Efficacy of Front-Line Ibrutinib and Rituximab Combination and the Impact of Treatment Discontinuation in Unfit Patients with Chronic Lymphocytic Leukemia: Results of the Gimema LLC1114 Study

Francesca Romana Mauro 1,*, Francesca Paoloni 2, Stefano Molica 3, Gianluigi Reda 4, Livio Trentin 5, Paolo Sportoletti 6, Monia Marchetti 7, Daniela Pietrasanta 8, Roberto Marasca 9, Gianluca Gaidano 10, Marta Coscia 11, Caterina Stelitano 12, Donato Mannina 13, Nicola Di Renzo 14, Fiorella Ilariucci 15, Anna Marina Liberati 16, Lorella Orszucci 17, Francesca Re 18, Monica Tani 19, Gerardo Musuraca 20, Daniela Gottardi 21, Pier Luigi Zinzani 22, Alessandro Gozzetti 23, Annalisa Chiarenza 24, Anna Guarini 1, Gian Matteo Rigolin 31, Alfonso Piccioci 2, Antonio Cuneo 31 and Robin Foà 1

Citation: Mauro, F.R.; Paoloni, F.; Molica, S.; Reda, G.; Trentin, L.; Sportoletti, P.; Marchetti, M.; Pietrasanta, D.; Marasca, R.; Gaidano, G.; et al. Efficacy of Front-Line Ibrutinib and Rituximab Combination and the Impact of Treatment Discontinuation in Unfit Patients with Chronic Lymphocytic Leukemia: Results of the Gimema LLC1114 Study. Cancers 2022, 14, 207. https://doi.org/10.3390/cancers1410207

Academic Editor: Davide Mahadevan

Received: 10 December 2021 | Accepted: 28 December 2021 | Published: 31 December 2021

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

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1 Hematology, Department of Translational and Precision Medicine, Sapienza University, Via Benevento 6, 00161 Rome, Italy; ruocco.1584726@studenti.uniroma1.it (V.R.); delgiudice@bce.uniroma1.it (I.D.G.); depropriis@bce.uniroma1.it (M.S.D.P.); dellastarza@bce.uniroma1.it (I.D.S.); rpanni@bce.uniroma1.it (M.N.); guarini@bce.uniroma1.it (A.G.); rfoa@bce.uniroma1.it (R.F.)
2 GIMEMA Foundation, 00187 Rome, Italy; f.paoloni@gimema.it (F.P.); p.fazi@gimema.it (P.F.); a.piciocchi@gimema.it (A.P.)
3 Department of Hematology, Pugliese Ciaccio Hospital, 88100 Catanzaro, Italy; smolica@libero.it
4 Hematology Department, Foundation IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, University of Milan, 20100 Milan, Italy; gianluigi.reda@policlinico.mi.it (G.R.); antonino.neri@unimi.it (A.N.)
5 Hematology Division, Department of Medicine, University of Padua, 35128 Padua, Italy; livio.trentin@unipd.it
6 Institute of Hematology-Centro di Ricerca Emato-Oncologica (CREO), Department of Medicine, University of Perugia, 06129 Perugia, Italy; paolo.sportoletti@unipg.it
7 Department of Hematology, SS. Antonio e Biagio e Cesare Arrigo Hospital and University of Eastern Piedmont, 15121 Alessandria, Italy; moniamarchettitamellini@gmail.com
8 Hematology Department, Azienda Ospedaliera SS Arrigo e Biagio e Cesare Arrigo, 15121 Alessandria, Italy; dptesrasanta@ospedale.al.it
9 Hematology Unit, Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, 41126 Modena, Italy; roberto.marasca@unimore.it
10 Division of Hematology, Department of Translational Medicine, University of Eastern Piedmont, AOU Maggiore della Carità, 28100 Novara, Italy; gianluca.gaidano@med.unipiem.it
11 Division of Hematology, A.O.U. Città della Salute e della Scienza di Torino and Department of Molecular Biotechnology and Health Sciences, University of Torino, 10100 Torino, Italy; martacoscia@unito.it
12 Division of Hematology, Azienda Ospedaliera Bianchi Melarkin Morelli, 89124 Reggio Calabria, Italy; caterinaselitano27@gmail.com
13 Division of Hematology, Azienda Ospedaliera Papardo, 98158 Messina, Italy; donamannini@gmail.com
14 Hematology and Stem Cell Transplant Unit, “Vito Fazzi” Hospital, 73100 Lecce, Italy; direnzo.ematolecce@gmail.com
15 Hematology, Azienda Ospedaliera Arcispedale Santa Maria Nuova IRCCS, 42123 Reggio Emilia, Italy; fiorella.ilariucci@ausl.re.it
16 Department of Hematology, Università degli Studi di Perugia, A.O.S., 05100 Terni, Italy; marina.liberati@unipg.it
17 Department of Hematology, Azienda Ospedaliera Universitaria Città della Salute e della Scienza di Torino, 10100 Torino, Italy; lorusucci@cittadellasalute.to.it
18 Hematology and Bone Marrow Transplant Center, Azienda Ospedaliera Universitaria di Parma, 43126 Parma, Italy; gerardo.musuraca@irst.emr.it
19 Istituto Scientifico Romagnoli per lo Studio e la Cura dei Tumori-IRST, 47014 Meldola, Italy; moniamarchettitamellini@gmail.com
20 A.O.U. S. Giovanni Battista A.O. Mauriziano-Umberto I, 10128 Torino, Italy; dgottardi@mauriziano.it

Cancers 2022, 14, 207. https://doi.org/10.3390/cancers1410207 https://www.mdpi.com/journal/cancers
Simple Summary: This prospective, multicenter study aimed to investigate the efficacy and safety of a front-line treatment with the ibrutinib and rituximab combination in 146 unfit patients with chronic lymphocytic leukemia (CLL). We observed an OR, CR, and 48-month PFS rates of 87%, 22.6%, and 77%, respectively. Responses with undetectable MRD were observed in 6.2% of all patients and 27% of CR patients. TP53 disruption and B-symptoms revealed a significant and independent impact on PFS. The 48-month cumulative treatment discontinuation rate due to adverse events in this patient population was 29.1%. It was significantly higher in male patients, in patients aged ≥70 years, and in those managed at centers that enrolled less than five patients. In conclusion, the ibrutinib and rituximab combination was an effective front-line treatment for unfit patients with CLL. However, a high rate of treatment discontinuations due to adverse events was observed in this unfit population.

Abstract: The GIMEMA group investigated the efficacy, safety, and rates of discontinuations of the ibrutinib and rituximab regimen in previously untreated and unfit patients with chronic lymphocytic leukemia (CLL). Treatment consisted of ibrutinib, 420 mg daily, and until disease progression, and rituximab (375 mg/sqm, given weekly on week 1–4 of month 1 and day 1 of months 2–6). This study included 146 patients with a median age of 73 years, with IGHV unmutated in 56.9% and TP53 disrupted in 22.2%. The OR, CR, and 48-month PFS rates were 87%, 22.6%, and 77%, respectively. Responses with undetectable MRD were observed in 6.2% of all patients and 27% of CR patients. TP53 disruption (HR 2.47; \(p = 0.03\)) and B-symptoms (HR 2.91; \(p = 0.02\)) showed a significant and independent impact on PFS. The 48-month cumulative rate of treatment discontinuations due to disease progression (DP) or adverse events (AEs) was 5.6% and 29.1%, respectively. AEs leading more frequently to treatment discontinuation were atrial fibrillation in 8% of patients, infections in 8%, and non-skin cancers in 6%. Discontinuation rates due to AEs were higher in male patients (HR: 0.46; \(p = 0.05\)), patients aged ≥70 years (HR 5.43, \(p = 0.0017\)), and were managed at centers that enrolled <5 patients (HR 5.1, \(p = 0.04\)). Patients who discontinued ibrutinib due to an AE showed a 24-month next treatment-free survival rate of 63%. In conclusion, ibrutinib and rituximab combination was an effective front-line treatment with sustained disease control in more than half of unfit patients with CLL. Careful monitoring is recommended to prevent and manage AEs in this patient population.

Keywords: chronic lymphocytic leukemia; treatment; ibrutinib; rituximab; unfit; adverse events

1. Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the adult population. About 21,250 new cases of CLL have been estimated in the United States...
for 2021. CLL mainly affects aged subjects, with an average age at diagnosis of around 70 years [1]. During the last years, relevant advances in the understanding of the biologic mechanisms associated with the proliferation and survival of CLL cells have led to the clinical use of ibrutinib, a small molecule that inhibits the Bruton tyrosine kinase (BTK). From the first studies, ibrutinib has been proven to be highly effective, regardless of age, prior treatment, and high-risk biologic features of the leukemic cell [2,3]. After that, several randomized trials demonstrated the superiority over chemoimmunotherapy of front-line ibrutinib as a single agent, or combined with an anti-CD20 monoclonal antibody [4–7]. The excellent therapeutic activity of this agent has revolutionized the treatment approach of CLL, and today, ibrutinib is a standard of care for CLL patients of all ages, both in the relapsed/refractory and in front-line settings. However, despite the excellent response rates and prolonged responses, treatment discontinuation, mainly due to adverse events (AE), is a relevant problem limiting the effectiveness of this agent [8–10].

Based on the improved outcomes observed with the addition of rituximab to chemotherapy [11–14] and on the efficacy of ibrutinib and rituximab [15], the GIMEMA (Gruppo Italiano Malattie EMatologiche dell’Adulto) group, in 2015, started a prospective, multicenter study to investigate the safety and efficacy of a front-line treatment, consisting of six courses of the ibrutinib and rituximab combination followed by ibrutinib single agent, in unfit patients with CLL. Herein, we report the long-term results of this schedule in 146 unfit patients with CLL, the safety profile of treatment, and the reasons and prognostic impact of treatment discontinuation.

2. Methods

2.1. Patients

Between March 2015 and April 2017, 159 unfit patients with CLL were enrolled in the GIMEMA LLC1114 study, a prospective, phase 2, multicenter, single-arm study. Inclusion criteria included previously untreated CLL requiring treatment according to the International Workshop on CLL (iwCLL) criteria [16]. Patients were defined as unfit in the presence of a Cumulative Illness Rating Scale (CIRS) [17] score >6, and or a creatinine clearance <70 mL/min.

In addition, the absence of Richter transformation, active infection, or secondary malignancy was also required in patients enrolled in the study. The assessment of the biologic profile included fluorescence-in-situ-hybridization (FISH) and the IGHV and TP53 mutation status as previously described [18,19].

2.2. Treatment

Treatment consisted of ibrutinib, 420 mg once daily given continuously, and rituximab, 375 mg/sqm, every week, on day 1 of month 1 and day 1 of months 2–6. Patients received ibrutinib single agent until one of the following events disease progression or severe toxicity, or for a maximum of 6 years.

All patients received Pneumocystis carinii prophylaxis with trimethoprim-sulfamethoxazole.

2.3. Response

The response was assessed according to the iwCLL criteria [16] 2 months after the last administration of rituximab. The response assessment included clinical examination, PB examination, BM aspirate and biopsy, and total body CT scan. In patients who achieved a complete response (CR), a centralized assessment of MRD, in both the PB and BM, was performed by an eight-color flow cytometry assay with a sensitivity of at least $10^{-4}$ according to the internationally standardized European Research Initiative on CLL criteria [20]. MRD was further assessed in PB and BM by allele-specific oligonucleotide polymerase chain reaction (PCR) in patients in complete remission (CR) with undetectable MRD (uMRD) by flow cytometry. The response was monitored every 6 months during the follow-up.
2.4. Study Endpoints

The primary endpoint of the study was progression-free survival (PFS). The secondary endpoints included the overall responses rate (ORR), the CR rate, the rate of CRs with undetectable MRD (uMRD) in the PB and BM, overall survival (OS), and survival outcomes according to the clinical and biological features of the patients. The safety profile and reasons for permanent discontinuation of treatment were also analyzed. AEs were graded according to the Common Terminology Criteria for Adverse Events, version 3 [21].

2.5. Statistical Analysis

Patients' characteristics were summarized using cross-tabulations for categorical variables or using quantiles for continuous variables. In the univariate analysis, non-parametric tests were performed for comparisons between groups (Chi-Squared and Fisher Exact test in the case of categorical variables or response rate; Mann–Whitney and Kruskal–Wallis tests in case of continuous variables). Survival distributions were estimated using the Kaplan–Meier Product Limit estimator. Differences in survival curves were evaluated using the Log-Rank test. Cox regression models were performed in univariate and multivariate analyses to assess the effect of clinical and biologic factors on PFS and OS. Hazard ratios (HR) and a 95% confidence interval were reported as parameter results of the Cox regression models. The multivariate models were all considered relevant.

Curves of the cumulative incidence of treatment discontinuations by specific causes (e.g., adverse events) were estimated using the proper non-parametric method. The Gray test was applied for comparing the curves of cumulative incidence and the Fine and Gray regression model was used in the univariate and multivariate analyses to assess the effects of covariates on the survival outcome in cases of competitive risks.

All analyses were analyzed on an Intention-To-Treat basis. All tests were two-sided, accepting \( p < 0.05 \) as indicating a statistically significant difference. Confidence intervals were calculated at the 95% level. All of the analyses were performed using the SAS software (release 9.4; SAS Campus Drive, Cary, NC 27513, USA) and R system software (R Foundation for Statistical Computing c/o Institute for Statistics and Mathematics, Wirtschaftsuniversität, 1020 Wien, Austria). Details about data collection are reported in Supplementary Figure S1.

2.6. Ethics

This study has been carried out according to the Helsinki Declaration and was approved by the Ethical Committees of all of the participating institutions. All of the participants gave their written informed consent. This study is registered at ClinicalTrials.gov, Identifier: NCT02232386.

3. Results

One hundred and fifty-nine CLL patients were enrolled in this study. The patient disposition is described in Supplementary Figure S1. Thirteen patients were considered not eligible and were excluded from the study before receiving any study drug (not eligible, 8; AE, 2; death, 1; refused treatment, 1; medical decision, 1). One hundred and forty-six patients with a median follow-up of 49.1 months (IQR, 39.4–54) represent the intention-to-treat population assessed for treatment response and safety. The baseline clinical and biological characteristics of patients are summarized in Table 1. Briefly, the median age was 73 years (range 37–88), the median CIKS score 6, the median creatinine clearance 62.7 mL/min, and 37.9% of the patients had an ECOG performance score of 1–2. Unmutated IGHV status was observed in 56.9% of patients and TP53 disruption (del17p and/or TP53 mutation) in 22.2%.
Table 1. Baseline clinical and biologic characteristics of patients.

| Heading                  | N (%) |
|--------------------------|-------|
| No of patients           | 146   |
| Gender                   |       |
| male                     | 88 (60.3) |
| female                   | 58 (39.7) |
| Age median (range)       | 73 (37–88) |
| <70 yrs                  | 47 (32.2) |
| ≥70 yrs                  | 99 (67.8) |
| B symptoms               |       |
| present                  | 26 (18.7) |
| absent                   | 113 (81.3) |
| Binet Stage              |       |
| A–B                      | 92 (63.0) |
| C                        | 54 (37.0) |
| B2M                      |       |
| normal                   | 27 (25.2) |
| increased                | 80 (74.8) |
| LDH                      |       |
| normal                   | 103 (71.0) |
| increased                | 42 (29.0) |
| CIRS median (range)      |       |
| ≥8                       | 6 (8) |
| CrCl, mL/min             | median (range) 62.7 (0.80–152.00) |
| <70 mL/min               | 87 (64.0) |
| ECOG PS                  |       |
| 0                        | 90 (62.1) |
| 1–2                      | 55 (37.9) |
| Patients with IgG levels |       |
| >400 mg/dL               | 24 (17.5) |
| ≤400                      | 113 (82.5) |
| FISH aberrations         |       |
| del13q                   | 42 (29.1) |
| del 11q                  | 21 (14.6) |
| trisomy 12               | 24 (16.7) |
| del 17q                  | 13 (9.0) |
| no aberrations           | 44 (30.6) |
| TP53 disruption a         |       |
| present                  | 32 (22.2) |
| absent                   | 112 (77.8) |
| IGHV                      |       |
| unmutated                | 83 (56.9) |
| mutated                  | 63 (43.1) |

B2M—β2-Microglobulin; LDH—lactate dehydrogenase; CIRS—Cumulative Illness Rating Scale score; CrCl—creatinine clearance; ECOG PS—Eastern Cooperative Oncology Group performance-status; FISH—fluorescence in situ hybridization; IGHV—immunoglobulin heavy-chain variable region gene; a Del17p and/or TP53 mutation.

3.1. Response to Treatment

Patients received a median number of six courses of the ibrutinib and rituximab combination (range 1–6), and 137 (94%) completed the planned six courses of treatment.

Here, 127/146 patients (87%) achieved a response at the end of the ibrutinib and rituximab combination. Responses were confirmed by CT scan and included a complete response (CR/CRi) in 33 (22.6%) patients, a partial response (PR) in 76 (52.1%), and a PR with lymphocytosis (PR-L) in 18 (12.3%).

In an ITT analysis, 9 of the 146 patients (6.2%) obtained a flow-cytometric uMRD at one or more time points, three at the ECOCT, and six during the follow-up. When the analysis was restricted to the 33 patients with CR, the rate of patients with uMRD was 27.3% (9/33). While uMRD was transient in five patients, in four (4/146, 2.7%; 4/33 patients with CR,
12.1%) it persisted for 6, 52, 52, and 54 months. Three of the nine patients with uMRD by flow-cytometry showed no residual disease in the PB also by ASO-PCR at one or more time points.

Ten (6.9%) patients showed stable disease, one progressed (0.6%) while eight (5.5%) discontinued the ibrutinib and rituximab combination (adverse event, 7; second malignancy, 1).

3.2. Survival Analysis

Ten (6.9%) patients developed disease progression (CLL progression, 9; Richter syndrome, 1) with a 48-month PFS of 77% (95% CI 70.2–85.0) (Figure 1A).

In the multivariate analysis, age (≥70 vs. <70 years), CIRS (≥8 vs. <8), Binet stage (C vs. A/B), CrCl, ml/min (≥70 vs. <70), LDH (increased vs. normal), IGHV (unmutated vs. mutated), and del 11q (present vs. absent) did not show a significant impact on PFS, while B-symptoms and TP53 disruption emerged as the only independent factors associated with a significantly shorter PFS (Table 2).

### Table 2. Impact of baseline factors on progression-free survival: univariate and multivariate analysis.

| Variables                      | Univariate Analysis | Multivariate Analysis |
|--------------------------------|---------------------|-----------------------|
|                                | Hazard Ratio (95% CI) | p-Value | Hazard Ratio (95% CI) | p-Value |
| Age ≥70 vs. <70 years           | 2.09 (0.91–4.82)     | 0.08     | 1.34 (0.51–3.54)     | 0.54    |
| CIRS: ≥8 vs. <8                 | 1.34 (0.54–3.31)     | 0.52     | -                     | -       |
| Binet stage: C vs. A/B          | 1.01 (0.50–2.07)     | 0.96     | 1.48 (0.63–3.52)     | 0.37    |
| B symptoms: present vs. absent  | 2.37 (1.10–5.11)     | 0.02     | 2.91 (1.18–7.17)     | 0.02    |
| CrCl, mL/min ≥70 vs. <70        | 0.47 (0.19–1.17)     | 0.10     | 0.52 (0.19–1.49)     | 0.23    |
| LDH increased vs. normal        | 1.46 (0.70–3.04)     | 0.30     | -                     | -       |
| IGHV unmutated vs. mutated      | 1.75 (0.83–3.71)     | 0.13     | 1.54 (0.63–3.78)     | 0.34    |
| Del 11q present vs. absent      | 2.34 (1.03–5.32)     | 0.04     | 2.13 (0.76–5.98)     | 0.15    |
| TP53 disruption present vs. absent | 1.74 (0.82–3.73)  | 0.15     | 2.47 (1.07–5.74)     | 0.03    |

CIRS—Cumulative Illness Rating Scale; CrCl—creatinine clearance; IGHV—immunoglobulin heavy-chain variable region gene.
Twelve patients died, seven because of an adverse event (AE; heart failure, 1; severe infection, 5; liver failure, 1), four due to a second malignancy, and one due to disease progression. The 48-month OS rate was 90% (95% CI 84.7–95.3; Figure 1B). High LDH levels ($p = 0.03$), B symptoms ($p = 0.03$), and TP53 disruption ($p = 0.04$) showed a significant impact on OS in the univariate analysis (Supplementary Table S1). However, none of these factors maintained significance in the multivariate analysis.

3.3. Adverse Events

The type and severity of the AEs recorded in this study are described in Supplementary Table S2. Grade 3–4 granulocytopenia, recorded in 27% of patients, was the most common AE leading to dose reduction or transient treatment interruption. At the last follow-up, the daily dose of ibrutinib received by the 80 patients still on treatment was 420 mg in 62 (77.5%), 280 mg in 14 (17.5%), and 140 mg in 5 (6%). Grade ≥3 infections were diagnosed in 18% of patients, and included lower respiratory tract infections in 8%, with three cases of lethal SARS-CoV-2 pneumonia. Any grade cardiovascular AEs were recorded in 30% of patients and included atrial fibrillation in 16% (grade 3–4 atrial fibrillation, 6%). New-onset hypertension was experienced by 13% of patients. Any grade bleeding disorders were observed in 23% of patients. However, severe bleeding events were uncommon (5%) and included cerebral hemorrhage in three cases. Other AEs frequently reported were any grade myalgias and arthralgias (16%), diarrhea (14%), and skin rash (10%). A non-skin second malignancy was diagnosed in 13 (9%) patients (gastric, 3; lung, 2; bladder, 2; breast, 1; mesothelioma, 1; neuroendocrine, 1; thyroid, 1; bowel, 1; hepatic, 1). No new safety signals or unknown/unwitnessed deaths were recorded.

3.4. Adverse Events Leading to Permanent Treatment Discontinuation

The main reason leading to the permanent discontinuation of treatment was represented by an AE, recorded in 44 (30.1%) patients. Treatment discontinuations rates due to AEs were 17.8% at 12 months, 23.3% at 24 months, 26.0% at 36 months, and 29.1% at 48 months. The median age of patients who discontinued ibrutinib permanently due to an AE was 78 years (range 56.8–90.2).

Cardiovascular disorders were a common AE, leading to treatment discontinuation (11% of the cases, including atrial fibrillation in 8%), followed by infections (8%), non-skin cancers (6%), and cerebral hemorrhage, 3% (Supplementary Table S2). In the multivariate analysis, the male gender was significantly and independently associated with a higher rate of treatment discontinuations due to AEs (HR: 0.46; $p = 0.05$; Supplementary Table S3). Two other factors showed a significant and independent impact on discontinuations caused by AEs, aged older than 70 years (HR: 5.43; $p = 0.002$), and treatment managed at centers that enrolled less than five patients (HR: 0.51, $p = 0.04$). Based on an age older than 70 years and less than five patients enrolled by the referral centers, we identified three groups of patients. In the low-risk group, which included patients with none of the above risk factors, the rate of discontinuations was 11.8%; in the intermediate-risk group that included patients with one of the two risk factors, the rate was 28.3%, while for patients of the high-risk group who showed both risk factors, the rate of discontinuations was 52.5% ($p = 0.001$; Figure 2).
3.5. Prognostic Impact of Treatment Discontinuation

At the time of the last follow-up, 80 (55%) patients, including 31 patients in CR (31/80, 39%; 31/146, 21.2%), were still on ibrutinib, while 66 (45%) discontinued treatment (Supplementary Figure S1). The 48-month cumulative rates of treatment discontinuation due to disease progression, AEs, and second malignancies were 5.6%, 29.1%, and 6%, respectively (Table 3 and Figure 3).
Table 3. Reasons for treatment discontinuation.

| Reason for Treatment Discontinuation | No. Patients | % Patients | 48-Months Cumulative Incidence (95% CI) | Median Age, Years (Range) |
|--------------------------------------|--------------|------------|---------------------------------------|--------------------------|
| Disease progression                   | 10           | 6.8%       | 5.6% (1.5–9.6)                        | 76 (57–85)               |
| Adverse events                        | 44           | 30.1%      | 29.1% (21.5–36.6)                     | 78 (57–90)               |
| Second malignancies                   | 9            | 6.2%       | 6.0% (1.9–10.1)                       | 76 (56–81)               |

The 12-month survival rates of patients who permanently discontinued treatment due to AE, second malignancy, and disease progression were 85% (95% CI: 74.6–96.9), 41.7% (95% CI: 14.7–100.0), and 33% (95% CI: 11.0–98.1), respectively ($p = 0.01$; Figure 4).

Figure 4. Survival probability from treatment discontinuation according to the reason for discontinuations, disease progression, adverse event, and second malignancy.

The 24-month next treatment-free survival rate of patients who discontinued ibrutinib due to AE was 63% (Supplementary Figure S2).

4. Discussion

We investigated the benefit and safety of a front-line treatment with the ibrutinib and rituximab combination in an unfit cohort of CLL patients, defined by a CIRS comorbidity score >6 and/or a reduced renal function. The results of this study confirm the efficacy of this schedule in 146 patients with CLL and with a median age of 73 years. Furthermore, 87% of patients achieved a response, which included a CR in 22.6% of the cases. Moreover, 6.2% of all patients and 27% of CR patients showed a response with uMRD by flow-cytometry. Although the absence of a control arm limits the results of this study, the relatively high CRs and PFS rates we observed, 77% at 48 months, were consistent with those of other trials investigating the efficacy of ibrutinib-based treatments in the front-line setting [4–7,15–23]. The modulation of molecules interacting with the microenvironment produced by the treatment may have favored the fast mobilization of CLL cells [24]. The low rate of disease progressions observed in our study, 10%, further confirmed that the emergence of ibrutinib-resistant subclones is rare in the front-line setting [4–7].
In this study, PFS was not significantly influenced by IGHV mutational status. Moreover, del (11q) or the achievement of CR did not exert the same beneficial impact on PFS described in other studies [25,26]. As observed in the Alliance trial [6], patients with TP53 showed an inferior PFS. That being said, the 48-month PFS of 65% was higher than observed in the past with chemoimmunotherapy in this subset of patients, and is in line with that of other studies with ibrutinib in patients carrying TP53 disruption [27,28]. A significantly lower PFS was associated with the presence of B symptoms. This finding underlines the unfavorable impact of symptomatic disease. In the study by Woyach et al., patients treated with ibrutinib and rituximab showed a PFS similar to those who received single-agent ibrutinib [6]. The PFS value we observed with ibrutinib and rituximab was not superior to that described in other studies with an ibrutinib single agent [4,23]. This observation further questions the benefit of adding rituximab to ibrutinib.

In this patient population, treatment discontinuations due to AE were frequent, with a 48-month cumulative rate of 29.1%. This was not an unexpected finding in patients already older and unfit at baseline. In two trials that included younger patients, the discontinuation rates due to AEs were 19.1% at 4 years and 21% at 5 years, respectively [8,22]. In a retrospective analysis that included 616 patients with CLL, toxicities were also the most common reason for treatment discontinuation [10]. Variable rates of ibrutinib discontinuations have also been reported in real-world studies [29–33]. It is noteworthy that treatment discontinuations rates due to AEs were lower with fixed-duration venetoclax combined with obinutuzumab or rituximab [34,35].

An intriguing finding was the relatively high 12-month survival rate, 85%, and the 24-month next treatment-free survival, 63%, of patients who discontinued treatment due to AE. Similar favorable outcomes have also been described in other studies [7,9,10].

Atrial fibrillation is a well-known AE associated with the use of ibrutinib [30]. The rate of any grade atrial fibrillation was 16%, similar to that of other studies that included older patients treated front-line with ibrutinib [4,6].

Atrial fibrillation was the reason for treatment discontinuation in 8% of patients, a higher rate than previously reported [5,6,22,36]. The characteristics of our patient population may have influenced the discontinuations rates due to atrial fibrillation and also to infections, in 8%. The impact of ibrutinib on cellular immunity has been extensively investigated, with conflicting results. While pre-clinical data described multiple inhibitory effects of ibrutinib on the activity of natural killer cells and macrophages [37,38], recent data suggest that ibrutinib may induce an in vivo immune modulation, with a TH2/TH1 shift in the peripheral blood lymphocytes that is more pronounced in IGHV unmutated and CR patients [39].

A higher rate of discontinuations due to AEs was observed among male patients. To the best of our knowledge, a relationship between sex and treatment discontinuation due to AEs has not been reported in patients treated with ibrutinib. The higher incidence of atrial fibrillation described in males may have had an impact on the increased rate of discontinuations. Older age, over 70 years, and treatment managed at centers that enrolled less than five patients were also associated with an increased rate of discontinuations due to AEs. The presence of both risk factors was associated with a 52.5% discontinuation rate. Older age plays an important role in developing AEs leading to treatment discontinuation. Increasing age is a risk factor for cardiovascular disorders, and the incidence of most cancers also increases with age. Moreover, functions of the immune system decline with age predisposing infections. As previously suggested [40,41], close collaboration with cardio-oncologists and infectious disease specialists should be considered in order to avoid treatment discontinuations due to the toxicities of the targeted agents.

Long-term follow-up data from studies will allow for evaluating whether second-generation BTK inhibitors or a time-limited therapy with venetoclax could be preferable in unfit and older patients.
5. Conclusions

In conclusion, this study shows that the ibrutinib and rituximab combination is an effective front-line treatment with sustained disease control in more than half of unfit and elderly patients with CLL. However, our data highlights the high rate of treatment discontinuations due to AEs and suggests careful monitoring to prevent and manage AEs in this patient population.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/cancers14010207/s1. Figure S1: Patients dispositions. Figure S2: Next treatment-free survival for patients who discontinued ibrutinib due to an adverse event. Table S1: Impact of baseline factors on overall survival: univariate analysis. Table S2: Adverse events. Table S3: Impact of clinical and biologic characteristics of patients on treatment discontinuation due to adverse events: univariate and multivariate analysis.

Author Contributions: F.R.M., F.P., A.P., A.C. (Antonio Cuneo), R.F.: conception, design of the work, analysis, interpretation of data; drafting the work, revising it critically for important intellectual content; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; final approval of the version to be published. S.M., G.R., LT., PS., M.M., D.P., R.M. (Roberto Marasca), G.G., M.C., C.S., D.M., N.D.R., F.I., A.M.L., L.O., F.R., M.T., G.M., D.G., P.L.Z., A.G. (Alessandro Gozzetti), A.M., M.G., A.C. (Annalisa Chiarenza), LL., M.V., A.I., R.M. (Roberta Murru), VR., I.D.G., M.S.D.P., I.D.S., S.R., M.N., P.F., A.N., A.G. (Anna Guaranì), G.M.R.: acquisition and interpretation of data; revising the paper critically for important intellectual content; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have read and agreed to the published version of the manuscript.

Funding: This study was partly supported by Janssen, who provided the study drug and the Associazione Italiana per la Ricerca sul Cancro (AIRC), Metastases Special Program, No 21198, Milan, Italy (RF).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Azienda Ospedaliero Universitaria Policlinico Umberto I, Rome; Italy; protocol code: Rif.3455; date of approval: 27 November 2014.

Informed Consent Statement: Informed written consent was obtained from all subjects involved in the study.

Data Availability Statement: Study data were collected and managed using REDCap (Research Electronic Data Capture; Supplementary Material) a web-based software platform designed to support data capture for research studies. The data presented in this study are available on request. Qualified researchers may request access to anonymized patient data and study documents. Details on sharing criteria and processes for requesting access to data can be required to a.piciocchi@gimema.it.

Acknowledgments: The authors thank the patients and their families and the investigators of the participating centers.

Conflicts of Interest: F.R.M.: research funding from Gilead, advisory board participation fees from AbbVie, Gilead, Janssen, AstraZeneca, Takeda, Roche; speakers bureau fees from Gilead, Janssen, and Abbvie. P.S.: advisory board participation fees from Janssen, Abbvie AstraZeneca. M.M.: consultancy: Gilead; paid speech: Novartis, Amgen; sponsored meetings: Abbvie, Takeda, Pfeizer. R.M. (Roberto Marasca): Janssen e Abbvie: honoraria and travel grant. AstraZeneca: honoraria. G.G.: Abbvie (Advisory Board, Speaker’s Bureau), Astra-Zeneca (Advisory Board), Beigene (Advisory Board), Incyte (Advisory Board), Janssen (Advisory Board, Speaker’s Bureau). M.C.: Janssen honoraria and research funding), Abbvie and AstraZeneca (honoraria). A.M.L.: Research grants from: Takeda, Servier, Roche, Celgene, Abbvie, Incyte, Janssen. Sanofi, Verastem, Novartis, Morphosys, GSK, Oncopeptides, Karyopharm, Onconova, Archigen, Pfizer, Fibrogen, Beigene. Consulting fees from Incyte. Speakers bureau: Takeda, Servier, Celgene, Abbvie, BMS, Janssen. Support for travel: Takeda, Roche, Janssen, BMS. M.T.: advisory board participation fees from Janssen and, Astra Zeneca. G.M.: Janssen: Consulting, Honoraria, Advisory role. Incyte: Consulting, Honoraria, Advisory role. Roche: Consulting, Honoraria, Advisory role. P.L.Z.: Advisory board participation fees from
Speakers bureau: Advisory board and speakers bureau: Verastem, Celltrion, Gilead, Janssen, BMS, Servier, Sandoz, MSD, TG Therap., Takeda, Roche, Eusapharma, Kyowa Kirin, Novartis, ADC therap., Incyte, Beigene. Consultant fees; Verastem, MSD, Eusapharma, Novartis. M.V.: Advisory board participation fees from Janssen, Beigene, Astrazeneca, Roche. R.M. (Roberta Murru): Advisory board participation fees from Janssen and AbbVie. G.M.R.: advisory board participation fees from AbbVie, Gilead, Janssen, Astra-Zeneca and research funding from Gilead. A.C. (Antonio Cuneo): advisory board participation fees from AbbVie, Gilead, Janssen, Astra-Zeneca and research funding from Gilead. No disclosures: D.M.; N.D.R., F.R., A.G. (Alessandro Gozzetti), M.G., L.L., A.I., V.R., I.D.G., M.S.D.P., I.D.S., S.R., M.N., F.P., G.R., L.T., M.M., D.P., C.S., F.I., L.O., D.G., A.M., A.C. (Annalisa Chiarenza), A.P., P.F., A.N., A.G. (Anna Guarini), R.F.

Abbreviations

- **CLL**: Chronic Lymphocytic Leukemia
- **OR**: Overall response rate
- **CR**: Complete Response
- **CRi**: Complete Response with incomplete bone marrow recovery
- **PR**: Partial response
- **PR-L**: Partial response with Lymphocytosis
- **MRD**: Minimal residual disease
- **UMRD**: Undetectable minimal residual disease
- **PFS**: Progression free survival
- **OS**: Overall survival
- **IGHV**: ImmunoGlobulin heavy-chain variable region gene
- **AE**: Adverse event
- **GIMEMA**: Gruppo Italiano Malattie EMatologiche dell’Aduloto
- **B2M**: β2-Mmcroglobulin
- **LDH**: Lactate DeHydrogenase
- **CIRS**: Cumulative Illness Rating Scale score
- **CrCl**: Creatinine clearance
- **ECOG PS**: Eastern Cooperative Oncology Group performance-status
- **FISH**: Fluorescence in situ hybridization
- **TP53**: Tumor protein p53
- **ITT**: Intention to treat
- **EOCT**: End of combination therapy
- **Ig**: Immunoglobulins

References

1. National Cancer Institute, Division of Cancer Control and Population Sciences (DCCP). 2021 Report of the Surveillance, Epidemiology, and End Results (SEER) Program. Available online: https://seer.cancer.gov/statfacts/html/clyl.html (accessed on 9 December 2021).
2. Byrd, J.C.; Furman, R.R.; Coutre, S.E.; Flinn, I.W.; Burger, J.A.; Blum, K.A.; Grant, B.; Sharman, J.P.; Coleman, M.; Wierda, W.G.; et al. Targeting BTK with Ibrutinib in Relapsed Chronic Lymphocytic Leukemia. *N. Engl. J. Med.* 2013, 369, 32–42. [CrossRef]
3. Byrd, J.C.; Brown, J.R.; O’Brien, S.; Barrientos, J.C.; Kay, N.E.; Reddy, N.M.; Coutre, S.; Tam, C.S.; Mulligan, S.P.; Jaeger, U.; et al. Ibrutinib versus Ofatumumab in Previously Treated Chronic Lymphoid Leukemia. *N. Engl. J. Med.* 2014, 371, 213–223. [CrossRef]
4. Burger, J.A.; Tedeschi, A.; Barr, P.M.; Robak, T.; Owen, C.; Ghia, P.; Bairey, O.; Hillmen, P.; Bartlett, N.L.; Li, J.; et al. Ibrutinib as Initial Therapy for Patients with Chronic Lymphocytic Leukemia. *N. Engl. J. Med.* 2015, 373, 425–437. [CrossRef]
5. Moreno, C.; Greil, R.; Demirkan, F.; Tedeschi, A.; Anz, B.; Larratt, L.; Šimković, M.; Samoilova, O.; Novak, J.; Ben-Yehuda, D.; et al. Ibrutinib plus obinutuzumab versus chlorambucil plus obinutuzumab in first-line treatment of chronic lymphocytic leukaemia (iLLUMINATE): A multicentre, randomised, open-label, phase3 trial. *Lancet Oncol.* 2019, 20, 43–56. [CrossRef]
6. Woyach, J.A.; Ruppert, A.S.; Heerema, N.A.; Zhao, W.; Booth, A.M.; Ding, W.; Bartlett, N.; Brander, D.M.; Barr, P.M.; Rogers, K.A.; et al. Ibrutinib regimens versus chemoimmunotherapy in older patients with untreated CLL. *N. Engl. J. Med.* 2018, 379, 2517–2528. [CrossRef]
7. Shanafelt, T.D.; Wang, X.V.; Kay, N.E.; Hanson, C.A.; O’Brien, S.; Barrientos, J.; Jelinek, D.F.; Braggio, E.; Leis, J.F.; Zhang, C.C.; et al. Ibrutinib-rituximab or chemoimmunotherapy for chronic lymphocytic leukemia. *N. Engl. J. Med.* 2019, 381, 432–443. [CrossRef]
8. Maddocks, K.J.; Ruppert, A.S.; Lozanski, G.; Heerema, N.A.; Zhao, W.; Abruzzo, L.V.; Lozanski, A.; Davis, M.; Gordon, A.L.; Smith, L.L.; et al. Etiology of ibrutinib Therapy Discontinuation and Outcomes in Patients With Chronic Lymphocytic Leukemia. *JAMA Oncol.* 2015, 1, 80–87. [CrossRef]

9. Jain, P.; Thompson, P.A.; Keating, M.; Estrov, Z.; Ferrajoli, A.; Jain, N.; Kantarjian, H.; Burger, J.A.; O’Brien, S.; Wierda, W.G. Long-term outcomes for patients with chronic lymphocytic leukemia who discontinue ibrutinib. *Cancer* 2017, 123, 2268–2273. [CrossRef]

10. Mato, A.R.; Nabhan, C.; Thompson, M.C.; Lamanna, N.; Brander, D.M.; Hill, B.; Howlett, C.; Skarbnik, A.; Cheson, B.D.; Zent, C.; et al. Toxicities and outcomes of 616 ibrutinib-treated patients in the United States: A real-world analysis. *Haematologica* 2018, 103, 874–879. [CrossRef]

11. Hallek, M.; Fischer, K.; Fingerle-Rowson, G.; Fink, A.; Busch, R.; Mayer, J.; Hensel, M.; Hopfinger, G.; Hess, G.; von Grünhagen, U.; et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukemia: A randomised, open-label, phase 3 trial. *Lancet* 2010, 376, 1164–1174. [CrossRef]

12. Foa, R.; Del Giudice, I.; Cuneo, A.; DEL Poeta, G.; Ciolli, S.; Di Raimondo, F.; Lauria, F.; Cencini, E.; Rigolin, G.M.; Cortelezzi, A.; et al. Chlorambucil plus rituximab with or without maintenance rituximab as first-line treatment for elderly chronic lymphocytic leukemia patients. *Am. J. Hematol.* 2014, 89, 480–486. [CrossRef]

13. Goede, V.; Fischer, K.; Busch, R.; Engelke, A.; Eichhorst, B.; Wendtner, C.-M.; Chagorova, T.; DE LA Serna, J.; Dilhuydy, M.-S.; Illner, T.; et al. Obinutuzumab plus Chlorambucil in Patients with CLL and Coexisting Conditions. *N. Engl. J. Med.* 2014, 370, 1101–1110. [CrossRef]

14. Eichhorst, B.; Fink, A.-M.; Bahlo, J.; Busch, R.; Kovacs, G.; Maurer, C.; Lange, E.; Köppler, H.; Kiehl, M.; Söklér, M.; et al. First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukemia (CLL10): An international, open-label, randomised, phase 3, non-inferiority trial. *Lancet Oncol.* 2016, 17, 928–942. [CrossRef]

15. Burger, J.A.; Keating, M.J.; Wierda, W.G.; Hartmann, E.; Hoellenriegel, J.; Rosin, N.Y.; de Weerdt, I.; Jeyakumar, G.; Ferrajoli, A.; Cardenas-Turanzas, M.; et al. Safety and activity of ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukemia: A single-arm, phase 2 study. *Lancet Oncol.* 2014, 15, 1090–1099. [CrossRef]

16. Hallek, M.; Cheson, B.D.; Catovsky, D.; Caligaris-Cappio, F.; Döhner, H.; Hillmen, P.; Keating, M.J.; Montserrat, E.; Rai, K.R.; et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: A report from the In-ternational Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute–Working Group 1996 guidelines. *Blood* 2008, 111, 5456–5456. [CrossRef]

17. Miller, M.D.; Paradis, C.F.; Houck, P.R.; Mazumdar, S.; Stack, J.A.; Rifai, A.; Mulsant, B.; Reynolds, C.F., 3rd. Rating chronic medical illness burden in geropsychiatric practice and research: Application of the Cumulative Illness Rating Scale. *Psychiatry Res.* 1992, 41, 237–248. [CrossRef]

18. Rosenquist, R.; Ghia, P.; Hadzidimitriou, A.; Sutton, L.-A.; Agathangelidis, A.; Baliakas, P.; Darzentas, N.; Giudicelli, V.; Lefranc, M.-P.; Langerak, A.W.; et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: Updated ERIC recommendations. *Leukemia* 2017, 31, 1477–1481. [CrossRef]

19. Malcikova, J.; Tausch, E.; Rossi, D.; Sutton, L.A.; Soussi, T.; Zenz, T.; Kater, A.P.; Niemann, C.U.; Gonzalez, D.; Davi, F.; et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia—Update on methodological ap-proaches and results interpretation. *Leukemia* 2018, 32, 1070–1080. [CrossRef]

20. Rawstron, A.C.; Böttcher, S.; Letestu, R.; Villamor, N.; Fazi, C.; Kartsios, H.; de Tute, R.M.; Shingles, J.; Ritgen, M.; Moreno, C.; et al. Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international har-monized approach for flow cytometric residual disease monitoring in CLL. *Leukemia* 2013, 27, 142–149. [CrossRef]

21. Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events. Version 3. Available online: https://ctep.cancer.gov/protocoldevelopment/electronic (accessed on 9 December 2021).

22. Burger, J.A.; Sivina, M.; Jain, N.; Kim, E.; Kadia, T.; Estrov, Z.; Nogueras-Gonzalez, G.M.; Huang, X.; Jorgensen, J.; Li, J.; et al. Long-term efficacy and safety of first-line ibrutinib plus rituximab in patients with chronic lymphocytic leukemia. *Blood* 2019, 133, 1011–1019. [CrossRef]

23. Burger, J.A.; Barr, P.M.; Robak, T.; Owen, C.; Ghia, P.; Tedeschi, A.; Bairey, O.; Hillmen, P.; Coulter, S.E.; Devereux, S.; et al. Randomized trial of ibrutinib vs ibrutinib plus rituximab in patients with chronic lymphocytic leukemia. *Blood* 2015, 126, 376—376. [CrossRef]

24. Peragine, N.; De Propis, M.S.; Intoppa, S.; Milani, M.L.; Mariglia, P.; Mauro, F.R.; Raponi, S.; Soddu, S.; Cuneo, A.; Rigolin, G.M.; et al. Modulated expression of adhesion, migration and activation molecules may predict the degree of response in chronic lymphocytic leukemia patients treated with ibrutinib plus rituximab. *Haematologica* 2020, 106, 1500–1503. [CrossRef]

25. Kipps, T.J.; Fraser, G.; Coutre, S.E.; Brown, J.R.; Barrientos, J.C.; Barr, P.M.; Byrd, J.C.; O’Brien, S.M.; Dilhuydy, M.-S.; Hill-men, P.; et al. Long-Term Studies Assessing Outcomes of Ibrutinib Therapy in Patients With Del(11q) Chronic Lymphocytic Leukemia. *Clin. Lymphoma Myeloma Leuk.* 2019, 19, 715–722.e6. [CrossRef]

26. Strati, P.; Schlette, E.J.; Soto, L.M.S.; Dueñas, D.; Sivina, M.; Kim, E.; Keating, M.J.; Wierda, W.G.; Ferrajoli, A.; Kantarjian, H.; et al. Achieving complete remission in CLL patients treated with ibrutinib: Clinical significance and predictive factors. *Blood* 2020, 135, 510–513. [CrossRef]
27. Ahn, I.E.; Tian, X.; Wiestner, A. Ibrutinib for Chronic Lymphocytic Leukemia with TP53 Alterations. *N. Engl. J. Med.* 2020, 383, 498–500. [CrossRef]  
28. Allan, J.N.; Shanafelt, T.; Wiestner, A.; Moreno, C.; O’Brien, S.M.; Li, J.; Kriegsfeld, G.; Dean, J.P.; Ahn, I.E. Long-term efficacy of first-line ibrutinib treatment for chronic lymphocytic leukaemia in patients with TP53 aberrations: A pooled analysis from four clinical trials. *Br. J. Haematol.* 2021. [CrossRef]  
29. Parikh, S.; Chaffee, K.; Call, T.; Ding, W.; Leis, J.F.; Chanan-Khan, A.; Bowen, D.A.; Conte, M.; Van Dyke, D.L.; Hanson, C.A.; et al. Ibrutinib therapy for chronic lymphocytic leukemia (CLL): An analysis of a large cohort of patients treated in routine clinical practice. *Blood* 2015, 126, 2935. [CrossRef]  
30. Sandoval-Sus, J.; Chavez, J.; Dalia, S.; Bello, C.M.; Shah, B.D.; Ho, V.Q.; Lodzon, L.; Kharfan-Dabaja, M.A.; Sotomayor, E.M.; Sokol, L.; et al. Outcomes of patients with relapsed/refractory chronic lymphocytic leukemia after ibrutinib discontinuation outside clinical trials: A single institution experience. *Blood* 2015, 126, 2945. [CrossRef]  
31. Brown, J.R.; Moslehi, J.; O’Brien, S.; Ghia, P.; Hillmen, P.; Cymbalista, F.; Shanafelt, T.D.; Fraser, G.; Rule, S.; Kipps, T.J.; et al. Characterization of atrial fibrillation adverse events reported in ibrutinib randomized controlled registration trials. *Haematologica* 2017, 102, 1796–1805. [CrossRef]  
32. Rigolin, G.M.; Cavazzini, F.; Piccioni, A.; Arena, V.; Visentin, A.; Reda, G.; Zamproga, G.; Cibien, F.; Vitagliano, O.; Coscia, M.; et al. Efficacy of idelalisib and rituximab in relapsed/refractory chronic lymphocytic leukemia treated outside of clinical trials. A report of the Gimema Working Group. *Hematol. Oncol.* 2021, 39, 326–335. [CrossRef]