CLL

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CLL is a hematological malignancy considered as the most frequent lymphoproliferative disease in the western world. It is characterized by high molecular heterogeneity and despite the available therapeutic options, there are many patient subgroups showing the insufficient effectiveness of disease treatment.

Keywords: CLL ; proteomics ; drug repurposing ; precision medicine ; malignancy

1. Introduction

1.1. Currently Known Pathophysiology, Molecular Diagnosis and Treatment Strategies in CLL

CLL is the most frequent lymphoproliferative disease in the western world [1][2] characterized by the clonal proliferation and progressive accumulation of mature, typically CD5-positive B-cells in the blood, bone marrow, and secondary lymphoid tissues [2][3][4]. It shows a high biological, genetical, molecular and clinical diversity [1][5], projecting its highly heterogenous pathophysiology (**Figure 1**). Among the known features with clinical relevance in the pathobiology of CLL are the highly genetic mutations acting either independently or in combination with chromosomal rearrangements [1][5]. Driver mutations have been associated with adverse clinical outcomes, and thus serve as biomarkers, indicators of therapeutic options or as potential therapeutic targets [2][4][5][6][7][8]. Somatic mutations in immunoglobulin heavy chain variable region gene (IGHV), activating B cell receptor (BCR)-signaling kinases lead to the lower survival and proliferation of CLL cells, providing patients with “mutated” M-CLL, which is a better clinical outcome vs. “unmutated” U-CLL patients [2][9]. It is important to mention that the signaling of UM-CLL is generally highly responsive to the antigenic stimulus, while M-CLL are anergic. Continual or repetitive BCR signaling adds further complexity in CLL pathogenesis, contributing to autophagy regulation, promoting tumor survival, proliferation, and consequently tumor progression [10]. Complex karyotype (CK), defined by the presence of at least three genetic abnormalities in the same clone, is detectable in 14–34% of CLL cases and it is recommended as a new negative prognostic biomarker associated with an adverse outcome and worse response to chemoimmunotherapy [11][12][13][14][15]. Other intriguing features of vital significance in the growth, survival, and drug resistance of CLL cells are metabolic plasticity and signals from the lymphoid tissue microenvironment (LTME) [2][4]. Metabolic plasticity involves the main metabolic pathways of mitochondrial biogenesis and bioenergetics, ROS production, and adaptation to intrinsic oxidative stress, found to be elevated in CLL [16]. LTME produces various essential proteins and metabolites [3][17] modulating the redox and metabolic state of CLL cells [18] and switching either to oxidative phosphorylation (OXPHOS) or glycolysis [19]. Furthermore, enhanced BCR signaling induces the metabolic activation of CLL cells through OXPHOS, energetically supporting the transcription and translation processes [20].

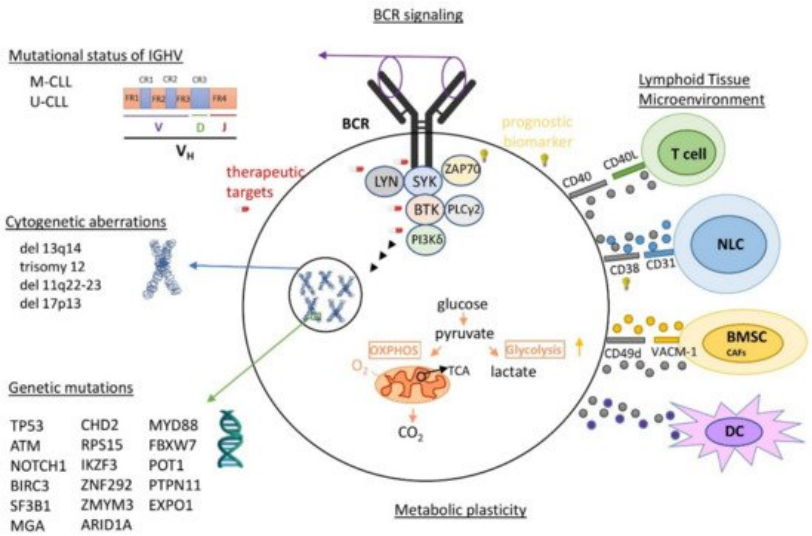

Figure 1. Currently known heterogeneity in the pathophysiology of CLL. Some of the most important elements of CLL pathophysiology are: (1) highly varying genomic mutations, (2) loss or addition of large amounts of chromosomal material, (3) mutational status of variable region of IGHV, (4) frequent activation of BCR signaling and (5) continuous proliferating signals from the cancerous microenvironment. IGHV: immunoglobulin heavy chain variable region genes; M-CLL: mutated CLL; U-CLL: unmutated CLL; BCR: B cell receptor; NLC: nurse-like cells; BMSC: bone marrow stromal cells; DC: dendritic cells; OXPHOS: oxidative phosphorylation; TCA: citric acid cycle.

The molecular diagnostic criteria in CLL guidelines and beyond traditional Rai or Binet staging [21] include (i) the co-expression of CD5 with the B-cell antigens CD19 and CD20, (ii) characteristically lower levels of surface immunoglobulin, CD20, and CD79b (vs. normal B cells), (iii) the expression of kappa or lambda immunoglobulin (Ig) light chains [3][22] and (iv) the identification of specific gene mutations and serum markers [2][3][22][23]. Additionally, the CLL International Prognostic Index (CLL-IPI) proposes a weighted grading of five parameters: (i) *TP53* dysfunction, (ii) mutational status of IGHV, (iii) serum level of β2-microglobulin, (iv) clinical stage, and (v) age [3]. Furthermore, an increasing number of studies are supporting the use of new biomarkers for the diagnosis, prognosis of clinical course and therapeutic decision, such as newly approved driver genes [5][24][25][26][27][28][29][30] serum micro-RNAs [31], etc. Interestingly, assessment of the minimal residual disease (MRD), referring to the small numbers of CLL cells that remain in patients in remission during or after treatment, is an emerging prognostic biomarker of progression-free and overall survival [23][32].

A plethora of pharmacological targets have been investigated in CLL. Patients, according to their clinical history, are prioritized to therapeutic options, including chemotherapy, immunotherapy (IT), chimeric antigen receptor and other targeted therapeutic strategies, used alone or in combination. More specifically, chemotherapeutic agents are still used in many cases as a first-line treatment. [2][3]. In chemo-immunotherapy (CIT), mAbs bind in the surface antigens of CLL cells, resulting in apoptosis, complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC). Different combinations of several agents have been reported and evaluated in several publications [2][3][22][33][34]. Inhibitors targeting the aberrantly regulated components of apoptosis, and of BCR signaling in CLL [2][3][22][33][35][36], have started to replace CIT, in first- and second-line indications [3][36] and many other new generations or under investigation agents [36][37][38][39]. The therapy using CAR-T cells represents a recent therapeutic option for some CLL patients [40][41]. Finally, among the promising CLL therapies under investigation targeting several deregulated pathways are the cross-talk between CLL cells, the tumor microenvironment [42][43], the Wnt signaling pathway [44], various miRNAs [45], the Notch2 signaling pathway [46], the mitochondrial metabolism [16] and the epigenetic modifications [47].

1.2. The Knowledge Gap in the Fight against CLL

There is still a translational gap between basic knowledge and clinical application in CLL. Despite current therapeutic strategies and improvements, there are an increasing number of deaths in accordance with the increasing incidence rates and the second primary malignancies (SPMs) [48][49][50][51][52][53]. Unknown genetic risk factors related to specific SMPs in patients, individual complex karyotypes, genetic mutations, altered signaling pathways, individual tumor microenvironments, recurrent expanded or diminished genetic alterations and “ad hoc” therapies, and drugs combinations without restrictive guideline based on characteristic biomarkers, are among some of the reasons [3][5][8][36][54]. The consequences are inadequate drug response, MRD and drug resistance [5][32][36][54]. To deal with the missing information regarding the molecular etiology, complexity, and heterogeneity of the disease traits, it is important to decode in detail the different molecular elements and their intricate interplay driving CLL phenotypes, to allow the selection of more effective and safe treatment options, as well as long-term remissions.

1.3. Proteomics and Drug Repurposing in the Fight against CLL

The springboard to a more precise and holistic molecular perspective of the pathobiology of CLL patients is through the contribution of omics and systems biology approaches that enable improved early and accurate diagnosis, prognosis, and therapeutic insights. The identification and validation of more specific signatures and drug targets elucidating the underlying mechanism of action, as well as the application of an individualized, well-tolerated, and safe therapeutic protocol, could ensure the long-term, good-quality survival of CLL patients [5][8]. Exploiting omics results, or high quality and well-documented omics data available in public repositories can be used for a comprehensive biological insight in CLL pathobiology. The meta- and re-analysis of such omics data can unravel characteristic differences responsible for the deregulation of important molecular networks and pathways in CLL. It is imperative that these differences are scrupulously investigated for their unique essentiality in different CLL phenotypes, categorizing patients into further subgroups, and identifying specific druggable targets for the selection of a more precise treatment. Furthermore, since the rate of FDA approvals is constantly decreasing and many resources and time are needed for conventional drug development, the combination of omics data with in silico and experimental drug repurposing approaches can be used for the repositioning

of FDA-approved drugs against druggable protein targets in CLL. All this information could be further integrated with other available data (clinical, pharmacovigilance, basic research) to prove the biological/clinical significance, as well as the rational existence of these findings. This review emphasizes both proteomics and drug repurposing approaches. Proteomics provide essential multi-level information on the structure and function of the whole proteome under specific conditions, which is closer to the actual phenotype of the biological system. Different state-of-the art proteomics approaches can unravel the complex and heterogeneous CLL molecular phenotype, providing new insights on the mechanisms of its initiation and progression, the identification of protein biomarkers and putative drug targets for drug repurposing for more effective therapeutic options. On the other hand, drug repurposing approaches are promising faster and more precise novel pharmaceutical strategies in comparison to traditional drug discovery approaches that could enhance the drug arsenal in CLL treatment.

2. Application of State-of-the-Art Mass Spectrometry-Based Proteomics in CLL Studies

2.1. The Powerful MS-Based Proteomics

Extensive research studies to characterize human molecular physiology in health and diseases have mostly focused on genomics, epigenomics and transcriptomics-based analyses, providing a prediction of a given cellular condition, overlooking proteins, the main effectors of cell phenotype and progression. The proteome is highly dynamic, fluctuating both spatially and temporally, mainly due to various endogenous and exogenous signaling events that regulate gene expression, protein maturation, structure, function and other mechanisms, including alternative splicing or/and post translation modifications (PTMs), that enhance proteome diversity and dynamics, producing, by far, a larger number of proteoforms than the predicted number of genes in a cell. Proteomics enables the large-scale characterization of the complete proteome of a cell, tissue, biological fluid, or organism, employing mainly state-of-the-art mass spectrometry (MS)-based and bioinformatics approaches. Thus, proteomics represents the best approach to assess major aspects of cellular biology in health and disease. Advances in the field allow approaches for the global or targeted comparative proteome and phosphoproteome profiling, the accurate detection of PTMs, and the analysis of protein interactions under a specific, well-defined set of conditions of interest [55]. Technological advances in the field, nowadays, allow the assessment of the whole proteome of complex eukaryotic cells in one experiment in a few hours. Methodological innovations allow multiplexing by enabling the simultaneous analysis of multiple samples in a single run, greatly improving the analytical power of the method. These advancements have made proteomics one of the most rapidly developing fields of cell and molecular biology [56][57][58]. The study of the proteome represents an invaluable piece of information for understanding complex features and mechanisms of the pathogenesis of diseases, including cancer. As the proteome reflects the physical condition of a patient at a specific time point, the proteomic data may enable better decisions on how to treat such a patient. Hence, proteomics represents a fundamental method enabling precision medicine for all patients worldwide [59].

2.2. Revelation of CLL through Proteomics

CLL pathogenesis is an outcome of both genetic predisposition and environmental impact, which generates extreme heterogeneity in disease behavior and clinical outcomes [55] that is reflected in the proteome of the patients. Traditionally, proteins involved in the progression of the CLL have mostly been studied using conventional biochemical approaches, focusing on one study of a single protein or a small group of proteins [60] providing significant mechanistic details and correlations, but failing to address the system-wide molecular and biochemical complexity of CLL. Nowadays, proteomics approaches have enabled the high-throughput investigation of the significantly altered abundance of proteins, their modifications, their topology, their function, structure, and interactions in CLL, offering valuable information on the disease regulation and progression, connecting the missing links of the available information. The next paragraphs review all the currently available proteomics studies in CLL and address how this data have been employed to understand the complex molecular mechanisms involved in CLL and identify novel therapeutic targets and biomarkers for diagnosis and prognosis.

3. Drug Repurposing in CLL

3.1. Drug Repurposing in Hematological Malignancies—The Performance of CLL

Drug repurposing is a strategy of identifying new therapeutic uses for pre-existing, FDA-approved or investigational drugs, that are outside the aim of the original medical indication. This approach is based either on the fact that different diseases may have similar molecular signatures and druggable targets, or that off-target drug effects may be useful for the treatment of other diseases (polypharmacology) [61][62][63]. In comparison to conventional drug discovery, the main

advantages of this process are that it requires almost half the years and one third of the money needed in the first method [64], while the safety and toxicity profile of repurposed drugs is completely established.

Even increasing data from multiple experimental studies and clinical observations have depicted that different non-neoplastic drugs have potential anticancer activity, including cardiovascular drugs, antipsychotics, antidepressants, microbiological agents, anti-viral drugs, antibiotics, non-steroidal anti-inflammatory drugs, antidiabetic, anti-emetic drugs, etc. [65][66][67][68][69][70]. There are many repurposed drugs that are already used in hematological malignancies. The discovery of these drugs is based on (i) clinical trials results, (ii) random observations, (iii) the biological background of a disease, or (iv) in vitro and (v) in silico high-throughput screenings [65]. There are also many studies investigating drug repurposing in different subtypes of hematological malignancies [71][72][73][74][75][76][77][78][79]. Concerning CLL, 2816 compounds were studied in vitro and 102 of them influenced the lymphocytes of all six CLL patients tested. Only five of them (auranofin, azacytidine, dimercaprol, podofilox, plicamycin) had no simultaneous effect on the respective cells of the five healthy volunteers, used as the control group [80]. Additionally, there are studies that support the repositioning of nelfinavir and chloroquine as a combinatorial therapy, as well as FDA-approved allergy medications (e.g., clemastine) with ibrutinib and roflumilast with idelalisib. These drug combinations showed great anti-cancer properties in CLL [81][82][83]. In **Table 1**, all the repurposed drugs in CLL derived either from proteomics studies or/and other studies are summarized.

Table 1. Repurposed drugs in CLL. Overview of already proposed repurposed drugs in CLL, showing the possible molecular targets (targeted proteins); the quality of scientific evidence to assess the drug repurposing evidence level (status of evidence); and the publication referring each repurposed drug (publication). The proteomics-based repurposed drugs are indicated in bold. CT: clinical trial; PhI/II: phase I/II.

Repurposed Drugs	Targeted Proteins	Status of Evidence	Publication
acitretin	**RXRA**	**in silico + experimental**	[56]
alitretinoin	**RXRA**	**in silico**	[56]
aplidine	**MAPK8**	**in silico**	[56]
arsenic trioxide	**PML**	**in silico + CT PhI/II**	[56]
auranofin	**IL-1β, TNF, IL-6, thioredoxin reductase**	**experimental + CT PhI/II**	[80]
azacytidine	**DNA methyltransferases, DNMT1**	**in silico + experimental + CT PhI/II**	[56][80]
belimumab	**BAFF**	**experimental**	[80]
belinostat	**HDAC8, HDAC3**	**in silico**	[56]
benoxaprofen	**ALOX6**	**in silico**	[56]
bexarotene	**RXRA**	**in silico**	[56]
chloroquine	**autophagy-related proteins**	**experimental**	[83]
cladribine	**RRM2B**	**in silico + experimental + CT PhI/II**	[56]
clemastine	**sphingosine**	**experimental**	[82]
dasatinib	**LCK**	**in silico + experimental + CT PhI/II**	[56]
decitabine	**DNMT1**	**in silico**	[56]
diclofenac	**ALOX7**	**in silico**	[56]
dimercaprol	**N/A**	**experimental**	[81]
elomotecan	**TOP1**	**in silico**	[56]
elsamitrucin	**TOP1**	**in silico + CT PhII**	[56]
estramustin	**MAP2**	**in silico**	[56]
etretinate	**RXRA**	**in silico**	[56]
gossypol	**BCL2**	**in silico + experimental + CT PhI/II**	[56]
hydroxyurea	**RRM2B**	**in silico**	[56]
MK 1775	**WEE1**	**in silico**	[56]

Repurposed Drugs	Targeted Proteins	Status of Evidence	Publication
nelfinavir	HIV protease	experimental	[83]
nintedanib	LCK	in silico	[56]
N-methyl-4-Ile-cyclosporin	PPIA	in silico	[56]
oblimersen	BCL2	in silico + PhI/II	[56]
paclitaxel	BCL2	in silico	[56]
pazopanib	LCK	in silico + experimental	[56]
pentosan polysulfate	FGF2	in silico	[56]
plicamycin	NA	experimental	[80]
podofilox	DNA topoisomerase II	experimental	[80]
pyroxamide	HDAC9, HDAC3	in silico	[56]
rasagiline	BCL2	in silico	[56]
roflumilast	PDE4	experimental	[81]
simvastatin	HMGCR, LFA-1	in silico + experimental + CT PhI	[84]
sucralfate	FGF4	in silico	[56]
suradista	FGF3	in silico	[56]
T 0128	TOP1	in silico	[56]
TA 270	ALOX5	in silico	[56]
talmapimod	MAPK13	in silico	[56]
tin mesoporphyrin	HMOX1/2	in silico	[56]
tretinoin	RXRA	in silico + CT PhI	[56]
triapine	RRM2B	in silico	[56]
tributyrin	HDAC7	in silico	[56]
tributyrin	HDAC3	in silico	[56]
valproic acid	ALDH5A1	in silico + experimental + CT PhI/II	[56]
vemurafenib	FGR	in silico	[56]

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