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Wpływ mikrobioty jelitowej na leczenie i jego powikłania u chorych na szpiczaka plazmocytowego

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu
w dyscyplinie nauki medyczne

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Spis treści

1. Wykaz publikacji

[1] Jasiński M, Biliński J, Maciejewska M, Ostrowska K, Rusicka-Krzewska P, Konarski W, Podsiadły E, Snarski E, Basak GW. Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma. Scientific Reports. 2024 Dec 28;14(1):31221.

IF 3.8 MNiSW 140 pkt

[2] Jasiński M, Maciejewska M, Brodziak A, Górka M, Skwierawska K, Jędrzejczak WW, Tomaszewska A, Basak GW, Snarski E. Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation. Scientific reports. 2021 Nov 18;11(1):22507.

IF 4.997 MNiSW 140 pkt

[3] Jasiński M, Biliński J, Basak GW. The role of the crosstalk between gut microbiota and immune cells in the pathogenesis and treatment of multiple myeloma. Frontiers in Immunology. 2022 Mar 31;13:853540.

IF 7.3 MNiSW 140 pkt

[4] Jasiński M, Biliński J, Basak GW. The role of the gut microbiome in pathogenesis, biology, and treatment of plasma cell dyscrasias. Frontiers in oncology. 2021 Oct 1;11:741376.

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2. Wykaz stosowanych skrótów

Skrót	Rozwinięcie w j. angielskim	Rozwinięcie w j. polskim
NGS	Next generation sequencing	Sekwencjonowanie następnej generacji
MM	Multiple myeloma	Szpiczak plazmocytowy
Auto-SCT	Autologous stem cell transplantation	Autologiczne przeszczepienie komórek krwiotwórczych
SCFA	Short-chain fatty acids	Krótkołańcuchowe kwasy tłuszczowe
Treg	T regulatory cells	Komórki T regulatorowe
IgH	Immunoglobulin Heavy-chain variable region	Rejon zmienny immunoglobulin
ARB	Antibiotic-resistant bacteria	Bakterie oporne na antybiotyki
OS	Overall Survival	Całkowity czas przeżycia
PFS	Progression-Free Survival	Czas przeżycia wolny od progresji
MRD	Minimal Residual Disease	Minimalna choroba resztkowa
GvHD	Graft-Versus-Host Disease	Choroba „przeszczep przeciwko gospodarzowi”
FMT	Fecal Microbiota Transplantation	Przeszczep mikrobioty kałowej
CRAB	Calcium, Renal failure, Anemia, Bone lesions	Hiperkalcemia, niewydolność nerek, anemia, zmiany kostne
ISS	International Staging System	Międzynarodowy system stopniowania
R-ISS	Revised International Staging System	Zrewidowany międzynarodowy system stopniowania
LDH	Lactate Dehydrogenase	Dehydrogenaza mleczanowa
FLC	Free Light Chains	Wolne łańcuchy lekkie
MRI	Magnetic Resonance Imaging	Rezonans magnetyczny
CT	Computed Tomography	Tomografia komputerowa
PET-CT	Positron Emission Tomography–Computed Tomography	Pozytonowa tomografia emisyjna połączona z tomografią komputerową
MGUS	Monoclonal Gammopathy of Undetermined Significance	Gammapatia monoklonalna o nieustalonym znaczeniu
SMM	Smoldering Multiple Myeloma	Szpiczak tlący się
BCMA	B-Cell Maturation Antigen	Antygen dojrzewania limfocytów B
CAR-T	Chimeric Antigen Receptor T-cell therapy	Terapia limfocytami T z chimerycznym receptorem antygenu
DRd	Daratumumab, Lenalidomide, Dexamethasone	Daratumumab, lenalidomid, deksametazon
Dara-VRd	Daratumumab, Bortezomib, Lenalidomide, Dexamethasone	Daratumumab, bortezomib, lenalidomid, deksametazon
Dara-VTD		Daratumumab, bortezomib, talidomid, deksametazon

Daratumumab, Bortezomib, Thalidomide,
Dexamethasone

3. Streszczenie w języku polskim

Mikrobiota jelitowa stanowi ogół mikroorganizmów (bakterii, archeonów, wirusów, grzybów i innych drobnoustrojów) zasiedlających przewód pokarmowy człowieka lub innego organizmu. Z kolei mikrobiom jelitowy jest to zbiór materiału genetycznego wszystkich mikroorganizmów obecnych w jelitach, obejmujący ich geny i produkty genowe.

W ostatnich dekadach rozwój badań nad mikrobiotą jelitową doprowadził do przełomowego zrozumienia roli, jaką odgrywa zespół drobnoustrojów zamieszkujących jelita w kształtowaniu odpowiedzi immunologicznej i w etiopatogenezie wielu chorób, w tym nowotworów hematologicznych. Pierwsze obserwacje wskazujące na związek między składnikami mikrobioty a układem immunologicznym pojawiły się już na początku XXI wieku, jednak dopiero wraz z rozwojem technik sekwencjonowania następnej generacji (NGS) i metagenomiki możliwe stało się precyzyjne scharakteryzowanie różnorodności gatunkowej oraz funkcjonalnej mikrobioty jelitowej u pacjentów.

W przypadku szpiczaka plazmocytowego (MM), nowotworu wywodzącego się z komórek plazmatycznych, badania mikrobioty jelitowej wciąż znajdują się na wczesnym etapie. Dotychczasowe prace dotyczyły analizy zmian mikrobiomu pod wpływem zastosowanego leczenia oraz wpływ tych zmian na rokowania chorych. U chorych z MM, młodszych i w dobrej formie klinicznej uznaną metodą leczenia jest konsolidacja uzyskanej remisji poprzez zastosowanie wysokodawkowanej chemioterapii wspomaganej podaniem autologicznych komórek krwiotwórczych (auto-SCT).

Niniejsza rozprawa doktorska składa się z cyklu czterech powiązanych tematycznie publikacji naukowych, ukazujących kompleksowe oddziaływanie mikrobioty jelitowej na progresję choroby, wyniki leczenia i powikłania – w szczególności powikłania związane z auto-SCT.

Rola mikrobioty jelitowej w kształtowaniu odpowiedzi immunologicznej jest dobrze udokumentowana w chorobach zapalnych jelit oraz chorobach autoimmunologicznych. Liczne badania eksperymentalne wykazały, że krótkołańcuchowe kwasy tłuszczowe (SCFA), produkty fermentacji wytwarzane przez bakterie z rodzajów *Clostridium* i *Bacteroides*, pełnią kluczową funkcję w utrzymaniu integralności bariery jelitowej, stymulują produkcję cytokin przeciwzapalnych (m.in. IL-10) oraz wspomagają różnicowanie komórek Treg (komórki regulatorowe), co przeciwdziała nadmiernej aktywacji limfocytów Th17. Z drugiej strony, przewlekła dysbioza charakteryzuje się często spadkiem populacji bakterii produkujących

SCFA i wzrostem bakterii z rodzajów *Enterobacteriaceae* czy *Streptococcus*. Prowadzi to do przewlekłego stanu zapalnego błony śluzowej, nadmiernej produkcji cytokin prozapalnych (m.in. IL-6, TNF-α) oraz potencjalnego stymulowania proliferacji klonalnej limfocytów B. W przypadku MM jest to szczególnie istotne, ponieważ rozwój choroby poprzedza stan MGUS (gammopatia monoklonalna o nieustalonym znaczeniu), w którym klonalne plazmocyty ulegają kolejnym mutacjom w rejonach zmiennych immunoglobulin (IgH) w odpowiedzi na antygeny zewnętrzne. Istnieje hipoteza wskazująca na możliwość inicjacji bądź przyspieszenia progresji choroby poprzez antygeny bakteryjne dostępne w świetle przewodu pokarmowego.

Cykl publikacji zawiera dwie prace oryginalne i dwie prace poglądowe.

W pierwszej pracy pt. *Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma* (https://doi.org/10.1038/s41598-024-82589-z) pokazano wpływ kolonizacji przewodu pokarmowego przez bakterie antybiotykooporne (ARB) przed auto-SCT na ryzyko powikłań infekcyjnych po wykonaniu procedury. Według obecnych danych kolonizacja ARB jest markerem zubożonej mikrobioty jelitowej. Ta z kolei może być zaangażowana w rozwój i progresję dyskrazji plazmocytowych. Praca przedstawia analizy przeprowadzone na 138 pacjentach, którzy zostali poddani łącznie 141 procedurom auto-SCT. Pacjenci skolonizowani ARB w przewodzie pokarmowym mieli znacznie wyższe ryzyko infekcji we wczesnym okresie po auto-SCT w porównaniu do grupy bez kolonizacji (52 vs. 26%, P = 0,02). Pomimo braku istotności statystycznej, zaobserwowano tendencję do krótszego przeżycia całkowitego (OS) w grupie pacjentów skolonizowanych.

Kolejna praca pt. *Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation* (https://doi.org/10.1038/s41598-021-02002-x) przedstawia rolę stosowania lodów jako profilaktyki rozwoju toksycznego uszkodzenia śluzówek jamy ustnej po auto-SCT. Autorzy przeprowadzili retrospektywną analizę 74 pacjentów, dowodząc, że spożywanie lodów podczas infuzji wysokodawkowanego melfalanu pozwala znacząco zmniejszyć odsetek uszkodzenia śluzówek jamy ustnej (OM). Metoda ta jest lepiej tolerowana niż tradycyjne płukanie ust zimnymi płynami. Częstość występowania OM w grupie otrzymującej lody wyniosła 28,84%, podczas gdy w grupie, która lodów nie otrzymywała wyniosła 59,09%. Prawdopodobnie zmniejszenie częstości występowania OM wpłynęło na skrócenie okresu hospitalizacji i obniżenie kosztów leczenia powikłań. Wspomniana praca wskazuje szerzej na wpływ roli integralności śluzówki przewodu pokarmowego na częstość występowania powikłań po auto-

SCT. Mikrobiota jelitowa jest jednym z kluczowych czynników dla zachowania integralności bariery jelito-krew.

W kolejnej pracy pt. *The Role of the Crosstalk Between Gut Microbiota and Immune Cells in the Pathogenesis and Treatment of Multiple Myeloma* (doi: 10.3389/fimmu.2022.853540) szczegółowo opisano rolę mikrobioty jelitowej i jej zmiany na przestrzeni całego życia, ze szczególnym uwzględnieniem jej wpływu na formowanie układu odpornościowego. W szczególności skupiono się na opisie przewlekłej stymulacji antygenowej limfocytów B jako mechanizmu rozwoju MM. Ukazano złożony system interakcji pomiędzy limfocytami B, mikrobiotą, komórkami dendrytycznymi, makrofagami, neutrofilami, komórkami plazmatycznymi i komórkami nabłonka jelit. Wskazana publikacja podsumowuje dotychczasową wiedzę na temat czynników inicjujących rozwój MM podkreślając rolę jaką pełnią mikroorganizmy w tym procesie.

Celem kolejnej pracy pt. *The Role of the Gut Microbiome in Pathogenesis, Biology, and Treatment of Plasma Cell Dyscrasias* (doi: 10.3389/fonc.2021.741376) było pokazanie czynników odpowiedzialnych za progresję dyskrazji plazmocytowych na szlaku od MGUS, przez SMM (szpiczak tlący się) ostatecznie do MM. Dodatkowo wskazano na wpływ mikrobioty jelitowej na odpowiedź na terapie u chorych z MM. Przedyskutowano rolę mikrobioty w rozwoju i progresji MM, odpowiedzi na leczenie u pacjentów z MM oraz potencjalną rolę przeszczepienia mikrobioty jelitowej (FMT) jako procedury modyfikującej ryzyko progresji bądź uwrażliwienia opornych na leczenie komórek.

Przedstawione wyniki prac oryginalnych i podsumowanie stanu wiedzy na czas opublikowania prac poglądowych przedstawiają razem spójny model wieloetapowego wpływu mikrobioty jelitowej na przebieg MM. Pierwszym etapem jest ukształtowanie mikrobiomu jelitowego na najwcześniejszych etapach życia i jego wpływ na funkcjonowanie układu immunologicznego w późniejszych etapach. Drugim – przewlekła stymulacja antygenowa limfocytów B antygenami bakteryjnymi i tworzenie mikrośrodowiska sprzyjającego rozwoju MGUS i progresji z MGUS do MM. Trzecim – promowanie określonego profilu wydzielanych cytokin i metabolitów w trakcie leczenia co wpływa na jego wyniki i rokowania chorych. Czwartym – potencjalny wpływ modyfikacji mikrobioty jelitowej na redukcję powikłań auto-SCT bądź nawet profilaktykę progresji MGUS do MM.

Podsumowując, niniejszy cykl badań stanowi wkład w zrozumienie roli mikrobioty jelitowej w patogenezie, leczeniu i powikłaniach MM oraz wskazuje na praktyczne interwencje, które mogą znacząco poprawić wyniki leczenia i jakość życia chorych. Przedstawione powyżej prace łączą badania podstawowe z klinicznymi zastosowaniami ich wyników.

4. Streszczenie w języku angielskim (wraz z angielską wersją tytułu rozprawy)

The impact of the gut microbiota on the treatment and complications in patients with multiple myeloma

Gut microbiota contains the community of microorganisms (bacteria, archaea, viruses, fungi, and other microbes) that inhabit the gastrointestinal tract of a human or other organism. On the other hand, gut microbiome is the collective genetic material of all the microorganisms present in the gut, including their genes and gene products.

In recent decades, the development of research on the gut microbiota has led to a breakthrough understanding of the role played by the community of microorganisms inhabiting the intestines in shaping the immune response and in the etiopathogenesis of many diseases, including hematologic malignancies. The first observations indicating a link between elements of the microbiota and the immune system appeared as early as the beginning of the 21st century, yet only with the advent of next-generation sequencing (NGS) techniques and metagenomics it became possible to precisely characterize the species-level and functional diversity of the gut microbiota in patients.

In the case of multiple myeloma (MM), a malignancy originating from plasma cells, studies of the gut microbiota are still at an early stage. The work conducted so far has concerned analysis of microbiome changes under the influence of therapy and the impact of those changes on patient prognosis. In MM patients who are younger and in good clinical condition, consolidation of the achieved remission with high-dose chemotherapy with subsequent administration of autologous hematopoietic stem cells (auto-SCT) is an established standard treatment.

This doctoral dissertation consists of a cycle of four thematically related scientific publications, showing the comprehensive influence of the gut microbiota on disease progression, treatment outcomes and complications—particularly complications associated with auto-SCT.

The role of the gut microbiota in shaping the immune response is well documented in inflammatory bowel diseases and autoimmune disorders. Numerous experimental studies have demonstrated that short-chain fatty acids (SCFAs), fermentation products of bacteria from the genera *Clostridium* and *Bacteroides*, play a key role in maintaining intestinal-barrier integrity, stimulate the production of anti-inflammatory cytokines (including IL-10) and support the differentiation of regulatory T cells (Tregs), which counteracts excessive activation of Th17

lymphocytes. Conversely, chronic dysbiosis is often marked by a decline in SCFA-producing bacteria and an increase in bacteria from the genera *Enterobacteriaceae* or *Streptococcus*. This leads to chronic mucosal inflammation, excessive production of pro-inflammatory cytokines (including IL-6, TNF-α) and potential stimulation of clonal B-cell proliferation. In MM this is particularly important, because disease development is preceded by MGUS (monoclonal gammopathy of undetermined significance), in which clonal plasma cells undergo further mutations in immunoglobulin heavy-chain variable regions (IgH) in response to external antigens. A hypothesis has been put forward that bacterial antigens present in the gastrointestinal lumen may initiate or accelerate disease progression.

The publication cycle contains two review papers and two original research articles.

In the first paper, entitled „*Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma*" (https://doi.org/10.1038/s41598-024-82589-z), the impact of colonization of the gastrointestinal tract by antibiotic-resistant bacteria (ARB) before auto-SCT on the risk of infectious complications after the procedure was demonstrated. According to current literature, ARB colonization is a marker of a depleted gut microbiota that, as other studies suggest, may be involved in the development and progression of plasma-cell dyscrasias. The paper analyzes 138 patients who underwent a total of 141 auto-SCT procedures. Patients colonized with ARB in the gastrointestinal tract had a markedly higher risk of infection after auto-SCT compared with the non-colonized group (52% vs 26%, P = 0.02). Although not statistically significant, a tendency toward shorter overall survival (OS) was observed in the colonized group.

The next paper entitled "*Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation*" (https://doi.org/10.1038/s41598-021-02002-x), presents the role of using ice-cream as prophylaxis against oral mucositis after auto-SCT. The authors carried out a retrospective analysis of 74 patients, demonstrating that eating ice-cream during the infusion of high-dose melphalan significantly reduces the incidence of oral mucositis (OM). The method is better tolerated than traditional rinsing of the mouth with cold fluids. The incidence of OM in the ice-cream group was 28.84%, whereas in the group that did not receive ice-cream it was 59.09%. The likely reduction in OM frequency shortened hospital stays and lowered the cost of treating complications. This study more broadly highlights the role of gastrointestinal-mucosal integrity in the incidence of complications after auto-SCT. The gut microbiota is one of the key factors in maintaining the blood-intestinal barrier.

The subsequent study, entitled "*The Role of the Crosstalk Between Gut Microbiota and Immune Cells in the Pathogenesis and Treatment of Multiple Myeloma*" (doi: 10.3389/fimmu.2022.853540), details the role of the gut microbiota and its changes throughout life, with particular emphasis on its influence on the formation of the immune system. It focuses especially on chronic antigenic stimulation of B lymphocytes as a mechanism in MM pathogenesis. A complex system of interactions among B lymphocytes, the microbiota, dendritic cells, macrophages, neutrophils, plasma cells and intestinal epithelial cells is presented. This publication summarizes the existing knowledge about factors initiating MM development, underscoring the role microorganisms play in that process.

The next paper, entitled "*The Role of the Gut Microbiome in Pathogenesis, Biology, and Treatment of Plasma Cell Dyscrasias*" (doi: 10.3389/fonc.2021.741376), aimed to show the factors responsible for the progression of plasma-cell dyscrasias along the pathway from MGUS, through SMM (smoldering myeloma), ultimately to MM. In addition, it highlighted the impact of the gut microbiota on therapeutic responses in MM patients. The role of the microbiota in MM development and progression, treatment response in MM patients, and the potential role of fecal microbiota transplantation (FMT) as a procedure that might modify progression risk or resensitize treatment-resistant cells was discussed.

Integration of the results of the original studies and the summaries of current knowledge in the review papers presents a coherent model of the multistage influence of the gut microbiota on the course of MM. The first stage is shaping the gut microbiome at the earliest stages of life and its influence on immune-system functioning throughout life. The second is chronic antigenic stimulation of B lymphocytes by bacterial antigens and the creation of a microenvironment conductive to MGUS development and progression from MGUS to MM. The third is promotion of a specific profile of secreted cytokines and metabolites during treatment, influencing therapy outcomes and patient prognosis. The fourth is the potential impact of modifying the gut microbiota on reducing auto-SCT complications or even on prophylaxis of progression from MGUS to MM.

In summary, this series of studies contributes to understanding the role of the gut microbiota in MM pathogenesis, treatment and its complications, and points to practical interventions that can markedly improve treatment outcomes and patients' quality of life. The works presented above combine basic research with clinical application of their results.

5. Wstęp uzasadniający połączenie wskazanych publikacji w jeden cykl, jak i komentujący osiągniecie naukowe kandydata na tle dotychczasowego stanu wiedzy.

5.1. Szpiczak plazmocytowy.

Szpiczak plazmocytowy (szpiczak mnogi, ang. *multiple myeloma*, MM) to złośliwy nowotwór układu krwiotwórczego wywodzący się z dojrzałych komórek plazmatycznych, odpowiedzialnych za produkcję przeciwciał. W warunkach patologicznych jeden z klonów tych komórek proliferuje w sposób niekontrolowany w szpiku kostnym, prowadząc do nadprodukcji nieprawidłowego białka jednego rodzaju – tzw. białka monoklonalnego (*M-protein*) [1]. Choroba ta powoduje liczne powikłania narządowe, w tym uszkodzenie kości, nerek, układu krwiotwórczego oraz zaburzenia gospodarki wapnia. Charakterystyczne dla zaawansowanego szpiczaka są objawy zgrupowane pod akronimem CRAB (hiperkalcemia, niewydolność nerek, niedokrwistość, zmiany kostne) [2].

Pod względem epidemiologicznym, szpiczak mnogi stanowi około 1% wszystkich nowotworów złośliwych oraz około 10% nowotworów układu krwiotwórczego [3]. Choroba ta występuje głównie u osób starszych – mediana wieku w momencie rozpoznania wynosi 69 lat. Przewagę zachorowań obserwuje się u mężczyzn (ok. 1,5-krotnie częściej niż u kobiet) oraz w populacji afroamerykańskiej, u której ryzyko zachorowania jest ponad dwukrotnie wyższe niż u rasy białej [4]. Czynnikami ryzyka są również występowanie choroby w rodzinie oraz obecność stanów przednowotworowych, takich jak MGUS (gammopatia monoklonalna o nieokreślonym znaczeniu) i tzw. szpiczak „tlący się" (ang. *smoldering multiple myeloma*), które mogą przejść w pełnoobjawowy MM [5].

Proces diagnostyczny rozpoczyna się zazwyczaj od badań laboratoryjnych krwi i moczu. Elektroforeza białek surowicy oraz oznaczenie stężeń wolnych łańcuchów lekkich (FLC) pozwalają na wykrycie obecności białka monoklonalnego. Kluczowe znaczenie ma również biopsja szpiku kostnego, która wykazuje naciek przez ≥10% plazmocytów [6]. Diagnostyka obrazowa, oparta na badaniach MRI, CT lub PET-CT, pozwala na ocenę uszkodzeń kostnych. Obecność objawów CRAB oraz stężenie białka monoklonalnego powyżej 3 g/dL różnicują MM od MGUS i SM [6].

W celu oceny stopnia zaawansowania choroby stosuje się *International Staging System* (ISS) oraz jego zmodyfikowaną wersję (R-ISS), uwzględniającą m.in. stężenie beta-2 mikroglobuliny, albuminy, dehydrogenazy mleczanowej (LDH) oraz obecność niekorzystnych aberracji chromosomalnych [7]. Systemy te pozwalają na określenie rokowania, a pięcioletnie

przeżycie znacznie różni się w zależności od stopnia zaawansowania (od 40% w III stadium do ponad 80% w I stadium według R-ISS) [8].

Leczenie szpiczaka plazmocytowego zależy od stanu klinicznego pacjenta, wieku, chorób współistniejących oraz czynników cytogenetycznych. Standardowa terapia pierwszego rzutu u chorych kwalifikujących się do przeszczepienia autologicznych komórek krwiotwórczych (auto-SCT) obejmuje leczenie indukujące remisję czterema lekami (najczęściej daratumumab, bortezomib, lenalidomid lub talidomid i deksametazon – tzw. schemat Dara-VR(lub T)d), a u pacjentów niekwalifikujących się – obecnie najczęściej schemat oparty na daratumumabie, lenalidomidzie i deksametazonie (DRd) [9]. Leczenie podtrzymujące, najczęściej z użyciem lenalidomidu, pozwala na przedłużenie czasu wolnego od choroby [10]. W przypadku nawrotu choroby stosuje się przeciwciała monoklonalne (np. daratumumab, elotuzumab), inhibitory proteasomu (karfilzomib, ixazomib), nowe leki immunomodulujące (pomalidomid) oraz od niedawna refundowane w Polsce przeciwciała bispecyficzne [11]. Obiecujące wyniki w terapii opornej choroby uzyskuje się również dzięki zastosowaniu terapii CAR-T – genetycznie modyfikowanych limfocytów skierowanych przeciwko antygenowi BCMA [12].

Terapia szpiczaka mnogiego wymaga również leczenia wspomagającego, obejmującego zapobieganie powikłaniom kostnym (bisfosfoniany, denosumab), profilaktykę przeciwwirusową (acyklowir), profilaktykę przeciwzakrzepową oraz modyfikację dawkowania leków w zależności od funkcji nerek. Leczenie wiąże się również z ryzykiem wystąpienia powikłań takich jak zakażenia, uszkodzenie nerek, neuropatia obwodowa, zaburzenia kognitywne, powikłania zakrzepowo-zatorowe, uszkodzenie śluzówek przewodu pokarmowego oraz kardiotoksyczność niektórych leków (np. karfilzomibu) [13].

5.2. Mikrobiota jelitowa – aktualny stan wiedzy.

5.2.1 Choroby zakaźne i zapalne.

Mikrobiota jelitowa, stanowiąca niezwykle złożony i dynamiczny ekosystem, odgrywa zasadniczą rolę w utrzymaniu homeostazy organizmu oraz w modulacji odpowiedzi immunologicznej [14]. W jej skład wchodzą bakterie, archeony, wirusy, grzyby i pierwotniaki, a największe zróżnicowanie i liczebność tych mikroorganizmów obserwuje się w jelicie grubym [15]. Coraz więcej dowodów naukowych wskazuje, że mikrobiota jelitowa uczestniczy w rozwoju oraz progresji licznych chorób infekcyjnych i zapalnych, zarówno o podłożu autoimmunologicznym, jak i związanych z zakażeniami wirusowymi lub bakteryjnymi [16, 17].

Skład mikrobioty jelitowej kształtowany jest zarówno przez czynniki endogenne (takie jak wiek, czynniki genetyczne gospodarza, odporność wrodzona i nabyta), jak i egzogenne (dieta, styl życia, region geograficzny, stosowanie antybiotyków) [18]. Zaburzenia w jej składzie, określane mianem dysbiozy, prowadzą do osłabienia funkcji ochronnych i immunomodulacyjnych mikrobioty. Dysbioza może skutkować nadmierną aktywacją układu odpornościowego, co przyczynia się do rozwoju stanów zapalnych i chorób autoimmunologicznych, takich jak nieswoiste zapalenia jelit (IBD), reumatoidalne zapalenie stawów (RZS), toczeń rumieniowaty układowy (SLE) czy zapalne choroby skóry [19, 20].

Mikroorganizmy jelitowe oddziałują na gospodarza poprzez produkcję metabolitów, wśród których szczególne znaczenie mają krótkołańcuchowe kwasy tłuszczowe (SCFA) – takie jak maślan, octan i propionian – powstające w wyniku fermentacji błonnika [21]. SCFA wywierają efekt przeciwzapalny poprzez hamowanie deacetylaz histonowych (HDAC) oraz aktywację receptorów sprzężonych z białkiem G (FFAR), co sprzyja powstawaniu komórek Treg i tłumieniu odpowiedzi prozapalnej (Th17) [22]. Ponadto, SCFA wpływają na stabilizację czynnika indukowanego hipoksją (HIF-1α), który reguluje integralność bariery nabłonkowej jelita, produkcję mucyn oraz ekspresję cytokin i peptydów przeciwdrobnoustrojowych.

Obok SCFA istotne znaczenie mają metabolity tryptofanu, takie jak pochodne indolu, które wpływają na różnicowanie makrofagów M2, komórek Treg oraz komórek limfoidalnych typu 3 (ILC3), odpowiedzialnych za produkcję interleukiny 22 (IL-22). IL-22 uczestniczy w regeneracji nabłonka jelitowego i obronie przed patogenami [23]. Inną ważną grupą związków są poliaminy, np. putrescyna, odgrywające rolę w proliferacji komórek nabłonkowych i limfocytów [24].

Mikrobiota jelitowa aktywnie moduluje profil cytokinowy gospodarza. Bakterie takie jak *L. rhamnosus* i *L. reuteri* stymulują produkcję przeciwzapalnej IL-10 przez komórki Treg [25], natomiast niektóre szczepy *Prevotella* nasilają produkcję IL-17A, prozapalnej cytokiny produkowanej przez komórki Th17 [26]. Z kolei *Bacteroides fragilis* promuje różnicowanie Treg kosztem komórek Th17, co prowadzi do rozwoju tolerancji immunologicznej [27]. Zaburzenia w składzie mikrobioty wpływają więc bezpośrednio na równowagę między tolerancją a stanem zapalnym.

W chorobach autoimmunologicznych, takich jak nieswoiste zapalenia jelit, reumatoidalne zapalenie stawów i toczeń rumieniowaty układowy, wykazano istotne zmiany w składzie mikrobioty. U pacjentów z nieswoistymi zapaleniami jelit obserwuje się zwiększoną liczebność bakterii z rodzaju *Escherichia coli*, zwłaszcza szczepów adherentno-inwazyjnych, które nasilają odpowiedź zapalną i uszkadzają barierę jelitową [28]. Jednocześnie obniża się liczba

bakterii produkujących maślan, takich jak *Faecalibacterium prausnitzii* [29]. W reumatoidalnym zapaleniu stawów stwierdzono zwiększoną obecność *Prevotella copri, Collinsella aerofaciens* oraz zmniejszenie różnorodności mikrobiologicznej [30]. W toczniu rumieniowatym układowym natomiast obserwuje się obniżony stosunek *Firmicutes/Bacteroidetes*, zmniejszenie liczebności *Bifidobacterium* oraz zwiększenie liczby bakterii o potencjale prozapalnym, takich jak *Rhodococcus, Eggerthella* i *Klebsiella* [20].

Znaczenie mikrobioty jelitowej uwidacznia się także w chorobach wirusowych. W przypadku zakażeń HBV i HCV stwierdza się zmniejszenie liczby bakterii z rodzaju *Bifidobacterium* i *Lactobacillus*, przy jednoczesnym wzroście bakterii z rodziny *Enterobacteriaceae* [31]. Dysbioza może sprzyjać przełamywaniu odpowiedzi immunologicznej przez wirusy (tzw. viral escape), pogarszając rokowanie i przyczyniając się do progresji do marskości lub raka wątrobowokomórkowego [32]. W kontekście COVID-19 zaobserwowano, że ciężkość choroby koreluje z zaburzeniami w mikrobiocie, m.in. spadkiem liczby bakterii z rodzaju *Faecalibacterium* oraz wzrostem *Streptococcus* i *Veillonella*, co może nasilać tzw. burzę cytokinową [33].

5.2.2 Onkologia

W ostatnich latach pojawia się coraz więcej dowodów naukowych na to, że skład i aktywność mikrobioty jelitowej może istotnie wpływać na inicjację, progresję oraz leczenie chorób nowotworowych [34]. Stan dysbiozy – zaburzenie równowagi w składzie mikrobioty – może prowadzić do przewlekłego stanu zapalnego, uszkodzenia bariery jelitowej i translokacji drobnoustrojów oraz ich toksyn do krążenia ogólnego, co sprzyja nowotworzeniu [35].

Istnieje wiele mechanizmów molekularnych, przez które mikroorganizmy jelitowe inicjują transformację nowotworową. Przykładem jest bakteria *Helicobacter pylori*, która poprzez produkcję onkoproteiny CagA indukuje stres oksydacyjny, destabilizuje genom gospodarza i promuje rozwój raka żołądka [36]. Podobnie *Fusobacterium nucleatum* wspiera rozwój raka jelita grubego przez aktywację szlaku sygnałowego β-kateniny oraz hamowanie aktywności komórek NK poprzez białko Fap2 [37, 38]. Inne bakterie, takie jak *Escherichia coli* (szczepy produkujące kolibaktynę) i *Bacteroides fragilis* (produkujące toksynę BFT), powodują uszkodzenia DNA, stres oksydacyjny oraz stymulację proliferacji komórek nabłonkowych [39].

Mikrobiota może także wpływać na odpowiedź organizmu na leczenie przeciwnowotworowe. Wykazano, że obecność określonych bakterii (np. *Bifidobacterium longum, Lactobacillus johnsonii*) zwiększa skuteczność chemioterapii i immunoterapii poprzez wzmacnianie aktywności komórek T cytotoksycznych i komórek dendrytycznych [40]. Przykładowo,

bakterie z rodzaju *Bacteroides* warunkują skuteczność inhibitorów punktów kontrolnych układu odpornościowego (CTLA-4 i PD-1), a ich obecność może decydować o odpowiedzi klinicznej pacjentów na leczenie [41].

Z drugiej strony, stosowanie antybiotyków może zaburzać skład mikrobioty, osłabiać odpowiedź immunologiczną i zmniejszać efektywność leczenia przeciwnowotworowego. W badaniach na myszach wykazano, że antybiotykoterapia obniża skuteczność immunoterapii i chemioterapii cyklofosfamidem, prawdopodobnie na skutek eliminacji bakterii Gram-dodatnich, które uczestniczą w stymulacji limfocytów Th17 [42].

Z perspektywy terapeutycznej, rosnące zainteresowanie budzi możliwość modulowania mikrobioty poprzez probiotyki, prebiotyki oraz transplantację mikrobioty jelitowej (FMT). Probiotyki, takie jak *Lactobacillus casei, Bifidobacterium breve* czy *Clostridium butyricum*, wykazują potencjał przeciwnowotworowy dzięki produkcji SCFA, regulacji ekspresji cytokin oraz stymulacji odpowiedzi immunologicznej [43]. Ich obecność koreluje z zahamowaniem wzrostu guza, poprawą tolerancji terapii i redukcją działań niepożądanych, takich jak biegunki indukowane chemioterapią [44]. Ponadto, badania kliniczne i przedkliniczne wskazują, że przeszczepienie mikrobioty od pacjentów dobrze odpowiadających na immunoterapię może przywrócić wrażliwość na leczenie u chorych pierwotnie opornych [45].

Mikrobiota jelitowa działa zatem jako czynnik dwufunkcyjny – może zarówno promować, jak i hamować rozwój nowotworów. Jej wpływ zależy od składu mikrobiologicznego, obecności określonych toksyn, interakcji z układem odpornościowym oraz stanu zapalnego. Kluczowym celem przyszłych badań pozostaje identyfikacja korzystnych i szkodliwych szczepów bakterii oraz opracowanie strategii terapeutycznych, które pozwolą modulować mikrobiotę w kierunku efektów przeciwnowotworowych.

5.2.3 Hematologia

Nowotwory hematologiczne charakteryzują się niekontrolowanym rozrostem komórek układu krwiotwórczego i limfoidalnego, któremu towarzyszy osłabienie odpowiedzi immunologicznej. Stan taki może sprzyjać zakłóceniom w składzie mikrobioty jelitowej, co z kolei prowadzi do namnażania patogenów oportunistycznych i dalszego osłabienia układu odpornościowego. Przykładowo, w białaczkach stwierdzono zmniejszoną obecność bakterii z rodzin *Lachnospiraceae* i *Ruminococcaceae*, które odpowiadają za produkcję maślanu – metabolitu o działaniu przeciwzapalnym i przeciwnowotworowym. Ich niedobór może pogarszać integralność bariery jelitowej i promować translokację lipopolisacharydów (LPS) do krążenia ogólnego, nasilając stan zapalny i progresję nowotworu [46].

W ostrej białaczce limfoblastycznej (ALL) obserwuje się zmniejszoną różnorodność mikrobiologiczną oraz spadek ilości *Faecalibacterium prausnitzii*, co koreluje z wyższym stężeniem interleukiny 6 (IL-6) i białka C-reaktywnego (CRP) [47]. Z kolei u chorych z przewlekłą białaczką limfocytową (CLL) w mikrobiocie jelitowej dominuje *Prevotella*, bakteria promująca różnicowanie limfocytów Th17 – komórek o działaniu prozapalnym i potencjalnie wspierających rozwój nowotworu [48]. W białaczkach szpikowych, zarówno ostrych (AML), jak i przewlekłych (CML), także odnotowano zmiany w mikrobiocie, m.in. wzrost liczby bakterii z rodzaju *Streptococcus* i *Actinobacteria* oraz spadek korzystnych bakterii produkujących SCFA [49].

W przypadku chłoniaków, zwłaszcza chłoniaka z dużych komórek B zaobserwowano zwiększoną obecność *Escherichia coli* i *Clostridium butyricum* [50]. *E. coli* może wytwarzać kolibaktynę – toksynę uszkadzającą DNA, natomiast *C. butyricum*, dzięki produkcji maślanu, może wywierać działanie przeciwnowotworowe [39]. Co ciekawe, zakażenie bakterią *Helicobacter pylori* jest istotnym czynnikiem rozwoju chłoniaka MALT (tkanki chłonnej związanej z błoną śluzową), a sama jej eradykacja może prowadzić do remisji nowotworu [51].

Liczne czynniki, takie jak droga porodu, sposób karmienia, dieta, styl życia, stosowanie antybiotyków, chemioterapia czy przeszczepienie komórek krwiotwórczych, istotnie wpływają na skład i funkcjonowanie mikrobioty jelitowej [52]. Chemioterapia i radioterapia mogą uszkadzać nabłonek jelitowy, prowadząc do translokacji bakterii i nasilenia stanu zapalnego. Przeszczepienie komórek krwiotwórczych może z kolei prowadzić do poważnych powikłań, takich jak choroba przeszczep przeciwko gospodarzowi (GvHD), której ciężkość koreluje z obecnością określonych taksonów bakteryjnych, m.in. z rodziny *Enterobacteriaceae* [53].

Z uwagi na rosnącą rolę mikrobioty w patogenezie i terapii nowotworów hematologicznych, coraz większe zainteresowanie budzi możliwość jej modulowania. Podobnie jak w innych chorobach nowotworowych rozważane są probiotyki, prebiotyki, dieta wysokobłonnikowa oraz FMT.

5.2.4 Neurologia.

Istnieje coraz więcej dowodów naukowych potwierdzających istnienie tzw. osi jelito- mózg (gut–brain axis, GBA), stanowiącej dwukierunkowy szlak komunikacyjny między mikrobiotą jelitową a ośrodkowym układem nerwowym (OUN), poprzez mechanizmy neuroendokrynne, immunologiczne i metaboliczne [54].

W prawidłowych warunkach mikrobiota wpływa korzystnie na rozwój i funkcjonowanie układu nerwowego, m.in. poprzez syntezę neurotransmiterów (takich jak GABA, serotonina, dopamina), SCFA, a także regulację ekspresji neurotrofin, w tym czynnika neurotroficznego

pochodzenia mózgowego (BDNF) [55]. Dysbioza, czyli zaburzenia w składzie i funkcjonowaniu mikrobioty, może jednak przyczyniać się do rozwoju licznych schorzeń neurologicznych, w tym choroby Parkinsona, choroby Alzheimera, stwardnienia rozsianego, padaczki, autyzmu, udaru mózgu czy stwardnienia zanikowego bocznego (ALS) [56].

W chorobie Parkinsona obserwuje się m.in. zwiększoną obecność bakterii z rodziny *Enterobacteriaceae* i zmniejszenie liczby bakterii *Prevotellaceae*. Zmiany te korelują z nasileniem objawów motorycznych oraz obecnością agregatów α-synukleiny w układzie nerwowym [57]. Badania z wykorzystaniem modeli zwierzęcych wykazały, że transplantacja kału od pacjentów z chorobą Parkinsona do myszy wywołuje u tych ostatnich objawy neurozapalne i zaburzenia ruchowe, co wskazuje na udział mikrobioty w patogenezie choroby [58]. Również SCFA, choć niezbędne w fizjologicznych ilościach, mogą w nadmiarze indukować neurozapalne procesy w mózgu [59].

W chorobie Alzheimera stwierdza się z kolei zwiększoną obecność bakterii prozapalnych, takich jak *Escherichia coli* i *Shigella*, oraz zmniejszenie liczby bakterii o działaniu przeciwzapalnym, takich jak *Eubacterium rectale* [60]. Zaburzenia w składzie mikrobioty prowadzą do zwiększonej produkcji cytokin zapalnych (IL-1β, TNF-α) oraz aktywacji inflammasomów, które promują odkładanie się blaszek amyloidowych i hiperfosforylację białka tau w mózgu [61]. Dodatkowo, mikrobiota wpływa na integralność bariery jelitowej i może oddziaływać na funkcjonowanie bariery krew-mózg, przyczyniając się do progresji neurodegeneracji [62].

W stwardnieniu rozsianym (MS) wykazano, że kolonizacja jelit przez bakterie segmentowane filamentowe (SFB) indukuje odpowiedź limfocytów Th17. Z drugiej strony, obecność bakterii z rodzaju *Bacteroides* czy *Prevotella* może wspierać aktywność komórek T regulatorowych (Treg) i hamować postęp choroby [63]. Zmiany w składzie mikrobioty jelitowej wpływają również na przepuszczalność bariery jelitowej oraz poziom cytokin prozapalnych, co przekłada się na nasilanie procesu demielinizacji i neurozapalnego charakteru choroby [64].

Autyzm (ASD) to kolejny przykład schorzenia, w którym mikrobiota odgrywa znaczącą rolę. Około 40% osób z ASD cierpi na zaburzenia żołądkowo-jelitowe, co sugeruje możliwy udział mikrobioty w patogenezie choroby [65]. Zmiany w składzie mikrobioty jelitowej mogą wpływać na rozwój neuropsychiatryczny dziecka poprzez mechanizmy immunologiczne i metaboliczne, prowadząc do zaburzeń funkcji poznawczych, nastroju i zachowania [66]. FMT od zdrowych dawców wykazała poprawę objawów behawioralnych u dzieci z autyzmem, choć dokładne mechanizmy pozostają jeszcze nie do końca poznane [67].

W przypadku udaru mózgu i urazowego uszkodzenia mózgu, dysbioza mikrobioty jelitowej może zwiększać przepuszczalność bariery jelitowej oraz promować migrację limfocytów T do mózgu, co potęguje procesy neurozapalne i powiększa objętość obszarów uszkodzenia [68]. Z kolei metabolity wydzielane przez składniki mikrobioty, takie jak TMAO (trimetyloamina-N-tlenek), pochodzące z diety bogatej w cholinę, są związane z ryzykiem miażdżycy i udaru [69]. W padaczce obserwuje się różnice w składzie mikrobioty pomiędzy pacjentami reagującymi i niereagującymi na leczenie. Dieta ketogenna, stosowana jako terapia wspomagająca w lekoopornej padaczce, wpływa korzystnie na skład mikrobioty (np. zwiększenie liczby *Akkermansia muciniphila, Parabacteroides*) i może redukować częstość napadów [70]. Istnieją również dowody na to, że probiotyki mogą modulować częstość napadów poprzez wpływ na metabolizm neuroprzekaźników.

W ALS, jak i w chorobie Huntingtona, obserwuje się zmniejszenie liczby bakterii produkujących maślan oraz zmiany w różnorodności mikrobiologicznej, które mogą wpływać na integralność nabłonka jelitowego, poziom cytokin zapalnych oraz rozwój neurodegeneracji [71]. W badaniach klinicznych z zastosowaniem probiotyków odnotowano pewne zmiany w składzie mikrobioty, jednak brak jeszcze wystarczających danych, które pozwoliłyby jednoznacznie potwierdzić skuteczność tego typu interwencji.

5.3.Rola mikrobioty jelitowej w patogenezie, leczeniu i powikłaniach szpiczaka plazmocytowego – opis aktualnego stanu wiedzy.

Szpiczak plazmocytowy rozwija się najczęściej ze stanów przednowotworowych, takich jak gammopatia monoklonalna o nieokreślonym znaczeniu (MGUS) oraz tzw. „tlący się" szpiczak mnogi (SMM). Choć transformacja MGUS do MM zachodzi jedynie u około 1% pacjentów rocznie, to mechanizmy odpowiedzialne za tę progresję pozostają nie do końca poznane [72]. W ostatnich latach coraz więcej uwagi poświęca się mikrośrodowisku szpiku kostnego oraz wpływowi mikrobioty jelitowej na immunologiczne i metaboliczne warunki sprzyjające klonalnej ekspansji komórek plazmatycznych.

Istnieją dowody, że komórki nowotworowe MM mogą kształtować skład mikrobioty jelitowej, jednocześnie będąc pod wpływem metabolitów bakteryjnych. Jednym z kluczowych mechanizmów jest nadprodukcja amoniaku (NH_4^+) przez komórki MM, wynikająca z ich zwiększonego zapotrzebowania na glutaminę. NH_4^+ przekształcany jest w wątrobie do mocznika, jednak przy niewydolności nerek, charakterystycznej dla wielu pacjentów z MM, następuje wzrost stężenia mocznika we krwi i jego zwiększony transport do światła jelita. To środowisko sprzyja kolonizacji przez bakterie odpowiedzialne za przemiany związków

azotowych, takie jak *Klebsiella pneumoniae* i *Streptococcus pneumoniae*, które przekształcają mocznik z powrotem do glutaminy – kluczowego aminokwasu wspierającego metabolizm komórek nowotworowych [73]. Równie istotną rolę odgrywają SCFA, takie jak maślan, propionian i octan, produkowane przez bakterie fermentujące błonnik. SCFA mają zdolność hamowania czynników prozapalnych, takich jak NF-κB, IL-6 i TNF-α, oraz promowania różnicowania komórek T regulatorowych (Treg) poprzez indukcję ekspresji FOXP3 i IL-10 [74]. Równocześnie mogą one ograniczać aktywność prozapalnych komórek Th17, co jest istotne, gdyż IL-17 – kluczowa cytokina produkowana przez Th17 – odgrywa rolę w aktywacji osteoklastów i tworzeniu ognisk litycznych w kościach, charakterystycznych dla MM. Badania wykazały, że poziom IL-17 jest podwyższony u pacjentów z MM oraz SMM, i może stanowić marker predykcyjny progresji choroby [75].

Kolonizacja jelita przez *Prevotella heparinolytica* prowadzi do różnicowania komórek Th17 i ich migracji do szpiku kostnego, gdzie nasilają proliferację komórek MM. Zahamowanie IL-17 lub jego receptora w modelach zwierzęcych opóźniało progresję choroby, co wskazuje na potencjalną wartość terapeutyczną ukierunkowaną na mikrobiotę jelitową i związane z nią szlaki immunologiczne [76].

Najnowsze badania genetyczne dostarczają kolejnych dowodów na związek przyczynowy między mikrobiotą a MM. Badanie oparte na randomizacji mendlowskiej z 2024 roku wykazało, że wyższa obfitość bakterii z grupy *Eubacterium ruminantium* jest przyczynowo związana ze zwiększonym ryzykiem rozwoju MM (OR=1.71). Z kolei obecność bakterii z rodzajów *Dorea* (OR=0.46) i *Coprococcus* (OR=0.47) oraz z grupy *Eubacterium rectale* (OR=0.37) ma działanie ochronne. Odkrycia te stanowią fundamentalny krok naprzód, przesuwając paradygmat z obserwacji korelacyjnych w kierunku zrozumienia przyczynowo-skutkowych mechanizmów leżących u podstaw wpływu mikrobioty na inicjację szpiczaka [77].

Skład mikrobioty jelitowej wpływa również na odpowiedź na leczenie MM. W szczególności, obecność bakterii produkujących maślan, takich jak *Faecalibacterium prausnitzii* i *Eubacterium hallii*, była skorelowana z osiągnięciem ujemnej minimalnej choroby resztkowej (MRD) po leczeniu indukującym remisję i przeszczepieniu komórek macierzystych [78]. Z kolei stosowanie antybiotyków o szerokim spektrum, zwłaszcza w okresie okołoprzeszczepowym, wiąże się z dysbiozą i gorszymi wynikami leczenia, w tym krótszym przeżyciem wolnym od progresji (PFS).

5.4.Uzasadnienie połączenia prac w cykl publikacji.

Przedłożony cykl publikacji tworzy spójną i wielopoziomową narrację naukową, badającą wpływ mikrobioty jelitowej na szpiczaka plazmocytowego od mechanizmów podstawowych po bezpośrednie implikacje kliniczne. Spójność cyklu opiera się na połączeniu prac przeglądowych, które budują fundament teoretyczny, z pracami oryginalnymi, które weryfikują postawione hipotezy w warunkach klinicznych. **Prace przeglądowe (Publikacja 3 i 4)** dogłębnie analizują aktualny dla czasu opublikowania stan wiedzy na temat roli mikrobioty w patogenezie dyskrazji plazmocytowych. Ukazują, w jaki sposób dysbioza, przewlekła stymulacja antygenowa i metabolity bakteryjne mogą przyczyniać się do progresji od stanu MGUS do pełnoobjawowego MM oraz wpływać na skuteczność terapii. Stanowią one szerokie tło biologiczne i uzasadnienie dla podjęcia badań klinicznych. **Prace oryginalne (Publikacja 1 i 2)** przenoszą te koncepcje do praktyki klinicznej, koncentrując się na kluczowym etapie leczenia młodszych i „fit" chorych, jakim jest auto-SCT.

W publikacji nr 1 skupiono się na zaburzeniach składu mikrobioty jelitowej skutkującej kolonizacją przewodu pokarmowego bakteriami antybiotykoopornymi. Zbadano wpływ tych kolonizacji na rozwój powikłań po auto-SCT, w szczególności powikłań infekcyjnych.

W publikacji nr 2 przedstawiono toksyczne uszkodzenie śluzówek jamy ustnej jako jedno z najczęstszych powikłań auto-SCT u chorych ze szpiczakiem plazmocytowym. Również to powikłanie może mieć związek z zaburzeniami mikrobioty bytującej w jamie ustnej.

W publikacji nr 3 przedstawiono na poziomie molekularnym szereg interakcji jakie powstają pomiędzy komórkami śluzówki przewodu pokarmowego a mikroorganizami wchodzącymi w skład mikrobioty jelitowej u chorych z dyskrazjami plazmocytowymi, wśród nich najczęstszą – szpiczakiem plazmocytowym.

W publikacji nr 4 pokazano rolę mikrobioty jelitowej w patogenezie, leczeniu i jego powikłaniach u chorych z dyskrazjami plazmocytowymi. Skupiono się szczególnie na czynnikach związanych z mikrobiotą mogących mieć związek z tempem progresji od MGUS przez SMM do pełnoobjawowego MM.

Razem, niniejszy cykl publikacji przedstawia holistyczne spojrzenie na problem: od zrozumienia fundamentalnych mechanizmów patogenetycznych, przez identyfikację klinicznie istotnych czynników ryzyka, aż po zaproponowanie praktycznych, opartych na dotychczasowych dowodach interwencji poprawiających bezpieczeństwo i wyniki leczenia pacjentów z MM.

6. Założenia i cel pracy.

Głównym celem projektu była ocena roli jaką odgrywa mikrobiota jelitowa w leczeniu i powikłaniach chorych ze szpiczakiem plazmocytowym, ze szczególnym uwzględnieniem chorych młodszych, w dobrej formie, którzy mogą być poddani procedurze auto-SCT.

W dwóch pracach oryginalnych o charakterze retrospektywnym przedstawiono dane wskazujące na realny wpływ bariery jelito-krew oraz składu mikrobioty jelitowej w kształtowaniu się odsetka poszczególnych powikłań po podaniu wysokodawkowanej chemioterapii wspomaganej przeszczepieniem autologicznych komórek krwiotwórczych.

W dwóch pracach przeglądowych dokonano gruntownej analizy literatury, przedstawiając dowody na realny i potencjalny wpływ mikrobioty jelitowej na wyniki leczenia i częstość powikłań u chorych z dyskrazjami plazmocytowymi.

7. Kopie opublikowanych prac

7.1. Publikacja 1

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Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma

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Patients undergoing autologous stem cell transplantation (auto-SCT) face elevated risks of infections. Additionally, patients colonized in the gastrointestinal tract with antibiotic-resistant bacteria (ARB) are at higher risk of infection with ARB and other infections. Therefore, patients colonized with ARB before auto-SCT should present with an exceptionally high incidence of infections. According to current literature, ARB colonization is the surrogate marker for dysbiosis, which is known to be associated with a diagnosis of multiple myeloma (MM). Given that, this retrospective study aimed to assess the influence of ARB colonization on infection rates, hematopoiesis regeneration, mucositis, overall survival, and progression-free survival following auto-SCT in MM. Data from 138 MM patients undergoing 141 auto-SCT were analyzed, with 15% showing ARB colonization. Among colonized patients, ESBL-producing gram-negative rods predominated. Patients with gut ARB colonization had significantly higher infection rates than non-colonized individuals (52 vs. 26%, $P = 0.02$), particularly bloodstream infections (43% vs. 14%, $P = 0.004$). Colonized patients also tended to exhibit shorter survival rates although there was no statistical significance (1-year and 2-year OS; non-colonized vs. colonized; 97 and 92% vs. 90 and 86%; $p = 0.054$). Based on our results, gut colonization before auto-SCT negatively affects treatment outcomes.

Keywords Autologous hematopoietic stem cell transplantation, Antibiotic-resistant bacteria, Gut colonization, Infection rates, Fecal microbiota transplantation

Resistance of bacteria against antibiotics is known as a global threat, and in 2019, approximately 5 million deaths globally were attributable to that phenomenon[1]. Predictions exist that antibiotic resistance will cause more deaths than cancer soon[2]. Therefore, patients' colonization with antibiotic-resistant bacteria (ARB) is sometimes called the state of a "ticking bomb". Although it is not a disease per se, ARB colonization increases the risk of mortality due to infections. That is particularly worrying in immunocompromised individuals, where mortality is reaching up to 95%[3,4].

An important reason for ARB colonization is the impairment of the gut microbiota composition, which creates niches for new bacteria. Such damage to the gut microbiota repertoire may result from the administration of antibiotics, chemotherapeutics, or immunosuppression[5]. The Bone Marrow Transplantation Units are locations where the admitted patients are frequently colonized with ARB. That is due to the profile of individuals with impaired gut microbiome, resulting from numerous antibiotics, long and numerous hospitalizations, and microbiota-damaging therapies before allogeneic hematopoietic stem cell transplantation (alloHSCT). We have

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previously shown that even 31% of all patients qualified for alloHSCT are colonized with ARB[3], but there are also centers where the colonization rate approaches 70%[6]. That alone increases the risk of systemic infections and acute graft-versus-host disease (aGHVD) incidence[3]. Our team and other authors proved that the ARB colonization before alloHSCT is also associated with the significant shortening of the overall survival (OS)[3,7–9].

Our group revealed that fecal microbiota transplantation (FMT) performed in patients with blood disorders promotes the eradication of ARB from the gastrointestinal tract[10]. Furthermore, in the recent work of Innes et al., it was shown that fecal microbiota transplantation (FMT) in ARB carriers could mitigate the adverse outcomes seen in alloHSCT recipients, such as fever and intensive care, however, without significant impact on OS[11]. The role of FMT in the treatment of aGvHD has also been documented by us and other teams[12,13].

Plasma cell dyscrasias are a group of diseases derived from a mutated clone of plasma cells producing the monoclonal protein. The most common disease in that group, and simultaneously the second most common hematologic malignancy, is multiple myeloma (MM). It is still an incurable disease, although considerable progress in treatment has been made in recent years due to new drug development[14]. Despite that, autologous stem cell transplantation (auto-SCT) preceded by high-dose melphalan infusion is still the most common consolidation therapy for younger and fit patients[15]. Melphalan used in conditioning is frequently associated with mucositis, typically affecting the patients a few days after the procedure[16]. Frequently, not only the oral mucosa is affected (which in particularly severe cases can lead to opioid requirement and parenteral nutrition) but the intestinal mucosa as well. That can cause the disintegration of the gut epithelium and mucosa-associated lymphoid tissue (MALT) disruption, dramatically increasing the risk of gut microbes' influx into the bloodstream, subsequent bacteriemia, and sepsis[17]. It was also shown that the gut microbiota of patients with plasma cell dyscrasias is frequently disrupted, mainly because of antibiotics frequently prescribed for these patients. Moreover, the plasma cells have numerous direct and indirect interactions with gut epithelium, shaping the gut microbiota composition[18,19]. Taking into consideration what was previously said about ARB and that patients on hematology wards are frequently being ARB-colonized, such patients are at significant risk of life-threatening infections.

Knowing that ARB colonization is the surrogate marker for gut microbiota disruption, we wanted to learn if gut ARB colonization alone influences the rate of infections and bacteremia after auto-SCT and how it affects mortality. Furthermore, the influence of ARB colonization on other events, such as regeneration of hematopoiesis, mucositis, overall survival, and progression-free survival potentially associated with that factor, was analyzed.

Methods

Study design

This retrospective, single-center study used clinical data on consecutive MM patients who underwent auto-SCT between August 2016 and June 2022 in the Department of Hematology, Transplantation, and Internal Medicine of the Medical University of Warsaw, Poland.

Materials and methods

Patients' gut colonization data were collected during a three-month period before auto-SCT. At least one positive isolate collected from a rectal swab (performed by qualified nurse) was enough to classify the patient as colonized. Moreover, results from blood, urine, and feces samples regarding the infectious sites during the three-month period after auto-SCT were included in the study. A total of 4776 clinical specimens were analyzed during the study period.

Data collection

Patient information was collected from the laboratory records, including species of bacteria and antibiotic sensitivity patterns.

Microbiological procedures

Bacteria were cultured using bacteriological media according to laboratory procedures. Blood cultures were performed with Plus Aerobic/F and Anaerobic/F culture bottles (Becton, Dickinson, and Company) and monitored using the Bactec FX system (Becton, Dickinson, and Company) for 7 days. Bacterial identification was performed with MALDI-TOF mass spectrometry with Microflex LT mass (Bruker, Germany) using the MBT Compass IVD software (Bruker Daltonics, Germany) according to manufacturer instructions. Antimicrobial susceptibility testing (AST) was performed with the Kirby-Bauer disk diffusion method for the following antibiotics: ertapenem, meropenem, imipenem, temocillin, vancomycin, cefoxitin and interpreted according to European Committee on Antimicrobial Susceptibility Testing guidelines (2012). Bacteremia was defined as at least one positive blood culture, irrespective of clinical symptoms. Two consecutive positive cultures were required to confirm bacteremia caused by coagulase-negative pathogens.

The ability of isolates to produce carbapenemases (MBL, KPC OXA-48) was investigated by DDST-EDTA – double-disk synergy with ethylenediaminetetraacetic acid - detected MBL and CDT – combined disk test – detected KPC. Produce OXA-48 detected using an antibiogram disc with temocillin. Rapidec Carba NP and/or the GeneXpert qualitative real-time PCR method (Cepheid, Sunnyvale, CA) were also done.

ESBL was detected with the phenotypic confirmation method – double-disk synergy test (DDST).

Procedures and definitions

All patients had standard anti-bacterial prophylaxis with quinolones from day 0 till engraftment. Engraftment day was defined as the day when values of white blood cells (WBC) > 1000/µl and neutrophils (NEU) > 0.5×10^9/L were reached for the first time and did not decrease during the two consecutive days. Neutropenic fever

was defined according to ESMO guidelines as an oral temperature of > 38.3 °C or two consecutive readings of > 38.0 °C for 2 h and an absolute neutrophil count (ANC) of $< 0.5 \times 10^9$/L, or expected to fall below that threshold[20]. In the case of febrile episodes during the neutropenic period, patients were treated with antibiotics targeting the gut-colonizing ARB and standard, broad-spectrum antibiotics. The severity of the disease and the patient's status were measured with HCT-CI, and the most important features before auto-SCT were listed.

ARB bacteria were defined as bacteria resistant to clinically relevant antibiotics, which mean that given antibiotic is the last from the repertoire of classical antibiotics capable of treating in a given group, or a given antibiotic is the last oral antibiotic in a given group, etc. Generally, in this work, when defining ARB, we meant primarily antibiotic resistance determined by resistance to at least 3 classes of antibiotics (MDR - multidrug resistance), mostly ESBL, VRE and CPE (CRE), and ARB colonization was stated if one of those three resistance mechanism were present.

All patients provided standard informed consent for auto-SCT, data analysis, and publication. According to the local guidelines, rectal swabs were collected as a standard practice in all hospitalized patients. According to Polish law, the approval of the institutional review board was not required because of the noninterventional nature of the study. The study was performed in accordance with the Declaration of Helsinki and approved by the internal review board of the Department of Hematology, Transplantation, and Internal Medicine of the Medical University of Warsaw.

Statistical analysis

The 1- and 2-year overall survival of patients after auto–SCT was analyzed. Surviving patients were censored at the last follow-up examination or the date of subsequent auto-SCT.

Patients' baseline characteristics were summarized and tested with appropriate statistical analyses (Mann-Whitney U-test for continuous variables, the Pearson chi-square test, and Fisher's exact test for categorical variables).

Transplant-related events and outcomes were tested with Fisher's exact test, and the log-rank test was used for survival analysis.

Results

Patients characteristics

The study included 138 patients who underwent 141 auto–SCTs. Clinical characteristics and demography are shown in Table 1. There was an equal sex distribution (52% men), and the median age at the time of auto-SCT was 60 years (range, 37 to 71). The median time from the diagnosis of MM to auto–SCT was 9 months (range, 1 to 144). The most commonly administered dose of melphalan was 200 mg/m^2 (72 auto-SCT, 51%), but the reduced dose of 140 mg/m^2 was also common (58 auto-SCT, 41%). Most of the patients reached very good partial response (VGPR) before the auto–SCT (67 patients, 48%) or better (VGPR + CR + sCR (94 patients, 67%). At the time of auto-SCT, 15% of patients ($n = 21$) were colonized by ARB in the gut. Twenty-four ARB species were isolated from rectal swabs (3 patients had 2 colonizing ARB species). Among the most common colonizing ARB, *Escherichia coli* ESBL + was present in 6.4% and *Klebsiella pneumoniae* ESBL + in 3.5% of patients (Fig. 1). There were no significant differences in patient characteristics between groups (colonized vs. non-colonized). Most importantly, there were no differences between patients' general statuses regarding HCT-CI.

Impact of the ARB colonization on the results and complications of autoSCT

The hematopoietic cell engraftment occurred after a median of 10 days post auto-SCT (range 5–25 days). The median time of neutropenia and thrombocytopenia following the transplantation was similar between the groups. Similarly, the difference in number of days with G-CSF support was not statistically significant. Oral mucositis (OM) occurred in 60 patients (46%) overall, and the rate of OM between the groups was not statistically significant (Table 2).

In three months following the transplantation, 30% of all patients ($n = 42$) experienced at least 1 infection. The most common etiology was *C. difficile*, responsible for pseudomembranous colitis ($N = 6$). Twenty-six (18%) had bacteriemia. Significant differences existed in the incidence of infections between the non-colonized and the gut-colonized groups (26 vs. 52%, $P = 0.02$) (Figs. 2 and 3). Moreover, there was a statistically significant difference between the non-colonized and gut-colonized groups regarding bacteremia incidence (14 vs. 43%, $P = 0.004$) (Figs. 4 and 5). What is particularly interesting, only in 2 patients in the colonized group the infection agent was the same as the gut-colonizing bacteria pre-auto-SCT (22%). In both cases, bacteriemia with a colonizing microbe was identified.

The rate of neutropenic fever in all patients was 12% ($n = 15$). The difference in neutropenic fever incidence between groups (colonized vs. non-colonized) was not statistically significant (11% vs. 20%; $p = 0.317$). The overall survival status showed no statistically significant differences between the groups. In the group of all patients, the 1-year and 2-year OS was 96 and 91%, respectively, in the median observation time of 43.0 months. There were no statistically significant differences in the case of 1-year and 2-year OS between the non-colonized vs. colonized group, respectively (97 and 92% vs. 90 and 86%; $p = 0.054$).

Discussion

This retrospective, single-center analysis showed that 15% of all auto-SCT procedures involved patients being colonized with ARB. That condition influenced the infection rate – bacteriemia occurred in 43% of colonized patients, whereas it occurred only in 14% of non-colonized individuals ($p = 0.004$). Additionally, the rate of all infections was also higher. In the colonized group, it reached 52%, while in the non-colonized group, it was 26% ($p = 0.02$).

Characteristics	All auto-SCT ($n=141$)	Non-colonized ($n=120$)	Colonized ($n=21$)	*P* value
Year of autoSCT, median (range)	2018 (2016–2022)	2018 (2016–2022)	2017 (2016–2021)	NS
Male sex (N, %)	73 (52)	62 (52)	11 (52)	NS
Age at autoSCT, yr, median (range)	60 (37–71)	61 (37–71)	60 (44–70)	NS
Time from diagnosis to autoSCT, mo, median (range)	9 (1-144)	9 (1-144)	9 (5–52)	NS
Stage of disease at diagnosis (ISS) (N, %) 1 2 3	$N=100$ 31 (31) 28 (28) 41 (41)	$N=83$ 26 (31) 23 (28) 34 (41)	$N=17$ 5 (29) 5 (29) 7 (42)	NS
HCT-CI 0 1–2 3 or higher	58 (41) 54 (38) 29 (21)	53 (44) 41 (34) 26 (22)	5 (24) 13 (62) 3 (14)	0.053
Melphalan dose (N, %) 50 mg/m^2 100 mg/m^2 140 mg/m^2 200 mg/m^2	$N=134$ 1 (1) 3 (2) 58 (43) 72 (54)	$N=113$ 1 (1) 2 (2) 49 (43) 61 (54)	$N=21$ 0 (0) 1 (5) 9 (43) 11 (52)	NS
Response before autoSCT (N, %) PD SD PR VGPR CR sCR	1 (1) 3 (2) 43 (30) 67 (48) 19 (13) 8 (6)	1 (1) 3 (3) 37 (30) 55 (46) 18 (15) 6 (5)	0 (0) 0 (0) 6 (29) 12 (57) 1 (5) 2 (9)	NS
Response to the therapy before autoSCT (N, %) ≤PR ≥VGPR	47 (33) 94 (67)	41 (34) 79 (66)	6 (29) 15 (71)	NS
Fresh/frozen cells (N, %) Fresh Frozen	$N=122$ 85 (70) 37 (30)	$N=104$ 75 (72) 29 (28)	$N=18$ 10 (56) 8 (44)	NS
Creatinine at the day of autoSCT, mmol/l, median (range)	0.94 (0.42–9.65)	0.93 (0.42–7.3)	1.27 (0.46–9.65)	NS
Isotype of IgH (N, %) IgG IgA IgD	$N=108$ 80 (63) 24 (19) 4 (3)	$N=93$ 70 (66) 20 (18) 3 (3)	$N=15$ 10 (67) 4 (27) 1 (6)	NS
LCD	58 (41)	50 (42)	8 (38)	NS
Number of cells infused (x 10^6/kg), median, range	4.76 (2.03-27)	4.77 (2.03-27)	4.53 (3.29–9.91)	NS

Table 1. Baseline characteristics of patients included in the study. LCD (light chain disease), PD (progressive disease), SD (stable disease), PR (partial response), VGPR (very good partial response), CR (complete response), sCR (stringent complete response).

The percentage of colonized patients before auto-SCT was significantly different compared to individuals undergoing alloHSCT (31%)[3]. Furthermore, the gut colonizing bacteria were different as well. Whereas in the population of patients before alloHSCT, the bacteria species were predominantly VRE and then ESBL-producing gram-negative rods, in our cohort, the main bacteria were the latter ones and *Stenotrophomonas maltophilia* (natural resistance against carbapenems and aminoglycosides). VRE was detected only in a minimal number of patients ($n=2$). Given that ARB colonization is the surrogate marker for poor gut microbiota repertoire (dysbiosis), it can be concluded that the impairment of gut microbiota composition is far more noticeable in the population of patients treated with alloHSCT than in the auto-SCT cohort. The reason may be the higher overall incidence of infections before alloHSCT and the more liberal usage of broad-spectrum antibiotics affecting the gut microbes (different than in an auto-SCT setting). Similar outcomes were shown in other publications, which showed the influence of ARB colonization on stem cell transplantation. In the paper published by Forcina et al., the multidrug-resistant gram-negative bacteria (MDR) colonized 16,9% and 9,6% of patients before allogeneic and autologous hematopoietic stem cell transplantation, respectively[21].

As mentioned before, patients with MM are particularly susceptible to infections. Given the fact that the time from the first symptoms till the diagnosis of malignancy is usually longer (in contrast to acute myeloid/lymphoblastic leukemia), they are typically treated with numerous antibiotics during that time. Despite that, in our and other works, the colonization rate before auto-SCT proved much lower than in alloHSCT recipients. That may be because the time from the start of MM treatment to auto-SCT is relatively short (median 9 months; range 1-144 months), and the treatment is less intensive than in the case of acute leukemia or other aggressive cancers. In addition, contrary to individuals with acute myeloid/lymphoblastic leukemia, patients with MM are usually treated as outpatients and have a lower risk of neutropenia and associated with that infections. Given that infections remain the leading cause of mortality in patients with plasma cell dyscrasias, it is essential to decrease the colonization rate before the auto-SCT to transform the abovementioned statistics into fewer infections during the time following the procedure. One of the most promising procedures is fecal microbiota transplantation and microbiota modulation.

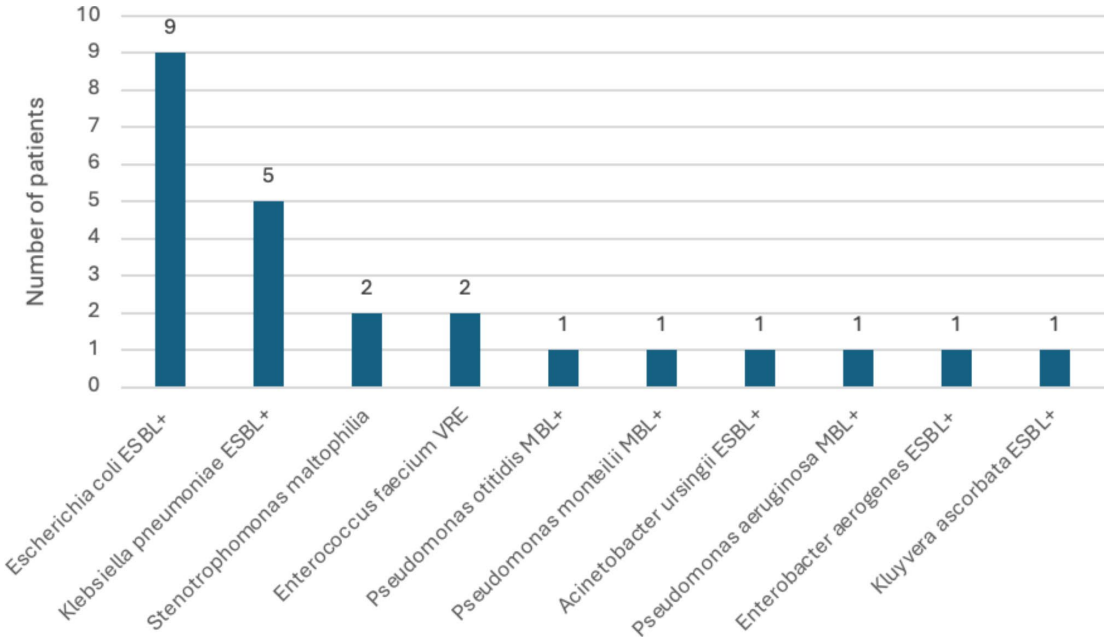

Fig. 1. Gut-colonizing ARB pathogens in the period of 3 months before auto-SCT.

Variable	All auto-SCT ($n=141$)	Non-colonized ($n=120$)	Colonized ($n=21$)	*P* value
Mucositis N (%)	$N=130$ 60 (46)	$N=113$ 52 (46)	$N=17$ 8 (47)	NS
Engraftment – day; median, range	10 (5–25)	10 (5–25)	10 (9–20)	NS
Neutropenia < 1,5 G/l, median days, range	5 (0–18)	5 (0–18)	5.5 (3–18)	NS
Thrombocytopenia < 20 G/l, median days, range	12 (1–49)	12 (1–41)	12 (1–49)	NS
G-CSF, days, median, range	10 (3–23)	10 (6–23)	10.5 (3–20)	NS
Neutropenic fever N (%)	$N=124$ 15 (12)	$N=109$ 12 (11)	$N=15$ 3 (20)	NS
Patients with at least 1 infection N (%)	42 (30)	31 (26)	11 (52)	$P=0.02$
Bacteriemia N (%)	26 (18)	17 (14)	9 (43)	$P=0.004$

Table 2. Transplant-related events and outcomes.

Analyzed data also showed no statistical differences between the groups regarding time to engraftment, days of G-CSF administration, the incidence of mucositis, days of neutropenia, or thrombocytopenia. On the contrary, it was showed that patients colonized in the gut tended to have a higher incidence of neutropenic fever following the procedure than the non-colonized individuals. However, these results proved not statistically significant (11 vs. 20%, $P=0.317$). As previously mentioned, particularly interesting results were seen in the case of infection rates in different groups. In the whole cohort, the infection rate was 30%, whereas 18% of patients had bacteriemia. That is far less than in the alloHSCT population, which had bacteriemia in 32% of all patients, citing our previous work[3]. The most frequent bloodstream infection agent was coagulase-negative staphylococci, which is common in patients undergoing autoSCT with central venous access. In the group with ARB gut colonization, 52% of patients had at least one episode of infection during the time of 3 months after the procedure, whereas in the non-colonized group, 26% ($P=0.02$). That shows the dominant role of gut colonization as the risk of infections after the procedure. The positive blood culture rate post-auto-SCT period showed even more interesting results. In the gut-colonized group, 43% had at least one episode of bacteremia, whereas in the non-colonized group, only 14% ($P=0.004$). Nevertheless, when the etiology of this bacteriemia was analyzed, only in 2 patients did the etiology factor correspond with ARB colonization before auto-SCT. It is considered as ARB colonization is a marker of dysbiosis, and this increases the rate of bacterial translocation, but ARB are not

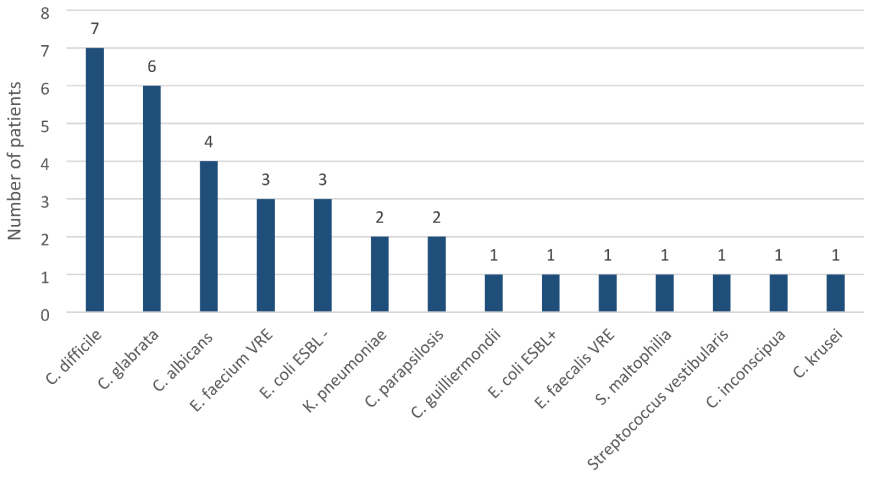

Fig. 2. Number of patients with other than bacteriemia infections 3 months after autoSCT and their etiologies (non-colonized group).

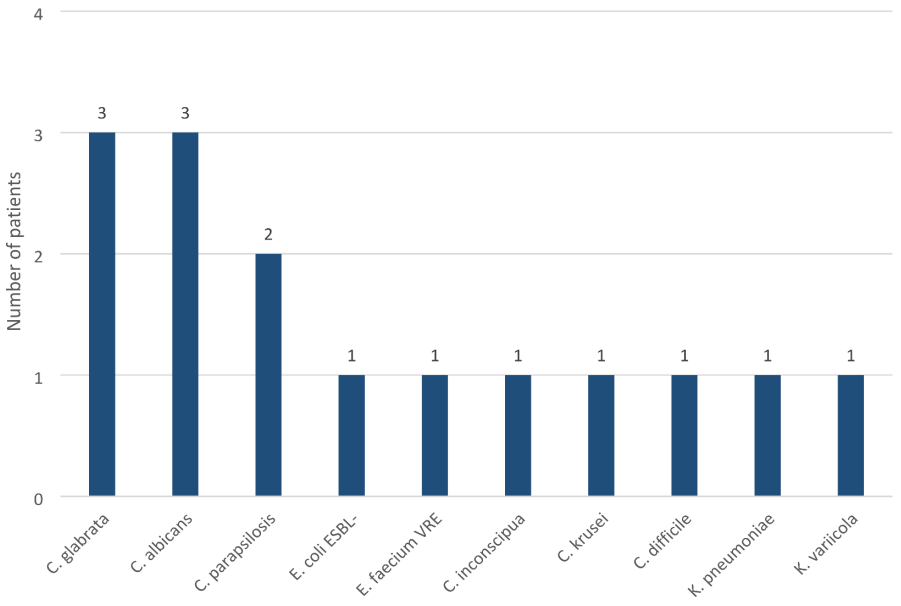

Fig. 3. Number of patients with other than bacteriemia infections 3 months after autoSCT and their etiologies (colonized group).

"in advantage" to be translocated – it is the result of biology and statistics. Most often, the translocating bacteria is "typical gut bacteria". The same observation was seen in our previous study with patients undergoing alloSCT[3].

Regarding OS analysis (1-year and 2-year OS; non-colonized vs. colonized group; 97 and 92% vs. 90 and 86%; $p = 0.054$), the difference was not statistically significant, although the tendency for shorter survival in the colonized group is visible.

Previous works on ARB colonization and its effects on the auto-SCT setting mainly addressed the disruption of the gut microbiota. The group of Peled et al. showed that the gut microbiota of auto-SCT recipients is significantly injured, which is associated with increased non-relapse mortality after the procedure[22]. Joks et

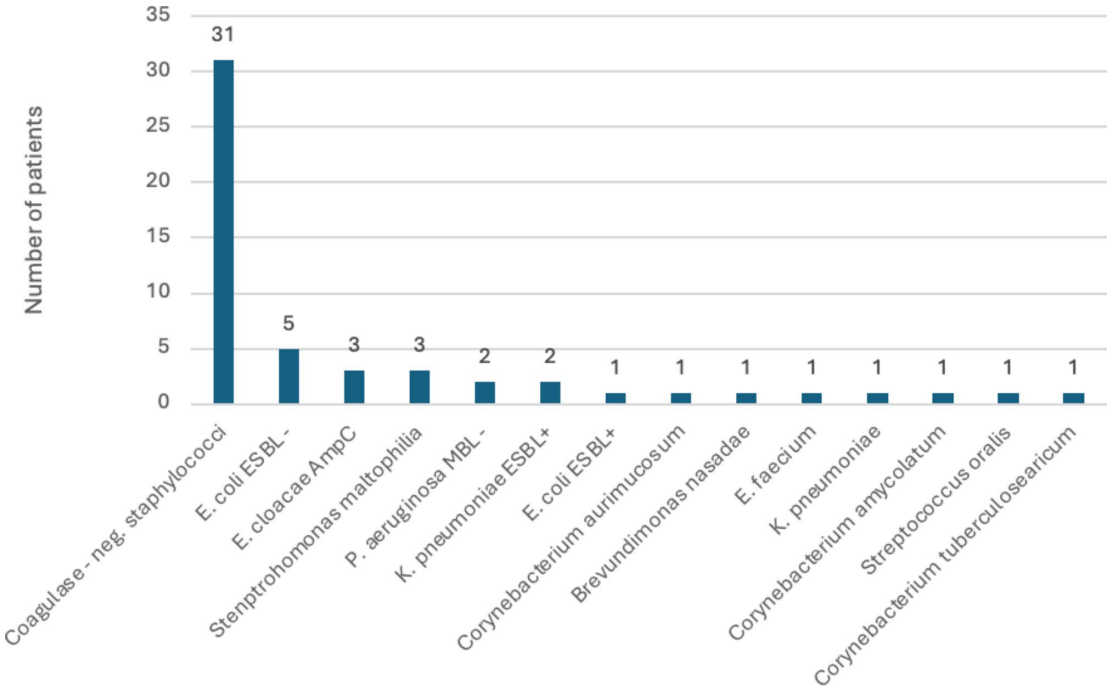

Fig. 4. Number of patients with positive blood cultures 3 months after autoSCT and their etiologies (non-colonized group).

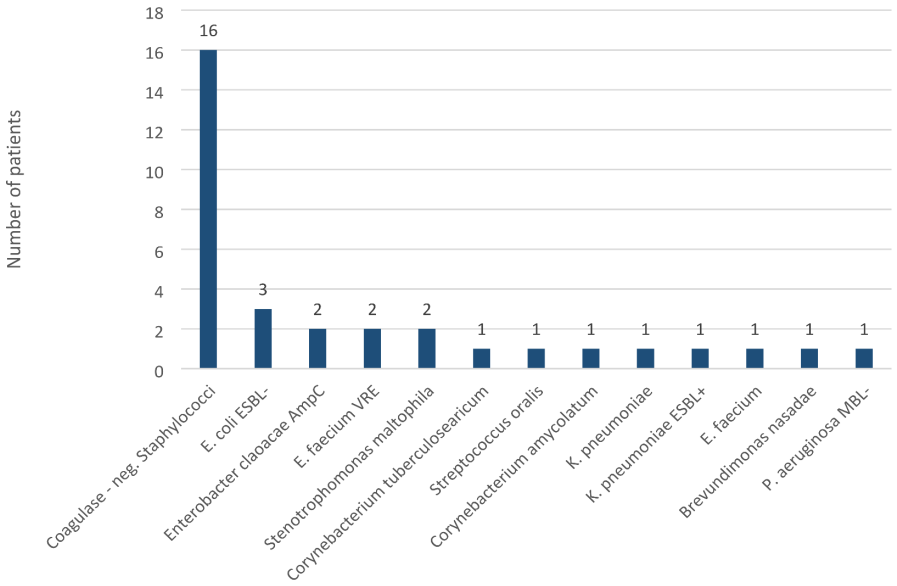

Fig. 5. Number of patients with positive blood cultures 3 months after autoSCT and their etiologies (colonized group).

al. presented a study assessing the impact of multidrug-resistant gram-negative bacteria (MDRG) colonization prior to transplant on auto-SCT results in lymphoma patients. They proved that colonization with MDRG is a risk factor for septic shock development[23].

In the previously mentioned work of Forcina et al., the MDR gram-negative bacteria colonizing the gut before auto-SCT did not significantly influence OS, transplant-related mortality (TRM), or infections-related mortality. The authors stated that mortality due to infections associated with MDR bacteria can be mitigated with pre-emptive antimicrobial treatment in cases of neutropenic fever[21].

In other work of Scheich et al., the opposite outcomes were noted. The OS of patients undergoing autoSCT and being colonized with MDR bacteria before the procedure was significantly shorter than in the non-colonized group. That was shown in univariate (61.7% versus 73.3%, $P=0.005$) and multivariate analysis (hazard ratio, 2.463; 95% confidence interval, 1.311 to 4.626; $P=0.005$). Altogether, 21.7% of patients were colonized with MDR bacteria before the transplantation. Worth mentioning is the fact that not multiple myeloma (43.5%) but lymphoma (53.3%) was the most common indication for auto-SCT, which constitutes a significant difference compared with our study[24].

Khan et al. recently showed a paper showing that patients undergoing auto-SCT had a loss of diversity of the gut microbiota after the procedure. Those patients with above-median peri engraftment diversity of fecal samples had decreased risk of progression (PFS hazard ratio, 0.46; 95% confidence interval, 0.26–0.82; $P=0.008$) even when adjusted for disease and its status. Additionally, when fecal diversity was shown as a $\log_{10}$-transformed continuous variable, those patients with greater fecal diversity had a decreased risk of death (OS HR 0.5; 95% CI, 0.29–0.87; $P=0.014$). However, after adjustment for disease status and its type, that association was not statistically significant (HR 0.58; 95% CI, 0.32–1.06; $P=0.079$)[22].

In another work of D'Angelo et al., they showed that lower alpha diversity at engraftment was associated with partial response rather than very good partial response or complete response (CR/VGPR vs. PR, $P<0.05$). That result stems from the population of patients with MM undergoing the autoSCT[25].

The limitations of our work should also be mentioned. First, the results are based on single-center, retrospective data. Second, groups are relatively small (particularly colonized group), and therefore, plausible conclusions cannot always be drawn. Third, the observation time of patients is relatively short, which does not allow us to draw any conclusions about the influence of gut colonization on survival time.

Conclusions

To sum up, our work shows that the ARB colonization in the gut three months before the auto-SCT transforms into a higher rate of all infections three months after the procedure. That was particularly emphasized in terms of bloodstream infections. Considering other similar works, it is warranted that actions be taken to reverse the gut colonization of patients before auto-SCT should be taken. One emerging treatment that can potentially restore gut microbiota diversity is FMT and microbiota-based restoration therapies.

Data availability

No datasets were generated or analysed during the current study.

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References

1. Antimicrobial Resistance, C. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* **399** (10325), 629–655 (2022).
2. Piddock, L. J. V. Reflecting on the final report of the O'Neill review on antimicrobial resistance. *Lancet Infect. Dis.* **16** (7), 767–768 (2016).
3. Bilinski, J. et al. Impact of Gut colonization by antibiotic-resistant Bacteria on the outcomes of allogeneic hematopoietic stem cell transplantation: a retrospective, single-center study. *Biol. Blood Marrow Transpl.* **22** (6), 1087–1093 (2016).
4. Kim, S. B. et al. Incidence and risk factors for carbapenem- and multidrug-resistant *Acinetobacter baumannii* bacteremia in hematopoietic stem cell transplantation recipients. *Scand. J. Infect. Dis.* **46** (2), 81–88 (2014).
5. Thursby, E. & Juge, N. Introduction to the human gut microbiota. *Biochem. J.* **474** (11), 1823–1836 (2017).
6. Valverde, A. et al. High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. *J. Clin. Microbiol.* **46** (8), 2796–2799 (2008).
7. Patriarca, F. et al. Risk factors and outcomes of infections by multidrug-resistant Gram-negative bacteria in patients undergoing hematopoietic stem cell transplantation. *Biol. Blood Marrow Transpl.* **23** (2), 333–339 (2017).
8. Sadowska-Klasa, A. et al. Colonization with multidrug-resistant bacteria increases the risk of complications and a fatal outcome after allogeneic hematopoietic cell transplantation. *Ann. Hematol.* **97** (3), 509–517 (2018).
9. Scheich, S. et al. Clinical impact of colonization with multidrug-resistant organisms on outcome after allogeneic stem cell transplantation in patients with acute myeloid leukemia. *Cancer* **124** (2), 286–296 (2018).
10. Bilinski, J. et al. Fecal microbiota transplantation in patients with blood disorders inhibits gut colonization with antibiotic-resistant bacteria: results of a prospective, single-center study. *Clin. Infect. Dis.* **65** (3), 364–370 (2017).
11. Innes, A. J. et al. Fecal microbiota transplant mitigates adverse outcomes seen in patients colonized with Multidrug-resistant organisms undergoing allogeneic hematopoietic cell transplantation. *Front. Cell. Infect. Microbiol.* **11**, 684659 (2021).
12. Bilinski, J., Jasinski, M. & Basak, G. W. The role of fecal microbiota transplantation in the treatment of Acute Graft-versus-host disease. *Biomedicines*, **10**(4), 1–16 (2022).
13. Bilinski, J. et al. Fecal microbiota transplantation with ruxolitinib as a treatment modality for steroid-refractory/dependent acute, gastrointestinal graft-versus-host disease: a case series. *Am. J. Hematol.* **96** (12), E461–E463 (2021).
14. Barlogie, B. et al. Curing myeloma at last: defining criteria and providing the evidence. *Blood* **124** (20), 3043–3051 (2014).
15. Devarakonda, S., Efebera, Y. & Sharma, N. Role stem cell transplantation multiple myeloma. *Cancers (Basel)*, **13**(4), 1–19 (2021).
16. Jasinski, M. et al. Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation. *Sci. Rep.* **11** (1), 22507 (2021).

17. Wardill, H. R. et al. Translational model of melphalan-induced gut toxicity reveals drug-host-microbe interactions that drive tissue injury and fever. *Cancer Chemother. Pharmacol.* **88** (2), 173–188 (2021).
18. Jasinski, M., Bilinski, J. & Basak, G. W. The role of the gut microbiome in pathogenesis, biology, and treatment of plasma cell dyscrasias. *Front. Oncol.* **11**, 741376 (2021).
19. Jasinski, M., Bilinski, J. & Basak, G. W. The role of the Crosstalk between Gut Microbiota and Immune cells in the Pathogenesis and treatment of multiple myeloma. *Front. Immunol.* **13**, 853540 (2022).
20. Klastersky, J. et al. Management of febrile neutropaenia: ESMO Clinical Practice guidelines. *Ann. Oncol.* **27** (suppl 5), v111–v118 (2016).
21. Forcina, A. et al. Clinical impact of Pretransplant Multidrug-resistant gram-negative colonization in autologous and allogeneic hematopoietic stem cell transplantation. *Biol. Blood Marrow Transpl.* **24** (7), 1476–1482 (2018).
22. Khan, N. et al. Fecal microbiota diversity disruption and clinical outcomes after auto-HCT: a multicenter observational study. *Blood* ,**137**, 1527–1537 (2020).
23. Joks, M. et al. Neutropenic enterocolitis and multidrug-resistant bacteria colonization in lymphoma patients undergoing autologous stem cell transplantation. *Acta Haematol. Pol.* **53**, 258–266 (2022).
24. Scheich, S. et al. Clinical impact of colonization with Multidrug-resistant organisms on outcome after autologous stem cell transplantation: a retrospective single-center study. *Biol. Blood Marrow Transpl.* **23** (9), 1455–1462 (2017).
25. D'Angelo, C. et al. Perturbation of the gut microbiome and association with outcomes following autologous stem cell transplantation in patients with multiple myeloma. *Leuk. Lymphoma.* **64** (1), 87–97 (2023).

Author contributions

J.B., E.P., and G.W.B. conceived and designed the study; M.J., M.M., K.O., and P.R. collected the data; M.J., J.B., W.K., and G.W.B. contributed to analysis and interpretation; M.J. and J.B. drafted the article; M.M., K.O., E.P., E.S., G.W.B. critically revised the article; M.J. and J.B. contributed to statistics.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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7.2.Publikacja 2

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Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation

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Oral mucositis (OM) is one of the most frequent adverse events of high-dose conditioning chemotherapy with melphalan prior to autologous hematopoietic stem cell transplantation (AHSCT). It significantly reduces the patients' quality of life. One of the preventive strategies for OM is cryotherapy. We retrospectively analyzed whether commercially available ice-cream could prevent OM during the melphalan infusion. We retrospectively analyzed 74 patients after AHSCT to see whether there is any correlation between OM and cryotherapy (ice-cream), melphalan dose (140 mg/m^2 or 200 mg/m^2). The incidence of OM in our study inversely correlated with cryotherapy in the form of ice-cream. Out of 74 patients receiving conditioning chemotherapy with high-dose melphalan, 52 received cryotherapy. Fifteen patients in the cryotherapy group (28.84%) developed OM, whereas 13 patients (59.09%) developed it in the group without cryotherapy. In a multiple linear regression test cryotherapy remained a significant protective factor against OM ($p = 0.02$) We have also seen the relationship between melphalan dose with OM ($p < 0.005$). Cryotherapy in the form of ice-cream is associated with a lower rate of OM and, therefore, could potentially be used as a cost-effective, less burdensome, and easy to implement method in prevention of oral mucositis.

Autologous hematopoietic stem cell transplantation (AHSCT) is part of standard therapy in patients with multiple myeloma, amyloidosis, systemic sclerosis or POEMS syndrome[1,2]. Due to the use of high-dose of conditioning chemotherapy regimens, patients experience a wide variety of toxicities. Oral mucositis (OM) is one of the most common side effects of this treatment, and is reported by patients as the most deteriorating and decreasing the quality of life[3]. The OM often causes the need for parenteral nutrition but also increases the risk of bacterial translocation and sepsis during cytopenia[4]. In the most severe cases it may require parenteral opioids to relieve the patients' pain what is associated with the risk of adverse events[5,6]. One of the strategies used to prevent OM is cryoprotection[7]. Typical cryoprotection protocols entail prolonged use of ice chips or ice cubes, which are not well received by patients[8]. While this approach is effective[9], it is still not widely applied in transplantation centers. The mechanism of ice chips' action is vasoconstriction, which stops the inflammation in the region of the oral cavity mucosa and reduces the contact of toxic drugs with the mucous layer[10]. The AHSCT in multiple myeloma might be a spot-on indication for cryotherapy as melphalan used as a standard conditioning regimen has a short half time and thus, the maximal toxicity is limited to the few hours after the start of infusion. In fact, it has been proven that cryotherapy during high-dose melphalan administration effectively reduces incidence and severity of OM in patients undergoing AHSCT[8,11]. However due to long time of melphalan infusion, some patients complain of a cold sensation and stop the use of ice chips before infusion ends[8].

Another, more patient-friendly and easy to comply approach to cryotherapy deployed by some centers is the use of ice cream. This is especially common in pediatric centers. Surprisingly, we could not identify in literature

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searches any references that could provide evidence that this procedure lowers the chances for oral mucositis in adult patients receiving chemotherapy.

In our work, we analyzed the impact of consuming commercially available ice-cream during the melphalan infusion on mucositis after the AHSCT.

Methods

We retrospectively analyzed the medical data of the patients who underwent AHSCT with non-cryopreserved hematopoietic stem cells in our clinic between November 2017 and December 2020. The inclusion criterium was the availability of the data on the cryotherapy in the medical records and 74 out of all 87 patients met the inclusion criteria. Sixty-three patients had multiple myeloma (MM), 4 patients had amyloidosis with MM (AL + MM), 3 patients had amyloidosis (AL), 3 patients had systemic sclerosis (SSc) and one patient had POEMS (Polyneuropathy, Organomegaly, Endocrinopathy, Myeloma protein, Skin changes) syndrome. As conditioning therapy, the patients received 200 mg/m^2 or 140 mg/m^2 melphalan, depending on the clinical picture of the disease and comorbidities. The dose was adjusted in accordance with EBMT CALM study results[12]. Patients without any of OM symptoms were classified as 0, while patients with the most severe, life-threatening OM were assessed as IV in CTC-AE v 5.0 scale. Oral mucositis assessment was done every day during the morning doctor's round by attending physician. The patient's highest recorded score was documented in the discharge letter and used for the analysis in this study. All methods were carried out in accordance with relevant guidelines and regulations. All patients provided informed consent for the treatment and for the use of medical data for scientific purposes.

Cryotherapy with ice-cream. The patients received cryotherapy with ice-cream on demand or as requested by the physician for the prevention of oral mucositis. The ice-cream was given from the beginning of the melphalan infusion. The protocol consisted of 3 ice-cream doses chosen by the patient from ice-cream commercially available at the hospital cafeteria. Patients received ice-cream in the form of popsicles and dairy-containing products as well. The consecutive ice-creams were given on patient demand. Patients were asked to eat slowly, thawing the ice-cream in the mouth. The compliance with the protocol was not measured. For the duration of neutropenia all patients received the same oral care which comprised of octenidine and calcium phosphate rinses.

Statistical analysis. To test whether cryotherapy remained a significant protective factor against OM after taking into account melphalan dose we used a multiple linear regression test. The comparison of age between groups with or without OM was made with the two-tailed Mann–Whitney U Test (Calculator: https://www.socscistatistics.com/tests/mannwhitney/).

Ethics approval. This research study was conducted retrospectively from data obtained for clinical purposes. Ethical approval was waived by the local Ethics Committee of the Medical University of Warsaw in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

Results

The mean age of patients was 58.1 years (Table 1). Overall, 28 out of 74 patients (37.84%) developed OM after AHSCT. Fifty-two patients received cryotherapy during infusion of melphalan, whereas 22 of them did not. In the group with cryotherapy, 15 of 52 (28.85%) patients developed OM, while in the group without cryotherapy, 13 of 22 (59.09%). In a multiple linear regression test cryotherapy remained a significant protective factor against OM ($p = 0.02$) (Fig. 1). No mucosal-injury-related sepsis was observed in the study population. The median length of hospitalization in patients without OM was 17 days whereas in patients with OM—19 days (whole population—18 days). In the studied population only 3 patients (4.1%) required total parenteral nutrition (TPN). Two of them received TPN because of the grade 3 and 4 OM in the CTC-AE v 5.0. scale and were fed parenterally for 21 and 12 days respectively. The third patient was given with TPN for 11 days because of subileus. It is also worth mentioning that in the control group 3 patients were classified as G3 and 1 patient was classified as G4 OM, whereas in the cryotherapy group 2 and 0 patients respectively.

Discussion

The study provides evidence that cryotherapy can prevent OM in patients undergoing AHSCT. In our analysis, we have demonstrated that the use of commercially available ice-cream in patients receiving high-dose chemotherapy conditioning regimens significantly reduces the risk of OM. The protocol used in our study is simple, easy to implement, cost-efficient, and less burdensome for the patients. Our findings are in line with the data on cryotherapy effectiveness as OM prophylaxis. A recent systematic review on cryotherapy in patients undergoing AHSCT using high-dose melphalan conditioning regimen upgraded this strategy's level of evidence to *recommendation*[13]. A meta-analysis of randomised controlled trials from 2015 comparing the effect of oral cryotherapy with no treatment in ASCT patients confirmed cryotherapy effectiveness in reducing incidence of severe OM, duration of use of parenteral nutrition and hospitalization length[14]. However, the aforementioned studies concentrated on cryotherapy protocols that included the use of ice chips. Therefore, our analysis adds additional data on the effectiveness of cryotherapy protocol with the use of commercially available ice cream. Considering the lack of studies on this type of cryotherapy strategy, our study fills a knowledge gap in this area. The relatively small groups and retrospective methodology are the most important limitations of this study. The results should be validated in a prospective randomized study comparing the effectiveness of different cryotherapy protocols

Variable	Group	Cryotherapy N = 52	No cryotherapy N = 22
Age in years	Mean	57.8	58.7
	Range	26–70	37–68
Indication for AHSCT n (%)	MM	44 (84.62)	19 (86.36)
	AL + MM	3 (5.77)	1 (4.55)
	AL	2 (3.85)	1 (4.55)
	SSc	3 (5.77)	0 (0)
	POEMS	0 (0)	1 (4.55)
Melphalan dose n (%)	140/m^2	33 (63.46)	11 (50)
	200/m^2	19 (36.54)	11 (50)
Grades of OM n (%)	G0	37 (71.15)	9 (40.91)
	G1	11 (21.15)	4 (18.18)
	G2	2 (3.85)	5 (22.73)
	G3	2 (3.85)	3 (13.64)
	G4	0 (0)	1 (4.55)

Table 1. Baseline characteristics of analysed patients with or without cryotherapy. *OM* oral mucositis, *AHSCT* autologous hematopoietic stem cell transplantation, *MM* multiple myeloma, *AL* light chain amyloidosis, *SSc* systemic sclerosis, *POEMS* polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin abnormalities.

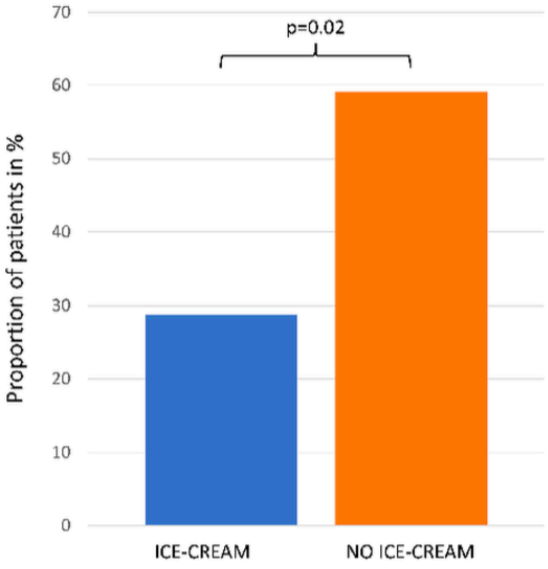

Figure 1. Proportion of patients with oral mucositis on and without ice-cream cryotherapy. 28.85% patients who received ice-cream cryotherapy developed OM, whereas 59.09% developed it in the group with no cryotherapy. In a multiple linear regression test cryotherapy remained a significant protective factor against OM ($p = 0.02$).

to confirm non-inferiority of the strategy based on the use of commercially available ice-cream. The influencing factor was not surprisingly higher mg/kg melphalan dose, which stays in line with previous findings[15].

In summary, we have shown that the application of commercially available ice-cream during short melphalan infusions can contribute to a lower rate and severity of mucositis in patients undergoing autologous HSCT.

Data availability

Data from the study can be obtained upon request by the corresponding author.

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References

1. Sureda, A. *et al.* Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2015. *Bone Marrow Transpl* **50**(8), 1037–1056. https://doi.org/10.1038/bmt.2015.6 (2015).
2. Kanate, A. S. *et al.* Indications for hematopoietic cell transplantation and immune effector cell therapy: guidelines from the American Society for transplantation and cellular therapy. *Biol Blood Marrow Transp* **26**(7), 1247–1256. https://doi.org/10.1016/j.bbmt.2020.03.002 (2020).
3. Sonis, S. T. *et al.* Oral mucositis and the clinical and economic outcomes of hematopoietic stem-cell transplantation. *J Clin Oncol* **19**(8), 2201–2205. https://doi.org/10.1200/JCO.2001.19.8.2201 (2001).
4. Lalla, R. V., Saunders, D. P. & Peterson, D. E. Chemotherapy or radiation-induced oral mucositis. *Dent Clin North Am* **58**(2), 341–349. https://doi.org/10.1016/j.cden.2013.12.005 (2014).
5. Bellm, L. A., Epstein, J. B., Rose-Ped, A., Martin, P. & Fuchs, H. J. Patient reports of complications of bone marrow transplantation. *Support Care Cancer* **8**(1), 33–39. https://doi.org/10.1007/s005209900095 (2000).
6. Coda, B. A. *et al.* Comparative efficacy of patient-controlled administration of morphine, hydromorphone, or sufentanil for the treatment of oral mucositis pain following bone marrow transplantation. *Pain* **72**(3), 333–346. https://doi.org/10.1016/s0304-3959(97)00059-6 (1997).
7. Riley, P. *et al.* Interventions for preventing oral mucositis in patients with cancer receiving treatment: cytokines and growth factors. *Cochrane Database Syst Rev* **11**, CD011990. https://doi.org/10.1002/14651858.CD011990.pub2 (2017).
8. Lilleby, K. *et al.* A prospective, randomized study of cryotherapy during administration of high-dose melphalan to decrease the severity and duration of oral mucositis in patients with multiple myeloma undergoing autologous peripheral blood stem cell transplantation. *Bone Marrow Transp* **37**(11), 1031–1035. https://doi.org/10.1038/sj.bmt.1705384 (2006).
9. Chen, J., Seabrook, J., Fulford, A. & Rajakumar, I. Icing oral mucositis: oral cryotherapy in multiple myeloma patients undergoing autologous hematopoietic stem cell transplant. *J Oncol Pharm Pract* **23**(2), 116–120. https://doi.org/10.1177/1078155215620920 (2017).
10. Karagozoglu, S. & Filiz Ulusoy, M. Chemotherapy: the effect of oral cryotherapy on the development of mucositis. *J Clin Nurs* **14**(6), 754–765. https://doi.org/10.1111/j.1365-2702.2005.01128.x (2005).
11. Tartarone, A., Matera, R., Romano, G., Vigliotti, M. L. & Di Renzo, N. Prevention of high-dose melphalan-induced mucositis by cryotherapy. *Leuk Lymphoma* **46**(4), 633–634. https://doi.org/10.1080/10428190400029957 (2005).
12. Auner, H. W. *et al.* Melphalan 140 mg/m(2) or 200 mg/m(2) for autologous transplantation in myeloma: results from the Collaboration to Collect Autologous Transplant Outcomes in Lymphoma and Myeloma (CALM) study: a report by the EBMT Chronic Malignancies Working Party. *Haematologica* **103**(3), 514–521. https://doi.org/10.3324/haematol.2017.181339 (2018).
13. Correa, M. E. P. *et al.* Systematic review of oral cryotherapy for the management of oral mucositis in cancer patients and clinical practice guidelines. *Support Care Cancer* **28**(5), 2449–2456. https://doi.org/10.1007/s00520-019-05217-x (2020).
14. Wang, L. *et al.* Efficacy of oral cryotherapy on oral mucositis prevention in patients with hematological malignancies undergoing hematopoietic stem cell transplantation: a meta-analysis of randomized controlled trials. *PLoS ONE* **10**(5), e0128763. https://doi.org/10.1371/journal.pone.0128763 (2015).
15. Grazziutti, M. L. *et al.* Oral mucositis in myeloma patients undergoing melphalan-based autologous stem cell transplantation: incidence, risk factors and a severity predictive model. *Bone Marrow Transpl* **38**(7), 501–506. https://doi.org/10.1038/sj.bmt.1705471 (2006).

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The Role of the Crosstalk Between Gut Microbiota and Immune Cells in the Pathogenesis and Treatment of Multiple Myeloma

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Around 10% of all hematologic malignancies are classified as multiple myeloma (MM), the second most common malignancy within that group. Although massive progress in developing of new drugs against MM has been made in recent years, MM is still an incurable disease, and every patient eventually has relapse refractory to any known treatment. That is why further and non-conventional research elucidating the role of new factors in MM pathogenesis is needed, facilitating discoveries of the new drugs. One of these factors is the gut microbiota, whose role in health and disease is still being explored. This review presents the continuous changes in the gut microbiota composition during our whole life with a particular focus on its impact on our immune system. Additionally, it mainly focuses on the chronic antigenic stimulation of B-cells as the leading mechanism responsible for MM promotion. The sophisticated interactions between microorganisms colonizing our gut, immune cells (dendritic cells, macrophages, neutrophils, T/B cells, plasma cells), and intestinal epithelial cells will be shown. That article summarizes the current knowledge about the initiation of MM cells, emphasizing the role of microorganisms in that process.

Keywords: multiple myeloma, gut microbiota, intestinal immune system, fecal microbiota transplantation, B cell, plasma cell

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1 INTRODUCTION

Multiple myeloma (MM) is a hematological neoplasm deriving from clonal plasma cells. In almost every case, it is preceded by a premalignant stage called monoclonal gammopathy of undetermined significance (MGUS) (1, 2). In 3-4% of the whole population over the age of 50, the diagnosis of MGUS could be stated (3). The median age at the time of diagnosis of MM is approximately 70 years (4). The global incidence of MM steadily increases, which can be only partly explained by aging, with the highest score in Western European, North American, and Australasian populations reaching in 2016 about 5 cases per 100 000 persons. In 2019 the global incidence of MM amounted to 155 688 cases, compared to 138 509 in the year 2016. The age-standardized incidence rate (ASIR) was 1.92/100 000 in 2019. During the 2019 year, 113 474 deaths were noted due to MM, whereas 98 437 were in 2016. That short period of three years shows the dynamics of the new MM cases

increase. From 1990 to 2016, the incidence of new MM cases increased by 126% (52.9% was attributed to aging, which is typical for cancers that mainly affect the older population), while deaths due to MM increased by 94% (5, 6). The incidence of MM in the population <30 years is infrequent (0.02-0.3%) (7). Fortunately, the prognosis for patients with MM significantly improved during the last years, which is due to many new drugs, better availability of autologous hematopoietic stem cell transplantation (ASCT), and constantly emerging new therapies such as CAR-T cells (8). To better illustrate the progress: the 5-year survival rate of MM in 1975-1977 was 25% and reached 49% in 2005-2011 (9).

As mentioned before, almost all cases of MM pass through an utterly asymptomatic phase referred to as MGUS, in which monoclonal, malignant in their nature plasma cells live in the patient's body (2). Normal plasma cells carry on their surface the following combination of antigens: $CD19^+/CD56^-/CD45^+/CD38^+$, while the malignant plasma cells are losing CD19 and CD45 and acquiring CD56 (10). The threshold, when the abnormal plasma cells are still in a pre-cancerous entity, MGUS, is set on less than 10% of all bone marrow mononuclear cells (11). The oncogenesis is usually initiated within germinal centers of the lymph node during the isotype class switching and somatic hypermutation (SHM) occurrence (12). The leading role in the normal plasma cells transformation into malignant ones is attributed to cyclin D family proteins mutations enabling G1/S transition (13). Only 1-2% of MGUS patients progress to symptomatic MM per year (14). To become malignant, plasma cells must gain the proliferation and growth potential by self-renewing clone.

The two oncogenes believed to play a critical role in that process are Ras and Myc (15, 16). Interestingly, the mutations found in MM cells are also largely present at the MGUS stage, suggesting that genetic mutations are necessary but insufficient for myeloma development (17). The bone marrow environment plays a complementary role in that process. In addition to genetic factors and aging, environmental factors appear critical to forming a cancerous cell in MM. During our lifetime, our body cells, especially immunocompetent cells located in the lymphatic tissues of the structures that separate us from the outside world, e.g., in the intestines, skin, or liver, interact millions of times with various environmental factors - animate and inanimate. The more environmental signals for recombination and proliferation, the greater the likelihood of mutation in plasma cells, as in any other. It seems logical that chronic antigenic stimulation provokes many rounds of proliferation and selection of B cells, which means an increased risk of mutational changes starting oncogenesis when not repaired. Finally, the last stage of the disease is associated with stroma-independent growth and results in extramedullary diseases or plasma cell leukemia (PCL). The main pathway in this process is characterized by constitutive NF-κB activation, which influences the expression of adhesion molecules, such as VLA-4 (18).

In our previous work, we have described the role of the gut microbiome in pathogenesis, biology, and treatment of plasma cell dyscrasias (19). This review will gather all the information about the sophisticated interplay between the immune cells and the gut microbiota and how this could potentially lead to MM development.

2 GUT MICROBIOTA – SIGNIFICANCE DURING OUR LIFE

One of the most surprising data regarding the first steps in gut colonization was that gut microbiota starts its development already *in utero*. Previously, the fetus's intestine was considered germ-free, but that view was challenged with the results of a few studies. The microorganisms were detected in the amniotic fluid (20, 21), umbilical cord (22), placenta (23), and the most critical – meconium, which is the first excretion that derives from all that has been ingested or secreted before the delivery (24, 25). What is particularly interesting, in the mice model, microorganisms within the fetus's gut resemble those which are colonizing the mother's intestine (24). Therefore, these microbes should efflux the mother's systemic circulation to reach the placenta.

Moreover, during the late pregnancy, the intestinal translocation of bacteria to the vessels is enhanced, which could play a role in the initial colonization of the fetus's gut (26). A study conducted by Gosalbes et al. showed that the gut microbiota of infants during their first weeks of life includes the microorganisms found in the meconium, which were still detectable even seven months after birth (27). In addition, Brosseau et al. recently presented the study results, which shows that supplementation of prebiotics for pregnant women leads to the transmission of specific microorganisms and immune factors from mother to fetus allowing the development of the tolerogenic immune system imprinting that influences other health outcomes (28). However, these data contradict the recently published work, which shows that gut colonization starts after birth and bacteria found in meconium were the effect of skin contamination (29).

Right after birth, the gut is being rapidly colonized, and during that period, the mode of delivery plays a crucial role in establishing gut microbiota composition. For example, infants delivered vaginally possess the gut microbiota, mainly consisting of lactobacilli living in high abundance in the vagina (30). On the other hand, infants born through C-section are frequently colonized by the microorganisms such as *Clostridium* species and facultative anaerobes. Moreover, infants delivered by C-section are colonized by the *Bacteroides* genus with delay (31), and only 41% of their fecal microbiota is identical to the mother's gut microbiota composition (72% in vaginally delivered infants) (32).

The gut microbiota composition during the first year of life changes, while the diversity of microorganisms colonizing the gut increases (33). Its composition resembles more and more of that seen in adults, but it takes another two years to establish a typical pattern of adult-like microbiota (34, 35). However, some studies showed that the maturation of human gut microbiota lasts for more than the first three years of life and can change its

composition even till 12 years (36). The whole process of intestinal colonization by newer and newer microorganisms is remarkably similar to the dynamic development and growth of the repertoire of immunocompetent cells. These are mechanisms that go hand in hand, at the same time, and are strongly interdependent.

The impact of proper gut microbiota development is evident regarding the risk of immune disorders. Lack of balanced gut microbiota can result in various autoimmune and atopic diseases (37, 38). It is not surprising given the fact that the largest area of contact between microbes and immune cells is within the intestine. Our immune system is constantly stimulated by the enormous plethora of ligands presented by microorganisms colonizing the gut, such as lipopolysaccharides (LPS), flagellin, or unmethylated CpG motifs (39). These ligands shape the further differentiation of naïve T cells into T regulatory type (Treg) or the Th1, Th2, and Th17 cells (40). Tregs can inhibit the differentiation of naïve T cells towards Th types (41), suppress eosinophils, basophils, mast cells (42), and the production of immunoglobulin (Ig) E (43). Conversely, different types of Th cells can inhibit the other ones amplifying through that process the immune response (44).

For a long time, the researchers were focused on the role of balance between Th1 and Th2 cells. Excessive activation of one type of cell causes autoimmune and chronic inflammatory diseases (Th1) or allergic diseases (Th2) (45, 46). The role of Th17 cells in diseases classically associated with an imbalance of Th1/Th2 activation was also shown (47). Taking into consideration the role of balance between Treg and Th cells and also the fact that such balance is closely related to the composition of gut microbiota, it leads to the conclusion that gut microbiota is the initial factor in the pathogenesis of a wide variety of chronic inflammatory, allergic and autoimmune disease (48, 49).

There is also one other proof of how vital well-balanced gut microbiota is for maintaining the immune system in shape. Experiments on germ-free mice, free of any microorganisms, showed that gut microbiota is obligatory for Tregs differentiation (50). Other experiments showed that different bacteria and their products induce the activation of Tregs in mice (51). On the other hand, segmented filamentous bacteria (SFB) facilitate the differentiation of naïve T cells towards proinflammatory Th17 cells in mice (52). Together, these experiments showed us the key role of balanced gut microbiota in health and disease.

3 THE ROLE OF THE B-CELL CHRONIC STIMULATION IN MULTIPLE MYELOMA PATHOGENESIS

The process of immunoglobulins (Ig) production starts within the germinal centers (GCs) of secondary lymphoid organs. This is where naïve B cells encounter T cells accountable for selecting B cells eligible for future combat against pathogens or antigens (53). Given that, one can easily conclude that the whole process of Ig production starts there – in the secondary lymphoid organs, especially in the gut-associated lymphoid tissue (GALT), and that is where the defense of the whole organism begins.

In the GCs, B cells are selected based on the higher affinity of B-cell receptors (BCR) towards the antigen. This is the initial step in immunity organization that will last for long years (53). During that process, the naïve B cells undergo two sophisticated DNA changes by which only B cells with the highest affinity against the antigen are selected. One of these processes is somatic hypermutation (SHM) with antigen selection, and the second one – immunoglobulin heavy chain (IgH) switch recombination. These two types of DNA modifications are the source of mutations and breaks of double-strand DNA, sometimes also in oncogenes (54). When the oncogene is positioned near the site of the Ig enhancer, then it results in dysregulation and potent proliferation of B cells. These are the initial cells that will constitute multiple myeloma (MM) (55). Many B cell neoplasms share the same feature, which are the translocations that are mediated by errors during recombination in V (variable), D (diversity), and J (joining) gene segments or the abovementioned two more subtle changes in DNA sequence (56).

The association between chronic intracellular infection with viruses [HCV, HSV, EBV (57)] or bacteria [*Helicobacter pylori* (58)] and the increased risk of neoplasms development was shown many years ago. It is now established that up to 20% of malignancies are microbiota-dependent (59). The transformation of a normal cell into malignant may occur indirectly *via* chronic antigenic stimulation of the BCR or directly *via* B cell infection and transformation (60). The main proof for the role of chronic antigenic stimulation in the pathogenesis of MGUS and MM is the specificity against some viruses of monoclonal Ig produced by mutated clone (61–63). Moreover, in some cases, the antiviral therapy against chronic HCV infection alone was sufficient to reach the regression in the MM that had features compatible with MGUS (64–66). On the other hand, patients with Gaucher's disease have an increased risk of transforming normal B cells into myeloma cells (67). Some reports indicate that lyso-glucosylceramide 1 (LGL1) and lyso-phosphatidylcholine (LPC), which are accumulated in Gaucher's disease, become antigens that drive the selection of B cells and therefore contribute to the pathogenesis of MM (68).

Although very intriguing, these reports require few words of explanation. Only in the subset of MM patients, the abnormal immune response to infection may play a role in the pathogenesis of MM (69). However, the infectious agent was not detected or remains unknown in the rest of them.

Considering the reports about the role of an abnormal immune response against infectious agents in the pathogenesis of MGUS and MM, it seems highly probable that the continuous and large-scale interactions between B cells and the gut microbiota could play a role in the pathogenesis of gammopathies. Therefore, it is crucial to detect whether the monoclonal Ig is targeting specifically against some bacteria that colonize the gastrointestinal tract and whether temporal changes in the composition of the gut microbiota could influence the initiation of gammopathies.

4 THE INTERACTION BETWEEN GUT MICROBIOTA AND OTHER CELLS

4.1 Epithelial Cells

Recent studies showed that gut epithelium plays a critical role in regulating the host immune system and the luminal microbiota. Intestinal epithelial cells (IECs) include Paneth cells, absorptive epithelial cells, and goblet cells, and their two central roles are to segregate and mediate between the microorganisms colonizing the gut and the immune system. The former function of IECs is possible because of the physical and chemical barriers which prevent the intestinal inflammation that could start because of conflict between these "two armies" of cells. The latter function means that IECs can forward signals deriving from the gut microbes and their metabolites and transform that signal for the language "understandable" for the immune cells (70). One example of such crosstalk where IECs play a crucial role was seen in mice when segmented filamentous bacteria (SFB) colonized the gastrointestinal tract of germ-free mice attached to the surface of the IECs and induced the production of serum amyloid A (SAA) (52). That, in turn, caused the facilitation of the Th17 differentiation and IL-23 receptor-dependent IL-22 production by innate lymphoid cells 3 (ILC3) (71). On the other hand, IL-17 and IL-22 from Th17 and ILC3 cells induce the production of antimicrobial molecules such as antimicrobial peptides (AMPs) and the regenerating islet-derived 3 (Reg3) family of proteins by epithelial cells, which control the composition of the gut microbiota (72).

What is particularly important from the point of view of this review is to know that IECs drive the IgA class switching in B cells, which are occupying the lamina propria, *via* the production of a proliferation-inducing ligand (APRIL) through toll-like receptor (TLR) signaling (73). The fraction of cells mainly engaged in that process is the M cells, which specialize in the uptake and delivery of antigens derived from the lumen to the antigen-presenting cells (APC) such as dendritic cells (74). Essential for that aim is glycoprotein A (GP2), a transcytotic receptor of M cells responsible for transporting antigens from the lumen to the other side of the wall (75).

Therefore, as was presented, the IECs are responsible for the transition of signals (by TLRs and other receptors and M cells) between the gut microbes and the immune cells that are staying on the two sides of the wall and by secretion of chemokines, cytokines, and hormones they maintain the balance between "both armies."

4.2 Immune Cells

Immune cells engaged in the crosstalk with the gut microbiota are predominantly seen in the lamina propria. The most common ones are T regulatory cells, NK cells, and invariant T cells. Dendritic cells infiltrate very deeply into villi and closely contact with the IECs (76) (**Figure 1**).

4.2.1 Dendritic Cells (DCs)

The immune cells at the site of the gut epithelium should generate tolerance to the antigens found in the food, but simultaneously they must be ready for immediate response to the emergence of pathogens. DCs are APCs known to be the central players in the immune system together with macrophages. These cells can uptake the antigens from the gut microbes with the mediation of epithelial cells or directly extend their dendrites through the inner mucosal lining to connect with the environment colonized by the microorganisms (77). Through those mechanisms, the DCs eventually shape the composition of the gut microbiota by sampling the microbes and then giving the special orders to activate appropriate response (78). The key role processes occur in the mesenteric lymph nodes where the antigens derived from the lumen are presented to the naïve T cells by the DCs (79). These DCs are characterized by the inability to leave the mesenteric lymph nodes and reach the spleen, thus preventing the organism from inducing a commensal-specific systemic response (80). The specific type of DCs, occupying the lamina propria is characterized by the expression of CD103 on its surface and the production of TGF-β, which causes the differentiation of naïve T cells into CD4+CD25+Foxp3+ T cell of regulatory phenotype (81). This is particularly peculiar given the fact that usually DCs release the inflammatory cytokines and drive the differentiation of Th1 cells. Therefore, researchers hypothesize that the local environment of IECs can stimulate this specific phenotype of DCs. That local environment means, for instance, the thymic stromal lymphopoietin (TSLP) released by the IECs, which was shown in humans to induce the release of the APRIL and BAFF by DCs and in turn supports the class switching of the B cells to IgA (82) or the switching of IgA1 to the IgA2 production which are characterized with protease-resistant phenotype (73). Nevertheless, as will be mentioned further, the epithelial cells are not the only ones to modulate the function of DCs because the other immune cells, like macrophages, can also regulate the function of that population.

4.2.2 Macrophages

Macrophages share some similarities with CD103+ DCs. One of them is the ability to induce the differentiation of the Treg cells (83). However, contrary to the DCs, macrophages' migration to the mesenteric lymph nodes has not been shown yet, so they probably do not induce oral tolerance (79). One of the existing hypotheses is that macrophages associated with the gut epithelium support the maintenance of Treg cells. Additionally, macrophages can tune the proinflammatory function of DCs by inhibiting their ability to drive Th17 differentiation (83).

Unlike the macrophages residing other than gut tissue, the subtype of macrophages associated with the gut does not possess the CD14 on their surface, responsible for the LPS-induced cell activation and proinflammatory cytokines production (84). Furthermore, these cells produce anti-inflammatory cytokines such as IL-10 and help the DCs maintain the population of Treg to prevent the mucosal auto-inflammation (83). However, although human gut macrophages are known for anti-inflammatory functions, they do not lose their ability to phagocyte and perform defense functions (84). There is also known that within the gut, the population of CD14+

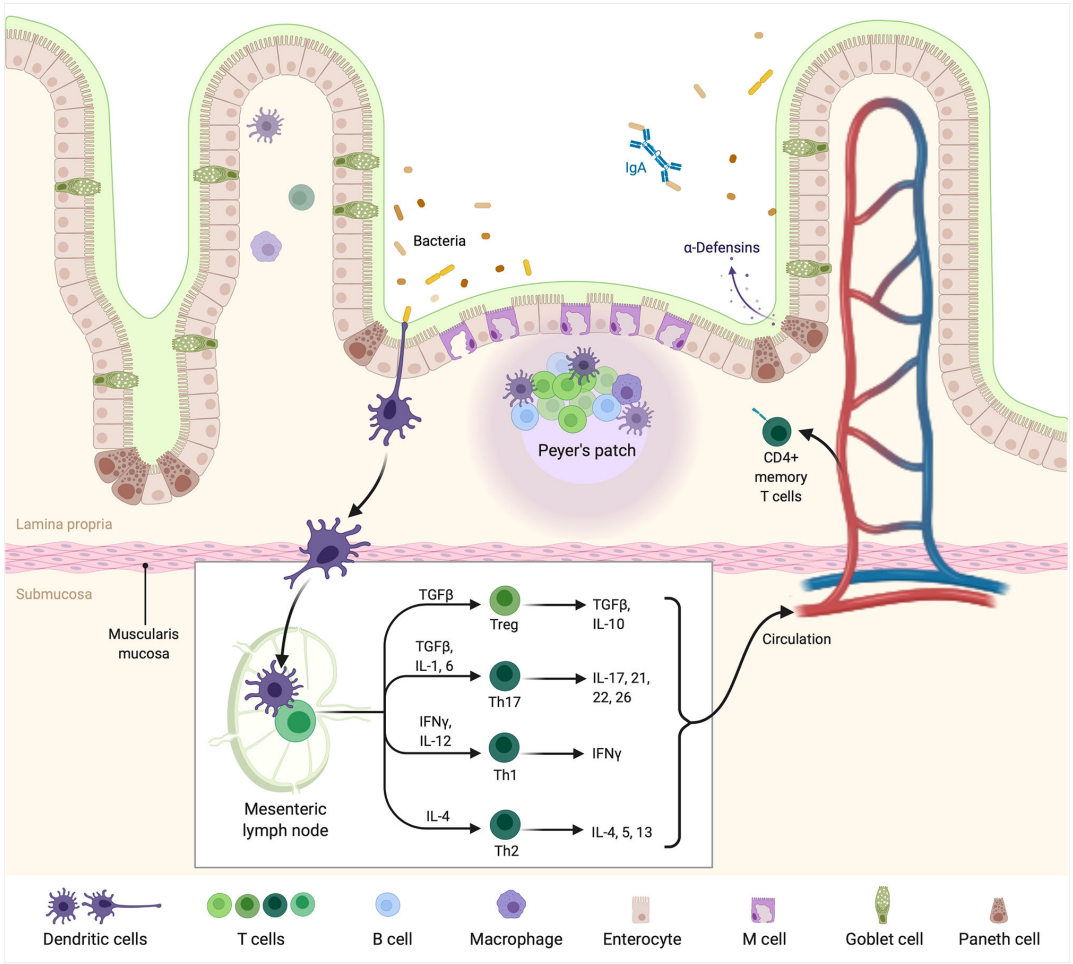

FIGURE 1 | The intestinal immune system. Bacteria currently colonizing the gut are sensed by DCs presenting their antigens in mesenteric lymph nodes or Peyer's patches. In lymph nodes, DCs are accountable for further differentiation of T cells into Treg, Th17, Th1, and Th2 cells producing pro or antiinflammatory "profile" of cytokines. From the "myeloma point of view" particularly important is the balance between Treg/Th17 cells. The latter is accountable for pro-inflammatory cytokines such as IL-17 production, which are known to facilitate the development of multiple myeloma. Created with BioRender.com.

macrophages reside and can produce proinflammatory cytokines such as IL-23 and TNF-α, leading to the further accumulation of similar cells (85).

4.2.3 Neutrophils

Flagellin is the protein of gram-negative bacteria such as *Proteus* or *Escherichia* that stimulates the TLR5/MyD88 signaling in IECs. This pathway promotes the production of IL-8 by IECs, which causes the recruitment of neutrophils to the lamina propria (86).

Neutrophils are known to promote or inhibit the growth of the tumor (87). Moreover, they can switch from promoting to the inhibiting mode and stop the tumor's progression (88). On the other hand, the interactions between neutrophils and the gut microbiota were shown to impact the tumor's growth rate. An example of that is a mouse model of serrated polyps, a premalignant lesion of the colon. Throughout the intestine, the endothelial growth factor receptor ligand is produced, but only the cecum is the site where that molecule promotes the development of polyps. That is because the growth of polyps

requires the specific gut microbiota composition in the cecal mucosa. Furthermore, it was shown that administration of antibiotics or depletion of neutrophils resulted in inhibition of serrated polyps' development, suggesting the crucial role of bacteria and neutrophils in that process (89).

4.2.4 T Cells

We limit the interplay between T cells and the gut microbiota to the role of the Th17/Treg cells balance because of their great importance in switching the mode of the immune system from pro to anti-inflammatory and vice versa. Such imbalance was reported to play a role in chronic inflammations (90), allergic diseases (91), cancers, and autoimmune diseases (92, 93). Germ-free mice were shown to have decreased number of both populations of cells (94), but it has also been shown that specific metabolites such as ATP and short-chain fatty acids (SCFA) can induce differentiation of Th17 and Treg cells, respectively (95, 96). SCFA are the bacterial products produced from the dietary fiber by the anaerobic gut microbiota (97).

4.2.5 B Cells and Plasmacytes

The gut microbiota is known for its impact on the development, differentiation, activation, and function of the B cells. Regarding the development of human innate-like B cells and marginal zone B cells, the gut-associated lymphoid tissue (GALT) may be the site of a growing repertoire of B cells (98). Interestingly, a subset of human immature B cells, known as transitional 2 (T2) B cells from the bone marrow, tend to reside in the intestine for their activation. The maturation process relies on eliminating self-reactive B cells from the developing repertoire. Failure in that process is seen in the systemic lupus erythematosus (SLE), which suggests that this site constitutes a checkpoint against autoimmunity (99).

The intestinal microbiota may influence the B cells through the direct and indirect modes. The former depends on B cells' direct activation *via* BCR recognition of the carbohydrates and proteins produced by the gut microbes, which act as antigens (100). That is T-dependent B-cell activation, but B cells can be activated T cell-independently. That is because they have TLRs on their surface, which are extremely important for their survival and function (101). For example, Oh et al. showed that mice lacking the TLR5 could not develop immunity against seasonal influenza vaccination because of the inability to sense the gut microbiota (102). Similarly, the metabolites of the bacteria can also activate the B cells. For instance, SCFAs may affect B-cell metabolism and facilitate the differentiation of B-cell, hence promoting immunoglobulin promotion (103).

Although some papers showed in recent years that a repertoire of B cells could develop within GALT with the help of the gut microbiota, there are still many gaps in that field. However, that potentially shed light on the possibility that the composition of the gut microbiota could shape the repertoire of B cells and that dysbiosis could be potentially accountable for the chronic antigenic stimulation of B cells and subsequent genesis of MGUS and MM (**Figure 2**).

5 POSSIBLE MECHANISTIC PATHWAY OF MYELOMA CELL INITIATION IN THE CONTEXT OF MICROBIOTA

It is well known that the IgA is produced in the intestine by B cells, but little is known about the production of other subtypes of immunoglobulins. However, there is already evidence that the gut microbiota may induce the TLR4-dependent production of IgG and that these antibodies are efficient in fighting against systemic infection (104). Furthermore, some other studies show that the SCFAs regulate the production of immunoglobulins in different ways. For example, it was shown that after the administration of cholera toxin, the SCFAs facilitate the production of BAFF and retinoic acid (RA) by DCs to upregulate the synthesis of IgG and IgA (105). Given that, it is reasonable to think that SCFA may play an active role in regulating the production of immunoglobulins.

Considering what was said before that the gut microbiota could potentially drive the repertoire of BCR, it seems probable that dysbiosis could affect that process. MGUS starts when the monoclonal globulin starts to be detectable and when there are less than 10% of clonal plasma cells within the bone marrow. Nevertheless, the first mutated cell is probably created a long time before the diagnosis of MGUS or MM. Therefore, we speculate that this first step towards entirely symptomatic MM could start within the gastrointestinal wall. Given that microbes, their antigens, and metabolites are recognized by B cells, activate them, and provoke proliferation, it seems reasonable to think that lack of balanced gut microbiota with overgrowth of sparse species of bacteria and then chronic antigen stimulation could lead to fully symptomatic MM. Thus, a mechanistic vision of gut microbiota-dependent myeloma formation could be like we deliberate below.

Because of acquired or resulting from genetic predispositions dysbiosis, there is an overgrowth of selected microorganisms in the gut. Sometimes, even subtler changes such as overgrowth of one bacteria species or even the presence of one antigen that is constantly produced within the gut by microbes could constantly stimulate the immune system of the GALT. Then DCs occupying this area are continuously activated with the help of IECs in that process. DCs, after first contact with antigen, are migrating to mesenteric lymph nodes, which is the place of the "crime," where B cells are stimulated by a minimal number of antigens presented by DCs. That stimulation leads to numerous rounds of proliferation done by B cells, during which they are accumulating mutations during the processes of SHM and class switching. Eventually, the first mutated cell emerges, but that does not necessarily mean that progression of MM is initiated here. Changed plasma cells after rounds of proliferation reach the bone marrow, where they will produce immunoglobulins. As said before, the additional events must occur within the bone marrow to facilitate the progression from MGUS, through SMM, to fully symptomatic MM, and eventually to PCL. That progression is supported by the proinflammatory cytokines, which are stimulating osteoclasts to destroy the bones

to "make space" for quicker and quicker proliferating myeloma cells (106). We postulate that this is another process in which the gut microbiota could play the role since papers show that the lack of balanced gut microbiota results in a more proinflammatory state of the immune system, and for instance, differentiation of T cells within the gut is skewed towards Th17 cells. Moreover, the work of Jian et al. showed that "crosstalk on distance" of myeloma cells and the gut microbes is possible and that these two groups of cells cooperate and support the growth mutually (107).

Considering that, it is worth asking whether there are any changes in the gut microbiota between different stages of the disease from MGUS to PCL? An initial, small study done by Pepeljugoski et al. proves that such changes occur (108) and that progression in the disease is associated with developing dysbiosis. Additionally, it would be very interesting to check if dysbiosis or even subtle changes in the gut microbiota composition could be a risk factor for MGUS. Perhaps, at least some of the cases of MGUS/MM are producing a monoclonal protein targeting antigens deriving from the gut. That hypothesis will be discussed further, but to show that dysbiosis is a critical player in the progression of the disease, a correlation study that will link the gut microbiota composition with the immune-related gene expression profile is needed. Our group has initiated a study on newly diagnosed MGUS, SMM, and MM patients recently, in which we are going to search whether there is such correlation and how it is changing with time and applied treatment.

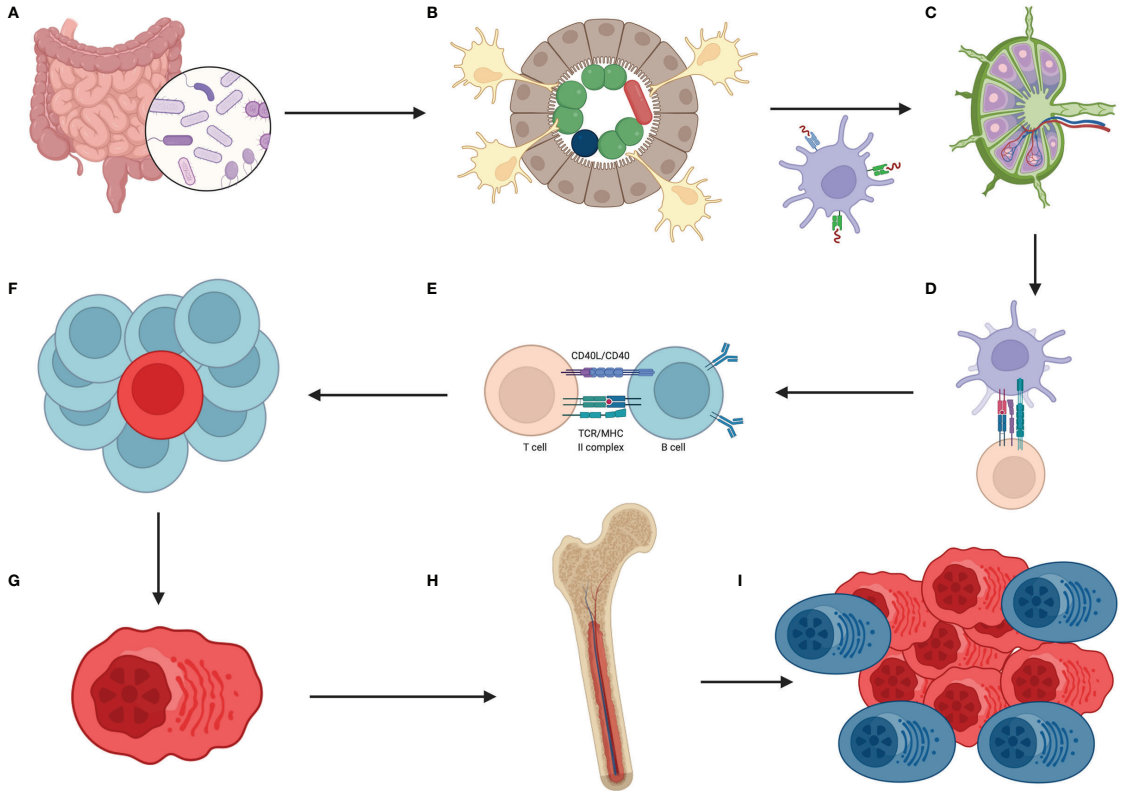

FIGURE 2 | How the hypothetical pathway from dysbiosis to multiple myeloma looks? The sequence of events is as follows **(A)** lack of balanced gut microbiota which means overgrowth of selected species of bacteria **(B)** these bacteria are accountable for constant, oligo- or even monoclonal stimulation of DCs (with help of IECs) which migrate to mesenteric lymph nodes and/or Peyer's patches **(C)** there, B cells, T cells and mentioned DCs meet each other **(D)** DCs are presenting this oligo-, monoclonal antigens to T cells **(E)** which then are responsible for selection of B cells that are going to have required features to combat the antigen **(F)** because of continuous stimulation in the gut the process of B cell selection is intense and these cells undergo numerous rounds of proliferation which are preceded by SHM and class switching, associated with DNA changes **(G)** one initial B cell with driver mutation emerges, transforms to plasma cell that produces oligo-, monoclonal antibodies against the antigen, and proliferates **(H)** then plasma cells migrate to bone marrow which is the site of constant immunoglobulins production **(I)** when mutated plasma cells acquire additional mutations and are surrounded by favorable milieu then initial state of MGUS changes into SMM, MM and eventually to PCL. Created with BioRender.com.

6 THE EFFECTS OF GUT MICROBIOTA COMPOSITION ON TREATMENT RESULTS IN MM

Lack of balanced gut microbiota can lead to the lack of "training" given to the immune system by microbes. That, in turn, can lead to the inhibition of the active immune system that can combat new cancer cells created every day. Therefore, along with changing the paradigm to an immune-dependent approach, the gut microbiota role in the effectiveness of immunotherapy or cellular therapy should be revised. For instance, it is probable that by restoring the balanced gut microbiota, the results of mentioned therapies could be enhanced. Such a prove we can learn from immune checkpoint inhibitors and cancer treatment (109). However, this could also lead to more pronounced adverse events (110).

Cyclophosphamide was shown with very low efficacy when mice were injected with tumor cells and then treated with antibiotics to achieve a germ-free microenvironment. Thus, a lack of balanced gut microbiota causes low sensitivity of tumor cells for cyclophosphamide (111).

Autologous stem cell transplantation (ASCT) is currently a standard of care for patients in good condition. Until the engraftment, the patients are in critical pancytopenia and prone to opportunistic infections and therefore very usually treated with antimicrobials. However, before that, patients receive conditioning therapy that influences the composition of the gut microbiota and has a gross impact on intestinal epithelium. That altogether leads to the dysbiosis and monodominance of microbes such as Enterobacteriaceae (112). Researchers have also shown that butyrate, one of the SCFAs produced by the *Eubacterium halii* and *Faecalibacterium prausnitzii*, is associated with minimal residual disease negativity after the induction therapy for MM (113).

Regarding the proteasome inhibitors (PIs), which are commonly used to treat MM, it is worth noting that their adverse event is diarrhea. Unfortunately, the pathophysiology of gastrointestinal toxicity of PIs is poorly understood. However, as it is known that the SCFAs and PIs regulate the NF-kB pathway, the gut microbiota probably influences the risk of adverse events after PIs (114).

7 NOVEL APPROACHES IN METHODS EXPLORING THE ROLE OF GUT MICROBIOTA IN MGUS AND MM DEVELOPMENT

Calcinotto et al. published a study on mice where they showed that one specific species of the microorganisms colonizing the gut, namely *Prevotella heparinolytica* induced differentiation of Th17 cells. They then migrated to the bone marrow of Vk*MYC mice (which are the transgenic mice that develop disease mimicking MM) and favored the progression of MM. That agrees with the notion that SMM patients with a higher level of IL-17 in the bone marrow have faster progression of the disease (115).

Moreover, the relationships between myeloma cells and the gut microbes should be elucidated by identifying the gut microbiota composition that predicts a higher probability of MGUS development. It would be essential to know the specificity of the monoclonal protein produced by the mutated clone and correlate the results with the gut microbiota. A hypothesis is that pathogenic species colonizing (even temporally) the gut or state of dysbiosis when particular species of bacteria overgrowth could be responsible for the chronic antigenic stimulation and development of at least part of MGUS and MM cases.

Additionally, it would be interesting to see whether the gut microbiota composition influences the cytokines produced by the leukocytes in the blood. For example, maybe the state of dysbiosis provokes the production of proinflammatory cytokines by leukocytes and, therefore, indirectly promotes the progression of MM or MGUS.

8 NEW POTENTIAL TARGETS OF TREATMENTS IN MULTIPLE MYELOMA

As it was said in the previous paragraph, it is worth checking whether the treatments targeting IL-17 or IL-17R could work by lowering the risk of progression of the MGUS/SMM/MM. Such drugs are already registered by the FDA (anti-IL-17A antibodies), making it even easier to conduct such a study (116).

Preclinical studies suggest that some species of bacteria colonizing the gut promote the progression of MGUS or MM. Papers are mounting about the role of the gut microbiota in the pathogenesis of many diseases, and great hope is seen in the procedure of fecal microbiota transplantation to restore the balanced gut microbiota. Maybe such a procedure or probiotics/prebiotics could lower the risk of progression of MGUS to more advanced stages of the disease. Currently, the patients with MGUS or SMM are offered with watchful waiting strategy since the risk of progression, especially in the case of MGUS, is particularly low. Perhaps in the future, the gut microbiota composition of these patients is going to be known in detail. The patients with an exceptionally high risk of progression could be treated with prebiotics/probiotics or even with fecal microbiota transplantation (FMT) to diminish the risk of progression completely.

Jian et al. showed recently that the bacterial diversity of the gut microbiota of newly diagnosed MM patients is significantly increased with enrichment of nitrogen-recycling bacteria such as *Klebsiella* and *Streptococcus*. The researchers assume this is because of the progression of MM associated with the excessive accumulation of urea. Then the urea reaches the intestinal wall and selects nitrogen-recycling bacteria for overgrowth. In turn, microbes can produce L-glutamine, which is then delivered to the host and promotes the proliferation of myeloma cells since they cannot produce it on their own. Thus, the authors propose that the gut microbiota alterations, namely reduction of *Klebsiella* and *Streptococcus* populations, which are also present

in the normal microflora, could lower the risk of progression of MM (107). Furthermore, MM patients are also prone to infections, for instance, pneumonia with a common etiology of *Klebsiella* or *Streptococcus* (117). Therefore, the reduction of these populations could additionally mitigate the risk of infections during the disease. Additionally, it was also found that SCFA-producing bacteria were depleted in MM, and the addition of such bacteria in mice resulted in the mitigation of tumor progression (107).

9 CONCLUSIONS

To sum up, it seems highly probable that there is a role of the gut microbiota in the pathogenesis and treatment of MM. With ever-growing numbers of papers published in that field, the hope for an entirely new type of prophylaxis of progression of MGUS or treatment of MM is growing in parallel. Our previous work shows the remarkable efficacy of FMT in preventing colonization of a single, in that case, antibiotic-resistant bacteria (118). Also, in the model of graft-versus-host disease, we have shown that it is possible to stop the inflammatory process in the gut by FMT, shedding new light on the immunomodulatory effect of the gut microbiota (119, 120). Given that single species of bacteria, *Klebsiella* and *Streptococcus* were shown to play a role in the progression of MM, it seems that further studies on gut microbiota in the treatment of MM are warranted. Additionally, these bacteria are often responsible for infections in that population of patients. Therefore, the possible efficacy of FMT in the elimination of these "microbial partners in crime" would be multidirectional.

AUTHOR CONTRIBUTIONS

MJ and JB wrote the paper, GWB reviewed the paper. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Landgren O, Kyle RA, Pfeiffer RM, Katzmann JA, Caporaso NE, Hayes RB, et al. Monoclonal Gammopathy of Undetermined Significance (MGUS) Consistently Precedes Multiple Myeloma: A Prospective Study. *Blood* (2009) 113(22):5412–7. doi: 10.1182/blood-2008-12-194241
2. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A Monoclonal Gammopathy Precedes Multiple Myeloma in Most Patients. *Blood* (2009) 113(22):5418–22. doi: 10.1182/blood-2008-12-195008
3. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of Monoclonal Gammopathy of Undetermined Significance. *N Engl J Med* (2006) 354(13):1362–9. doi: 10.1056/NEJMoa054494
4. Kristinsson SY, Landgren O, Dickman PW, Derolf AR, Bjorkholm M. Patterns of Survival in Multiple Myeloma: A Population-Based Study of Patients Diagnosed in Sweden From 1973 to 2003. *J Clin Oncol* (2007) 25 (15):1993–9. doi: 10.1200/JCO.2006.09.0100
5. Cowan AJ, Allen C, Barac A, Basaleem H, Bensenor I, Curado MP, et al. Global Burden of Multiple Myeloma: A Systematic Analysis for the Global Burden of Disease Study 2016. *JAMA Oncol* (2018) 4(9):1221–7. doi: 10.1001/jamaoncol.2018.2128
6. Zhou L, Yu Q, Wei G, Wang L, Huang Y, Hu K, et al. Measuring the Global, Regional, and National Burden of Multiple Myeloma From 1990 to 2019. *BMC Cancer* (2021) 21(1):606. doi: 10.1186/s12885-021-08280-y
7. Kazandjian D. Multiple Myeloma Epidemiology and Survival: A Unique Malignancy. *Semin Oncol* (2016) 43(6):676–81. doi: 10.1053/j.seminoncol.2016.11.004
8. Jasinski M, Basak GW, Jedrzejczak WW. Perspectives for the Use of CAR-T Cells for the Treatment of Multiple Myeloma. *Front Immunol* (2021) 12:632937. doi: 10.3389/fimmu.2021.632937
9. Teras LR, DeSantis CE, Cerhan JR, Morton LM, Jemal A, Flowers CR, et al. 2016 US Lymphoid Malignancy Statistics by World Health Organization Subtypes. *CA Cancer J Clin* (2016) 66(6):443–59. doi: 10.3322/caac.21357
10. Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding Multiple Myeloma Pathogenesis in the Bone Marrow to Identify New Therapeutic Targets. *Nat Rev Cancer* (2007) 7(8):585–98. doi: 10.1038/nrc2189
11. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group Updated Criteria for the Diagnosis of Multiple Myeloma. *Lancet Oncol* (2014) 15(12):e538–48.
12. Walker BA, Wardell CP, Johnson DC, Kaiser MF, Begum DB, Dahir NB, et al. Characterization of IGH Locus Breakpoints in Multiple Myeloma Indicates a Subset of Translocations Appear to Occur in Pregerminal Center B Cells. *Blood* (2013) 121(17):3413–9. doi: 10.1182/blood-2012-12-471888
13. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J Jr, et al. Cyclin D Dysregulation: An Early and Unifying Pathogenic Event in Multiple Myeloma. *Blood* (2005) 106(1):296–303. doi: 10.1182/blood-2005-01-0034
14. Turesson I, Kovalchik SA, Pfeiffer RM, Kristinsson SY, Goldin LR, Drayson MT, et al. Monoclonal Gammopathy of Undetermined Significance and Risk of Lymphoid and Myeloid Malignancies: 728 Cases Followed Up to 30 Years in Sweden. *Blood* (2014) 123(3):338–45. doi: 10.1182/blood-2013-05-505487
15. Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, Schinzel AC, et al. Initial Genome Sequencing and Analysis of Multiple Myeloma. *Nature* (2011) 471(7339):467–72. doi: 10.1038/nature09837
16. Lohr JG, Stojanov P, Carter SL, Cruz-Gordillo P, Lawrence MS, Auclair D, et al. Widespread Genetic Heterogeneity in Multiple Myeloma: Implications for Targeted Therapy. *Cancer Cell* (2014) 25(1):91–101.
17. Xiao R, Cerny J, Devitt K, Dresser K, Nath R, Ramanathan M, et al. MYC Protein Expression is Detected in Plasma Cell Myeloma But Not in Monoclonal Gammopathy of Undetermined Significance (MGUS). *Am J Surg Pathol* (2014) 38(6):776–83. doi: 10.1097/PAS.0000000000000213
18. Demchenko YN, Glebov OK, Zingone A, Keats JJ, Bergsagel PL, Kuehl WM, et al. Classical and/or Alternative NF-kappaB Pathway Activation in Multiple Myeloma. *Blood* (2010) 115(17):3541–52. doi: 10.1182/blood-2009-09-243535
19. Jasinski M, Bilinski J, Basak GW. The Role of the Gut Microbiome in Pathogenesis, Biology, and Treatment of Plasma Cell Dyscrasias. *Front Oncol* (2021) 11:741376. doi: 10.3389/fonc.2021.741376
20. Lewis JF, Johnson P, Miller P. Evaluation of Amniotic Fluid for Aerobic and Anaerobic Bacteria. *Am J Clin Pathol* (1976) 65(1):58–63. doi: 10.1093/ajcp/65.1.58
21. Gibbs RS, Blanco JD, St Clair PJ, Castaneda YS. Quantitative Bacteriology of Amniotic Fluid From Women With Clinical Intraamniotic Infection at Term. *J Infect Dis* (1982) 145(1):1–8. doi: 10.1093/infdis/145.1.1
22. Jimenez E, Fernandez L, Marin ML, Martin R, Odriozola JM, Nueno-Palop C, et al. Isolation of Commensal Bacteria From Umbilical Cord Blood of Healthy Neonates Born by Cesarean Section. *Curr Microbiol* (2005) 51 (4):270–4. doi: 10.1007/s00284-005-0020-3

23. Roos PJ, Malan AF, Woods DL, Botha P, Hyland J, Heese Hde V, et al. The Bacteriological Environment of Preterm Infants. *S Afr Med J* (1980) 57 (10):347–50.
24. Jimenez E, Marin ML, Martin R, Odriozola JM, Olivares M, Xaus J, et al. Is Meconium From Healthy Newborns Actually Sterile? *Res Microbiol* (2008) 159(3):187–93. doi: 10.1016/j.resmic.2007.12.007
25. Moles L, Gomez M, Heilig H, Bustos G, Fuentes S, de Vos W, et al. Bacterial Diversity in Meconium of Preterm Neonates and Evolution of Their Fecal Microbiota During the First Month of Life. *PloS One* (2013) 8(6):e66986. doi: 10.1371/journal.pone.0066986
26. Perez PF, Dore J, Leclerc M, Levenez F, Benyacoub J, Serrant P, et al. Bacterial Imprinting of the Neonatal Immune System: Lessons From Maternal Cells? *Pediatrics* (2007) 119(3):e724–32. doi: 10.1542/peds.2006-1649
27. Gosalbes MJ, Llop S, Valles Y, Moya A, Ballester F, Francino MP, et al. Meconium Microbiota Types Dominated by Lactic Acid or Enteric Bacteria are Differentially Associated With Maternal Eczema and Respiratory Problems in Infants. *Clin Exp Allergy* (2013) 43(2):198–211. doi: 10.1111/cea.12063
28. Brosseau C, Selle A, Duval A, Misme-Aucouturier B, Chesneau M, Brouard S, et al. Prebiotic Supplementation During Pregnancy Modifies the Gut Microbiota and Increases Metabolites in Amniotic Fluid, Driving a Tolerogenic Environment In Utero. *Front Immunol* (2021) 12:712614. doi: 10.3389/fimmu.2021.712614
29. Kennedy KM, Gerlach MJ, Adam T, Heimesaat MM, Rossi L, Surette MG, et al. Fetal Meconium Does Not Have a Detectable Microbiota Before Birth. *Nat Microbiol* (2021) 6(7):865–73. doi: 10.1038/s41564-021-00904-0
30. Avershina E, Storro O, Oien T, Johnsen R, Pope P, Rudi K, et al. Major Faecal Microbiota Shifts in Composition and Diversity With Age in a Geographically Restricted Cohort of Mothers and Their Children. *FEMS Microbiol Ecol* (2014) 87(1):280–90. doi: 10.1111/1574-6941.12223
31. Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C, et al. Decreased Gut Microbiota Diversity, Delayed Bacteroidetes Colonisation and Reduced Th1 Responses in Infants Delivered by Caesarean Section. *Gut* (2014) 63(4):559–66. doi: 10.1136/gutjnl-2012-303249
32. Backhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and Stabilization of the Human Gut Microbiome During the First Year of Life. *Cell Host Microbe* (2015) 17(6):852. doi: 10.1016/j.chom.2015.05.012
33. Valles Y, Artacho A, Pascual-Garcia A, Ferrus ML, Gosalbes MJ, Abellan JJ, et al. Microbial Succession in the Gut: Directional Trends of Taxonomic and Functional Change in a Birth Cohort of Spanish Infants. *PloS Genet* (2014) 10(6):e1004406. doi: 10.1371/journal.pgen.1004406
34. Vaishampayan PA, Kuehl JV, Froula JL, Morgan JL, Ochman H, Francino MP, et al. Comparative Metagenomics and Population Dynamics of the Gut Microbiota in Mother and Infant. *Genome Biol Evol* (2010) 2:53–66. doi: 10.1093/gbe/evp057
35. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human Gut Microbiome Viewed Across Age and Geography. *Nature* (2012) 486(7402):222–7. doi: 10.1038/nature11053
36. Hollister EB, Riehle K, Luna RA, Weidler EM, Rubio-Gonzales M, Mistretta TA, et al. Structure and Function of the Healthy Pre-Adolescent Pediatric Gut Microbiome. *Microbiome* (2015) 3:36. doi: 10.1186/s40168-015-0101-x
37. Noverr MC, Huffnagle GB. The 'Microflora Hypothesis' of Allergic Diseases. *Clin Exp Allergy* (2005) 35(12):1511–20. doi: 10.1111/j.1365-2222.2005.02379.x
38. Bjorksten B. Environment and Infant Immunity. *Proc Nutr Soc* (1999) 58 (3):729–32. doi: 10.1017/S0029665199000956
39. Platt AM, Mowat AM. Mucosal Macrophages and the Regulation of Immune Responses in the Intestine. *Immunol Lett* (2008) 119(1-2):22–31. doi: 10.1016/j.imlet.2008.05.009
40. Romagnani S. Regulation of the T Cell Response. *Clin Exp Allergy* (2006) 36 (11):1357–66. doi: 10.1111/j.1365-2222.2006.02606.x
41. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, et al. A CD4+ T-Cell Subset Inhibits Antigen-Specific T-Cell Responses and Prevents Colitis. *Nature* (1997) 389(6652):737–42. doi: 10.1038/39614
42. Akdis M. Healthy Immune Response to Allergens: T Regulatory Cells and More. *Curr Opin Immunol* (2006) 18(6):738–44. doi: 10.1016/j.coi.2006.06.003
43. Meiler F, Klunker S, Zimmermann M, Akdis CA, Akdis M. Distinct Regulation of IgE, IgG4 and IgA by T Regulatory Cells and Toll-Like Receptors. *Allergy* (2008) 63(11):1455–63. doi: 10.1111/j.1398-9995.2008.01774.x
44. Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinni MP, et al. IL-4 and IFN (Alpha and Gamma) Exert Opposite Regulatory Effects on the Development of Cytolytic Potential by Th1 or Th2 Human T Cell Clones. *J Immunol* (1992) 149(9):2977–83.
45. Oboki K, Ohno T, Saito H, Nakae S. Th17 and Allergy. *Allergol Int* (2008) 57 (2):121–34. doi: 10.2332/allergolint.R-07-160
46. Abbas AK. Die and Let Live: Eliminating Dangerous Lymphocytes. *Cell* (1996) 84(5):655–7. doi: 10.1016/S0092-8674(00)81042-9
47. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-Specific T Cell Sensitization is Impaired in IL-17-Deficient Mice, Causing Suppression of Allergic Cellular and Humoral Responses. *Immunity* (2002) 17(3):375–87. doi: 10.1016/S1074-7613(02)00391-6
48. Rook GA, Brunet LR. Microbes, Immunoregulation, and the Gut. *Gut* (2005) 54(3):317–20. doi: 10.1136/gut.2004.053785
49. Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, Parasites, and the Hygiene Hypothesis. *Science* (2002) 296(5567):490–4. doi: 10.1126/science.296.5567.490
50. Strauch UG, Obermeier F, Grunwald N, Gurster S, Dunger N, Schultz M, et al. Influence of Intestinal Bacteria on Induction of Regulatory T Cells: Lessons From a Transfer Model of Colitis. *Gut* (2005) 54(11):1546–52. doi: 10.1136/gut.2004.059451
51. Baba N, Samson S, Bourdet-Sicard R, Rubio M, Sarfati M. Commensal Bacteria Trigger a Full Dendritic Cell Maturation Program That Promotes the Expansion of Non-Tr1 Suppressor T Cells. *J Leukoc Biol* (2008) 84 (2):468–76. doi: 10.1189/jlb.0108017
52. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell* (2009) 139(3):485–98. doi: 10.1016/j.cell.2009.09.033
53. Victora GD, Nussenzweig MC. Germinal Centers. *Annu Rev Immunol* (2012) 30:429–57. doi: 10.1146/annurev-immunol-020711-075032
54. Pasqualucci L, Neumeister P, Goossens T, Nanjangud G, Chaganti RS, Kuppers R, et al. Hypermutation of Multiple Proto-Oncogenes in B-Cell Diffuse Large-Cell Lymphomas. *Nature* (2001) 412(6844):341–6. doi: 10.1038/35085588
55. Avet-Loiseau H, Facon T, Grosbois B, Magrangeas F, Rapp MJ, Harousseau JL, et al. Oncogenesis of Multiple Myeloma: 14q32 and 13q Chromosomal Abnormalities are Not Randomly Distributed, But Correlate With Natural History, Immunological Features, and Clinical Presentation. *Blood* (2002) 99 (6):2185–91. doi: 10.1182/blood.V99.6.2185
56. Fonseca R, Blood E, Rue M, Harrington D, Oken MM, Kyle RA, et al. Clinical and Biologic Implications of Recurrent Genomic Aberrations in Myeloma. *Blood* (2003) 101(11):4569–75. doi: 10.1182/blood-2002-10-3017
57. Babel N, Schwarzmann F, Pruss A, Volk HD, Reinke P, et al. Monoclonal Gammopathy of Undetermined Significance (MGUS) is Associated With an Increased Frequency of Epstein-Barr Virus (EBV) Latently Infected B Lymphocytes in Long-Term Renal Transplant Patients. *Transplant Proc* (2004) 36(9):2679–82. doi: 10.1016/j.transproceed.2004.09.046
58. Rajkumar SV, Kyle RA, Plevak MF, Murray JA, Therneau TM. Helicobacter Pylori Infection and Monoclonal Gammopathy of Undetermined Significance. *Br J Haematol* (2002) 119(3):706–8. doi: 10.1046/j.1365-2141.2002.03912.x
59. de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, et al. Global Burden of Cancers Attributable to Infections in 2008: A Review and Synthetic Analysis. *Lancet Oncol* (2012) 13(6):607–15. doi: 10.1016/S1470-2045(12)70137-7
60. Bosseboeuf A, Feron D, Tallet A, Rossi C, Charlier C, Garderet L, et al. Monoclonal IgG in MGUS and Multiple Myeloma Targets Infectious Pathogens. *JCI Insight* (2017) 2(19):6–11. doi: 10.1172/jci.insight.95367
61. Hermouet S, Corre I, Gassin M, Bigot-Corbel E, Sutton CA, Casey JW, et al. Hepatitis C Virus, Human Herpesvirus 8, and the Development of Plasma-Cell Leukemia. *N Engl J Med* (2003) 348(2):178–9. doi: 10.1056/NEJM200301093480219
62. Harb J, Mennesson N, Lepetit C, Fourny M, Louvois M, Bosseboeuf A, et al. Comparison of Monoclonal Gammopathies Linked to Poliovirus or

Coxsackievirus vs. Other Infectious Pathogens. *Cells* (2021) 10(2):12–3. doi: 10.3390/cells10020438
63. Bosseboeuf A, Mennesson N, Allain-Maillet S, Tallet A, Piver E, Decaux O, et al. Characteristics of MGUS and Multiple Myeloma According to the Target of Monoclonal Immunoglobulins, Glucosylsphingosine, or Epstein-Barr Virus EBNA-1. *Cancers (Basel)* (2020) 12(5). doi: 10.3390/cancers12051254
64. Panfilio S, D'Urso P, Annechini G, D'Elia GM, De Angelis F, Stefanizzi C, et al. Regression of a Case of Multiple Myeloma With Antiviral Treatment in a Patient With Chronic HCV Infection. *Leuk Res Rep* (2013) 2(1):39–40. doi: 10.1016/j.lrr.2013.01.002
65. Rodriguez-Garcia A, Linares M, Morales ML, Allain-Maillet S, Mennesson N, Sanchez R, et al. Efficacy of Antiviral Treatment in Hepatitis C Virus (HCV)-Driven Monoclonal Gammopathies Including Myeloma. *Front Immunol* (2021) 12:797209. doi: 10.3389/fimmu.2021.797209
66. Rodríguez García A, Linares M, Mennesson N, Pérez-Revilla A, Sanchez R, Leivas A, et al. The Role of Antiviral Treatment in Hepatitis C Virus (HCV)-Driven Monoclonal Gammopathies. *Blood* (2019) 134:5560. doi: 10.1182/blood-2019-126549
67. Mistry PK, Taddei T, vom Dahl S, Rosenbloom BE. Gaucher Disease and Malignancy: A Model for Cancer Pathogenesis in an Inborn Error of Metabolism. *Crit Rev Oncog* (2013) 18(3):235–46. doi: 10.1615/CritRevOncog.2013006145
68. Nair S, Branagan AR, Liu J, Boddupalli CS, Mistry PK, Dhodapkar MV, et al. Clonal Immunoglobulin Against Lysolipids in the Origin of Myeloma. *N Engl J Med* (2016) 374(6):555–61. doi: 10.1056/NEJMoa1508808
69. Bigot-Corbel E, Gassin M, Corre I, Le Carrer D, Delaroche O, Hermouet S, et al. Hepatitis C Virus (HCV) Infection, Monoclonal Immunoglobulin Specific for HCV Core Protein, and Plasma-Cell Malignancy. *Blood* (2008) 112(10):4357–8. doi: 10.1182/blood-2008-07-167569
70. Okumura R, Takeda K. Roles of Intestinal Epithelial Cells in the Maintenance of Gut Homeostasis. *Exp Mol Med* (2017) 49(5):e338. doi: 10.1038/emm.2017.20
71. Sano T, Huang W, Hall JA, Yang Y, Chen A, Gavzy SJ, et al. An IL-23r/IL-22 Circuit Regulates Epithelial Serum Amyloid A to Promote Local Effector Th17 Responses. *Cell* (2016) 164(1-2):324. doi: 10.1016/j.cell.2015.12.047
72. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, et al. Interleukin (IL)-22 and IL-17 are Coexpressed by Th17 Cells and Cooperatively Enhance Expression of Antimicrobial Peptides. *J Exp Med* (2006) 203(10):2271–9. doi: 10.1084/jem.20061308
73. He B, Xu W, Santini PA, Polydorides AD, Chiu A, Estrella J, et al. Intestinal Bacteria Trigger T Cell-Independent Immunoglobulin A(2) Class Switching by Inducing Epithelial-Cell Secretion of the Cytokine APRIL. *Immunity* (2007) 26(6):812–26. doi: 10.1016/j.immuni.2007.04.014
74. Mabbott NA, Donaldson DS, Ohno H, Williams IR, Mahajan A. Microfold (M) Cells: Important Immunosurveillance Posts in the Intestinal Epithelium. *Mucosal Immunol* (2013) 6(4):666–77. doi: 10.1038/mi.2013.30
75. Hase K, Kawano K, Nochi T, Pontes GS, Fukuda S, Ebisawa M, et al. Uptake Through Glycoprotein 2 of FimH(+) Bacteria by M Cells Initiates Mucosal Immune Response. *Nature* (2009) 462(7270):226–30. doi: 10.1038/nature08529
76. Niess JH, Brand S, Gu X, Landsman L, Jung S, McCormick BA, et al. CX3CR1-Mediated Dendritic Cell Access to the Intestinal Lumen and Bacterial Clearance. *Science* (2005) 307(5707):254–8. doi: 10.1126/science.1102901
77. Chieppa M, Rescigno M, Huang AY, Germain RN. Dynamic Imaging of Dendritic Cell Extension Into the Small Bowel Lumen in Response to Epithelial Cell TLR Engagement. *J Exp Med* (2006) 203(13):2841–52. doi: 10.1084/jem.20061884
78. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, et al. Dendritic Cells Express Tight Junction Proteins and Penetrate Gut Epithelial Monolayers to Sample Bacteria. *Nat Immunol* (2001) 2(4):361–7. doi: 10.1038/86373
79. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, et al. Oral Tolerance Originates in the Intestinal Immune System and Relies on Antigen Carriage by Dendritic Cells. *J Exp Med* (2006) 203(3):519–27. doi: 10.1084/jem.20052016
80. Macpherson AJ, Uhr T. Induction of Protective IgA by Intestinal Dendritic Cells Carrying Commensal Bacteria. *Science* (2004) 303(5664):1662–5. doi: 10.1126/science.1091334
81. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A Functionally Specialized Population of Mucosal CD103+ DCs Induces Foxp3+ Regulatory T Cells via a TGF-Beta and Retinoic Acid-Dependent Mechanism. *J Exp Med* (2007) 204(8):1757–64. doi: 10.1084/jem.20070590
82. Xu W, He B, Chiu A, Chadburn A, Shan M, Buldys M, et al. Epithelial Cells Trigger Frontline Immunoglobulin Class Switching Through a Pathway Regulated by the Inhibitor SLPI. *Nat Immunol* (2007) 8(3):294–303. doi: 10.1038/ni1434
83. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina Propria Macrophages and Dendritic Cells Differentially Induce Regulatory and Interleukin 17-Producing T Cell Responses. *Nat Immunol* (2007) 8 (10):1086–94. doi: 10.1038/ni1511
84. Smythies LE, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH, et al. Human Intestinal Macrophages Display Profound Inflammatory Anergy Despite Avid Phagocytic and Bacteriocidal Activity. *J Clin Invest* (2005) 115(1):66–75. doi: 10.1172/JCI200519229
85. Bain CC, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O, et al. Resident and Pro-Inflammatory Macrophages in the Colon Represent Alternative Context-Dependent Fates of the Same Ly6Chi Monocyte Precursors. *Mucosal Immunol* (2013) 6(3):498–510. doi: 10.1038/mi.2012.89
86. Yu Y, Zeng H, Lyons S, Carlson A, Merlin D, Neish AS, et al. TLR5-Mediated Activation of P38 MAPK Regulates Epithelial IL-8 Expression *via* Posttranscriptional Mechanism. *Am J Physiol Gastrointest Liver Physiol* (2003) 285(2):G282–90. doi: 10.1152/ajpgi.00503.2002
87. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A. Neutrophil Function: From Mechanisms to Disease. *Annu Rev Immunol* (2012) 30:459–89. doi: 10.1146/annurev-immunol-020711-074942
88. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, et al. Polarization of Tumor-Associated Neutrophil Phenotype by TGF-Beta: "N1" Versus "N2" TAN. *Cancer Cell* (2009) 16(3):183–94. doi: 10.1016/j.ccr.2009.06.017
89. Bongers G, Pacer ME, Geraldino TH, Chen L, He Z, Hashimoto D, et al. Interplay of Host Microbiota, Genetic Perturbations, and Inflammation Promotes Local Development of Intestinal Neoplasms in Mice. *J Exp Med* (2014) 211(3):457–72. doi: 10.1084/jem.20131587
90. Mou H, Wu S, Zhao G, Wang J. Changes of Th17/Treg Ratio in the Transition of Chronic Hepatitis B to Liver Cirrhosis and Correlations With Liver Function and Inflammation. *Exp Ther Med* (2019) 17(4):2963–8. doi: 10.3892/etm.2019.7299
91. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg Induction by a Rationally Selected Mixture of Clostridia Strains From the Human Microbiota. *Nature* (2013) 500(7461):232–6. doi: 10.1038/nature12331
92. Shahid A, Bharadwaj M. The Connection Between the Th17 Cell Related Cytokines and Cancer Stem Cells in Cancer: Novel Therapeutic Targets. *Immunol Lett* (2019) 213:9–20. doi: 10.1016/j.imlet.2019.07.001
93. Zhang H, Liao X, Sparks JB, Luo XM. Dynamics of Gut Microbiota in Autoimmune Lupus. *Appl Environ Microbiol* (2014) 80(24):7551–60. doi: 10.1128/AEM.02676-14
94. Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, et al. Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine. *Cell Host Microbe* (2008) 4 (4):337–49. doi: 10.1016/j.chom.2008.09.009
95. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM, et al. The Role of Short-Chain Fatty Acids in the Interplay Between Diet, Gut Microbiota, and Host Energy Metabolism. *J Lipid Res* (2013) 54 (9):2325–40. doi: 10.1194/jlr.R036012
96. Proietti M, Cornacchione V, Rezzonico Jost T, Romagnani A, Faliti CE, Perruzza L, et al. ATP-Gated Ionotropic P2X7 Receptor Controls Follicular T Helper Cell Numbers in Peyer's Patches to Promote Host-Microbiota Mutualism. *Immunity* (2014) 41(5):789–801. doi: 10.1016/j.immuni.2014.10.010
97. Morrison DJ, Preston T. Formation of Short Chain Fatty Acids by the Gut Microbiota and Their Impact on Human Metabolism. *Gut Microbes* (2016) 7 (3):189–200. doi: 10.1080/19490976.2015.1134082
98. Weill JC, Weller S, Reynaud CA. A Bird's Eye View on Human B Cells. *Semin Immunol* (2004) 16(4):277–81. doi: 10.1016/j.smim.2004.08.007

99. Vossenkamper A, Blair PA, Safinia N, Fraser LD, Das L, Sanders TJ, et al. A Role for Gut-Associated Lymphoid Tissue in Shaping the Human B Cell Repertoire. *J Exp Med* (2013) 210(9):1665–74. doi: 10.1084/jem.20122465
100. Kim M, Kim CH. Regulation of Humoral Immunity by Gut Microbial Products. *Gut Microbes* (2017) 8(4):392–9. doi: 10.1080/19490976.2017.1299311
101. Sharifi L, Mirshafiey A, Rezaei N, Azizi G, Magaji Hamid K, Amirzargar AA, et al. The Role of Toll-Like Receptors in B-Cell Development and Immunopathogenesis of Common Variable Immunodeficiency. *Expert Rev Clin Immunol* (2016) 12(2):195–207. doi: 10.1586/1744666X.2016.1114885
102. Oh JZ, Ravindran R, Chassaing B, Carvalho FA, Maddur MS, Bower M, et al. TLR5-Mediated Sensing of Gut Microbiota is Necessary for Antibody Responses to Seasonal Influenza Vaccination. *Immunity* (2014) 41(3):478–92. doi: 10.1016/j.immuni.2014.08.009
103. Kim M, Qie Y, Park J, Kim CH. Gut Microbial Metabolites Fuel Host Antibody Responses. *Cell Host Microbe* (2016) 20(2):202–14. doi: 10.1016/j.chom.2016.07.001
104. Zeng MY, Cisalpino D, Varadarajan S, Hellman J, Warren HS, Cascalho M, et al. Gut Microbiota-Induced Immunoglobulin G Controls Systemic Infection by Symbiotic Bacteria and Pathogens. *Immunity* (2016) 44(3):647–58. doi: 10.1016/j.immuni.2016.02.006
105. Yang W, Xiao Y, Huang X, Chen F, Sun M, Bilotta AJ, et al. Microbiota Metabolite Short-Chain Fatty Acids Facilitate Mucosal Adjuvant Activity of Cholera Toxin Through GPR43. *J Immunol* (2019) 203(1):282–92. doi: 10.4049/jimmunol.1801068
106. Giuliani N, Colla S, Rizzoli V. New Insight in the Mechanism of Osteoclast Activation and Formation in Multiple Myeloma: Focus on the Receptor Activator of NF-kappaB Ligand (RANKL). *Exp Hematol* (2004) 32(8):685–91. doi: 10.1016/j.exphem.2004.03.015
107. Jian X, Zhu Y, Ouyang J, Wang Y, Lei Q, Xia J, et al. Alterations of Gut Microbiome Accelerate Multiple Myeloma Progression by Increasing the Relative Abundances of Nitrogen-Recycling Bacteria. *Microbiome* (2020) 8(1):74. doi: 10.1186/s40168-020-00854-5
108. Antoine Pepeljugoski C, Morgan G, Braunstein M. Analysis of Intestinal Microbiome in Multiple Myeloma Reveals Progressive Dysbiosis Compared to MGUS and Healthy Individuals. *Blood* (2019) 134(Supplement_1):3076–6. doi: 10.1182/blood-2019-130643
109. Huang J, Jiang Z, Wang Y, Fan X, Cai J, Yao X, et al. Modulation of Gut Microbiota to Overcome Resistance to Immune Checkpoint Blockade in Cancer Immunotherapy. *Curr Opin Pharmacol* (2020) 54:1–10. doi: 10.1016/j.coph.2020.06.004
110. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al. Gut Microbiome Influences Efficacy of PD-1-Based Immunotherapy Against Epithelial Tumors. *Science* (2018) 359(6371):91–7. doi: 10.1126/science.aan3706
111. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillere R, Hannani D, et al. The Intestinal Microbiota Modulates the Anticancer Immune Effects of Cyclophosphamide. *Science* (2013) 342(6161):971–6. doi: 10.1126/science.1240537
112. Andermann TM, Peled JU, Ho C, Reddy P, Riches M, Storb R, et al. The Microbiome and Hematopoietic Cell Transplantation: Past, Present, and Future. *Biol Blood Marrow Transplant* (2018) 24(7):1322–40. doi: 10.1016/j.bbmt.2018.02.009
113. Pianko MJ, Devlin SM, Littmann ER, Chansakul A, Mastey D, Salcedo M, et al. Minimal Residual Disease Negativity in Multiple Myeloma is Associated With Intestinal Microbiota Composition. *Blood Adv* (2019) 3(13):2040–4. doi: 10.1182/bloodadvances.2019032276
114. Al-Homsi AS, Feng Y, Duffner U, Al Malki MM, Goodyke A, Cole K, et al. Bortezomib for the Prevention and Treatment of Graft-Versus-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation. *Exp Hematol* (2016) 44(9):771–7. doi: 10.1016/j.exphem.2016.05.005
115. Calcinotto A, Brevi A, Chesi M, Ferrarese R, Garcia Perez L, Grioni M, et al. Microbiota-Driven Interleukin-17-Producing Cells and Eosinophils Synergize to Accelerate Multiple Myeloma Progression. *Nat Commun* (2018) 9(1):4832. doi: 10.1038/s41467-018-07305-8
116. Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K, et al. Secukinumab in Plaque Psoriasis–Results of Two Phase 3 Trials. *N Engl J Med* (2014) 371(4):326–38. doi: 10.1056/NEJMoa1314258
117. Nucci M, Anaissie E. Infections in Patients With Multiple Myeloma. *Semin Hematol* (2009) 46(3):277–88. doi: 10.1053/j.seminhematol.2009.03.006
118. Bilinski J, Grzesiowski P, Sorensen N, Madry K, Muszynski J, Robak K, et al. Fecal Microbiota Transplantation in Patients With Blood Disorders Inhibits Gut Colonization With Antibiotic-Resistant Bacteria: Results of a Prospective, Single-Center Study. *Clin Infect Dis* (2017) 65(3):364–70. doi: 10.1093/cid/cix252
119. Bilinski J, Jasinski M, Tomaszewska A, Lis K, Kacprzyk P, Chmielewska L, et al. Fecal Microbiota Transplantation With Ruxolitinib as a Treatment Modality for Steroid-Refractory/Dependent Acute, Gastrointestinal Graft-Versus-Host Disease: A Case Series. *Am J Hematol* (2021) 96(12):E461–3. doi: 10.1002/ajh.26365
120. Bilinski J, Lis K, Tomaszewska A, Grzesiowski P, Dzieciatkowski T, Tyszka M, et al. Fecal Microbiota Transplantation in Patients With Acute and Chronic Graft-Versus-Host Disease-Spectrum of Responses and Safety Profile. Results From a Prospective, Multicenter Study. *Am J Hematol* (2021) 96(3):E88–91. doi: 10.1002/ajh.26077

Conflict of Interest: JB and GWB are the founders of the fecal microbiota bank and laboratory named the Human Biome Institute.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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7.4.Publikacja 4

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REVIEW
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The Role of the Gut Microbiome in Pathogenesis, Biology, and Treatment of Plasma Cell Dyscrasias

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In response to emerging discoveries, questions are mounting as to what factors are responsible for the progression of plasma cell dyscrasias and what determines responsiveness to treatment in individual patients. Recent findings have shown close interaction between the gut microbiota and multiple myeloma cells. For instance, that malignant cells shape the composition of the gut microbiota. We discuss the role of the gut microbiota in (i) the development and progression of plasma cell dyscrasias, and (ii) the response to treatment of multiple myeloma and highlight faecal microbiota transplantation as a procedure that could modify the risk of progression or sensitize refractory malignancy to immunotherapy.

Keywords: plasma cell dyscrasias, gut microbiome, multiple myeloma, microbiota, short-chain fatty acids

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INTRODUCTION – PATHOGENESIS OF PLASMA CELL DYSCRASIAS

Typical genetic alterations in plasma cell dyscrasias are IgH translocations, hyperdiploidy, and cyclin D dysregulation. These are responsible for initiating changes in B-cell postgerminal centres, which result in the transformation of normal cells into benign tumour cells that cause monoclonal gammopathy of undetermined significance (MGUS) (1). This condition is the preclinical stage of multiple myeloma (MM) and occurs in ~3.2% of the population aged over 50 years (2). MGUS is an asymptomatic condition with elevated serum concentration of M protein. Only rarely does it progress to symptomatic MM (1% of patients/year) (3), which can be associated with symptoms that manifest as a result of hypercalcaemia, renal failure, anaemia, and bone lesions. Smouldering MM (SMM) is an asymptomatic, intermediate stage between MGUS and MM, that carries a 10% risk of progression to symptomatic MM per year during the first five years after diagnosis (4). If it is to be possible to screen intensively, perform prophylactic investigations on, and treat in the early stages only those patients who are most at risk of disease progression, accurate prognostic markers of progression of MGUS or SMM to MM are needed.

During the past few years, evidence has emerged that human gut microbiota play an important role in the progression of MM (5–7). The gut microbiota influence the course of MM and the disease shapes the composition of the bacteria in the intestines (6). These interactions, as described below, are based on the strong reliance of MM cells on proinflammatory cytokines [interleukin (IL)-6, tumour necrosis factor (TNF)-α, IL-13] and the ability of bacteria to recycle nitrogen (8).

Recent studies have yielded plenty of information on the differences in microbiota among MM patients and about longitudinal changes acquired during the treatment as well (9). Some recently identified gut microbes are responsible for inducing an inflammatory environment, both within the gut layer and throughout the whole body. These proinflammatory microbes might contribute to the progression of MGUS to MM (5). If they do, the microbiome composition could be used as a prognostic factor for assessing the risk of MGUS transformation or MM progression.

GUT MICROBIOTA AND IMMUNE SYSTEM IN HEALTH AND DISEASE, SPECIFICALLY INFECTIONS

The colonization of the intestine by microbes plays a key role in the maturation of the host's immune system (10). Current knowledge about crosstalk between gut microbiota and immune cells derives mainly from experiments conducted on germ-free animals (11). For instance, in germ-free mice the population of αβ and γδ intra-epithelial lymphocytes is significantly reduced (12), there is no production of IgA antibodies (13) and Th17 cells are absent (14). One example of a complicated interplay between gut microbiota and immune cells is the following. Polysaccharide A produced by *Bacteroides fragilis* binds to TLR2/TLR1 (Toll-like receptor) heterodimer connected with Dectin-1 (15). Then, the phosphoinositide 3-kinase (PI3K) pathway is activated, glycogen synthase kinase 3β inactivated, which eventually induces cAMP response element-binding protein expression of anti-inflammatory genes (15). Finally, the secretion of polysaccharide A by *Bacteroides fragilis* leads to the differentiation of Treg cells and influences the balance between Th1 and Th2 populations. On the other hand, butyrate produced by the gut microbiota can promote macrophage differentiation from monocytes through histone deacetylase 3 (HDAC3) inhibition that leads to enhanced antimicrobial host defense (16). These are only a few examples of how intricate the crosstalk on the line gut microbiota - immune cells is.

Gut microbiota can also predict responses to therapies administered in oncology. Chaput et al. showed that the presence of *Faecalibacterium* spp. increases the efficacy of anti-CTLA-4 immunotherapy while probably the *Bacteroides* spp. is associated with inferior responses in metastatic melanoma (17). Moreover, it is recently hypothesized that gut microbiota composition can influence the responses to the CAR-T therapy (18), and bearing in mind recent papers about the efficacy of such therapy in multiple myeloma the discussion about gut microbiota as a predictive marker of response is warranted (19).

The impact of the interplay between the immune system and gut microbiota in the context of infections cannot be forgotten as patients with multiple myeloma are far more prone to infections than the healthy population (20). The ability of microbes to release signaling molecules into the bloodstream can modulate the host's response to infections *via* the regulation of immune cell development (21). For instance, butyrate secreted by bacteria promotes the differentiation of monocytes in the bone marrow to a tolerogenic phenotype (22). Moreover, it was recently showed that some bacterial species could decrease the level of corticosterone in the blood which could improve the function of the immune system during the infection (23).

GUT MICROBIOTA AND TUMOURIGENESIS

The available data show that the gut microbiota are more numerous than genes, cells, and enzymatic reactions in the host organism, which suggest their importance for its health. In healthy persons, microorganisms are responsible for production of vitamins K, B_2 (riboflavin), B_{12} (cobalamin), folates, and biotin (24), metabolism of indigestible compounds, and protection from colonisation by opportunistic bacteria (25), and are necessary for the development of the humoral and cellular mucosal immune systems (26) (**Figure 1**). Along with these advantages of the gut microbiome, there are also some disadvantages. It is well established that dysbiosis, which is an imbalance in the proportion of microbes compared to a healthy state, plays a role in the pathogenesis of colorectal cancer (CRC) (27). Wang et al. showed that there is a difference in the composition of gut microbiota between patients with CRC and healthy individuals (28). A similar influence of microbial dysbiosis, *via* proinflammatory microbe-associated molecular patterns (MAMPs) and bacterial metabolites, has been shown in liver (29) and pancreatic (30) cancer.

The gut microbiota are accompanied by gut-associated lymphoid tissue (GALT), which is the largest peripheral immune organ (31). As many as 60–70% of peripheral lymphocytes are localised within the gut mucosa, so it is not surprising that the number of interactions between immune cells and the gut microbiota is high (32). There are numerous examples of how the gut microbiota and immune system influence each other within the gut mucosa. Brandsma et al. showed that the transplantation of proinflammatory faecal microbiota from *Casp1*$^{-/-}$ mice to *Ldlr*$^{-/-}$ mice resulted in systemic inflammation and promoted atherogenesis (33). In contrast, Mason et al. reported that reduced anti-inflammatory gut microbiota was correlated positively with depression. This correlation could be explained by inflammation playing a role in the pathogenesis of depression (34). The crosstalk from microbes to immune cells can be forwarded directly through their metabolites used as messengers, such as MAMPs or damage-associated molecular patterns (DAMPs), or through activation of Toll-like receptors (TLRs) that in turn cause the activation of immune cells (35, 36). Some metabolites, such as short-chain fatty acids (SCFAs), can directly promote the generation of T regulatory (Treg) cells (37) or are responsible for transforming growth factor-β production in epithelial cells within the gut. This in turn promotes Treg-cell confluence in the gut mucosa, which inhibits the activation of immune cells (38). Germ-free (GF) mice that are deprived completely of gut microbiota comprise excellent examples of the importance of gut bacteria for efficient immune function (26). In GF mice, Treg cell function is impaired, which suggests that gut microflora are necessary for the development of a fully functional

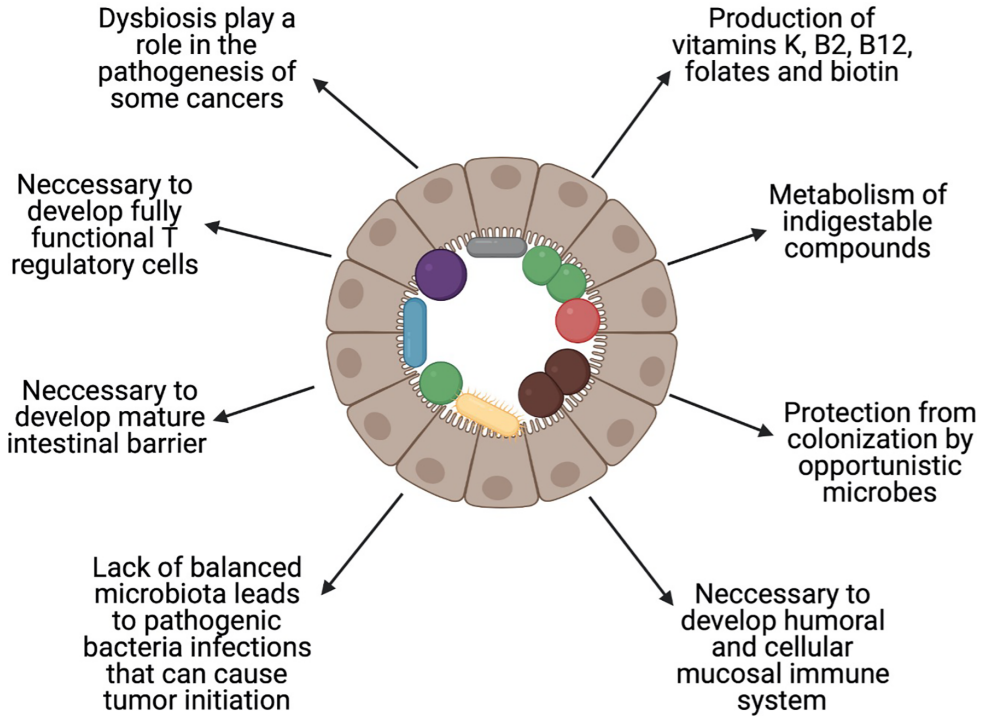

FIGURE 1 | Role of the balanced gut microbiota. Healthy gut microbiota are important in maintaining health. The figure shows the most important roles that are played by the human gut microbiota. Created with BioRender.com.

Treg cell population (39). In GF mice, the intestinal barrier is immature, which results in increased mucosal permeability (40). This is a key mechanism that leads to the development of inflammatory bowel disease or enteric infections (40). Colonisation of GF animals with normal gut microbiota leads to increased systemic immunological capacity, different patterns of migration of immune cells, significant changes in the production of specific antibodies, a general increase of immunoglobulin production, and changes in mucosal-associated lymphocyte tissues and cell populations (41–43).

In summary, in general, the micro-organisms in the gut are beneficial, but under certain conditions can have a damaging effect, in severe cases promoting the growth of cancer cells.

COMPARISON OF THE GUT MICROBIOME IN PATIENTS WITH PLASMA CELL DYSCRASIAS AND HEALTHY INDIVIDUALS

In recent years, scientists have confirmed the link between certain kinds of tumours and the composition of gut microbiota. For example, in CRC, many changes in the composition of bacterial species that colonise the gut have been identified and their contribution to tumourigenesis confirmed. Specific bacterial species colonizing the gut have even been indicated as possible markers of early diagnosis of CRC (44).

Regarding plasma cell dyscrasias and the gut microbiome, recent evidence shows metagenomic changes in the composition of commensal bacteria and frequent colonisation by opportunistic bacteria. Jian et al. performed a study on samples collected from 19 patients who had been newly diagnosed with MM and 18 healthy controls (6). They observed significant differences in the composition of bacteria in the gut between these two groups. One of the main changes was the increase of nitrogen-recycling bacteria, such as *Klebsiella* and *Streptococcus*, which are opportunistic pathogens that are responsible for infections associated with high mortality in this immunocompromised population. It has been suggested that this change might be due to the high serum concentration of urea in patients with MM, which results from increased production of NH_4^+ by tumour cells and restricted secretion of urea due to impaired renal function (45). The mechanism presented above is responsible attracting nitrogen-recycling bacteria to the gut. Changes in diversity in gut

microbiota have been reported, which indicates that samples from MM patients are characterised by increased diversity and poorer interactions between genera (6), although other studies have produced results that indicate contrary phenomena (46, 47). Furthermore, samples from MM patients included a reduced number of SCFA-producing bacteria, which affect tumourigenesis in plasma cell dyscrasias (see below) (6). Other changes in the composition of commensal bacteria, and colonisation with opportunistic pathogens, occur because of the treatment of MM. Unfortunately, research in this field is limited to the study of bacterial composition only. Further research, which studies differences in the balance and numbers, etc., of fungi, viruses, and eukaryotic organisms are needed (**Table 1**).

INFLUENCE OF THE GUT MICROBIOME ON THE DEVELOPMENT AND PROGRESSION OF PLASMA CELL DYSCRASIAS

As mentioned previously, MGUS is an asymptomatic state that occurs in ~3.2% of people aged over 50 (1). Only a small percentage of patients progress to symptomatic MM. For many years, researchers have wanted to identify the factors responsible for the development of plasma cell dyscrasias, and the reasons why some patients progress to MM whereas others do not.

Researchers have shown that there are no significant genetic differences between MGUS and MM cells. This suggests that environmental conditions could be an important factor in determining the risk for progression, although such factors are not necessarily present at the time at which MGUS develops. Therefore, tumour microenvironment seems to be a strong predictor of MGUS progression. Given the high degree of heterogeneity between clones in plasma cell dyscrasias, it is probable that only clones that are developing in a favourable niche will become an initiation point for further progression. As mentioned previously, proinflammatory TME in the bone marrow is needed for successful progression from MGUS to symptomatic MM, but it is a further issue how the gut microbiota can influence this microenvironment and contribute to tumour progression.

Short-Chain Fatty Acids

SCFAs are bacterial products that are responsible for ion absorption, gut motility, and modulation of immune responses (48). SCFAs can inhibit the nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) and such proinflammatory cytokines as IL-6 and TNF-α which are playing the role in activating osteoclasts to create niches for myeloma cells and additionally promote differentiation of Th17 cell (49). In contrast, SCFAs may also increase the level of IL-10 and induce expression of FoxP3 which in turn leads to differentiation of immunosuppressive $CD4^+$ T cell subset (Treg) (48). Eventually, both Treg (IL-10 and TGF-β) and Th17 (IL-17) cells secrete cytokines that promote MM cell proliferation *via* positive feedback loop (50). One SCFA, butyrate, is reported to increase T-cell apoptosis by HDAC-dependent Fas upregulation and consequent Fas-mediated apoptosis of T cells. That in turn inhibit T-cell accumulation within inflamed colonic mucosa which could prevent antigenic stimulation known for its role in multiple myeloma development (51). Furthermore, Jian et al. showed that SCFA-producing bacteria such as *Anaerostipes hadrus, Clostridium butyricum, and Clostridium saccharobutylicum* were reduced in patients with MM, and that the addition of *Clostridium butyricum* in a mouse model of MM resulted in mitigation of tumour progression (6). SCFAs are also involved in the response to treatment. Small, uncontrolled studies have indicated that SCFA-producing bacteria play a significant role in reducing the level of proinflammatory cytokines, thereby protecting the host from tumour progression. Loss of SCFA-producing bacteria can result in a higher risk of tumour progression. Bearing in mind that specific diets can increase the population of SCFA-producing bacteria, studies are needed to investigate whether changes in diet in patients with MGUS can influence the risk of tumour progression.

TABLE 1 | Summary of the alterations of the gut microbiota in MM patients.

Gut microbiota of MM patients
Frequently colonised with opportunistic bacteria (6)
Increase in the number of bacteria involved in nitrogen recycling, such as *K. pneumoniae* or *S, pneumoniae* (6)
Increased diversity and poorer interactions between genera (6)
Reduced number of SCFA-producing bacteria (6)
Changes resulting from applied treatments especially antibiotics

L-Glutamine

Jian et al. showed that stool samples from MM patients had higher concentrations than in healthy patients of bacteria that are involved in nitrogen utilisation and recycling, such as *Klebsiella* and *Streptococcus* (6). The following mechanism has been proposed to explain this phenomenon (6). MM cells are known producers of NH_4^+ (52), which results from uptake of glutamine (53). This NH_4^+ then accumulates in the bone marrow and is released into the blood. In a healthy organism, the liver successfully converts NH_4^+ into urea in the urea cycle. However, MM patients experience a high increase in blood NH_4^+ level that exceeds the capacity of the liver to convert it to urea and can even result occasionally in hyperammonaemic encephalopathy (54). In addition, monoclonal protein renal deposition and consequent reduction in renal function mean that the process of urea excretion is impaired severely (55). Taken together, these factors lead to an increased concentration of urea in the blood, such that excessive amounts of urea reach the intestinal lumen. The presence of urea in the gut layer causes the selection of nitrogen-recycling bacteria, such as *Klebsiella* and *Streptococcus.* These bacteria are involved in the hydrolysis of urea and synthesis of L-glutamine that is taken up by MM cells, which promotes tumour progression. It is probable that MM cells harness the gut microbiota of the host as a recycler of $NH4^+$ to deliver the necessary L-glutamine. In light of this, we speculate that targeting human microbiota with natural methods, or

antibiotics, if necessary, could be an attractive strategy to stop this vicious cycle.

Th17 Cells

The differentiation of Th17 in GF mice is inhibited (14). Microbial colonization, especially with segmented filamentous bacteria (SFB) promotes induction of Th17 cells (56). Furthermore, it is already known that Th17 elicited by SFB are of non-inflammatory phenotype while Th17 cells induced by other bacteria *Citrobacter* are secreting plenty of proinflammatory cytokines (57).

Plasma cells express IL-17 receptors on their surface and are stimulated *in vitro* and *in vivo via* IL-17 produced by Th17 cells (58). Of note, IL-6-STAT3 signalling pathway activated by IL-17 is relevant both for tumour (59) and plasma cell (60) growth which suggests the role of IL-17 during different stages of MM. IL-17 causes the upregulation of the receptor activator of the NF-κB ligand, which results in the activation of osteoclasts (61) and eosinophils that are producing IL-6 and TNF-α (5). Hence, IL-17 is the cytokine that bears the principal responsibility for bone lesions in plasma cell dyscrasias. Stromal cells respond to IL-17 as well by producing IL-6 (62). Moreover, the interplay between IL-6 and TGF-β, that are highly expressed in the bone marrow of patients with MM, is influencing the generation of Th17 cells (49).

Prevotella heparinolytica is responsible for the differentiation of Th17 cells and their migration to the bone marrow in the Vk*MYC mouse model of MM (5). In mice that lacked IL-17, the progression of plasma cell dyscrasias was delayed. Inhibition of IL-17, IL-17 receptor A, and IL-5 in a Vk*MYC model with monoclonal antibodies results in reduced accumulation of Th17 cells and eosinophils in the bone marrow, which results in delayed tumour progression (5).

Patients with MM have elevated serum level of IL-17 but interestingly after therapy with bis-phosphonate level of that cytokine is reduced (63). A higher level of IL-17 is also seen in the blood of patients with SMM and is a predictor of rapid progression of tumour growth. Therefore the level of IL-17 could be used as a potential marker of high-risk SMM patients (64). Similar to the Vk*MYC model, it would be useful to initiate studies on patients to determine which bacteria are involved in Th17 differentiation. Using this approach, bacteria that are involved indirectly in the development of bone lytic lesions, which is one of the main causes of morbidity in MM patients, could be eradicated (**Figure 2**).

THE LINK BETWEEN THE GUT MICROBIOME AND TREATMENT IN PLASMA CELL DYSCRASIAS

It is known that different results of treatment and toxicity profiles are associated with the gut microbiome (65, 66). For instance, a specific composition of gut microbiota is required for an optimal response to treatment with immune checkpoint inhibitors (67). Baruch et al. conducted a phase I study on faecal microbiota transplantation from complete responders to treatment for metastatic melanoma to 10 non-responders, which resulted in

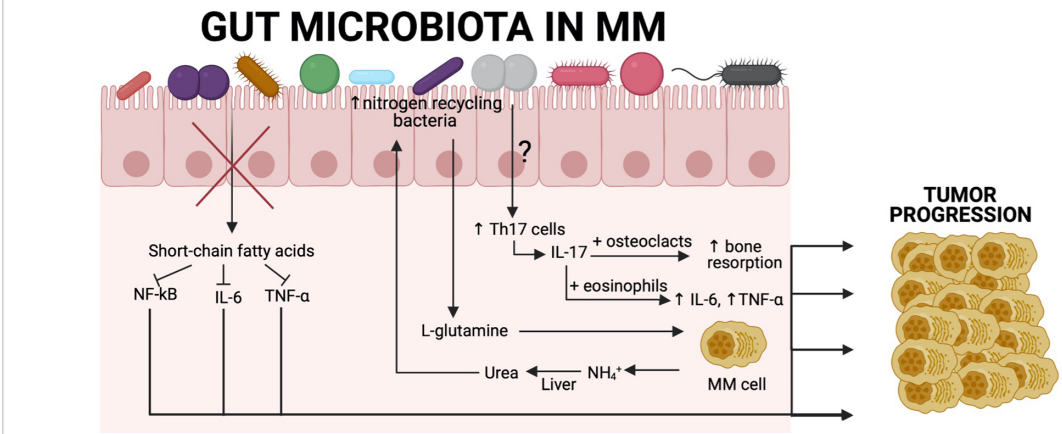

FIGURE 2 | Association between the gut microbiota and tumour progression in MM patients. Recent findings show a close relationship between gut commensal microbiota and MM cells. SCFA-producing bacteria are significantly reduced, resulting in increased levels of NF-κB, IL-6, and TNF-α, which are known to contribute to tumor progression in MM. Another example derives from the fact that MM patients have increased nitrogen-recycling bacteria. These bacteria are involved in L-glutamine production, which is an essential amino acid for MM cells. MM cells produce high amounts of $NH4^+$, which is transformed in the liver into urea and reaches high concentrations in the blood and can select nitrogen-recycling bacteria such as *K. pneumoniae* or *S. pneumoniae*. The influence of the gut microbiota on Th17 cell differentiation in MM patients remains to be characterised, although we know that in a Vk*MYC mouse model, *P. heparinolytica* was responsible for that. Patients with MM have significantly higher level of IL-17 in the blood, which is produced by Th17 cells and causes bone resorption, resulting in bone lesions that are the main symptom of this malignancy. Additionally, IL-17 activates eosinophils that are consequently producing proinflammatory cytokines (IL-6 and TNF-α) that are involved in tumor progression. Created with BioRender.com.

partial responses in three patients and a complete response in one (68). The gut microbiome can influence the results of treatment, especially in respect of adverse events, and treatment can modulate the gut microbiome.

During the last decade, new treatments for plasma cell dyscrasias have been introduced, including immunomodulatory drugs (thalidomide, lenalidomide, and pomalidomide), proteasome inhibitors, and monoclonal antibodies. These have improved the length and quality of life of patients with MM (69). To emphasise the role of the gut microbiome in plasma cell dyscrasias, we describe how microbes can affect the outcomes of treatment in plasma cell malignancies. Their role is particularly visible in respect of possible infectious complications after treatment that are due to infection. It was recently confirmed that treatment of MM changes the composition of the gut microbiome in respect of diversity (70).

Pianko et al. showed that MM patients with no minimal residual disease (MRD) after completion of upfront therapy had greater numbers of butyrate-producing *Eubacterium halii* than MRD-positive patients (71). Similarly, another butyrate producer, *Faecalibacterium prausnitzii*, was associated with an absence of MRD (71). Moreover, Peled et al. showed that intestinal *Eubacterium limosum* was associated with decreased risk of MM relapse after allogeneic haematopoietic cell transplantation (72). These observations suggest that changes in commensal microbiota caused by MM treatment could influence the entire process of therapy or be a predictor of a better response. Gopalakrishnan et al. showed how significant the impact of the changes in the gut microflora on the response to treatment can be. They showed that melanoma patients who responded well to immunotherapy with anti-PD-1 agents had a relative abundance of *Ruminococcaceae* family and higher alpha diversity (diversity within one sample) in faecal microbiome samples (73). Thus, it is possible that the composition of gut microbiota in MM patients has a major influence on the outcomes of immunotherapy, especially taking into account that MM, similarly to melanoma, is closely related to immune response.

Proteasome Inhibitors

PIs, such as bortezomib or carfilzomib are commonly used in primary and relapsed MM. One common adverse effect is gastrointestinal (GI) toxicity that results in diarrhoea. First, it was thought that PIs alter gut motility or cause neurotoxicity, resulting in autonomic neuropathy. The molecular reason for GI toxicity is now established as an increase in TNF-α receptor 1 expression on intestinal cells and higher concentrations of IL-6, TNF-α and IL-1β (74). However, there is a lack of evidence that PIs influence composition of the gut microbiota. It might be that inhibition of the NF-κB pathway is responsible for GI toxicity of PIs (75). SCFAs can suppress the NF-κB pathway, which could augment GI toxicity of PIs (76).

Steroids

Steroids are among the most commonly used anti-inflammatory drugs. They are used in chemotherapy regimens for MM, as well as in the treatment of a wide range of rheumatoid diseases. Huang et al. showed that mice that had been subjected to chronic exposure to steroids differed in the composition of their gut microbiota compared with their healthy counterparts (77). Steroid-treated mice had an increase in *Bifidobacterium* and *Lactobacillus*, which are both associated with anti-inflammatory effects, whereas they noted an absence of *Mucospirillum*, which is responsible for degradation of colonic mucin. This effect might be explained by the decrease of mucin production in mice treated chronically with steroids. Dexamethasone exerts its anti-inflammatory effects by blocking the NF-κB pathway (78). Furthermore, mice that were treated with dexamethasone produced less IL-17 than healthy mice (77). This may be another case in which steroids reshape the intestinal flora, since IL-17 production depends on Th17 cell differentiation, which is associated with specific gut microbiota. However, not only chronic exposure to, but also acute treatment with, steroids affected gut microbiota in mice (77). Ünsal et al. showed that rodents that were injected with a single, strong dose of dexamethasone underwent an increase in the number of ileal anaerobic bacteria. Moreover, a single injection of a low dose of dexamethasone resulted in an increase in the population of coliform bacteria (79). However, the long-term effect of these changes remains to be determined.

Antimicrobials

The link between antibiotics and the gut microbiome seems to be the most examined and the influence of this group of drugs on commensal bacteria is well established. However, although this link has been studied intensively in healthy volunteers, there remains a lack of wider studies with many groups of antibiotics in MM patients. Ziegler et al. showed that levofloxacin, which is the most commonly prescribed drug for bloodstream infections and neutropenic fever prophylaxis, had a less damaging effect on intestinal microbiota than broad-spectrum β-lactam (BSBL) antibiotics (80). The latter group reduced alpha diversity. The former was not associated with specific changes in the gut microbiome that had been found to be associated with poor clinical results (decrease in populations responsible for protection against *C. difficile*; increase in non-Bacteroidetes taxa, and reduction of alpha diversity). In light of their results, the authors emphasised that fluoroquinolone antibiotics protected patients from the negative effects of BSBLs (80). In MM patients who had been newly diagnosed and who were at particular risk of infection, the effect of prophylactic antibiotics was small and there was no decrease in early mortality (81). However, Valkovic et al. reported that MM diagnosis or progression was frequently preceded by infection (82). That could have been because bacterial infections are associated with robust production of proinflammatory cytokines and TLR activation on MM cells (83, 84). This is why prophylactic broad-spectrum antibiotics can result in a delay in disease progression. In respect of allogeneic stem-cell transplantation (alloSCT), Weber et al. showed that early use of broad-spectrum antibiotics that are active against commensal organisms, such as *Clostridiales* was associated with increased transplant-related mortality and decreased overall survival (85). Administration of imipenem–cilastatin or piperacillin–tazobactam for neutropenic

fever resulted in gut microbial perturbation and increased graft-*versus*-host disease-related mortality compared with aztreonam or cefepime, both of which decreased activity against commensal, anaerobic bacteria (86). Such observations of antibiotic effects on the response to treatment of MM need to be investigated in patients who are treated with autologous stem-cell transplantation (ASCT). There is also a recently published systematic review of infections associated with selinexor in patients with relapsed/refractory MM that also compares the risk of infections with other novel agents. It is already known that selinexor could prevent viral infections through blocking of XPO1 - mediated nuclear transport which facilitates the export of viral proteins. The authors state that randomized clinical trials are needed to fully understand the risk of infections associated with selinexor (87).

Autologous Stem-Cell Transplantation

D'Angelo showed that after ASCT, patients showed significantly decreased diversity of the microbial gut population (88). El Jurdi et al. showed an association between baseline microbiota of patients undergoing ASCT with further regimen-related toxicities and with the rate of neutrophil engraftment (89). They found that bacterial diversity after ASCT recovered within 1 month after the procedure, but that fungal populations constantly decreased, which suggests that a longer time is needed for the reconstitution of the mycobiome. Although the prospective study included only 15 patients, the results were encouraging for further studies. This group recognised several links between the composition of the microbiota and effects on ASCT-related toxicity and outcomes. One of the links relied on identifying an increased population of *Bacteroides* at day +7 in patients with less severe diarrhoea, while more severe diarrhoea, nausea, and vomiting occurred in patients with a higher prevalence of the stool populations of *Blautia* and *Ruminococcus*. They also identified a negative correlation between fungal phyla *Glomerella* presence in stools and neutrophil engraftment (89). Similar conclusions were drawn from the results of the small pilot study with 15 patients, showing that baseline microbiota were associated with subsequent incidence and severity of nausea, vomiting, neutropenic fever, and rate of neutrophil engraftment (90). Khan et al. showed recently that 534 adult recipients of high-dose chemotherapy with ASCT had significantly decreased alpha diversity at early pretransplant stages than healthy individuals and that this reduction in diversity tended to be more marked in the course of the procedure (9). The pattern of this loss of diversity and dominance of specific taxa were similar to those seen in patients after alloSCT. In addition, they showed that the greater the diversity of the gut microbiota, the lower risk of progression or death. Our group showed in a retrospective, single-centre study that colonisation with antibiotic-resistant bacteria had a significant influence on the outcomes of alloSCT (91). The main finding was that the overall survival of patients who were colonised by antibiotic-resistant bacteria was estimated to be half that of the noncolonised group. A similar conclusion was reached by Scheich et al. concerning the effect of colonisation by multidrug-resistant organisms on the results of ASCT (92).

Other Treatments

There is little information on the possible influence of other treatments, such as immunomodulatory drugs and monoclonal antibodies, on plasma cell dyscrasias (**Table 2**).

CONCLUSIONS

Despite some progress in the outcomes of treatment of MM, it remains a disease that cannot currently be cured, due to relapse or refractoriness to any available therapy. An emerging factor that could influence not only the refractoriness of MM but also a progression from asymptomatic MGUS to MM is the gut microbiota. We see that changes in the composition of

TABLE 2 | Relationship between the gut microbiota and treatment of plasma cell dyscrasias.

Treatment	How it affects the gut microbiota in plasma cell dyscrasias?
PIs	• There is no evidence proving the influence of PIs on gut microbiota
Steroids	• Mice treated with steroids had increased *Bifidobacterium* and *Lactobacillus* population and the absence of *Mucospirillum* bacteria (77)
	• Mice treated with dexamethasone had decreased production of IL-17 compared with an untreated group. IL-17 production is strictly related to the presence of Th17 cells, whose differentiation in the gut was recently proved in the Vk*MYC mouse model. This indicates some relationship (77)
	• Not only chronic exposure but also acute treatment resulted in alteration of the gut microbiota in rodents (79)
Antimicrobials	• Levofloxacin had no significant impact on the human gut microbiota, while BSBL antibiotics caused a reduction of alpha diversity (80)
	• Administration of broad-spectrum antibiotics efficient against commensal microbiota resulted in higher transplant-related mortality and decreased overall survival (85)
	• Patients treated with imipenem–cilastatin or piperacillin–tazobactam had increased risk of GVHD-related mortality compared with aztreonam or cefepime (86)
ASCT	• Patients after ASCT had decreased diversity of microbial populations in the gut and the normal composition was rebuilt within 1 month after the procedure (89)
	• There is a strong relationship between baseline microbiota of MM patients and severity of toxicity related to the procedure and with the rate of neutrophil engraftment (89)
	• Patients after high-dose chemotherapy before ASCT had significantly decreased alpha diversity of the gut microbiota compared with healthy individuals (9)
Other treatments	• Little is known about possible influence of gut microbiome on treatment outcomes with immunomodulatory drugs or monoclonal antibodies

commensal bacteria can affect the process of transforming MGUS to MM. Further, these changes are associated with colonisation with opportunistic pathogens that can become an aetiological agent of complications due to infection that are associated with treatment. Probably, in the future, it will be possible to identify patients who have an especially high risk of progression to MM, or even to modulate intestinal microflora to reduce the risk of progression of MGUS. It is also possible that the gut microbiota will be modulated to reduce complications that are due to treatment and disease, or to improve treatment outcomes. However, the field of microbiota in MM is still in its infancy and further work is required to gain a fuller understanding of the phenomena.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

REFERENCES

1. Agarwal A, Ghobrial IM. Monoclonal Gammopathy of Undetermined Significance and Smoldering Multiple Myeloma: A Review of the Current Understanding of Epidemiology, Biology, Risk Stratification, and Management of Myeloma Precursor Disease. *Clin Cancer Res* (2013) 19 (5):985–94. doi: 10.1158/1078-0432.CCR-12-2922
2. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of Monoclonal Gammopathy of Undetermined Significance. *N Engl J Med* (2006) 354(13):1362–9. doi: 10.1056/NEJMoa054494
3. Kyle RA, Therneau TM, Rajkumar SV, Offord JR, Larson DR, Plevak MF, et al. A Long-Term Study of Prognosis in Monoclonal Gammopathy of Undetermined Significance. *N Engl J Med* (2002) 346(8):564–9. doi: 10.1056/NEJMoa01133202
4. Kyle RA, Remstein ED, Therneau TM, Dispenzieri A, Kurtin PJ, Hodnefield JM, et al. Clinical Course and Prognosis of Smoldering (Asymptomatic) Multiple Myeloma. *N Engl J Med* (2007) 356(25):2582–90. doi: 10.1056/NEJMoa070389
5. Calcinotto A, Brevi A, Chesi M, Ferrarese R, Garcia Perez L, Grioni M, et al. Microbiota-Driven Interleukin-17-Producing Cells and Eosinophils Synergize to Accelerate Multiple Myeloma Progression. *Nat Commun* (2018) 9(1):4832. doi: 10.1038/s41467-018-07305-8
6. Jian X, Zhu Y, Ouyang J, Wang Y, Lei Q, Xia J, et al. Alterations of Gut Microbiome Accelerate Multiple Myeloma Progression by Increasing the Relative Abundances of Nitrogen-Recycling Bacteria. *Microbiome* (2020) 8 (1):74. doi: 10.1186/s40168-020-00854-5
7. D'Angelo CR, Sudakaran S, Callander NS. Clinical Effects and Applications of the Gut Microbiome in Hematologic Malignancies. *Cancer* (2021) 127 (5):679–87. doi: 10.1002/cncr.33400
8. Ahmed N, Ghannoum M, Gallogly M, de Lima M, Malek E. Influence of Gut Microbiome on Multiple Myeloma: Friend or Foe? *J Immunother Cancer* (2020) 8(1):1–3. doi: 10.1136/jitc-2020-000576
9. Khan N, Lindner S, Gomes ALC, Devlin SM, Shah G, Sung AD, et al. Fecal Microbiota Diversity Disruption and Clinical Outcomes After Auto-HCT: A Multicenter Observational Study. *Blood* (2020) 137:13–5. doi: 10.1182/blood.2020006923
10. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How Colonization by Microbiota in Early Life Shapes the Immune System. *Science* (2016) 352 (6285):539–44. doi: 10.1126/science.aad9378
11. Bauer H, Horowitz RE, Levenson SM, Popper H. The Response of the Lymphatic Tissue to the Microbial Flora. Studies on Germfree Mice. *Am J Pathol* (1963) 42:471–83.
12. Umesaki Y, Setoyama H, Matsumoto S, Okada Y . Expansion of Alpha Beta T-Cell Receptor-Bearing Intestinal Intraepithelial Lymphocytes After Microbial Colonization in Germ-Free Mice and its Independence From Thymus. *Immunology* (1993) 79(1):32–7.
13. Hapfelmeier S, Lawson MA, Slack E, Kirundi JK, Stoel M, Heikenwalder M, et al. Reversible Microbial Colonization of Germ-Free Mice Reveals the Dynamics of Iga Immune Responses. *Science* (2010) 328(5986):1705–9. doi: 10.1126/science.1188454
14. Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, et al. Specific Microbiota Direct the Differentiation of IL-17-Producing T-Helper Cells in the Mucosa of the Small Intestine. *Cell Host Microbe* (2008) 4(4):337–49. doi: 10.1016/j.chom.2008.09.009
15. Erturk-Hasdemir D, Oh SF, Okan NA, Stefanetti G, Gazzaniga FS, Seeberger PH, et al. Symbionts Exploit Complex Signaling to Educate the Immune System. *Proc Natl Acad Sci USA* (2019) 116:26157–66. doi: 10.2139/ssrn.3362573
16. Schulthess J, Pandey S, Capitani M, Rue-Albrecht KC, Arnold I, Franchini F, et al. The Short Chain Fatty Acid Butyrate Imprints an Antimicrobial Program in Macrophages. *Immunity* (2019) 50(2):432–445 e7. doi: 10.1016/j.immuni.2018.12.018
17. Chaput N, Lepage P, Coutzac C, Soularue E, Le Roux K, Monot C, et al. Baseline Gut Microbiota Predicts Clinical Response and Colitis in Metastatic Melanoma Patients Treated With Ipilimumab. *Ann Oncol* (2017) 28(6):1368–79. doi: 10.1093/annonc/mdx108
18. Abid MB, Shah NN, Maatman TC, Hari PN. Gut Microbiome and CAR-T Therapy. *Exp Hematol Oncol* (2019) 8:31. doi: 10.1186/s40164-019-0155-8
19. Jasinski M, Basak GW, Jedrzejczak WW. Perspectives for the Use of CAR-T Cells for the Treatment of Multiple Myeloma. *Front Immunol* (2021) 12:632937. doi: 10.3389/fimmu.2021.632937
20. Blimark C, Holmberg E, Mellqvist UH, Landgren O, Bjorkholm M, Hultcrantz M, et al. Multiple Myeloma and Infections: A Population-Based Study on 9253 Multiple Myeloma Patients. *Haematologica* (2015) 100(1):107–13. doi: 10.3324/haematol.2014.107714
21. Khosravi A, Yanez A, Price JG, Chow A, Merad M, Goodridge HS, et al. Gut Microbiota Promote Hematopoiesis to Control Bacterial Infection. *Cell Host Microbe* (2014) 15(3):374–81. doi: 10.1016/j.chom.2014.02.006
22. Gao Y, Zhou J, Qi H, Wei J, Yang Y, Yue J, et al. Lncrna Lncly6c Induced by Microbiota Metabolite Butyrate Promotes Differentiation of Ly6C(High) to Ly6C(Int/Neg) Macrophages Through Lncly6c/C/Ebpbeta/Nr4A1 Axis. *Cell Discov* (2020) 6(1):87. doi: 10.1038/s41421-020-00211-8
23. Menezes-Garcia Z, Do Nascimento Arifa RD, Acurcio L, Brito CB, Gouvea JO, Lima RL, et al. Colonization by Enterobacteriaceae is Crucial for Acute Inflammatory Responses in Murine Small Intestine via Regulation of Corticosterone Production. *Gut Microbes* (2020) 11(6):1531–46. doi: 10.1080/19490976.2020.1765946
24. Hill MJ. Intestinal Flora and Endogenous Vitamin Synthesis. *Eur J Cancer Prev* (1997) 6(Suppl 1):S43–5. doi: 10.1097/00008469-199703001-00009
25. Round JL, Mazmanian SK. The Gut Microbiota Shapes Intestinal Immune Responses During Health and Disease. *Nat Rev Immunol* (2009) 9(5):313–23. doi: 10.1038/nri2515
26. Cebra JJ. Influences of Microbiota on Intestinal Immune System Development. *Am J Clin Nutr* (1999) 69(5):1046S–51S. doi: 10.1093/ajcn/69.5.1046s
27. Zackular JP, Baxter NT, Iverson KD, Sadler WD, Petrosino JF, Chen GY, et al. The Gut Microbiome Modulates Colon Tumorigenesis. *mBio* (2013) 4(6): e00692–13. doi: 10.1128/mBio.00692-13
28. Wang T, Cai G, Qiu Y, Fei N, Zhang M, Pang X, et al. Structural Segregation of Gut Microbiota Between Colorectal Cancer Patients and Healthy Volunteers. *ISME J* (2012) 6(2):320–9. doi: 10.1038/ismej.2011.109
29. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, Mederacke I, et al. Promotion of Hepatocellular Carcinoma by the Intestinal Microbiota and TLR4. *Cancer Cell* (2012) 21(4):504–16. doi: 10.1016/j.ccr.2012.02.007
30. Ochi A, Nguyen AH, Bedrosian AS, Mushlin HM, Zarbakhsh S, Barilla R, et al. Myd88 Inhibition Amplifies Dendritic Cell Capacity to Promote Pancreatic Carcinogenesis via Th2 Cells. *J Exp Med* (2012) 209(9):1671–87. doi: 10.1084/jem.20111706

31. Ohno H. Intestinal M Cells. *J Biochem* (2016) 159(2):151–60. doi: 10.1093/jb/mvv121
32. Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P. The Immune Geography of Iga Induction and Function. *Mucosal Immunol* (2008) 1 (1):11–22. doi: 10.1038/mi.2007.6
33. Brandsma E, Kloosterhuis NJ, Koster M, Dekker DC, Gijbels MJJ, van der Velden S, et al. A Proinflammatory Gut Microbiota Increases Systemic Inflammation and Accelerates Atherosclerosis. *Circ Res* (2019) 124(1):94–100. doi: 10.1161/CIRCRESAHA.118.313234
34. Mason BL, Li Q, Minhajuddin A, Czysz AH, Coughlin LA, Hussain SK, et al. Reduced Anti-Inflammatory Gut Microbiota are Associated With Depression and Anhedonia. *J Affect Disord* (2020) 266:394–401. doi: 10.1016/j.jad.2020.01.137
35. Chassaing B, Ley RE, Gewirtz AT. Intestinal Epithelial Cell Toll-Like Receptor 5 Regulates the Intestinal Microbiota to Prevent Low-Grade Inflammation and Metabolic Syndrome in Mice. *Gastroenterology* (2014) 147(6):1363–77 e17. doi: 10.1053/j.gastro.2014.08.033
36. Sanchez JMS, Doty DJ, DePaula-Silva AB, Brown DG, Bell R, Klag KA, et al. Molecular Patterns From a Human Gut-Derived Lactobacillus Strain Suppress Pathogenic Infiltration of Leukocytes Into the Central Nervous System. *J Neuroinflamm* (2020) 17(1):291. doi: 10.1186/s12974-020-01959-2
37. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal Microbe-Derived Butyrate Induces the Differentiation of Colonic Regulatory T Cells. *Nature* (2013) 504(7480):446–50. doi: 10.1038/nature12721
38. Martin-Gallausiaux C, Beguet-Crespel F, Marinelli L, Jamet A, Ledue F, Blottiere HM, et al. Butyrate Produced by Gut Commensal Bacteria Activates TGF-Beta1 Expression Through the Transcription Factor SP1 in Human Intestinal Epithelial Cells. *Sci Rep* (2018) 8(1):9742. doi: 10.1038/s41598-018-28048-y
39. Ostman S, Rask C, Wold AE, Hultkrantz S, Telemo E. Impaired Regulatory T Cell Function in Germ-Free Mice. *Eur J Immunol* (2006) 36(9):2336–46. doi: 10.1002/eji.200535244
40. Ukena SN, Singh A, Dringenberg U, Engelhardt R, Seidler U, Hansen W, et al. Probiotic Escherichia Coli Nissle 1917 Inhibits Leaky Gut by Enhancing Mucosal Integrity. *PloS One* (2007) 2(12):e1308. doi: 10.1371/journal.pone.0001308
41. Stepankova R, Sinkora J, Hudcovic T, Kozakova H, Tlaskalova-Hogenova H. Differences in Development of Lymphocyte Subpopulations From Gut-Associated Lymphatic Tissue (GALT) of Germfree and Conventional Rats: Effect of Aging. *Folia Microbiol (Praha)* (1998) 43(5):531–4. doi: 10.1007/BF02820814
42. Tlaskalova-Hogenova H, Sterzl J, Stepankova R, Dlabac V, Veticka V, Rossmann P, et al. Development of Immunological Capacity Under Germfree and Conventional Conditions. *Ann N Y Acad Sci* (1983) 409:96–113. doi: 10.1111/j.1749-6632.1983.tb26862.x
43. Tlaskalova-Hogenova H, Cerna J, Mandel L. Peroral Immunization of Germfree Piglets: Appearance of Antibody-Forming Cells and Antibodies of Different Isotypes. *Scand J Immunol* (1981) 13(5):467–72. doi: 10.1111/j.1365-3083.1981.tb00158.x
44. Dai Z, Coker OO, Nakatsu G, Wu WKK, Zhao L, Chen Z, et al. Multi-Cohort Analysis of Colorectal Cancer Metagenome Identified Altered Bacteria Across Populations and Universal Bacterial Markers. *Microbiome* (2018) 6(1):70. doi: 10.1186/s40168-018-0451-2
45. Howman R, Thakerer A, Pitman M, Ding N, Thompson PA, Khot A, et al. Bortezomib, Cyclophosphamide, and Dexamethasone: Highly Effective for Rapid Reversal of Myeloma-Associated Hyperammonemic Encephalopathy. *Leuk Lymphoma* (2010) 51(12):2299–302. doi: 10.3109/10428194.2010.518654
46. Antoine Pepeljugoski C, Morgan G, Braunstein M. Analysis of Intestinal Microbiome in Multiple Myeloma Reveals Progressive Dysbiosis Compared to MGUS and Healthy Individuals. *Blood* (2019) 134(Supplement_1):3076–6. doi: 10.1182/blood-2019-130643
47. Zhang B, Gu J, Liu J, Huang B, Li J. Fecal Microbiota Taxonomic Shifts in Chinese Multiple Myeloma Patients Analyzed by Quantitative Polimerase Chain Reaction (QPCR) and 16S Rrna High-Throughput Sequencing. *Med Sci Monit* (2019) 25:8269–80. doi: 10.12659/MSM.919988
48. Kim CH, Park J, Kim M. Gut Microbiota-Derived Short-Chain Fatty Acids, T Cells, and Inflammation. *Immune Netw* (2014) 14(6):277–88. doi: 10.4110/in.2014.14.6.277
49. Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC, et al. Understanding Multiple Myeloma Pathogenesis in the Bone Marrow to Identify New Therapeutic Targets. *Nat Rev Cancer* (2007) 7(8):585–98. doi: 10.1038/nrc2189
50. Manzo VE, Bhatt AS. The Human Microbiome in Hematopoiesis and Hematologic Disorders. *Blood* (2015) 126(3):311–8. doi: 10.1182/blood-2015-04-574392
51. Zimmerman MA, Singh N, Martin PM, Thangaraju M, Ganapathy V, Waller JL, et al. Butyrate Suppresses Colonic Inflammation Through HDAC1-Dependent Fas Upregulation and Fas-Mediated Apoptosis of T Cells. *Am J Physiol Gastrointest Liver Physiol* (2012) 302(12):G1405–15. doi: 10.1152/ajpgi.00543.2011
52. Otsuki T, Yamada O, Sakaguchi H, Ichiki T, Kouguchi K, Wada H, et al. In Vitro Excess Ammonia Production in Human Myeloma Cell Lines. *Leukemia* (1998) 12(7):1149–58. doi: 10.1038/sj.leu.2401077
53. Bolzoni M, Chiu M, Accardi F, Vescovini R, Airoldi I, Storti P, et al. Dependence on Glutamine Uptake and Glutamine Addiction Characterize Myeloma Cells: A New Attractive Target. *Blood* (2016) 128(5):667–79. doi: 10.1182/blood-2016-01-690743
54. Pham A, Reagan JL, Castillo JJ. Multiple Myeloma-Induced Hyperammonemic Encephalopathy: An Entity Associated With High in-Patient Mortality. *Leuk Res* (2013) 37(10):1229–32. doi: 10.1016/j.leukres.2013.07.014
55. Kourelis TV, Nasr SH, Dispenzieri A, Kumar SK, Gertz MA, Fervenza FC, et al. Outcomes of Patients With Renal Monoclonal Immunoglobulin Deposition Disease. *Am J Hematol* (2016) 91(11):1123–8. doi: 10.1002/ajh.24528
56. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of Intestinal Th17 Cells by Segmented Filamentous Bacteria. *Cell* (2009) 139 (3):485–98. doi: 10.1016/j.cell.2009.09.033
57. Omenetti S, Bussi C, Metidji A, Iseppon A, Lee S, Tolaini M, et al. The Intestine Harbors Functionally Distinct Homeostatic Tissue-Resident and Inflammatory Th17 Cells. *Immunity* (2019) 51(1):77–89 e6. doi: 10.1016/j.immuni.2019.05.004
58. Prabhala RH, Pelluru D, Fulciniti M, Prabhala HK, Nanjappa P, Song W, et al. Elevated IL-17 Produced by TH17 Cells Promotes Myeloma Cell Growth and Inhibits Immune Function in Multiple Myeloma. *Blood* (2010) 115(26):5385–92. doi: 10.1182/blood-2009-10-246660
59. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can Promote Tumor Growth Through an IL-6-Stat3 Signaling Pathway. *J Exp Med* (2009) 206(7):1457–64. doi: 10.1084/jem.20090207
60. Palumbo A, Anderson K. Multiple Myeloma. *N Engl J Med* (2011) 364 (11):1046–60. doi: 10.1056/NEJMra1011442
61. Noonan K, Marchionni L, Anderson J, Pardoll D, Roodman GD, Borrello I. A Novel Role of IL-17-Producing Lymphocytes in Mediating Lytic Bone Disease in Multiple Myeloma. *Blood* (2010) 116(18):3554–63. doi: 10.1182/blood-2010-05-283895
62. Prabhala RH, Fulciniti M, Pelluru D, Rashid N, Nigroiu A, Nanjappa P, et al. Targeting IL-17A in Multiple Myeloma: A Potential Novel Therapeutic Approach in Myeloma. *Leukemia* (2016) 30(2):379–89. doi: 10.1038/leu.2015.228
63. Oteri G, Allegra A, Bellomo G, Alonci A, Nastro E, Penna G, et al. Reduced Serum Levels of Interleukin 17 in Patients With Osteonecrosis of the Jaw and in Multiple Myeloma Subjects After Bisphosphonates Administration. *Cytokine* (2008) 43(2):103–4. doi: 10.1016/j.cyto.2008.05.010
64. Rajkumar SV, Landgren O, Mateos MV. Smoldering Multiple Myeloma. *Blood* (2015) 125(20):3069–75. doi: 10.1182/blood-2014-09-568899
65. Hooper LV, Littman DR, Macpherson AJ. Interactions Between the Microbiota and the Immune System. *Science* (2012) 336(6086):1268–73. doi: 10.1126/science.1223490
66. Ivanov II, Honda K. Intestinal Commensal Microbes as Immune Modulators. *Cell Host Microbe* (2012) 12(4):496–508. doi: 10.1016/j.chom.2012.09.009
67. Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. The Commensal Microbiome is Associated With Anti-PD-1 Efficacy in Metastatic Melanoma Patients. *Science* (2018) 359(6371):104–8. doi: 10.1126/science.aao3290
68. Baruch EN, Youngster I, Ben-Betzalel G, Ortenberg R, Lahat A, Katz L, et al. Fecal Microbiota Transplant Promotes Response in Immunotherapy-Refractory Melanoma Patients. *Science* (2021) 371(6529):602–9. doi: 10.1126/science.abb5920

69. Naymagon L, Abdul-Hay M. Novel Agents in the Treatment of Multiple Myeloma: A Review About the Future. *J Hematol Oncol* (2016) 9(1):52. doi: 10.1186/s13045-016-0282-1
70. Ashraf A, Shaha N, Zahoor A, Arafat AF, Saif UR, Syeda SB, et al. Intestinal Microbiome Diversity and Clinical Outcomes With Multiple Myeloma: A Systematic Review. *J Clin Oncol* (2020) 38(15_suppl):e20542–2. doi: 10.1200/JCO.2020.38.15_suppl.e20542
71. Pianko MJ, Devlin SM, Littmann ER, Chansakul A, Mastey D, Salcedo M, et al. Minimal Residual Disease Negativity in Multiple Myeloma is Associated With Intestinal Microbiota Composition. *Blood Adv* (2019) 3(13):2040–4. doi: 10.1182/bloodadvances.2019032276
72. Peled JU, Devlin SM, Staffas A, Lumish M, Khanin R, Littmann ER, et al. Intestinal Microbiota and Relapse After Hematopoietic-Cell Transplantation. *J Clin Oncol* (2017) 35(15):1650–9. doi: 10.1200/JCO.2016.70.3348
73. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut Microbiome Modulates Response to Anti-PD-1 Immunotherapy in Melanoma Patients. *Science* (2018) 359(6371):97–103. doi: 10.1126/science.aan4236
74. Stansborough RL, Gibson RJ. Proteasome Inhibitor-Induced Gastrointestinal Toxicity. *Curr Opin Support Palliat Care* (2017) 11(2):133–7. doi: 10.1097/SPC.0000000000000266
75. Al-Homsi AS, Feng Y, Duffner U, Al Malki MM, Goodyke A, Cole K, et al. Bortezomib for the Prevention and Treatment of Graft-*Versus*-Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation. *Exp Hematol* (2016) 44(9):771–7. doi: 10.1016/j.exphem.2016.05.005
76. Alkharabsheh O, Sidiqi MH, Aljama MA, Gertz MA, Frankel AE. The Human Microbiota in Multiple Myeloma and Proteasome Inhibitors. *Acta Haematol* (2020) 143(2):118–23. doi: 10.1159/000500976
77. Huang EY, Inoue T, Leone VA, Dalal S, Touw K, Wang Y, et al. Using Corticosteroids to Reshape the Gut Microbiome: Implications for Inflammatory Bowel Diseases. *Inflamm Bowel Dis* (2015) 21(5):963–72. doi: 10.1097/MIB.0000000000000332
78. Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin AS Jr. Characterization of Mechanisms Involved in Transrepression of NF-Kappa B by Activated Glucocorticoid Receptors. *Mol Cell Biol* (1995) 15(2):943–53. doi: 10.1128/MCB.15.2.943
79. Unsal H, Balkaya M, Unsal C, Biyik H, Basbulbul G, Poyrazoglu E. The Short-Term Effects of Different Doses of Dexamethasone on the Numbers of Some Bacteria in the Ileum. *Dig Dis Sci* (2008) 53(7):1842–5. doi: 10.1007/s10620-007-0089-6
80. Ziegler M, Han JH, Landsburg D, Pegues D, Reesey E, Gilmar C, et al. Impact of Levofloxacin for the Prophylaxis of Bloodstream Infection on the Gut Microbiome in Patients With Hematologic Malignancy. *Open Forum Infect Dis* (2019) 6(7):ofz252. doi: 10.1093/ofid/ofz252
81. Mohyuddin GR, Aziz M, McClune B, Abdallah AO, Qazilbash M. Antibiotic Prophylaxis for Patients With Newly Diagnosed Multiple Myeloma: Systematic Review and Meta-Analysis. *Eur J Haematol* (2020) 104(5):420–6. doi: 10.1111/ejh.13374
82. Valkovic T, Nacinovic AD, Petranovic D. Prophylactic Broad Spectrum Antibiotics as a New Anti-Myeloma Therapy. *Med Hypotheses* (2013) 81(6):1137–40. doi: 10.1016/j.mehy.2013.10.021
83. Jego G, Bataille R, Geffroy-Luseau A, Descamps G, Pellat-Deceunynck C. Pathogen-Associated Molecular Patterns are Growth and Survival Factors for Human Myeloma Cells Through Toll-Like Receptors. *Leukemia* (2006) 20(6):1130–7. doi: 10.1038/sj.leu.2404226
84. Bohnhorst J, Rasmussen T, Moen SH, Flottum M, Knudsen L, Borset M, et al. Toll-Like Receptors Mediate Proliferation and Survival of Multiple Myeloma Cells. *Leukemia* (2006) 20(6):1138–44. doi: 10.1038/sj.leu.2404225
85. Weber D, Jenq RR, Peled JU, Taur Y, Hiergeist A, Koestler J, et al. Microbiota Disruption Induced by Early Use of Broad-Spectrum Antibiotics is an Independent Risk Factor of Outcome After Allogeneic Stem Cell Transplantation. *Biol Blood Marrow Transplant* (2017) 23(5):845–52. doi: 10.1016/j.bbmt.2017.02.006
86. Shono Y, Docampo MD, Peled JU, Perobelli SM, Velardi E, Tsai JJ, et al. Increased GVHD-Related Mortality With Broad-Spectrum Antibiotic Use After Allogeneic Hematopoietic Stem Cell Transplantation in Human Patients and Mice. *Sci Transl Med* (2016) 8(339):339ra71. doi: 10.1126/scitranslmed.aaf2311
87. Abid H, Wu JF, Abid MB. Risk for Infections With Selinexor in Patients With Relapsed/Refractory Multiple Myeloma: A Systematic Review of Clinical Trials. *Eur J Cancer* (2021) 154:7–10.
88. D'Angelo C, Sudakaran S, Hematti P, Asimakopoulos F, Augustine C, Safdar N, et al. Impact of Antibiotics on Gut Microbiota Diversity and the Results of a Prospective Dietary Assessment in Patients With Multiple Myeloma Undergoing Autologous Hematopoietic Stem Cell Transplantation. *Blood* (2019) 134(Supplement_1):4653–3. doi: 10.1182/blood-2019-123530
89. El Jurdi N, Filali-Mouhim A, Salem I, Retuerto M, Dambrosio NM, Baer L, et al. Gastrointestinal Microbiome and Mycobiome Changes During Autologous Transplantation for Multiple Myeloma: Results of a Prospective Pilot Study. *Biol Blood Marrow Transplant* (2019) 25(8):1511–9. doi: 10.1016/j.bbmt.2019.04.007
90. Kusakabe S, Fukushima K, Maeda T, Motooka D, Nakamura S, Fujita J, et al. Pre- and Post-Serial Metagenomic Analysis of Gut Microbiota as a Prognostic Factor in Patients Undergoing Haematopoietic Stem Cell Transplantation. *Br J Haematol* (2020) 188(3):438–49. doi: 10.1111/bjh.16205
91. Bilinski J, Robak K, Peric Z, Marchel H, Karakulska-Prystupiuk E, Halaburda K, et al. Impact of Gut Colonization by Antibiotic-Resistant Bacteria on the Outcomes of Allogeneic Hematopoietic Stem Cell Transplantation: A Retrospective, Single-Center Study. *Biol Blood Marrow Transplant* (2016) 22(6):1087–93. doi: 10.1016/j.bbmt.2016.02.009
92. Scheich S, Reinheimer C, Brandt C, Wichelhaus TA, Hogardt M, Kempf VAJ, et al. Clinical Impact of Colonization With Multidrug-Resistant Organisms on Outcome After Autologous Stem Cell Transplantation: A Retrospective Single-Center Study. *Biol Blood Marrow Transplant* (2017) 23(9):1455–62. doi: 10.1016/j.bbmt.2017.05.016

Conflict of Interest: JB and GWB are the founders of the faecal microbiota bank and laboratory named the Human Biome Institute.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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8. Podsumowanie

Niniejsza rozprawa doktorska obejmuje zarówno prace oryginalne, jak i przeglądowe, których wspólnym tematem jest rola mikrobioty jelitowej w rozwoju, leczeniu i powikłaniach leczenia szpiczaka plazmocytowego.

W pracy przeglądowej pt. „The Role of the Gut Microbiome in Pathogenesis, Biology, and Treatment of Plasma Cell Dyscrasias" (Frontiers in Oncology, 2021) podsumowano aktualną wiedzę na temat związku mikrobiomu jelitowego z patogenezą, biologią i leczeniem dyskrazji plazmocytowych. Wskazano, że komórki nowotworowe mogą kształtować skład mikrobioty, a zaburzenia jej równowagi sprzyjają progresji od MGUS do pełnoobjawowego szpiczaka plazmocytowego. Szczególne znaczenie przypisano bakteriom zaangażowanym w przemiany azotu oraz zmniejszonej liczbie bakterii produkujących SCFA, które pośrednio modulują mikrośrodowisko szpiku i wpływają na proliferację komórek nowotworowych. Podkreślono także, że mikrobiota może modulować odpowiedź na terapie, w tym immunoterapie nowej generacji, co otwiera perspektywę zastosowania interwencji takich jak przeszczepienie mikrobioty jelitowej czy ukierunkowane wsparcie dietetyczne.

W drugiej pracy przeglądowej pt. „The Role of the Crosstalk Between Gut Microbiota and Immune Cells in the Pathogenesis and Treatment of Multiple Myeloma" (Frontiers in Immunology, 2022) nacisk położono na szczegółowe przedstawienie złożonych interakcji pomiędzy mikroorganizmami jelitowymi, a komórkami układu odpornościowego w kontekście patogenezy i leczenia szpiczaka plazmocytowego. Szczególną uwagę zwrócono na przewlekłą stymulację antygenową limfocytów B jako mechanizm sprzyjający transformacji plazmocytów, a także na rolę równowagi między subpopulacjami limfocytów T (Treg i Th17) w utrzymaniu homeostazy immunologicznej. W pracy ukazano, że mikrobiota oddziałuje nie tylko poprzez metabolity takie jak SCFA, ale również poprzez wpływ na dojrzewanie komórek dendrytycznych, makrofagów, neutrofili i limfocytów, a tym samym reguluje procesy sprzyjające lub hamujące rozwój nowotworu. Podkreślono, że mikrobiota odgrywa istotną rolę w inicjacji zmian prekursorowych, jak i w dalszej progresji choroby, a jej modulacja może w przyszłości stać się elementem terapii wspierających.

Pierwsza praca o charakterze oryginalnym pt. „Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation" (Scientific Reports 2021) dotyczyła praktycznego problemu klinicznego, jakim jest zapobieganie toksycznemu uszkodzeniu śluzówek błony śluzowej jamy ustnej u pacjentów

poddawanych procedurze auto-SCT. Zbadano skuteczność zastosowania komercyjnie dostępnych lodów jako formy krioterapii i wykazano, że metoda ta istotnie zmniejsza częstość i nasilenie zmian zapalnych w jamie ustnej. Pokazano, że częstość występowania OM w grupie otrzymującej lody wyniosła 28,84%, podczas gdy w grupie, która lodów nie otrzymywała wyniosła 59,09%. Jest to prosta, tania, dobrze tolerowana przez pacjentów strategia i łatwa do wdrożenia w warunkach klinicznych. Wyniki tej pracy mają bezpośrednie znaczenie praktyczne i stanowią przykład badań ukierunkowanych na poprawę jakości życia pacjentów w trakcie intensywnego leczenia onkohematologicznego.

Druga praca oryginalna pt. „Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma" (Scientific Reports 2024) dotyczyła wpływu kolonizacji przewodu pokarmowego przez bakterie antybiotykooporne (ARB) przed auto-SCT na ryzyko powikłań infekcyjnych po wykonaniu procedury. Wykazano, że kolonizacja ARB wiąże się ze znacznie wyższym ryzykiem infekcji po auto-SCT w porównaniu do grupy bez kolonizacji. Częściej dochodziło do rozwoju sepsy bakteryjnej u chorych skolonizowanych. Biorąc pod uwagę fakt, iż dane literaturowe wskazują na związek kolonizacji ARB ze zubożeniem mikrobioty jelitowej można wysnuć wniosek, iż kolonizacja ARB jest markerem nieprawidłowej mikrobioty, która z kolei wpływa na przepuszczalność bariery krew-jelito. Pomimo braku istotności statystycznej, zaobserwowano tendencję do krótszego przeżycia całkowitego (OS) w grupie pacjentów skolonizowanych, na co wpływ może mieć śmiertelność wynikająca z wczesnych powikłań przeszczepienia krwiotwórczych komórek macierzystych (wydłużenie okresu obserwacji mogłoby uzyskać istotność statystyczną).

Podsumowując, przedstawione publikacje ukazują szerokie spektrum badań - prac oryginalnych i przeglądowych, które rzucają nowe światło na udział mikrobioty jelitowej i jej interakcje z układem odpornościowym w rozwoju i leczeniu szpiczaka plazmocytowego. Należy jednak z pokorą zaznaczyć, że badania oryginalne wskazują przede wszystkim na istotne asocjacje, lecz nie dowodzą mechanizmów przyczynowo-skutkowych. Z kolei prace przeglądowe odzwierciedlają stan wiedzy w momencie ich publikacji i wymagają stałej aktualizacji w obliczu szybko postępujących badań w tej dziedzinie.

9. Wnioski

Na podstawie wyników badań przedstawionych w ramach niniejszego cyklu publikacji sformułowano następujące wnioski:

1. Kolonizacja przewodu pokarmowego przez bakterie antybiotykooporne (ARB) przed procedurą auto-SCT stanowi istotny czynnik ryzyka rozwoju powikłań infekcyjnych, w szczególności bakteriemii (43% vs 14%, p=0.004), u pacjentów ze szpiczakiem plazmocytowym.
2. Zastosowanie krioterapii w formie lodów spożywczych podczas wlewu wysokodawkowanego melfalanu jest skuteczną, dobrze tolerowaną i efektywną kosztowo metodą profilaktyki zapalenia błon śluzowych jamy ustnej (OM), znamiennie redukującą częstość tego powikłania (28.8% vs 59.1%, p=0.02).
3. Mikrobiota jelitowa, poprzez mechanizmy takie jak przewlekła stymulacja antygenowa limfocytów B oraz produkcja metabolitów wpływających na mikrośrodowisko guza, odgrywa kluczową rolę w patogenezie dyskrazji plazmocytowych i może modulować progresję od stanów przednowotworowych do aktywnego szpiczaka plazmocytowego.
4. Skład i funkcja mikrobioty jelitowej wpływają na odpowiedź na leczenie przeciwszpiczakowe, a jej celowana modulacja, np. poprzez transplantację mikrobioty jelitowej (FMT) lub interwencje dietetyczne, może stanowić obiecującą strategię terapeutyczną w celu poprawy wyników leczenia i przezwyciężania lekooporności.

10. Piśmiennictwo

1. Kyle, R.A. and S.V. Rajkumar, *Multiple myeloma.* N Engl J Med, 2004. **351**(18): p. 1860-73.
2. Rajkumar, S.V., *Multiple myeloma: 2020 update on diagnosis, risk-stratification and management.* Am J Hematol, 2020. **95**(5): p. 548-567.
3. Siegel, R.L., K.D. Miller, H.E. Fuchs, and A. Jemal, *Cancer statistics, 2022.* CA Cancer J Clin, 2022. **72**(1): p. 7-33.
4. Waxman, A.J., et al., *Racial disparities in incidence and outcome in multiple myeloma: a population-based study.* Blood, 2010. **116**(25): p. 5501-6.
5. Mateos, M.V. and O. Landgren, *MGUS and Smoldering Multiple Myeloma: Diagnosis and Epidemiology.* Cancer Treat Res, 2016. **169**: p. 3-12.
6. Rajkumar, S.V., et al., *International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma.* Lancet Oncol, 2014. **15**(12): p. e538-48.
7. Palumbo, A., et al., *Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group.* J Clin Oncol, 2015. **33**(26): p. 2863-9.
8. Fonseca, R., et al., *International Myeloma Working Group molecular classification of multiple myeloma: spotlight review.* Leukemia, 2009. **23**(12): p. 2210-21.
9. Moreau, P., 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**(10192): p. 29-38.

10. McCarthy, P.L., et al., *Lenalidomide after stem-cell transplantation for multiple myeloma.* N Engl J Med, 2012. **366**(19): p. 1770-81.
11. Boussi, L.S., Z.M. Avigan, and J. Rosenblatt, *Immunotherapy for the treatment of multiple myeloma.* Front Immunol, 2022. **13**: p. 1027385.
12. Munshi, N.C., et al., *Idecabtagene Vicleucel in Relapsed and Refractory Multiple Myeloma.* N Engl J Med, 2021. **384**(8): p. 705-716.
13. Bringhen, S., et al., *Cardiovascular adverse events in modern myeloma therapy - Incidence and risks. A review from the European Myeloma Network (EMN) and Italian Society of Arterial Hypertension (SIIA).* Haematologica, 2018. **103**(9): p. 1422-1432.
14. Thursby, E. and N. Juge, *Introduction to the human gut microbiota.* Biochem J, 2017. **474**(11): p. 1823-1836.
15. Qin, J., et al., *A human gut microbial gene catalogue established by metagenomic sequencing.* Nature, 2010. **464**(7285): p. 59-65.
16. Belkaid, Y. and T.W. Hand, *Role of the microbiota in immunity and inflammation.* Cell, 2014. **157**(1): p. 121-41.
17. Honda, K. and D.R. Littman, *The microbiota in adaptive immune homeostasis and disease.* Nature, 2016. **535**(7610): p. 75-84.
18. Zuo, T. and S.C. Ng, *The Gut Microbiota in the Pathogenesis and Therapeutics of Inflammatory Bowel Disease.* Front Microbiol, 2018. **9**: p. 2247.
19. Moiseev, S., V. Rameev, E. Karovaikina, and L. Lysenko Kozlovskaya, *Gut microbiome in rheumatic diseases.* Ann Rheum Dis, 2021. **80**(11): p. e176.
20. Hevia, A., et al., *Intestinal dysbiosis associated with systemic lupus erythematosus.* mBio, 2014. **5**(5): p. e01548-14.
21. Koh, A., F. De Vadder, P. Kovatcheva-Datchary, and F. Backhed, *From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites.* Cell, 2016. **165**(6): p. 1332-1345.
22. Smith, P.M., et al., *The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis.* Science, 2013. **341**(6145): p. 569-73.
23. Zelante, T., et al., *Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22.* Immunity, 2013. **39**(2): p. 372-85.
24. Matsumoto, M., et al., *Impact of intestinal microbiota on intestinal luminal metabolome.* Sci Rep, 2012. **2**: p. 233.
25. Smits, H.H., et al., *Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin.* J Allergy Clin Immunol, 2005. **115**(6): p. 1260-7.
26. Scher, J.U., et al., *Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis.* Elife, 2013. **2**: p. e01202.
27. Round, J.L. and S.K. Mazmanian, *Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota.* Proc Natl Acad Sci U S A, 2010. **107**(27): p. 12204-9.
28. Darfeuille-Michaud, A., et al., *High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease.* Gastroenterology, 2004. **127**(2): p. 412-21.
29. Sokol, H., et al., *Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients.* Proc Natl Acad Sci U S A, 2008. **105**(43): p. 16731-6.

30. Zhang, X., et al., *The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment.* Nat Med, 2015. **21**(8): p. 895-905.
31. Wang, J., et al., *Gut Microbial Dysbiosis Is Associated with Altered Hepatic Functions and Serum Metabolites in Chronic Hepatitis B Patients.* Front Microbiol, 2017. **8**: p. 2222.
32. Tripathi, A., et al., *The gut-liver axis and the intersection with the microbiome.* Nat Rev Gastroenterol Hepatol, 2018. **15**(7): p. 397-411.
33. Zuo, T., et al., *Alterations in Gut Microbiota of Patients With COVID-19 During Time of Hospitalization.* Gastroenterology, 2020. **159**(3): p. 944-955 e8.
34. Garrett, W.S., *Cancer and the microbiota.* Science, 2015. **348**(6230): p. 80-6.
35. Zitvogel, L., et al., *Cancer and the gut microbiota: an unexpected link.* Sci Transl Med, 2015. **7**(271): p. 271ps1.
36. Hatakeyama, M., *Oncogenic mechanisms of the Helicobacter pylori CagA protein.* Nat Rev Cancer, 2004. **4**(9): p. 688-94.
37. Kostic, A.D., et al., *Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment.* Cell Host Microbe, 2013. **14**(2): p. 207-15.
38. Gur, C., et al., *Fusobacterium nucleatum supresses anti-tumor immunity by activating CEACAM1.* Oncoimmunology, 2019. **8**(6): p. e1581531.
39. Nougayrede, J.P., et al., *Escherichia coli induces DNA double-strand breaks in eukaryotic cells.* Science, 2006. **313**(5788): p. 848-51.
40. Sivan, A., et al., *Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy.* Science, 2015. **350**(6264): p. 1084-9.
41. Vetizou, M., et al., *Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota.* Science, 2015. **350**(6264): p. 1079-84.
42. Iida, N., et al., *Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment.* Science, 2013. **342**(6161): p. 967-70.
43. Matsuki, T., et al., *Epithelial cell proliferation arrest induced by lactate and acetate from Lactobacillus casei and Bifidobacterium breve.* PLoS One, 2013. **8**(4): p. e63053.
44. Redman, M.G., E.J. Ward, and R.S. Phillips, *The efficacy and safety of probiotics in people with cancer: a systematic review.* Ann Oncol, 2014. **25**(10): p. 1919-1929.
45. Baruch, E.N., et al., *Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients.* Science, 2021. **371**(6529): p. 602-609.
46. Galloway-Pena, J.R., et al., *The role of the gastrointestinal microbiome in infectious complications during induction chemotherapy for acute myeloid leukemia.* Cancer, 2016. **122**(14): p. 2186-96.
47. Oldenburg, M., et al., *The Microbiome in Childhood Acute Lymphoblastic Leukemia.* Cancers (Basel), 2021. **13**(19).
48. Faitova, T., et al., *The gut microbiome in patients with chronic lymphocytic leukemia.* Haematologica, 2022. **107**(9): p. 2238-2243.
49. Yu, D., et al., *Profiling of gut microbial dysbiosis in adults with myeloid leukemia.* FEBS Open Bio, 2021. **11**(7): p. 2050-2059.
50. Lyko, M., J. Maj, and A. Jankowska-Konsur, *The Role of the Gut Microbiome in Non-Hodgkin Lymphoma (NHL): A Focus on Diffuse Large B-Cell Lymphoma, Follicular Lymphoma, Cutaneous T-Cell Lymphoma, and NK/T-Cell Lymphoma.* Cancers (Basel), 2025. **17**(10).
51. Wotherspoon, A.C., C. Ortiz-Hidalgo, M.R. Falzon, and P.G. Isaacson, *Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma.* Lancet, 1991. **338**(8776): p. 1175-6.

52. Tanaka, M. and J. Nakayama, *Development of the gut microbiota in infancy and its impact on health in later life.* Allergol Int, 2017. **66**(4): p. 515-522.
53. Gavriilaki, E., et al., *Alteration of Gut Microbiota Composition and Diversity in Acute and/or Chronic Graft-versus-Host Disease Following Hematopoietic Stem Cell Transplantation: A Prospective Cohort Study.* Int J Mol Sci, 2024. **25**(11).
54. Carabotti, M., A. Scirocco, M.A. Maselli, and C. Severi, *The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems.* Ann Gastroenterol, 2015. **28**(2): p. 203-209.
55. Diaz Heijtz, R., et al., *Normal gut microbiota modulates brain development and behavior.* Proc Natl Acad Sci U S A, 2011. **108**(7): p. 3047-52.
56. Sharon, G., T.R. Sampson, D.H. Geschwind, and S.K. Mazmanian, *The Central Nervous System and the Gut Microbiome.* Cell, 2016. **167**(4): p. 915-932.
57. Scheperjans, F., et al., *Gut microbiota are related to Parkinson's disease and clinical phenotype.* Mov Disord, 2015. **30**(3): p. 350-8.
58. Sampson, T.R., et al., *Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease.* Cell, 2016. **167**(6): p. 1469-1480 e12.
59. Erny, D., et al., *Host microbiota constantly control maturation and function of microglia in the CNS.* Nat Neurosci, 2015. **18**(7): p. 965-77.
60. Vogt, N.M., et al., *Gut microbiome alterations in Alzheimer's disease.* Sci Rep, 2017. **7**(1): p. 13537.
61. Harach, T., et al., *Reduction of Abeta amyloid pathology in APPPS1 transgenic mice in the absence of gut microbiota.* Sci Rep, 2017. **7**: p. 41802.
62. Braniste, V., et al., *The gut microbiota influences blood-brain barrier permeability in mice.* Sci Transl Med, 2014. **6**(263): p. 263ra158.
63. Berer, K., et al., *Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination.* Nature, 2011. **479**(7374): p. 538-41.
64. Jangi, S., et al., *Alterations of the human gut microbiome in multiple sclerosis.* Nat Commun, 2016. **7**: p. 12015.
65. Li, Z., et al., *Gut microbiota and autism spectrum disorders: a bidirectional Mendelian randomization study.* Front Cell Infect Microbiol, 2023. **13**: p. 1267721.
66. Kang, D.W., et al., *Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study.* Microbiome, 2017. **5**(1): p. 10.
67. Kang, D.W., et al., *Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota.* Sci Rep, 2019. **9**(1): p. 5821.
68. Pluta, R., S. Januszewski, and S.J. Czuczwar, *The Role of Gut Microbiota in an Ischemic Stroke.* Int J Mol Sci, 2021. **22**(2).
69. Tang, W.H., et al., *Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk.* N Engl J Med, 2013. **368**(17): p. 1575-84.
70. Olson, C.A., et al., *The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet.* Cell, 2018. **174**(2): p. 497.
71. Rowin, J., Y. Xia, B. Jung, and J. Sun, *Gut inflammation and dysbiosis in human motor neuron disease.* Physiol Rep, 2017. **5**(18).
72. Rajkumar, S.V., *Updated Diagnostic Criteria and Staging System for Multiple Myeloma.* Am Soc Clin Oncol Educ Book, 2016. **35**: p. e418-23.
73. Jian, X., et al., *Alterations of gut microbiome accelerate multiple myeloma progression by increasing the relative abundances of nitrogen-recycling bacteria.* Microbiome, 2020. **8**(1): p. 74.

74. Furusawa, Y., et al., *Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells.* Nature, 2013. **504**(7480): p. 446-50.
75. Prabhala, R.H., et al., *Elevated IL-17 produced by TH17 cells promotes myeloma cell growth and inhibits immune function in multiple myeloma.* Blood, 2010. **115**(26): p. 5385-92.
76. Calcinotto, A., et al., *Microbiota-driven interleukin-17-producing cells and eosinophils synergize to accelerate multiple myeloma progression.* Nat Commun, 2018. **9**(1): p. 4832.
77. Zhang, C.Y., et al., *Causal associations between the gut microbiota and multiple myeloma: a two-sample Mendelian randomization study.* Front Nutr, 2024. **11**: p. 1400116.
78. Pianko, M.J., et al., *Minimal residual disease negativity in multiple myeloma is associated with intestinal microbiota composition.* Blood Adv, 2019. **3**(13): p. 2040-2044.

11. Oświadczenia współautorów

Dr n. med. i n. o zdr. Martyna Maciejewska

Warszawa, 18.08.2025

OŚWIADCZENIE

Jako współautor pracy pt. „Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma. Scientific Reports. 2024 Dec 28;14(1):31221." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie danych do analiz, krytyczna rewizja manuskryptu. Mój udział procentowy w przygotowanie publikacji określam jako 10%.

Wkład Marcina Jasińskiego określam jako 60%, obejmował on przygotowanie koncepcji i metodyki, przegląd literatury oraz przygotowanie manuskryptu.

Jako współautor pracy pt. „Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation. Scientific reports. 2021 Nov 18;11(1):22507." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie danych do analiz, krytyczna rewizja manuskryptu. Mój udział procentowy w przygotowanie publikacji określam jako 5%.

Wkład Marcina Jasińskiego określam jako 70%, obejmował on przygotowanie danych do analiz, analizę wyników i ich interpretację, analizę statystyczną, napisanie manuskryptu.

Wyrażam zgodę na wykorzystanie wyżej wymienionych prac jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

Lek. Michał Górka

Warszawa, 18.08.2025

OŚWIADCZENIE

Jako współautor pracy pt. „Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation. Scientific reports. 2021 Nov 18;11(1):22507." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie danych do analiz, krytyczna rewizja i ostateczne poprawki pracy. Mój udział procentowy w przygotowanie publikacji określam jako 2%.

Wkład Marcina Jasińskiego określam jako 70%, obejmował on przygotowanie danych do analiz, analizę wyników i ich interpretację, analizę statystyczną, napisanie manuskryptu.

Wyrażam zgodę na wykorzystanie pracy jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

Lekarz Michał Górka
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Dr n. med. Agnieszka Tomaszewska

Warszawa, 18.08.2025

OŚWIADCZENIE

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dr n. med. Agnieszka Tomaszewska
Specjalista chorób wewnętrznych
hematologii, immunologii
i transplantologii klinicznej
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Prof. dr hab. med. Grzegorz Władysław Basak Warszawa, 18.08.2025

OŚWIADCZENIE

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Wkład Marcina Jasińskiego określam jako 60%, obejmował on przygotowanie koncepcji i metodyki, przegląd literatury oraz przygotowanie manuskryptu.

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Wkład Marcina Jasińskiego określam jako 75%, obejmował on analizę danych dostępnych w literaturze, napisanie manuskryptu.

Jako współautor pracy pt. „The role of the gut microbiome in pathogenesis, biology, and treatment of plasma cell dyscrasias. Frontiers in oncology. 2021 Oct 1;11:741376." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: analiza danych dostępnych w literaturze, napisanie manuskryptu, krytyczna rewizja i ostateczne poprawki pracy. Mój udział procentowy w przygotowanie publikacji określam jako 10%.

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Wyrażam zgodę na wykorzystanie wyżej wymienionych prac jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

prof. dr hab. n. med. Grzegorz W. Basak
Specjalista chorób wewnętrznych
hematologii i transplantologii klinicznej
4887664

Prof. dr hab. med. Wiesław Wiktor Jędrzejczak

Warszawa, 18.08.2025

OŚWIADCZENIE

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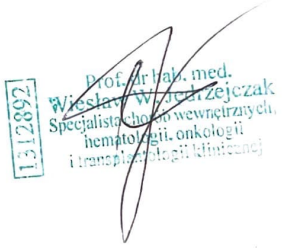
Wyrażam zgodę na wykorzystanie pracy jako części rozprawy doktorskiej lek. Marcina Jasińskiego.



Lek. Kamila Skwierawska

Warszawa, 18.08.2025

OŚWIADCZENIE

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Wyrażam zgodę na wykorzystanie pracy jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

Kamila Skwierawska

Prof. dr hab. n. med. Emilian Snarski

Warszawa, 18.08.2025

OŚWIADCZENIE

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Wkład Marcina Jasińskiego określam jako 60%, obejmował on przygotowanie koncepcji i metodyki, przegląd literatury oraz przygotowanie manuskryptu.

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Wkład Marcina Jasińskiego określam jako 70%, obejmował on przygotowanie danych do analiz, analizę wyników i ich interpretację, analizę statystyczną, napisanie manuskryptu.

Wyrażam zgodę na wykorzystanie wyżej wymienionych prac jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

EMILIAN SNARSKI

Dr hab. med. Jarosław Biliński Warszawa, 18.08.2025

OŚWIADCZENIE

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Wkład Marcina Jasińskiego określam jako 60%, obejmował on przygotowanie koncepcji i metodyki, przegląd literatury oraz przygotowanie manuskryptu.

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Wkład Marcina Jasińskiego określam jako 75%, obejmował on analizę danych dostępnych w literaturze, napisanie manuskryptu.

Jako współautor pracy pt. „The role of the gut microbiome in pathogenesis, biology, and treatment of plasma cell dyscrasias. Frontiers in oncology. 2021 Oct 1;11:741376." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: analiza danych dostępnych w literaturze, napisanie manuskryptu, krytyczna rewizja i ostateczne poprawki pracy. Mój udział procentowy w przygotowanie publikacji określam jako 20%.

Wkład Marcina Jasińskiego określam jako 70%, obejmował on analizę danych dostępnych w literaturze, napisanie manuskryptu.

Wyrażam zgodę na wykorzystanie wyżej wymienionych prac jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

Mgr Karolina Ostrowska

Warszawa, 18.08.2025

OŚWIADCZENIE

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Wkład Marcina Jasińskiego określam jako 60%, obejmował on przygotowanie koncepcji i metodyki, przegląd literatury oraz przygotowanie manuskryptu.

Wyrażam zgodę na wykorzystanie pracy jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

Karolina Ostrowska

Dr n. med. Wojciech Konarski

Warszawa, 18.08.2025

OŚWIADCZENIE

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Wyrażam zgodę na wykorzystanie pracy jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

Wojciech Konarski

Lek. Anna Brodziak Warszawa, 18.08.2025

OŚWIADCZENIE

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Wyrażam zgodę na wykorzystanie pracy jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

Dr hab. n. med. Edyta Podsiadły

Warszawa, 18.08.2025

OŚWIADCZENIE

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Edyta Podsiadły

Dr n. med. Patrycja Rusicka - Krzewska Warszawa, 18.08.2025

OŚWIADCZENIE

Jako współautor pracy pt. „Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma. Scientific Reports. 2024 Dec 28;14(1):31221.” oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie danych do analiz. Mój udział procentowy w przygotowanie publikacji określam jako 2%.

Wkład Marcina Jasińskiego określam jako 60%, obejmował on przygotowanie koncepcji i metodyki, przegląd literatury oraz przygotowanie manuskryptu.

Wyrażam zgodę na wykorzystanie pracy jako części rozprawy doktorskiej lek. Marcina Jasińskiego.

Lek. Marcin Jasiński Warszawa, 18.08.2025

OŚWIADCZENIE

Jako współautor pracy pt. „Impact of gut colonization by antibiotic-resistant bacteria on the outcomes of autologous stem cell transplantation in multiple myeloma. Scientific Reports. 2024 Dec 28;14(1):31221." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie danych do analiz, analiza wyników i ich interpretacja, analiza statystyczna, napisanie manuskryptu.

Mój udział procentowy w przygotowanie publikacji określam jako 60%.

Jako współautor pracy pt. „Ice-cream used as cryotherapy during high-dose melphalan conditioning reduces oral mucositis after autologous hematopoietic stem cell transplantation. Scientific reports. 2021 Nov 18;11(1):22507." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie danych do analiz, analiza wyników i ich interpretacja, analiza statystyczna, napisanie manuskryptu.

Mój udział procentowy w przygotowanie publikacji określam jako 70%.

Jako współautor pracy pt. „The role of the crosstalk between gut microbiota and immune cells in the pathogenesis and treatment of multiple myeloma. Frontiers in Immunology. 2022 Mar 31;13:853540." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: analiza danych dostępnych w literaturze, napisanie manuskryptu.

Mój udział procentowy w przygotowanie publikacji określam jako 75%.

Jako współautor pracy pt. „The role of the gut microbiome in pathogenesis, biology, and treatment of plasma cell dyscrasias. Frontiers in oncology. 2021 Oct 1;11:741376." oświadczam, że mój wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: analiza danych dostępnych w literaturze, napisanie manuskryptu.

Mój udział procentowy w przygotowanie publikacji określam jako 70%.

Marcin Jasiński