Impact of age on genetics and treatment efficacy in follicular lymphoma

Defining the impact of age on treatment outcome in patients with follicular lymphoma (FL) is challenging. Age >60 years is used as a risk factor in commonly applied risk scores.[1,2] However, older patients are, per se, at an increased risk of death due to the natural limits of human lifespan. Thus, age-related deaths blur commonly used treatment endpoints like progression-free survival (PFS), failure-free survival (FFS), and overall survival (OS). Conversely, progression-of-disease (POD), i.e., refractory, progressive, or relapsed FL, may more accurately represent treatment efficacy (see *Online Supplementary Methods*). Furthermore, aging may impact lymphoma biology. Here, we report genetic and clinical analyses indicating that older age is not associated with higher risk disease or inferior treatment efficacy.

To assess the impact of age on FL genetics, we re-analyzed DNA sequencing data from diagnostic biopsies of 258 patients with advanced disease (*Online Supplementary Table S1 and Figure S1*).[3] The number of gene mutations within coding regions of all 74 targeted genes increased with age. Specifically, the targeted mutational burden (TMB) increased by 13% per decade (95%-confidence interval (CI) [8;19], P=7.8 x10^{-7}, Figure 1A). This increase was caused by silent mutations (+16%/decade, 95%-CI [8;24], P=8.5 x10^{-5}) and missense mutations predicted to have low/intermediate functional impact by analysis of evolutionary conservation patterns (+19%/decade, 95%-CI [9;29], P=0.00011), whereas disruptive mutations (+4%/decade, 95%-CI [-3;11], P=0.31) and missense mutations predicted to have high functional impact (+8%/decade, 95%-CI [-3;20], P=0.16, Figure 1B) did not correlate with age. We found no impact of age on the fraction of single nucleotide variants consistent with aberrant somatic hypermutation (aSHM, Figure 1C). Univariate analysis of significantly mutated genes revealed that non-silent mutations in *MEF2B* (Odds ratio (OR) 1.41/decade, P=0.042), *TP53* (OR 1.82/decade, P=0.028) and *NOTCH2* (OR 3.69/decade, P=0.0041) were more common in older patients, while mutations in CD79A were more common in younger patients (OR 0.49/decade, P=0.039). However, after correction for multiple testing, no single non-silently mutated gene was significantly associated with age (*Online Supplementary Tables S2 and S3*). Also, the cumulative risk score calculated from gene mutations of the clinicogenetic risk model m7-FLIPI[3] did not indicate higher-risk disease with rising age (r_s=-0.11, P=0.076, *Online Supplementary Figure S2B*).

To assess the impact of age on treatment outcome, we analyzed patients who uniformly received R-CHOP for advanced FL within the GLSG2000 trial (*Online Supplementary Methods and Table S4*). Among 755 evaluable patients (Table 1 and *Online Supplementary Figure S3*), 9% were 18-40 years (n=65), 22% >40-50 years (n=163), 35% >50-60 years (n=261), 28% >60-70 years (n=208), and 8% >70 years (n=58, Figure 2A). The overall response rates were 98% for patients 18-40 years (62/63), 97% for >40-50 years (154/159), 96% for >50-60 years (248/258), 94% for >60-70 years (194/206), and 81% for >70 years (47/58; *Online Supplementary Table S5*). After a median follow up of 6.0 years (95%-CI [5.6;6.3]), the 5-year OS rates were 97% (18-40 years), 91% (>40-50 years), 90% (>50-60 years), 85% (>60-70 years), and 53% (>70 years; Figure 2B); 5-year FFS rates were 82% (18-40 years), 62% (>40-50 years), 62% (>50-60 years), 55% (>60-70 years), and 42% (>70 years), respectively (Figure 2C). We used the largest cohort (>50-60 years) as a reference. Patients <40 years showed a non-significant trend towards a more favorable OS (Hazard ratio (HR) 0.15, 95%-CI [0.02;1.12], P=0.065) and FFS (HR 0.63, 95%-CI [0.35;1.14], P=0.13), and a longer time to POD (HR 0.66, 95%-CI [0.38;1.16], P=0.15, Figure 2B-D and *Online Supplementary Table S6*). Patients >60-70 years had shorter OS (HR 1.88, 95%-CI [1.14;3.10], P=0.014), but FFS was not different compared to patients >50-60 years (HR 1.21, 95%-CI [0.89;1.64], P=0.24). Patients >70 years had both shorter OS (HR 7.24, 95%-CI [4.21;12.46], P=8.9 x10^{-13}) and FFS (HR 2.15, 95%-CI [1.44;3.22], P=0.00020; Figure 2B-C and *Online Supplementary Table S6*). To further delineate the impact of age on FFS, we separated the two mutually exclusive types of FFS events: POD and death w/o POD. Competing risk analysis demonstrated that shorter FFS of patients >70 years did not result from increased POD (HR 1.19, 95%-CI [0.75;1.89], P=0.47), but from a higher rate of death w/o POD (HR 24.65, 95%-CI [5.34;113.81], P=4.0 x10^{-5}, Figure 2D and *Online Supplementary Table S6*). Likewise, age as continuous variable was predictive for OS (HR

Table 1. Patient characteristics of the clinical cohort.

Characteristics		Entire cohort n=755	Age (years) 18-40 n=65	>40-50 n=163	>50-60 n=261	>60-70 n=208	>70 n=58	P#
Male sex		344/755 (46 %)	32/65 (49 %)	91/163 (56 %)	124/261 (48 %)	77/208 (37 %)	20/58 (34 %)	0.002
Clinical risk factors	>2 FLIPI risk-factors*	213/698 (31 %)	20/60 (33 %)	45/149 (30 %)	54/236 (23 %)	67/200 (34 %)	27/53 (51 %)	0.001
	Nodal sites >4	421/679 (62 %)	47/60 (78%)	104/142 (73 %)	139/237 (59 %)	104/192 (54 %)	27/48 (56 %)	<0.001
	LDH elevated	239/727 (33 %)	16/63 (25 %)	46/156 (29 %)	70/250 (28 %)	76/203 (37 %)	31/55 (56 %)	<0.001
	Hb <120g/l	146/728 (20 %)	12/62 (19 %)	29/158 (18 %)	38/249 (15 %)	50/203 (25 %)	17/56 (30 %)	0.037
	Stage III/IV	734/755 (97 %)	65/65 (100 %)	160/163 (98 %)	256/261 (98 %)	197/208 (95 %)	56/58 (97 %)	0.090
	ECOG PS >1	50/734 (7 %)	2/65 (3 %)	11/156 (7 %)	12/254 (5 %)	15/201 (7 %)	10/58 (17 %)	0.010
Histologic FL grade	1/2	586/607 (97 %)	55/57 (96 %)	140/142 (99 %)	203/209 (97 %)	145/152 (95 %)	43/47 (91 %)	0.19
	3	21/607 (3 %)	2/57 (4 %)	2/142 (1 %)	6/209 (3 %)	7/152 (5 %)	4/47 (9 %)	

FLIPI: Follicular lymphoma international prognostic index; LDH: lactate dehydrogenase; Hb: hemoglobin; ECOG PS: Eastern Cooperative Oncology Group Performance Score; FL: Follicular lymphoma; *excluding age; #Pearson's χ^2 test across all age cohorts

2.04/decade, 95%-CI [1.67;2.48], P=2.3 x10^{-12}), FFS (HR 1.21/decade, 95%-CI [1.07;1.36], P=0.0017) and death w/o POD (HR 3.72/decade, 95%-CI [2.48;5.59], P=2.2 x10^{-10}), but not for POD (HR 1.05/decade, 95%-CI [0.94;1.18], P=0.39, *Online Supplementary Table S6*). The cumulative incidence of death w/o POD in patients >70 years was 21% (n=12/58, Figure 2E). Eight of 12 events occurred within 1 year from treatment initiation. Thereof, 5 deaths were related to infections (Figure 2F).

To the best of our knowledge, this is the first study to explore the impact of age on the genetics of FL. We show that the number of gene mutations increases with age at diagnosis, as a result of more silent mutations and mutations predicted to have low functional impact. This is most consistent with the hypothesis that long-lived hematopoietic progenitor cells continuously acquire mutations during lifetime, most of which are not directly impacting lymphoma biology.[4,5] Non-silent mutations in individual genes were not significantly associated with age after adjusting for multiple testing. However, our molecular studies are certainly limited by the fact that we analyzed a targeted set of genes known to be recurrently mutated in FL, rather than whole exomes/genomes. Omics-wide studies are required to ultimately address the link between age and lymphoma biology. Still, it is an intriguing –and testable– hypothesis that the age-associated increase in mutational burden might translate into better responses to immune checkpoint inhibition in older patients.[6]

Our clinical analysis comprised mature data from a large and well-defined cohort of patients who uniformly received R-CHOP for advanced FL within a prospective clinical trial.[7] In line with a previous report,[8] patients >70 years had shorter FFS. However, by carefully dissecting causes of treatment failure we show that higher age was not associated with decreased treatment efficacy, as demonstrated by a similar time to POD, but with an increased rate of deaths without POD, i.e., higher non-relapse mortality. These findings question the value of age as disease-specific risk factor in FL as used in commonly applied risk classifiers.

While lymphoma continues to be the leading cause of

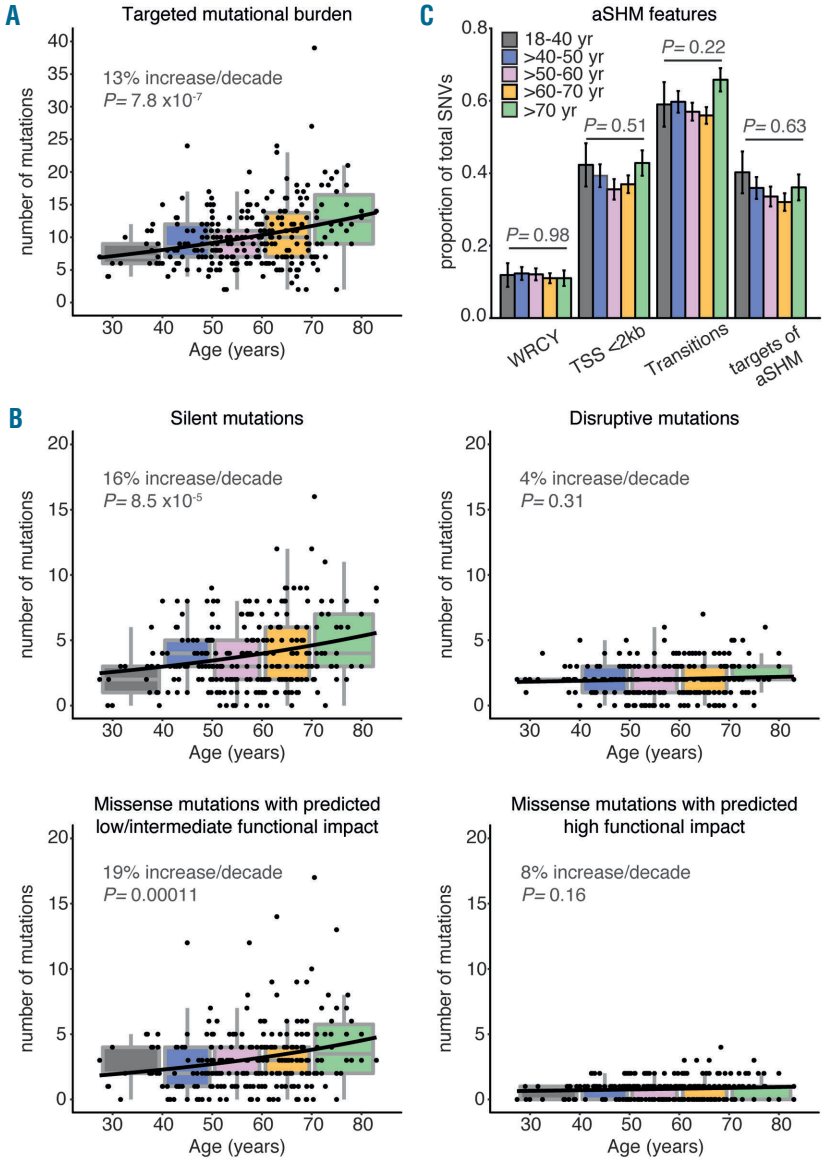

Figure 1. Targeted mutational burden (TMB) by age. (A) TMB in all 74 genes depicted as a dotplot by age (Quasi-Poisson regression). Boxplots indicate median number of gene mutations with 1st and 3rd quartiles, whiskers represent 1.5x interquartile range. (B) TMB by mutually exclusive types of gene mutations. (C) Fraction of single nucleotide variants (SNV) with features of aberrant somatic hypermutation (aSHM): "WRCY" denotes mutations within this hotspot motif. "TSS <2kb" denotes mutations within 2kb from the transcriptional start sites. "Transitions" denotes transition mutations. "Targets of aSHM" denotes mutations within known target genes of aSHM (see *Online Supplementary Methods*). Error bars show standard error of the mean. Color legend: gray for 18-40 years; blue for >40-50 years; purple for >50-60 years; orange for >60-70 years; green for >70 years. SNV: single nucleotide variant; aSHM: aberrant somatic hypermutation; TSS: transcriptional start site; yr: year

death in patients with FL among all age cohorts, recent data show that other causes of death are increasing with age.[9] While late non-relapse mortality is indicative of effective treatment, early non-relapse mortality can –at least partially– be attributed to treatment-related toxicity. In our study, patients >70 years had a 1-year non-relapse mortality of 14%. More than half of these patients died from infectious complications. This observation is even more remarkable as patients enrolled in clinical trials are often medically less compromised compared to real-world populations. Although this certainly requires independent validation in larger cohorts, a subset of older patients may indeed be at increased risk to not tolerate standard immunochemotherapy. Still, the majority of older patients gain similar benefit from standard treatment compared to younger cohorts. Thus, it is essential to prospectively identify the subset of patients at increased risk of early non-relapse death, e.g., by geriatric and functional assessments. These patients could benefit from growth factor support and/or anti-infective prophylaxis, which has been shown to reduce grade 3/4 infections in elderly patients receiving R-CHOP for aggressive B-cell lymphoma.[10]

Bendamustine plus rituximab is now widely used at

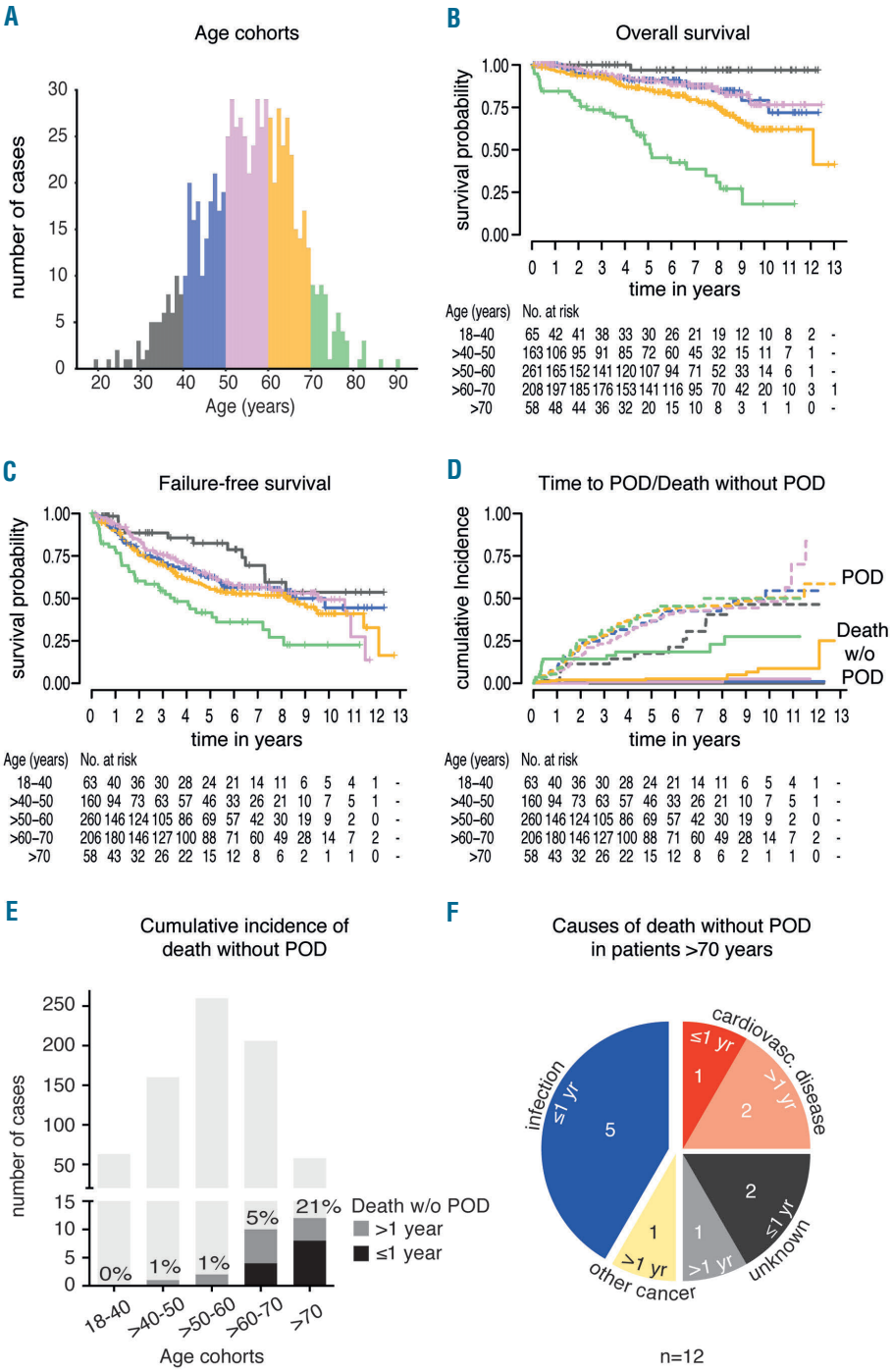

Figure 2. Treatment outcome of GLSG2000 patients who received R-CHOP for advanced FL according to age cohorts. A) Age distribution and definition of distinct age cohorts. (B) Kaplan–Meier curves for overall survival (OS). (C) Kaplan–Meier curve for failure-free survival (FFS). (D) Cumulative incidence analysis of failure-free survival with progression of disease (POD; dashed lines) as primary event, and death w/o POD (solid lines) as competing event. Analyses for progression-free survival (PFS) analogous to C and D are shown in *Online Supplementary Figure S4*. (E) Cumulative incidence of death w/o POD by age group after a median follow up of 5.7 years (95%-CI [5.1;6.1]). Black bars indicate deaths w/o POD within the first year after treatment initiation, gray bars indicate deaths beyond one year. (F) Causes of death w/o POD in patients >70 years. Causes of deaths within the first year after treatment initiation are shown in darker colors, those occurring after the first year in lighter colors. Color legend: gray for 18-40 years; blue for >40-50 years; purple for >50-60 years; orange for >60-70 years; green for >70 years. POD: progression of disease; w/o: without; yr: year; cardiovasc. disease: cardiovascular disease

many centers for frontline treatment of follicular lymphoma after initial studies have shown a favorable toxicity profile.[11,12] However, recent data raised safety concerns when standard-dose bendamustine was combined with either rituximab or obinutuzumab followed by antibody maintenance: more fatal adverse events occurred in the bendamustine arms -mostly due to infections during maintenance treatment- as compared to CHOP or CVP regimens.[13] Dose-reduced or chemotherapy-sparing approaches may be an option for medically unfit patients.

The relatively low number of patients >70 years is a limitation of our study. Other studies have also analyzed the impact of older age on treatment outcome. The Follicular Lymphoma Analysis of Surrogate Hypothesis (FLASH) group conducted a meta-analysis on patients from 18 different trials who received a variety of different treatment regimens. Data presented in abstract form similarly showed a higher incidence of "non-lymphoma deaths" in 542 patients >70 years, and –analogous to our study– no difference in the cumulative incidence of POD compared to patients <70 years.[14] The National LymphoCare Study analyzed 209 patients >80 years.[15] Patients in this longitudinal observation study also received different therapies, including watchful-waiting, rituximab-monotherapy or immunochemotherapy. The 5-year OS rate in this elderly patient population was 59% –comparable to our study– and no particular treatment regimen provided superior outcome.

In summary, we find that FL of older patients have a higher mutational burden, but are not enriched for gene mutations associated with higher risk disease. Likewise, we do not find inferior antilymphoma activity of R-CHOP in older patients. Instead, shorter survival in patients with advanced age results from an increased rate of non-relapse deaths. Thus, age itself should not guide treatment decisions in FL. Instead, future studies are needed to better identify and treat patients at increased risk of non-relapse death.

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