; Popat, R.; Oriol, A.; et al. First-in-human phase I study of the novel CELMoD agent CC-92480 combined with dexamethasone (DEX) in patients (pts) with relapsed/refractory multiple myeloma (RRMM). *J. Clin. Oncol.* **2020**, *38*, 8500. [CrossRef]
90. van de Donk, N.W.; Popat, R.; Larsen, J.; Minnema, M.C.; Jagannath, S.; Oriol, A.; Zonder, J.; Richardson, P.G.; Rodriguez-Otero, P.; Badros, A.Z.; et al. First Results of Iberdomide (IBER.;CC-220) in Combination with Dexamethasone (DEX) and Daratumumab (DARA) or Bortezomib (BORT) in Patients with Relapsed/Refractory Multiple Myeloma (RRMM). *Blood* **2020**, *136*, 16–17. [CrossRef]
91. van de Donk, N.W.C.J.; Popat, R.; Hulin, C.; Jagannath, S.; Oriol, A.; Richardson, P.G.; Facon, T.; Weisel, K.; Larsen, J.T.; Minnema, M.; et al. P07: RESULTS FROM THE CC-220-MM-001 DOSE-EXPANSION PHASE OF IBERDOMIDE PLUS DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA. *HemaSphere* **2022**, *6*, 14–15. [CrossRef]
92. Rasco, D.W.; Papadopoulos, K.P.; Pourdehnad, M.; Gandhi, A.K.; Hagner, P.R.; Li, Y.; Wei, X.; Chopra, R.; Hege, K.; DiMartino, J.F.; et al. A First-in-Human Study of Novel Cereblon Modulator Avadomide (CC-122) in Advanced Malignancies. *Clin. Cancer Res.* **2019**, *25*, 90–98. [CrossRef] [PubMed]
93. Nastoupil, L.J.; Kuruvilla, J.; Chavez, J.C.; Bijou, F.; Witzig, T.E.; Santoro, A.; Flinn, I.W.; Boccomini, C.; Kenkre, V.P.; Corradini, P.; et al. Phase Ib study of avadomide (CC-122) in combination with rituximab in patients with relapsed/refractory diffuse large B-cell lymphoma and follicular lymphoma. *eJHaem* **2022**, *3*, 394–405. [CrossRef] [PubMed]
94. Michot, J.-M.; Bouabdallah, R.; Vitolo, U.; Doorduijn, J.K.; Salles, G.; Chiappella, A.; Zinzani, P.L.; Bijou, F.; Kersten, M.J.; Sarmiento, R.; et al. Avadomide plus obinutuzumab in patients with relapsed or refractory B-cell non-Hodgkin lymphoma (CC-122-NHL-001): A multicentre, dose escalation and expansion phase 1 study. *Lancet Haematol.* **2020**, *7*, e649–e659. [CrossRef]
95. Li, L.; Xue, W.; Shen, Z.; Liu, J.; Hu, M.; Cheng, Z.; Wang, Y.; Chen, Y.; Chang, H.; Liu, Y.; et al. A Cereblon Modulator CC-885 Induces CRBN- and p97-Dependent PLK1 Degradation and Synergizes with Volasertib to Suppress Lung Cancer. *Mol. Ther. Oncolytics* **2020**, *18*, 215–225. [CrossRef] [PubMed]
96. Winter, G.E.; Buckley, D.L.; Paulk, J.; Roberts, J.M.; Souza, A.; Dhe-Paganon, S.; Bradner, J.E. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* **2015**, *348*, 1376–1381. [CrossRef]
97. Zhang, X.; Lee, H.C.; Shirazi, F.; Baladandayuthapani, V.; Lin, H.; Kuiatse, I.; Wang, H.; Jones, R.J.; Berkova, Z.; Singh, R.K.; et al. Protein targeting chimeric molecules specific for bromodomain and extra-terminal motif family proteins are active against pre-clinical models of multiple myeloma. *Leukemia* **2018**, *32*, 2224–2239. [CrossRef]
98. Lim, S.L.; Damnernsawad, A.; Shyamsunder, P.; Chng, W.J.; Han, B.C.; Xu, L.; Pan, J.; Pravin, D.P.; Alkan, S.; Tyner, J.W.; et al. Proteolysis targeting chimeric molecules as therapy for multiple myeloma: Efficacy, biomarker and drug combinations. *Haematologica* **2019**, *104*, 1209–1220. [CrossRef]
99. Steinebach, C.; Ng, Y.L.D.; Sosič, I.; Lee, C.-S.; Chen, S.; Lindner, S.; Vu, L.P.; Bricelj, A.; Haschemi, R.; Monschke, M.; et al. Systematic exploration of different E3 ubiquitin ligases: An approach towards potent and selective CDK6 degraders. *Chem. Sci.* **2020**, *11*, 3474–3486. [CrossRef]
100. Ng, Y.L.D.; Ramberger, E.; Bohl, S.R.; Dolnik, A.; Steinebach, C.; Conrad, T.; Müller, S.; Popp, O.; Kull, M.; Haji, M.; et al. Proteomic profiling reveals CDK6 upregulation as a targetable resistance mechanism for lenalidomide in multiple myeloma. *Nat. Commun.* **2022**, *13*, 1–13. [CrossRef]
101. Papatzimas, J.W.; Gorobets, E.; Maity, R.; Muniyat, M.I.; Maccallum, J.L.; Neri, P.; Bahlis, N.J.; Derksen, D.J. From Inhibition to Degradation: Targeting the Antiapoptotic Protein Myeloid Cell Leukemia 1 (MCL1). *J. Med. Chem.* **2019**, *62*, 5522–5540. [CrossRef]
102. Medvar, B.; Raghuram, V.; Pisitkun, T.; Sarkar, A.; Knepper, M.A. Comprehensive database of human E3 ubiquitin ligases: Application to aquaporin-2 regulation. *Physiol. Genom.* **2016**, *48*, 502–512. [CrossRef] [PubMed]
103. Shirasaki, R.; Matthews, G.M.; Gandolfi, S.; Simoes, R.D.M.; Buckley, D.L.; Vora, J.R.; Sievers, Q.L.; Brüggenthies, J.B.; Dashevsky, O.; Poarch, H.; et al. Functional Genomics Identify Distinct and Overlapping Genes Mediating Resistance to Different Classes of Heterobifunctional Degraders of Oncoproteins. *Cell Rep.* **2021**, *34*, 108532. [CrossRef] [PubMed]
104. He, Y.; Khan, S.; Huo, Z.; Lv, D.; Zhang, X.; Liu, X.; Yuan, Y.; Hromas, R.; Xu, M.; Zheng, G.; et al. Proteolysis targeting chimeras (PROTACs) are emerging therapeutics for hematologic malignancies. *J. Hematol. Oncol.* **2020**, *13*, 1–24. [CrossRef] [PubMed]
105. Qi, S.-M.; Dong, J.; Xu, Z.-Y.; Cheng, X.-D.; Zhang, W.-D.; Qin, J.-J. PROTAC: An Effective Targeted Protein Degradation Strategy for Cancer Therapy. *Front. Pharmacol.* **2021**, *12*. [CrossRef]
106. Li, X.; Pu, W.; Zheng, Q.; Ai, M.; Chen, S.; Peng, Y. Proteolysis-targeting chimeras (PROTACs) in cancer therapy. *Mol. Cancer* **2022**, *21*, 1–30. [CrossRef]
107. Gao, X.; Burris, H.A., III; Vuky, J.; Dreicer, R.; Sartor, A.O.; Sternberg, C.N.; Percent, I.J.; Hussain, M.H.A.; Kalebasty, A.R.; Shen, J.; et al. Phase 1/2 study of ARV-110, an androgen receptor (AR) PROTAC degrader, in metastatic castration-resistant prostate cancer (mCRPC). *J. Clin. Oncol.* **2022**, *40*, 17. [CrossRef]
108. Hamilton, E.; Vahdat, L.; Han, H.S.; Ranciato, J.; Gedrich, R.; Keung, C.F.; Chirnomas, D.; Hurvitz, S. Abstract PD13-08: First-in-human safety and activity of ARV-471, a novel PROTAC®estrogen receptor degrader, in ER+/HER2- locally advanced or metastatic breast cancer. *Cancer Res.* **2022**, *82*, PD13-08. [CrossRef]
109. Starodub, A.; Gollerkeri, A.; De Savi, C.; Dey, J.; Agarwal, S.; Donohue, S.; Perea, R.; Klaus, C.; Gollob, J. Phase 1 study of KT-333, a targeted protein degrader, in patients with relapsed or refractory lymphomas, large granular lymphocytic leukemia, and solid tumors. *J. Clin. Oncol.* **2022**, *40*, TPS3171. [CrossRef]

BIBLIOGRAFIA

[1] P. Moreau *et al.*, "Multiple myeloma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up," *Ann. Oncol.*, vol. 28, pp. iv52–iv61, Jul. 2017, doi: 10.1093/annonc/mdx096.

[2] S. V. Rajkumar, "Multiple Myeloma: 2022 update on Diagnosis, Risk-stratification and Management," *Am. J. Hematol.*, vol. 97, no. 8, p. 1086, Aug. 2022, doi: 10.1002/AJH.26590.

[3] "Myeloma — Cancer Stat Facts." https://seer.cancer.gov/statfacts/html/mulmy.html (accessed Oct. 06, 2022).

[4] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2019," *CA. Cancer J. Clin.*, vol. 69, no. 1, pp. 7–34, Jan. 2019, doi: 10.3322/CAAC.21551.

[5] M. A. Dimopoulos *et al.*, "Multiple myeloma: EHA-ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†," *Ann. Oncol.*, vol. 32, no. 3, pp. 309–322, Mar. 2021, doi: 10.1016/j.annonc.2020.11.014.

[6] S. K. Kumar *et al.*, "NCCN Guidelines Version 1.2023 Multiple Myeloma NCCN Guidelines Index Table of Contents Discussion NCCN Guidelines Panel Disclosures ξ Bone marrow transplantation ‡ Hematology Þ Internal medicine † Medical oncology ¥ Patient advocacy * Discussion section ," 2022, Accessed: Oct. 23, 2022. [Online]. Available: https://www.nccn.org/.

[7] M. Dimopoulos *et al.*, "Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma," *N. Engl. J. Med.*, vol. 357, no. 21, pp. 2123–2132, Nov. 2007, doi: 10.1056/NEJMOA070594.

[8] D. S. Siegel *et al.*, "Improvement in Overall Survival With Carfilzomib, Lenalidomide, and Dexamethasone in Patients With Relapsed or Refractory Multiple Myeloma," *J. Clin. Oncol.*, vol. 36, no. 8, pp. 728–734, Mar. 2018, doi: 10.1200/JCO.2017.76.5032.

[9] P. Moreau *et al.*, "Oral Ixazomib, Lenalidomide, and Dexamethasone for Multiple Myeloma," *N. Engl. J. Med.*, vol. 374, no. 17, pp. 1621–1634, Apr. 2016, doi: 10.1056/NEJMOA1516282.

[10] N. J. Bahlis *et al.*, "Daratumumab plus lenalidomide and dexamethasone in relapsed/refractory multiple myeloma: extended follow-up of POLLUX, a randomized, open-label, phase 3 study," *Leukemia*, vol. 34, no. 7, pp. 1875–1884, Jul. 2020, doi: 10.1038/S41375-020-0711-6.

[11] M. A. Dimopoulos *et al.*, "Elotuzumab, lenalidomide, and dexamethasone in RRMM: final overall survival results from the phase 3 randomized ELOQUENT-2 study," *Blood Cancer J.*, vol. 10, no. 9, Sep. 2020, doi: 10.1038/S41408-020-00357-4.

[12] D. S. Siegel *et al.*, "Pomalidomide plus low-dose dexamethasone in relapsed refractory multiple myeloma after lenalidomide treatment failure," *Br. J. Haematol.*, vol. 188, no. 4, pp. 501–510, Feb. 2020, doi: 10.1111/BJH.16213.

[13] M. A. Dimopoulos *et al.*, "Daratumumab plus pomalidomide and dexamethasone versus pomalidomide and dexamethasone alone in previously treated multiple myeloma (APOLLO): an open-label, randomised, phase 3 trial," *Lancet. Oncol.*, vol. 22, no. 6, pp. 801–812, Jun. 2021, doi: 10.1016/S1470-2045(21)00128-5.

[14] P. G. Richardson *et al.*, "Isatuximab plus pomalidomide and low-dose dexamethasone versus pomalidomide and low-dose dexamethasone in patients with relapsed and refractory multiple myeloma (ICARIA-MM): follow-up analysis of a randomised, phase 3 study," *Lancet Oncol.*, vol. 23, no. 3, pp. 416–427, Mar. 2022, doi: 10.1016/S1470-2045(22)00019-5.

[15] M. A. Dimopoulos *et al.*, "Elotuzumab plus Pomalidomide and Dexamethasone for Multiple Myeloma," *N. Engl. J. Med.*, vol. 379, no. 19, pp. 1811–1822, Nov. 2018, doi: 10.1056/NEJMOA1805762/SUPPL_FILE/NEJMOA1805762_DATA-SHARING.PDF.

[16] P. G. Richardson *et al.*, "Pomalidomide, bortezomib, and dexamethasone for patients with relapsed or refractory multiple myeloma previously treated with lenalidomide (OPTIMISMM): a randomised, open-label, phase 3 trial," *Lancet. Oncol.*, vol. 20, no. 6, pp. 781–794, Jun. 2019, doi: 10.1016/S1470-2045(19)30152-4.

[17] J. J. Shah *et al.*, "Carfilzomib, pomalidomide, and dexamethasone for relapsed or refractory myeloma," *Blood*, vol. 126, no. 20, pp. 2284–2290, Nov. 2015, doi: 10.1182/BLOOD-2015-05-643320.

[18] A. Krishnan *et al.*, "Phase I/II trial of the oral regimen ixazomib, pomalidomide, and dexamethasone in relapsed/refractory multiple myeloma," *Leukemia*, vol. 32, no. 7, pp. 1567–1574, Jul. 2018, doi: 10.1038/S41375-018-0038-8.

[19] L. Garderet *et al.*, "Pomalidomide, cyclophosphamide, and dexamethasone for relapsed multiple myeloma," *Blood*, vol. 132, no. 24, pp. 2555–2563, Dec. 2018, doi: 10.1182/BLOOD-2018-07-863829.

[20] H. Quach *et al.*, "Mechanism of action of immunomodulatory drugs (IMiDS) in multiple myeloma," *Leukemia*, vol. 24, no. 1, p. 22, 2010, doi: 10.1038/LEU.2009.236.

[21] S. A. Holstein and P. L. McCarthy, "Immunomodulatory Drugs in Multiple Myeloma: Mechanisms of Action and Clinical Experience," *Drugs 2017 775*, vol. 77, no. 5, pp. 505–520, Feb. 2017, doi: 10.1007/S40265-017-0689-1.

[22] T. Ito *et al.*, "Identification of a primary target of thalidomide teratogenicity," *Science*, vol. 327, no. 5971, pp. 1345–1350, Mar. 2010, doi: 10.1126/SCIENCE.1177319.

[23] G. Lu *et al.*, "The myeloma drug lenalidomide promotes the cereblon-dependent destruction of ikaros proteins," *Science (80-.).*, vol. 343, no. 6168, pp. 305–309, Jan. 2014, doi: 10.1126/SCIENCE.1244917/SUPPL_FILE/LU.SM.PDF.

[24] J. Krönke *et al.*, "Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells," *Science (80-.).*, vol. 343, no. 6168, pp. 301–305, Jan. 2014, doi: 10.1126/SCIENCE.1244851/SUPPL_FILE/KRONKE-SM-REV.PDF.

[25] C. C. Bjorklund *et al.*, "Rate of CRL4CRBN substrate Ikaros and Aiolos degradation underlies differential activity of lenalidomide and pomalidomide in multiple myeloma cells by regulation of c-Myc and IRF4," *Blood Cancer J. 2015*

510, vol. 5, no. 10, pp. e354–e354, Oct. 2015, doi: 10.1038/bcj.2015.66.

[26] A. K. Gandhi *et al.*, "Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4CRBN," *Br. J. Haematol.*, vol. 164, no. 6, pp. 811–821, Mar. 2014, doi: 10.1111/BJH.12708.

[27] Y. X. Zhu *et al.*, "Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide," *Blood*, vol. 118, no. 18, pp. 4771–4779, Nov. 2011, doi: 10.1182/BLOOD-2011-05-356063.

[28] A. Lopez-Girona *et al.*, "Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide," *Leukemia*, vol. 26, no. 11, pp. 2326–2335, 2012, doi: 10.1038/LEU.2012.119.

[29] A. Broyl *et al.*, "High cereblon expression is associated with better survival in patients with newly diagnosed multiple myeloma treated with thalidomide maintenance," *Blood*, vol. 121, no. 4, pp. 624–627, Jan. 2013, doi: 10.1182/BLOOD-2012-06-438101.

[30] D. Heintel *et al.*, "High expression of cereblon (CRBN) is associated with improved clinical response in patients with multiple myeloma treated with lenalidomide and dexamethasone," *Br. J. Haematol.*, vol. 161, no. 5, pp. 695–700, Jun. 2013, doi: 10.1111/BJH.12338.

[31] I. Misiewicz-Krzeminska *et al.*, "Quantitative expression of Ikaros, IRF4, and PSMD10 proteins predicts survival in VRD-treated patients with multiple myeloma," *Blood Adv.*, vol. 4, no. 23, pp. 6023–6033, Dec. 2020, doi: 10.1182/BLOODADVANCES.2020002711.

STRESZCZENIE

Leki immunomodulujące (ang. *immunomodulatory drugs*, IMiDs) stanowią podstawę schematów terapeutycznych stosowanych w leczeniu nowo rozpoznanego i nawrotowego szpiczaka plazmocytowego (ang. *multiple myeloma*, MM). Istotny postęp, jaki dokonał się w leczeniu chorych na MM w ciągu ostatnich dwóch dekad jest przypisywany m.in. wprowadzeniu do rutynowej praktyki klinicznej talidomidu oraz jego nowszych pochodnych, tj. lenalidomidu i pomalidomidu. Jedyny dotychczas opisany, molekularny mechanizm działania IMiDs polega na bezpośredniej interakcji z białkiem cereblon (CRBN), które współtworzy kompleks o aktywności ligazy E3 ubikwityny ($CRL4^{CRBN}$). W wyniku przyłączenia IMiDs do CRBN dochodzi do zmiany profilu białek, które są ubikwitynowane przez $CRL4^{CRBN}$ i kierowane na drogę protasomalnej degradacji. Pod wpływem IMiDs dochodzi do nasilonej proteasomalnej degradacji czynników transkrypcyjnych (IZKF1, IKZF3) kluczowych dla przeżycia i proliferacji MM oraz pośrednio modulujących aktywność układu odpornościowego.

Celem niniejszej pracy była ocena zależności pomiędzy ekspresją składowych kompleksu ligazy E3 ubikwityny ($CRL4^{CRBN}$) a przebiegiem klinicznym i rokowaniem u chorych na MM poddanych leczeniu z wykorzystaniem IMiDs.

Za pomocą barwień immunohistochemicznych oceniono ekspresję składowych kompleksu $CRL4^{CRBN}$ oraz białek zależnych od jego aktywności, w archiwalnych trepanobioptatach pobranych od pacjentów przed rozpoczęciem leczenia opartego na IMiDs. Dokonano analizy statystycznej w zakresie uzyskanych wyników immunohistochemicznych oraz danych laboratoryjnych i klinicznych dotyczących pacjentów leczonych IMiDs.

Do badania włączono 130 pacjentów, w tym 81 (62%) pacjentów leczeniu pierwszej linii z wykorzystaniem talidomidu i 49 (38%) pacjentów, którzy otrzymali leczenie oparte na lenalidomidzie. Zidentyfikowano obecność białek CRBN, CUL4A, DDB1, IKZF1 oraz IKZF3 odpowiednio w 54%, 51%, 49%, 71% i 54% analizowanych trepanobioptatach. W wyniku przeprowadzonej analizy statystycznej stwierdzono zależność pomiędzy ekspresją białek CRBN i DDB1 a parametrami klinicznymi odpowiadającymi wyższemu zaawansowaniu MM w momencie rozpoczęcia leczenia IMiDs. Dodatkowo potwierdzono, że ekspresja CRBN koreluje z uzyskiwaną odpowiedzią na leczenie ($\geq$PR vs. <PR, p=0,012; $\geq$VGPR vs. <VGPR, p=0,032) oraz zidentyfikowano po raz pierwszy tożsamą zależność dla ekspresji CUL4A ($\geq$ PR vs. <PR, p=0,007; $\geq$VGPR vs. <VGPR p=0,027). Ekspresja CUL4A charakteryzowała również grupę pacjentów z mniejszym ryzykiem progresji MM (HR=0,66; 95% CI 0,44--0,99; p=0,046). W analizie jednoczynnikowej zidentyfikowano niekorzystne czynniki prognostyczne dla OS (ekspresja DDB1, obecność zmian osteolitycznych, starszy wiek pacjenta oraz wyższe wyjściowo stężenie β2-mikroglobuliny we krwi obwodowej). W analizie wieloczynnikowej potwierdzono niezależny, niekorzystny wpływ stężenia

β2-mikroglobuliny, obecności zmian osteolitcznych oraz ekspresji DDB1 na prawdopodobieństwo całkowitego przeżycia chorych na MM leczonych IMiDs.

Uzyskane wyniki przemawiają za predykcyjnym znaczeniem ekspresji CRBN i CUL4A oraz prognostycznym znaczeniem ekspresji DDB1 u chorych na MM leczonych IMiDs oraz mogą w przyszłości przyczynić się do identyfikacji pacjentów, którzy odniosą największą korzyść z leczenia opartego na aktywności kompleksu $CRL4^{CRBN}$.

ABSTRACT

The impact of the E3 ubiquitin ligase complex components on the clinical course and prognosis of patients with multiple myeloma treated with immunomodulatory drugs.

Immunomodulatory drugs (IMiDs) are the backbone of most combination regimens used in the treatment of multiple myeloma (MM) in newly diagnosed and relapsed or refractory settings. The introduction of thalidomide, a first-in-class IMiD, along with its newer derivatives (lenalidomide and pomalidomide), was a milestone in MM therapy leading to a significant improvement in patients' prognosis. Nowadays, it is known that IMiDs exert their anti-myeloma activity mainly by binding cereblon (CRBN), the substrate receptor protein of the CRL4 E3 ubiquitin ligase ($CRL4^{CRBN}$) complex. By binding CRBN, IMiDs alter their substrate specificity, leading to the change of proteins profile, which is ubiquitinated by $CRL4^{CRBN}$ and subsequently degraded by the proteasome. IMiDs lead to the enhanced degradation of transcriptional factors (IKZF1, IKZF3), which are crucial for MM survival and indirectly modulate immune system activity.

The study aimed to analyze the association between the expression of $CRL4^{CRBN}$ complex proteins and the clinical course of MM patients undergoing IMiDs-based treatment.

We evaluated the expression of $CRL4^{CRBN}$ complex proteins and their downstream targets with immunohistochemistry (IHC) staining in 130 bone marrow samples from MM patients treated with thalidomide (n=81, 62%) or lenalidomide-based (n=49, 38%) regimens. According to predefined cut-off values, the positivity (+) of CRBN, CUL4A, DDB1, IKZF1 and IKZF3 was observed in 54%, 51%, 49%, 71% and 54% of cases, respectively. We found that expression of CRBN and DDB1 was associated with higher burden of the MM at the initiation of IMiDs therapy. We confirmed that CRBN expression correlates with response to IMIDs treatment ($\geq$ PR vs. < PR, p=0,012; $\geq$ VGPR vs. < VGPR, p=0,032). The expression of CUL4A was also associated with response rate ($\geq$ PR vs. < PR, p=0,007; $\geq$ VGPR vs. < VGPR p=0,027) and improved PFS (HR = 0.66, 95% CI 0.44–0.99; p = 0.046). Moreover, DDB1 expression along with β2-microglobulin serum concentration and presence of the osteolytic lesions, showed a negative impact on OS both in univariate and multivariate analysis.

The study's results suggest that expression of CRBN, CUL4A and DDB1 may predict the clinical course of MM patients and identify patients with a high probability of response to IMiD-based therapy. These novel findings are the starting point for future research in personalized therapeutic approaches based on $CRL4^{CRBN}$ activity.

OŚWIADCZENIA WSPÓŁAUTORÓW

Warszawa, dn. 03.10.2022 r.

lek. Joanna Barankiewicz
Klinika Hematologii
Instytut Hematologii i Transfuzjologii
02-776 Warszawa, ul. Indiry Gandhi 14

OŚWIADCZENIE

Oświadczam, że w publikacji: Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683, mój wkład w przygotowanie pracy polegał na:

- współudziale w planowaniu metodyki badania
- przeprowadzeniu przeglądu literatury
- współudziale w realizacji zadań badawczych
- współudziale w gromadzeniu danych klinicznych
- systematyzacji uzyskanych wyników i zebranych danych
- współudziale w analizie statystycznej
- współudziale w interpretacji wyników przeprowadzonych badań
- napisaniu pierwotnej wersji manuskryptu
- współudziale w edycji manuskryptu
- wysłaniu manuskryptu do redakcji oraz odpowiedzi na uzyskane recenzje
- akceptacji ostatecznej formy manuskryptu

Oświadczam, że w publikacji: Barankiewicz J, Salomon-Perzyński A, Misiewicz-Krzemińska I, Lech-Marańda E. CRL4^{CRBN} E3 Ligase Complex as a Therapeutic Target in Multiple Myeloma. Cancers (Basel). 2022;14(18):4492. doi:10.3390/cancers14184492, mój wkład w przygotowanie pracy polegał na:

- zaplanowaniu tematyki pracy
- współudziale w przeprowadzeniu przeglądu literatury
- napisaniu pierwotnej wersji manuskryptu
- współudziale w edycji manuskryptu
- wysłaniu manuskryptu do redakcji oraz odpowiedzi na uzyskane recenzje
- akceptacji ostatecznej formy manuskryptu

podpis

Warszawa, dnia 03.10.2022 r.

dr hab. n. med. Anna Szumera-Ciećkiewicz[1,2]

[1]Zakład Diagnostyki Hematologicznej

Instytut Hematologii i Transfuzjologii

02-776 Warszawa, ul. Indiry Gandhi 14

[2]Zakład Patomorfologii Nowotworów

Narodowy Instytut Onkologii im. Marii Skłodowskiej – Curie

- Państwowy Instytut Badawczy

02-781 Warszawa, ul. W.K. Roentgena 5

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w przeprowadzeniu zadań badawczych
- współudziale w edycji manuskryptu
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

Anna Szumera-Ciećkiewicz

.....

Podpis

Warszawa, dn. 03.10.2022 r.

lek. Aleksander Salomon-Perzyński

Klinika Hematologii

Instytut Hematologii i Transfuzjologii

02-776 Warszawa, ul. Indiry Gandhi 14

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w gromadzeniu danych klinicznych,
- współudziale w edycji manuskryptu,
- akceptacji ostatecznej formy manuskryptu

Oświadczam, że w publikacji:

Barankiewicz J, Salomon-Perzyński A, Misiewicz-Krzemińska I, Lech-Marańda E. CRL4CRBN E3 Ligase Complex as a Therapeutic Target in Multiple Myeloma. Cancers (Basel). 2022;14(18):4492. Published 2022 Sep 16. doi:10.3390/cancers14184492

mój wkład w przygotowanie pracy polegał na:

- współudziale w przeprowadzeniu przeglądu literatury,
- współudziale w edycji manuskryptu,
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

.....

Podpis

Warszawa, dn. 03.10.2022 r.

dr n. med. Paulina Wieszczy

Klinika Gastroenterologii, Hepatologii i Onkologii Klinicznej

Centrum Medyczne Kształcenia Podyplomowego

02-781 Warszawa, ul. Roentgena 5

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w analizie statystycznej danych
- współudziale w edycji manuskryptu
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

Podpis

Warszawa, dn. 03.10.2022r.

dr n. med. Agata Malenda

Oddział Chorób Wewnętrznych z Pododdziałem Geriatrycznym

Szpital Zachodni im. Św. Jana Pawła II

05-825 Grodzisk Mazowiecki, ul. Daleka 11

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w planowaniu metodyki badania
- współudziale w gromadzeniu danych klinicznych
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

Agata Malenda

.....

Podpis

Warszawa, dn. 05.10.2022 r.

lek. Filip Garbicz

Zakład Hematologii Eksperymentalnej

Instytut Hematologii i Transfuzjologii

02-776 Warszawa, ul. Indiry Gandhi 14

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w realizacji zadań badawczych,
- współudziale w edycji manuskryptu,
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

Signed by / Podpisano przez: Filip Aleksander Garbicz

Date / Data: 2022-10-05 20:17

Podpis

Warszawa, dn. 03.10.2022 r.

prof. dr hab. n. med. Monika Prochorec-Sobieszek

Zakład Diagnostyki Hematologicznej

Instytut Hematologii i Transfuzjologii

02-776 Warszawa, ul. Indiry Gandhi 14

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w przeprowadzeniu zadań badawczych
- współudziale w edycji manuskryptu
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

Podpis

Warszawa, dn. 03.10.2022 r.

dr hab. Irena Misiewicz-Krzemińska

Profesor Instytutu

Zakład Hematologii Eksperymentalnej

Instytut Hematologii i Transfuzjologii

02-776 Warszawa, ul. Indiry Gandhi 14

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w interpretacji wyników
- współudziale w edycji manuskryptu
- akceptacji ostatecznej formy manuskryptu

Oświadczam, że w publikacji:

Barankiewicz J, Salomon-Perzyński A, Misiewicz-Krzemińska I, Lech-Marańda E. $CRL4^{CRBN}$ E3 Ligase Complex as a Therapeutic Target in Multiple Myeloma. Cancers (Basel). 2022;14(18):4492. Published 2022 Sep 16. doi:10.3390/cancers14184492

mój wkład w przygotowanie pracy polegał na:

- współudziale w przeprowadzeniu przeglądu literatury
- współudziale w edycji manuskryptu
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

.

Irena Misiewicz-Krzemińska

Elektronicznie podpisany przez Irena Misiewicz-Krzemińska
Data: 2022.10.03 12:19:33 +02'00'

Podpis

Warszawa, dn. 03.10.2022 r.

prof. dr hab. n. med. Przemysław Juszczyński

Zakład Hematologii Eksperymentalnej

Instytut Hematologii i Transfuzjologii

02-776 Warszawa, ul. Indiry Gandhi 14

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w planowaniu zadań badawczych,
- współudziale w edycji manuskryptu,
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

Przemysław Juszczyński

Digitally signed by Przemysław Juszczyński
DN: c=PL, serialNumber=PNOPL-75031506452, cn=Przemysław Juszczyński, givenName=Przemysław, sn=Juszczyński
Date: 2022.10.10 15:11:16 +02'00'

Podpis

Warszawa, dn. 03.10.2022 r.

prof. dr hab. n. med. Ewa Lech-Marańda

Klinika Hematologii

Instytut Hematologii i Transfuzjologii

02-776 Warszawa, ul. Indiry Gandhi 14

OŚWIADCZENIE

Oświadczam, że w publikacji:

Barankiewicz, J.; Szumera-Ciećkiewicz, A.; Salomon-Perzyński, A.; Wieszczy, P.; Malenda, A.; Garbicz, F.; Prochorec-Sobieszek, M.; Misiewicz-Krzemińska, I.; Juszczyński, P.; Lech-Marańda, E. The CRBN, CUL4A and DDB1 Expression Predicts the Response to Immunomodulatory Drugs and Survival of Multiple Myeloma Patients. J. Clin. Med. 2021, 10, 2683. https://doi.org/10.3390/jcm10122683

mój wkład w przygotowanie pracy polegał na:

- współudziale w planowaniu metodyki badania
- współudziale w interpretacji wyników przeprowadzonych badań
- współudziale w edycji manuskryptu
- akceptacji ostatecznej formy manuskryptu

Oświadczam, że w publikacji:

Barankiewicz J, Salomon-Perzyński A, Misiewicz-Krzemińska I, Lech-Marańda E. $CRL4^{CRBN}$ E3 Ligase Complex as a Therapeutic Target in Multiple Myeloma. Cancers (Basel). 2022;14(18):4492. Published 2022 Sep 16. doi:10.3390/cancers14184492

mój wkład w przygotowanie pracy polegał na:

- współudziale w edycji manuskryptu
- akceptacji ostatecznej formy manuskryptu

Jednocześnie wyrażam zgodę na przedłożenie przez lek. Joannę Barankiewicz ww. publikacji do rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów naukowych.

Signed by / Podpisano przez:
Ewa Lech-Marańda
Date / Data: 2022-10-03 09:05
podpis