



Article

Next-Generation-Sequencing of the Human B-Cell Receptor Improves Detection and Diagnosis and Enhances Disease Monitoring in Patients with Gastric Mucosa-Associated Lymphoid Tissue Lymphoma

Chidimma Agatha Akpa ^{1,*}, Cora Husemann ¹, Chris Allen ², Ann-Christin von Brünneck ¹, Jana Ihlow ^{1,3} and Michael Hummel ¹

¹ Department of Experimental Hematopathology, Institute of Pathology, Charité Medical University, 10117 Berlin, Germany; husemann.cora@gmail.com (C.H.); bruenneck@hp-hamburg.de (A.-C.v.B.); jana.ihlow@charite.de (J.I.); michael.hummel@charite.de (M.H.)

² Thermo Fisher Scientific, Paisley PA4 9RF, UK; chris.allen1@thermofisher.com

³ Berlin Institute of Health (BIH), Charité-Universitätsmedizin Berlin, 10117 Berlin, Germany

* Correspondence: chidimma.akpa@charite.de



Citation: Akpa, C.A.; Husemann, C.; Allen, C.; von Brünneck, A.-C.; Ihlow, J.; Hummel, M.
Next-Generation-Sequencing of the Human B-Cell Receptor Improves Detection and Diagnosis and Enhances Disease Monitoring in Patients with Gastric Mucosa-Associated Lymphoid Tissue Lymphoma. *J. Mol. Pathol.* **2024**, *5*, 292–303. <https://doi.org/10.3390/jmp5030021>

Academic Editor: Giancarlo Troncone

Received: 29 April 2024

Revised: 5 June 2024

Accepted: 1 July 2024

Published: 4 July 2024



Copyright: © 2024 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/>).

Abstract: Mucosa-associated lymphoid tissue (MALT) lymphomas are slow-growing B-cell lymphomas mainly diagnosed in the stomach and termed gastric MALT lymphoma (G-MALT). Despite histological evaluation, immunostaining, and additional B-cell clonality analysis by fragment analysis, a clear-cut diagnosis is not feasible in all cases, especially for clinical follow-up of patients after treatment. We examined clonally rearranged immunoglobulin heavy- and light-chain gene sequences of 36 genomic DNA samples from six different patients obtained at different time points over the course of several years using the OncoPrint™ B-cell receptor pan-clonality next-generation sequencing (NGS) assay. Each case consisted of samples diagnosed with G-MALT and samples without evidence of lymphoma, based on histological examinations. We show a robust correlation (100%) of the results between the applied NGS method and histology-diagnosed G-MALT-positive patients. We also detected malignant clonotypes in samples where histology assessment failed to provide clear evidence of G-MALT (15 out of 19 samples). Furthermore, this method revealed malignant clonotypes much earlier in the disease course, with NGS of the immunoglobulin light chain being crucial in complementing immunoglobulin heavy-chain analysis. Hence, the value of NGS in routine lymphoma diagnostics is greatly significant and can be explored in order to provide better diagnoses and proffer the early detection of lymphoma relapse.

Keywords: B-cell receptor; next-generation sequencing; gastric mucosa-associated lymphoid tissue lymphoma; minimal residual disease

1. Introduction

An efficient diagnosis of 10–15% of B-cell lymphomas remains difficult when using conventional diagnostic procedures, encompassing clinical evaluation, immunophenotyping, and histology [1–3]. Gastric MALT lymphoma (G-MALT), which comprises about 7–9% of all B-cell lymphomas, is a non-Hodgkin lymphoma originating from the marginal zone B-cells of the stomach, whose pathogenesis has been strongly linked to infection with *Helicobacter pylori* [4,5]. Routine diagnosis of this lymphoma subtype is made by an examination of all features of endoscopic biopsies, and, interestingly, many patients display a history of long-lasting gastritis [6,7].

G-MALT arises from a single aberrant B-cell leading to the expansion of identical clonal B-cells. Thus, the detection of uniquely rearranged (clonal) populations of B-cells would be beneficial in aiding diagnosis in cases with unclear histological and immunophenotypical findings. The human B-cell receptor (BCR) complex, comprising the membrane

immunoglobulin (IG), consists of two IG heavy chains (IGH) and two IG light chains (IGL). The IGH locus comprises several V (variable), D (diversity), and J (joining) gene segments, while the IGL (Kappa (k) and Lambda (λ)) loci are composed of V and J gene segments. Rearrangement of these gene segments during the process of V(D)J recombination occurs during the earliest stages of B-cell development [8]. Initially, one D gene segment randomly rearranges to one J gene segment, and in a second recombination step, one V gene segment rearranges to an already rearranged DJ gene segment, creating the functional VDJ exon that codes for the antigen-binding component of the IG chain [9]. In addition to the classical VJ recombination events of the IGL, rearrangement could also occur between IG Kappa (k) V genes and a purported kappa-deleting element (kde), or to a recombination signal sequence present in the IGk J-IGk C intron, rendering the IGk allele functionally inactive [10,11]. When specific primers are used to amplify the V(D)J exon, healthy cells display amplicons with various lengths, implying a polyclonal pattern of rearrangement, while malignant cells predominantly display amplicons of identical sizes, implying a monoclonal rearrangement [12]. Upon antigenic stimulation, B-cells become activated and proliferate rapidly in the germinal centers of secondary lymphoid tissues. This is accompanied by somatic hypermutations (SHMs) in the IG V genes, aimed at increasing diversity of the BCR repertoire. Moreover, a large proportion of MALT lymphoma cases have been shown to be targets of aberrant SHMs [13,14].

The Euroclonality BIOMED 2 multiplex PCR method developed over 20 years ago has been utilized as the gold standard for the detection of clonally rearranged IGH and IGL genes, as well as minimal residual disease in B-cell malignancies [1]. This method, however, poses technical and biologic limitations, as well as subjectivity, with result interpretation in borderline cases [9,15]. Next-generation sequencing (NGS) provides a robust and sensitive method for detecting BCR rearrangements with single base-pair resolution. The OncomineTM BCR pan-clonality assay (research use only) (ThermoFisher Scientific, Dreieich, Germany) has been demonstrated to be valuable for clonality detection, concurrently in IGH and IGL [16–19]. This assay interrogates the BCR heavy- and light-chain complementarity determining region 3 (CDR3) sequences, as well as rearrangements involving kde using multiplexed Ion AmpliSeqTM primers (ThermoFisher Scientific, Dreieich, Germany) in genomic DNA (gDNA). In this work, we aim to investigate this NGS-based assay for the detection of clonally rearranged BCR heavy and light chains in G-MALT lymphoma. We analyzed samples obtained from patients with a history of G-MALT at different time points/different body sites over the course of several years. We sought to examine the sensitivity and specificity of this method in detecting malignant clonotypes early on before disease onset, as well as to what extent. We also explored the re-emergence of novel clonotypes following therapy and probed for the exclusion of minimal residual disease in the stomach or other body sites. The lymphoma-specific IG rearrangements were depicted from histologically confirmed lymphoma samples.

2. Materials and Methods

2.1. Patient Identification and Sample Selection

We screened a local pathology data bank (Nexus) at the Institute of Pathology, Charité—Universitätsmedizin Berlin for patients with an unequivocal diagnosis of G-MALT at least at one time point. We sought patients who were biopsied over the course of several years with or without the manifestation of G-MALT. We then selected samples for each of these patients whereby following therapy, there was no hint of the presence of the pre-diagnosed G-MALT. Data curation and a review of the cases were performed by two certified pathologists. Age ranges for the patients were between 41 and 66 years. In total, 36 samples from 6 eligible patients were identified. A summary of the patient characteristics is shown (Table 1). Approval for the use of patient samples was obtained from the Charité ethics commission, Berlin (EA1/294/15).

Table 1. Summary of patient characteristics. NOS—not otherwise specified; R-CHOP—rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; BM—Bone marrow; MZL—marginal zone lymphoma; N—negative; P—positive; MRD—minimal residual disease; B-CLL—B-cell chronic lymphocytic leukemia.

Patient (Age)	Sample	Date of Sam-pling	Initial Diagno-sis	Sample Site	<i>H. pylori</i>	Other Manifesta-tions	G-MALT Therapy	BM Infil-tration	Relapse Comments
Patient A (41)	2003/01	21.01.2003	21.01.2003	Gastric mucosa	N	Stomach, ileum, mesentery, lung	2010—Field radiation (stomach and gastric lymph nodes)	No	1st relapse 26.11.2010
	2003/02	28.01.2003		Gastric mucosa					
	2010/01	18.05.2010		Colon mucosa					
	2010/02	26.11.2010		Gastric mucosa					
	2013/01	11.12.2013		Gastric mucosa					
	2017/01	04.12.2017		Colon mucosa					2nd relapse 11.12.2017
	2017/02	27.12.2017		Lung					
Patient B (66)	2001/01	08.08.2001	14.06.2001	Gastric mucosa	N	Stomach	Chemotherapy, NOS	No	1st relapse 21.03.2002 from then on MRD+ until 2009
	2001/02	22.08.2001		Ileum and colon mucosa					
	2001/03	12.11.2001		Gastric mucosa					
	2002/01	11.10.2002		Gastric mucosa					
	2004/01	29.09.2004		Gastric mucosa					
	2006/01	23.08.2006		Gastric mucosa					
	2009/01	23.03.2009		Colon mucosa					
	2010/01	08.07.2010		Gastric mucosa					
Patient C (65)	2003/01	16.10.2003	16.10.2003	Gastric mucosa	N	Stomach, Coecum	12/03–02/04 chemotherapy: 8× R-CHOP	Yes	-
	2004/01	26.07.2004		Gastric mucosa					
	2005/01	24.08.2005		Gastric mucosa					
	2006/01	03.07.2006		Ileum and colon mucosa					
	2007/01	26.10.2007		Duodenum and gastric mucosa					
	2009/01	26.05.2009		Duodenum and gastric mucosa					
	2011/01	26.01.2011		Gastric mucosa					
	2014/01	07.03.2014		Gastric mucosa					
	2019/01	03.09.2019		Gastric mucosa					
Patient D (58)	2008/01	09.06.2008	14.04.2008	Ileum and colon mucosa (MZL)	Initially N, switch to P in 2008	Stomach, ileum, colon, jugular lymph node	2008: Effective antibiotic therapy against Helicobacter pylori	B-CLL: Yes	Relapse 08.09.2010
	2009/01	18.03.2009		Gastric mucosa (MZL)					
	2009/02	02.12.2009		Gastric mucosa (MZL)					
	2011/01	07.07.2011		Blood (both MZL and CLL)					
Patient E (52)	2004/01	23.08.2004	23.08.2004	Gastric mucosa	N	Stomach	Resection (2/3 gastrectomy 2006)	No	1st relapse 12.05.2005
	2004/02	08.11.2004		Gastric mucosa					2nd relapse 13.11.2007
	2008/01	05.02.2008		Ileum mucosa					

Table 1. Cont.

Patient (Age)	Sample	Date of Sampling	Initial Diagnosis	Sample Site	<i>H. pylori</i>	Other Manifestations	G-MALT Therapy	BM Infiltration	Relapse Comments
Patient F (55)	2017/01	15.06.2017	01.08.1989	Colon mucosa	N	Stomach, sigma, liver	At initial diagnosis: COP (Cyclophosphamide, Vincristine, Prednisolone 3 cycles)	No	1st relapse 01.02.2017
	2017/02	16.06.2017		Gastric mucosa			Chlorambucil and Prednisolone 7 cycles		
	2017/03	30.06.2017		Colon mucosa			1st relapse (1st relapse 01.02.2017): 4x Rituximab		
	2017/04	30.11.2017		Gastric mucosa			2nd relapse (2nd relapse 01.04.2022): Rituximab		
	2022/01	25.04.2022		Colon mucosa					

2.2. Tissue Sectioning, Staining and gDNA Extraction

Archived formalin-fixed paraffin-embedded (FFPE) tissue blocks for each of the samples were sectioned (3 µm) and stained for H&E (to examine tissue morphology), CD3 (Dako/Agilent, polyclonal, 1:100, to determine the T-cell composition of the tissue), and CD20/PAX5 (CD20: Dako/Agilent, clone L26, 1:750, PAX5: Leica, clone 24/PAX-5, 1:10 to determine the B-cell content) using a Leica Bond Master (Leica Biosystems, Nussloch, Germany), following the manufacturer's instructions. gDNA was extracted from the tissue sections (10 µm) with the support of the Central Biobank Charité/BIH (ZeBanC), Berlin, using Maxwell® (Promega, Walldorf, Germany) and following the manufacturer's instructions. Quantification of the DNA samples was performed by spectrofluorometry using Quantus™ Fluorometer (Promega, Walldorf, Germany).

2.3. Library Preparation, Sequencing and Clonality Analysis

High-throughput libraries were created using the Oncomine™ BCR pan-clonality assay (ThermoFisher Scientific, Dreieich, Germany) following the manufacturer's protocol. We used gDNA input ranging from 15–30 ng, and the number of PCR cycles was increased by +3 during target amplification. Library enrichment following the standard protocol for library amplification and 2× purification was also performed. A summary of the library preparation protocol is depicted in Figure 1. Each of the generated libraries were diluted to a concentration of 30 pM. Then, 10–12 samples were then pooled to a concentration of 30 pM and loaded onto the Ion Chef™ system (ThermoFisher Scientific, Dreieich, Germany) for template preparation. The samples were sequenced on an Ion 530™ Chip (ThermoFisher Scientific, Dreieich, Germany) using the Ion GeneStudio™ S5 series system (ThermoFisher Scientific, Dreieich, Germany). The number of reads for each IGH and IGL are included (Supplementary Table S1). Clonality analysis was conducted with Ion Reporter™ 5.20 software (ThermoFisher Scientific, Dreieich, Germany) using the Oncomine IGH and IGL single-sample workflows that perform read mapping, filtering, and output frequencies of clones identified in the BCR repertoire [19]. A summarized depiction of the data output from the software is shown (Figure 2). Clonotypes with frequencies below the threshold of 0.001% of the total reads were not included for clonality assessment, as these were considered too-weak signals or background noise [20].

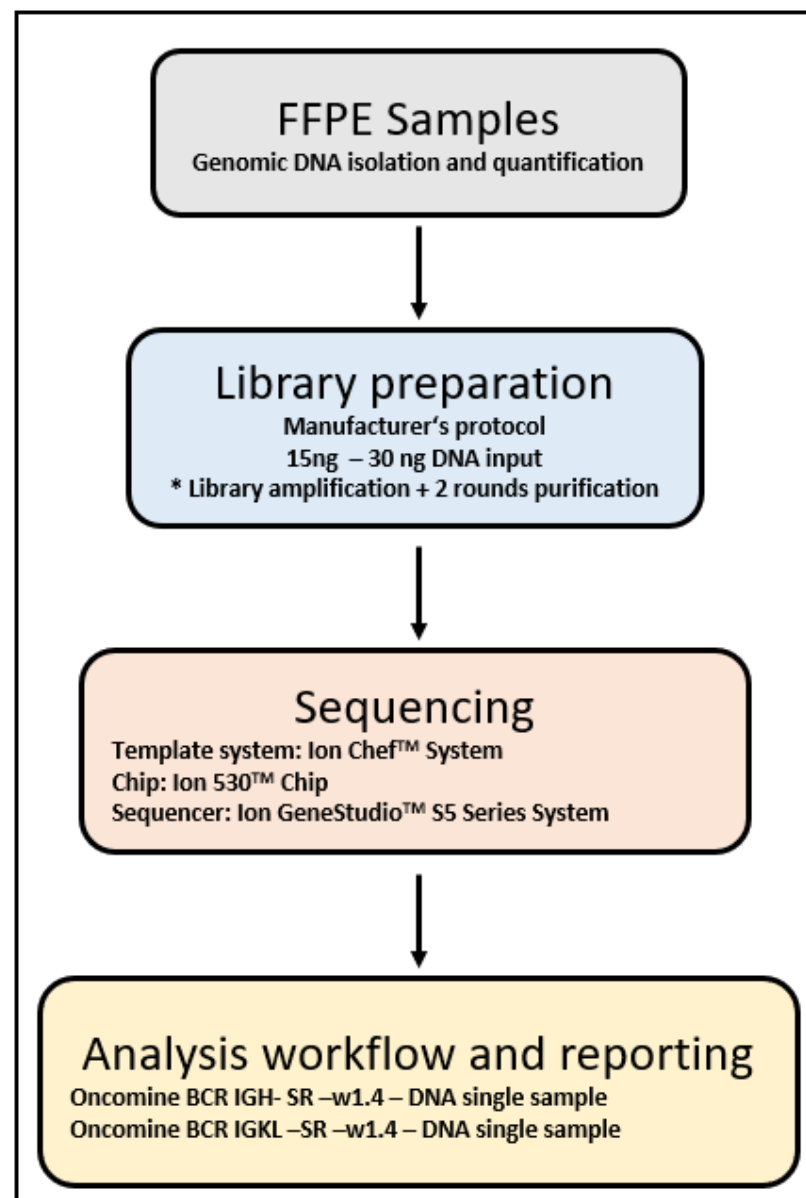


Figure 1. Flow chart showing summary of sample library preparation. FFPE: formalin-fixed paraffin-embedded. * Additional manufacturer's protocol for samples with low input quantity.

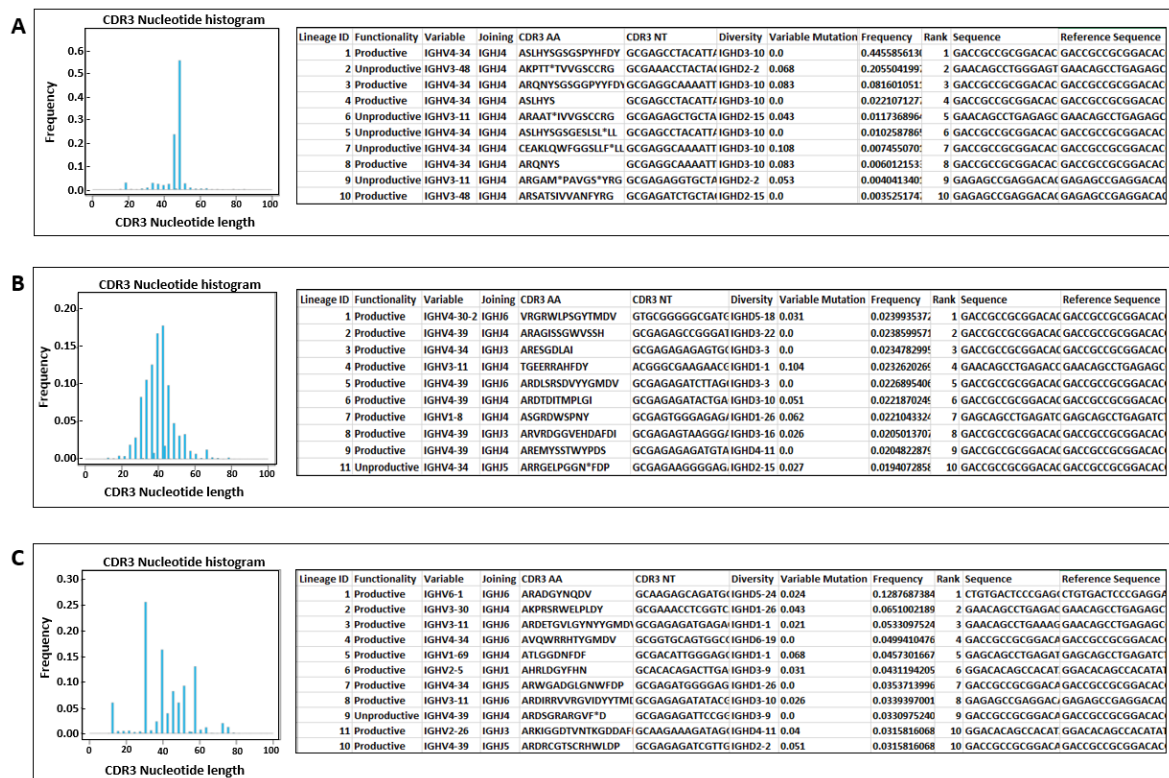


Figure 2. Output from the Ionreporter software with histogram showing how the CDR3 lengths of the clonotypes in the sample are distributed. (A) Clonal (biallelic) IGH rearrangement, (B) polyclonal IGH rearrangement, and (C) oligoclonal IGH rearrangement. CDR3 nucleotide lengths of the clonotypes are represented and ranked according to their frequency of occurrence in the sample, with 1 being the most dominant clonotype. A summary of the list with the top 10 clonotypes is shown.

2.4. Statistical Analysis

McNemar's test for paired data was used for statistical analysis to compare the difference in sensitivity between histology and NGS, as well as to examine the correlation between clonality detection by analyzing IGH versus analyzing IGL. p -values < 0.05 were considered statistically significant.

3. Results

Correlation of Results between the Applied NGS Method and Results from Histological Diagnosis

Of the 36 samples (6 patients) analyzed, 17 samples were diagnosed with G-MALT lymphoma based on histology assessment. A tumor-specific clonotype was also detected in these 17 samples (100%) using the applied NGS assay. Also, in 15/19 (78%) samples where a histology examination suggested no evidence of lymphoma, we detected the tumor-specific clonotype by means of NGS (Figure 3). The difference between the sensitivity of the NGS method applied and histology was statistically significant ($p = 0.0003$). Moreover, in 4/19 (21%) samples without evidence of G-MALT based on histology data, we were also unable to detect the malignant clonotype using NGS (Figure 3). We observed this in patient A, patient B, and patient C (Table 2). In none of the cases with clinical and histological proof of G-MALT were we unable to detect the malignant clonotype by way of NGS. The dominant clonotypes and their occurrence in the individual patients are shown (Supplementary Table S1). Frequencies of the tumor-specific clonotypes are shown (Supplementary Table S1).

Table 2. Clonality results based on NGS and correlation with histology/clinical diagnosis. Samples were defined as clonal in each case where the clonotype was found above threshold ($>0.001\%$). n.d.—not detectable.

Patient (Gender)	Samples Analyzed	Lymphoma Diagnosed (Histology)	IGH NGS Clonality	IGL NGS Clonality
Patient A (Female)	2003/01	+	Clonal/bi-allelic	Clonal
	2003/02	+	Clonal/bi-allelic	Clonal
	2010/01	—	Polyclonal	Polyclonal
	2010/02	+	Clonal/bi-allelic	Clonal
	2013/01	—	n.d.	Clonal
	2017/01	—	n.d.	Clonal
	2017/02	+	Clonal	Clonal
Patient B (Female)	2001/01	+	Oligoclonal	Clonal
	2001/02	—	Oligoclonal	Polyclonal
	2001/03	—	Clonal	Clonal
	2002/01	+	Clonal	Clonal
	2004/01	+	Clonal	Clonal
	2006/01	+	Clonal	Clonal
	2009/01	—	n.d.	Clonal
	2010/01	—	n.d.	Polyclonal
Patient C (Male)	2003/01	+	Clonal	Clonal
	2004/01	+	Oligoclonal	Clonal
	2005/01	—	Oligoclonal	Clonal
	2006/01	—	Oligoclonal	Clonal
	2007/01	—	Oligoclonal	Clonal
	2009/01	—	Oligoclonal	Clonal
	2011/01	—	Oligoclonal	Clonal
	2014/01	—	Clonal	Clonal
Patient D (Female)	2008/01	—	Clonal	Clonal
	2009/01	+	n.d.	Clonal
	2009/02	+	Clonal	Clonal
	2011/01	+	Clonal	Clonal
Patient E (Male)	2004/01	+	Oligoclonal	Clonal
	2004/02	—	Clonal	Clonal
	2008/01	—	Polyclonal	Clonal
Patient F (Female)	2017/01	—	Clonal	Clonal
	2017/02	+	Clonal	Clonal
	2017/03	—	Oligoclonal	Clonal
	2017/04	+	Clonal	Clonal
	2022/01	+	Clonal	Clonal

For patient A, we noted two dominant IGH clonotypes, with one being productive and the other non-productive (a bi-allelic case). The presence of SHMs in IGH was also evident from minor changes in the amino acid sequence for the productive and unproductive clonotype (Supplementary Table S1). For IGL, fewer amino acid changes in comparison with the IGH were observed in the malignant clonotype. No changes in the malignant clonotype amino acid sequence were noted for IGk.

We observed that in certain instances, although the malignant clonotype was not identifiable in the IGH, it was detected in the IGL at different frequencies using NGS (see Table 2 and Supplementary Table S1), with some samples exhibiting oligoclonal IGH rearrangements [1,9].

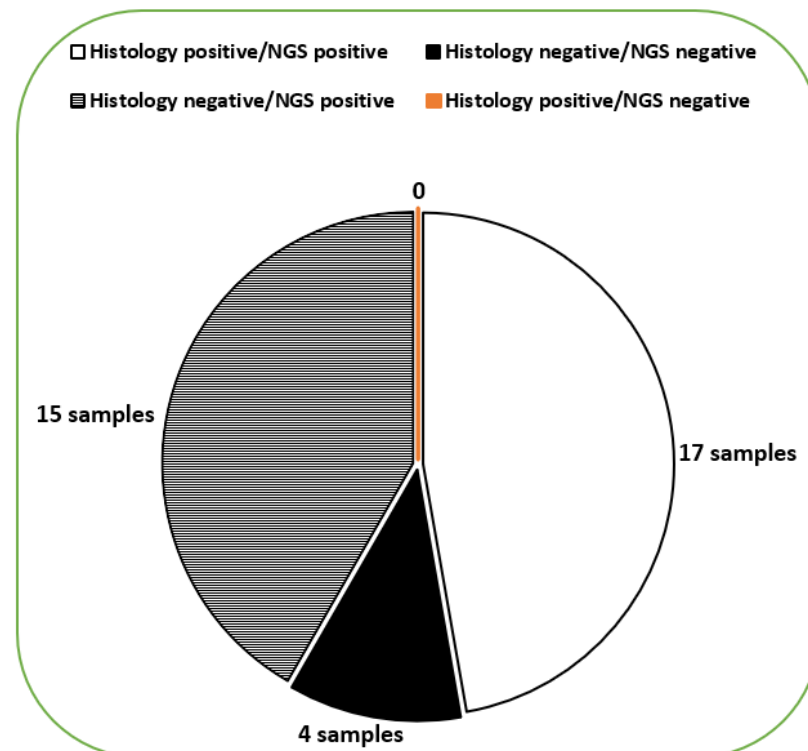


Figure 3. Pie chart showing correlation between histology /clinically diagnosed G-MALT and NGS assay results for the 36 patient samples. Histology /clinically diagnosed G-MALT is herein simply referred to as histology.

Furthermore, all patient cases diagnosed with G-MALT were *H. pylori*-negative except one (patient D) where the organism was detected later on in the disease (Table 1).

For Patient F, samples were collected at almost the same time point (one-day interval) but from the colonic mucosa and gastric mucosa, respectively (Tables 1 and 2; sample 2017/01 and 2017/02). In this patient, the lymphoma was undetected in the colon mucosa but diagnosed in the gastric mucosa by means of histology examination. By way of NGS, however, we showed—in addition to gastric mucosa association—colon involvement by detecting the malignant clonotype in the colonic mucosa at a low frequency.

4. Discussion

A diagnosis of G-MALT may be difficult when only based on clinical evaluations of patients, their history, and assessments of tissue histology and immunostaining. Hence, the necessity for molecular methods that interrogate the B-cell repertoire to detect clonally rearranged BCRs would play a pivotal role in aiding diagnosis. Moreover, in cases where the amount/proportion of infiltrating tumor B-cells is low, the need for a method with high sensitivity and specificity cannot be overemphasized. NGS provides a robust method for the detection of clonal and sub-clonal populations of malignant cells and for disease monitoring owing to its low limit of detection, high resolution, and high specificity in comparison with other non-NGS-based methods, including histology [12,18,21,22]. The demonstration of clonotypes on a single-nucleotide level by NGS can also be harnessed for clonal tracking. We exploited this potential by using the Oncomine™ BCR pan-clonality assay.

We analyzed a cohort of 36 samples obtained at different time points from six patients. Each patient had, at one time point, at least one histologically assessed G-MALT-positive sample. The correlation between the BCR pan-clonality NGS assay and G-MALT-positive results, based on clinical evidence and histology, highlights the value of this assay and the detection of clonality as a useful diagnostic tool. We also reveal the high sensitivity of this assay in detecting clones much earlier in the course of the lymphoma, even at time points where tissue histology yielded no evidence of malignancy. This indicates an additional

benefit of NGS in complementing routine diagnostic methods in assessing the lymphoma status of patients.

SHMs involving point mutations that occur in the V-regions of the IGH and IGL genes increases the affinity and diversity of activated B-cells [1,23], with clonally related cells frequently sharing SHMs [24]. We also note this in the IGH and IGL clonotypes of the samples of one patient (patient A), where the CDR3 amino acid sequences showed variations. Evidence that the clonotypes showing mutated sequences stem from one clone was confirmed by comparing the nucleic acid sequences from each of the clonotypes with those of the germline reference sequence using the IMGT blast tool https://www.imgt.org/IMGT_vquest/input [25] accessed on 3 January 2024. Here we found that the compared sequences had the same V- and J- gene and allele. We also detected these point mutations in the nucleic acid sequences (Supplementary Data S2). For IGL, fewer SHMs in comparison with the IGH were observed in the malignant clonotype, which is in agreement with another study [26]. Moreover, based on IGH analysis, clonality was detected in 26/32 (81%) samples, while IGL analysis revealed clonality in all 32 samples (100%). The difference in the number of samples in which clonality was detected in IGL as compared to IGH was statistically significant ($p = 0.0005$). Our ability to detect clonality in IGL even when it was undetectable in IGH using NGS underscores the necessity of incorporating IGL assessments alongside IGH analysis during clonality testing. It is crucial to note that relying solely on IGH analysis may lead to false negative results where the clonal population may be missed during analysis. This is because the more frequent somatic hypermutations (SHMs) in IGH genes can impede efficient primer binding to the target sequences [27].

Disease monitoring in the context of minimal residual disease (MRD), which is characterized by the minor representation of a known clonal population in the body following disease treatment, was examined in the patients of this study. Here, we defined MRD as the occurrence of known clonotypes with a frequency of more than 0.05% and less than 5% of the total reads in the sample. Following therapy, histology/clinical assessment may suggest no evidence of the previously diagnosed lymphoma type. However, the tumor-specific clonotype may still persist in tissues, and the assessment of MRD would be a prognostic determinant for relapse in such patients [28]. This was evident in some of the patients we examined, for example patient A, who, after receiving field radiation therapy in 2010, was excluded from a positive G-MALT diagnosis in 2013 based on histology and clinical assessment, whereas the malignant clone was present and detectable by NGS. Another good example is patient B, who, after receiving chemotherapy in 2001, showed no obvious signs of lymphoma based on histology and clinical examination in the subsequent months of that year. Meanwhile, the tumor-specific clonotype was still present in the patient. It is very likely that this additional information would have resulted in a more rigorous follow-up schedule for the patient prior to the relapse in 2002. A similar scenario also presented in patients C, E and F. Although there is no currently known treatment approach for G-MALT patients with MRD other than watchful waiting [29], knowledge that the malignant clonotype still exists in the patient could alter treatment course and disease surveillance in these patients [30], particularly with regard to risk assessment for later aggressive transformation. Furthermore, with proper awareness of their MRD status, patients could continue making healthier life choices, which could drastically impact their long-term survival. In light of precision medicine, our approach might also be of value for the current classification system for MRD in extranodal marginal zone lymphoma, which is currently based on histological/immunohistochemical criteria alone [31] and could aid in the risk stratification of MALT lymphoma after initial therapy.

We did not observe the emergence of any new clonotype distinct from the one present prior to therapy in any patient with relapse, whether in the gastric mucosa or at any other body site. For patient D, however, the blood sample examined in 2011, where there was also a clinical report of B-cell chronic lymphocytic leukemia (CLL), revealed the same clonotype in the ileum, colon, and gastric mucosa. This could mean that the malignant clone in the gastrointestinal tissues expanded via blood. Additionally, although the co-

occurrence of more than one clonally distinct B-cell lymphoid malignancy is a very rare but possible phenomenon, in one case, G-MALT was shown to be a secondary event in a CLL patient [32]. In another patient, CLL was diagnosed 16 months following the initial diagnosis of G-MALT [33]. Whether the clone that we detected was that which resulted in the CLL diagnosis remains to be clarified. However, the high frequency of this clonotype in the blood (21–89%) in comparison to gastrointestinal tissues (0.4–9.2%) could be reflective of the higher number of tumor B-cells in blood relative to the tissues.

It is surprising that in some samples of patient C, where the histology assessment showed the absence of B-cells in the tissues, we detected clonality in both IGH and IGL. This could be attributed to the low limit of detection and high sensitivity of NGS [34,35]. That we detected the malignant clonotype in nearby tissues by means of NGS where this was missed based on histology shows the usefulness and accuracy of this technique in the early detection of malignancy and nearby tissue involvement, which could assist in timely institution of further relevant therapeutic strategies to inhibit the spread of the tumor.

There have been many reports on the strong association and frequency of occurrence of *H. pylori* infection in patients diagnosed with G-MALT [36–39]. The incidence of *Helicobacter pylori*-negative MALT lymphoma from chronic gastritis was historically approximately 20% but has been increasing consistently over the past 20 years [40]. Out of the six patients examined in our study, only one was positive with *H. pylori*—the infection being initially absent from the patient following an initial diagnosis of G-MALT. Therapy for *H. pylori* was associated with clinical remission in this patient, which was in agreement with previous reports [5,41].

5. Conclusions

Our findings show the advantage of utilizing the Oncomine™ B-cell receptor pan-clonality NGS assay in detecting BCR clonality, as well as MRD in G-MALT, especially in situations where routine clinical assessment of the patient and immunostaining are insufficient to make a diagnosis. The early detection and tracking of BCR clonotypes could also serve as a prognostic tool for determining G-MALT patients at risk for relapse. This would provide clinicians with information to bear in mind while making precise treatment decisions for patients.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/jmp5030021/s1>, Supplementary Table S1: Frequency of individual clonotypes identified by NGS. Supplementary Data S2: Somatic hypermutation analysis.

Author Contributions: Conceptualization, M.H. and C.A.A.; methodology, C.A.A. and C.H.; software, C.A.A. and C.A.; validation, C.A.A.; formal analysis, C.A.A. and J.I.; investigation, C.A.A., J.I., and A.-C.v.B.; resources, M.H.; data curation, C.A.A. and C.H.; writing—original draft preparation, C.A.A.; writing—review and editing, C.A.A., C.H., J.I., C.A., and M.H.; visualization, C.A.A.; supervision, M.H.; funding acquisition, M.H. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported via intramural funding from Charité Medical University, Berlin.

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the Charité ethics commission, Berlin (A1/294/15, 24 November 2015).

Data Availability Statement: All data and protocols are available within this manuscript. Further inquiries can be directed to the corresponding author.

Acknowledgments: We thank Edda von der Wall and Anke Sommerfeld for their technical support.

Conflicts of Interest: Chris Allen was employed by the company Thermo Fisher Scientific. 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.

References

1. van Dongen, J.J.; Langerak, A.W.; Bruggemann, M.; Evans, P.A.; Hummel, M.; Lavender, F.L.; Delabesse, E.; Davi, F.; Schuurin, E.; García-Sanz, R.; et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia* **2003**, *17*, 2257–2317. [\[CrossRef\]](#)
2. Slater, D. Histopathological aspects of cutaneous lymphoma. *J. R. Soc. Med.* **2001**, *94*, 337–340. [\[CrossRef\]](#)
3. Groenen, P.J.; Langerak, A.W.; van Dongen, J.J.; van Krieken, J.H. Pitfalls in TCR gene clonality testing: Teaching cases. *J. Hematop.* **2008**, *1*, 97–109. [\[CrossRef\]](#)
4. Violeta Filip, P.; Cuciureanu, D.; Sorina Diaconu, L.; Maria Vladareanu, A.; Silvia Pop, C. MALT lymphoma: Epidemiology, clinical diagnosis and treatment. *J. Med. Life* **2018**, *11*, 187–193. [\[CrossRef\]](#)
5. Nakamura, S.; Hojo, M. Diagnosis and Treatment for Gastric Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma. *J. Clin. Med.* **2023**, *12*, 120. [\[CrossRef\]](#)
6. Park, B.S.; Lee, S.H. Endoscopic features aiding the diagnosis of gastric mucosa-associated lymphoid tissue lymphoma. *Yeungnam Univ. J. Med.* **2019**, *36*, 85–91. [\[CrossRef\]](#)
7. Zenzri, Y.; Charfi, L.; Sahraoui, G.; Yahyaoui, Y.; Mrad, K.; Boujelbene, N.; Doghri, R. Gastric mucosa-associated lymphoid tissue (MALT) lymphoma: Clinicopathological study and treatment outcome in 50 patients. *Pan Afr. Med. J.* **2020**, *37*, 372. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Perez-Vera, P.; Reyes-Leon, A.; Fuentes-Panana, E.M. Signaling proteins and transcription factors in normal and malignant early B cell development. *Bone Marrow Res.* **2011**, *2011*, 502751. [\[CrossRef\]](#)
9. Langerak, A.W.; Groenen, P.J.T.A.; Brüggemann, M.; Beldjord, K.; Bellan, C.; Bonello, L.; Boone, E.; Carter, G.I.; Catherwood, M.; Davi, F.; et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia* **2012**, *26*, 2159–2171. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Collins, A.M.; Watson, C.T. Immunoglobulin Light Chain Gene Rearrangements, Receptor Editing and the Development of a Self-Tolerant Antibody Repertoire. *Front Immunol.* **2018**, *9*, 2249. [\[CrossRef\]](#)
11. Brüggemann, M.; Kotrova, M.; Knecht, H.; Bartram, J.; Boudjogha, M.; Bystry, V.; Fazio, G.; Froňková, E.; Giraud, M.; Grioni, A.; et al. Standardized next-generation sequencing of immunoglobulin and T-cell receptor gene recombinations for MRD marker identification in acute lymphoblastic leukaemia; a EuroClonality-NGS validation study. *Leukemia* **2019**, *33*, 2241–2253. [\[CrossRef\]](#)
12. Shin, S.; Hwang, I.S.; Kim, J.; Lee, K.A.; Lee, S.T.; Choi, J.R. Detection of Immunoglobulin Heavy Chain Gene Clonality by Next-Generation Sequencing for Minimal Residual Disease Monitoring in B-Lymphoblastic Leukemia. *Ann. Lab. Med.* **2017**, *37*, 331–335. [\[CrossRef\]](#)
13. Troppan, K.; Wenzl, K.; Neumeister, P.; Deutsch, A. Molecular Pathogenesis of MALT Lymphoma. *Gastroenterol. Res. Pract.* **2015**, *2015*, 102656. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Qin, Y.; Greiner, A.; Trunk, M.J.; Schmausser, B.; Ott, M.M.; Muller-Hermelink, H.K. Somatic hypermutation in low-grade mucosa-associated lymphoid tissue-type B-cell lymphoma. *Blood* **1995**, *86*, 3528–3534. [\[CrossRef\]](#)
15. Groenen, P.J.T.A.; van Raaij, A.; van Altena, M.C.; Rombout, P.M.; van Krieken, J.M.H. A practical approach to diagnostic Ig/TCR clonality evaluation in clinical pathology. *J. Hematop.* **2012**, *5*, 17–25. [\[CrossRef\]](#)
16. Lowman, G.M.; Pastushok, L.; Mochoruk, K.; Hill, W.; Toro, M.; Pickle, L.; Gonzalez, C.; Ostresh, S.; Sarda, S.; Yang, C.; et al. Evaluation of multiple myeloma research samples by analysis of B cell heavy and light chain receptors in a single NGS assay. *Cancer Res.* **2022**, *82* (Suppl. S12), 2293. [\[CrossRef\]](#)
17. Lowman, G.; Pastushok, L.; Mochoruk, K.; Hill, W.; Toro, M.; Pickle, L.; Gonzalez, C.; Ostresh, S.; Sarda, S.; Yang, C.; et al. Immune Repertoire Analysis of Multiple Myeloma Research Samples Using NGS Characterization of Multiple B Cell Receptors in a Single Reaction. *Blood* **2021**, *138*, 1881. [\[CrossRef\]](#)
18. Lowman, G.M.; Toro, M.; Pickle, L.; Ostresh, S.; Sarda, S.; Yang, C. NGS characterization of multiple immune receptors from a single multiplex PCR reaction. *Cancer Res.* **2021**, *81* (Suppl. S13), 2779. [\[CrossRef\]](#)
19. Pastushok, L.; Sarda, S.; Mochoruk, K.; Hill, W.; Pickle, L.T.; Toro, M.; Gonzalez, C.; Ostresh, S.; Looney, T.J.; Yang, C.; et al. A Novel Single-Tube Next Generation Sequencing Assay for B-Cell Receptor Clonality Testing. *J. Mol. Pathol.* **2024**, *5*, 45–65. [\[CrossRef\]](#)
20. Seitz, V.; Gennermann, K.; Elezkurtaj, S.; Groth, D.; Schaper, S.; Dröge, A.; Lachmann, N.; Berg, E.; Lenze, D.; Köhl, A.; et al. Specific T-cell receptor beta-rearrangements of gluten-triggered CD8(+) T-cells are enriched in celiac disease patients' duodenal mucosa. *Clin. Immunol.* **2023**, *256*, 109795. [\[CrossRef\]](#)
21. Van der Straeten, J.; De Brouwer, W.; Kabongo, E.; Dresse, M.F.; Fostier, K.; Schots, R.; Van Riet, I.; Bakkus, M. Validation of a PCR-Based Next-Generation Sequencing Approach for the Detection and Quantification of Minimal Residual Disease in Acute Lymphoblastic Leukemia and Multiple Myeloma Using gBlocks as Calibrators. *J. Mol. Diagn.* **2021**, *23*, 599–611. [\[CrossRef\]](#)
22. Ho, C.C.; Tung, J.K.; Zehnder, J.L.; Zhang, B.M. Validation of a Next-Generation Sequencing-Based T-Cell Receptor Gamma Gene Rearrangement Diagnostic Assay: Transitioning from Capillary Electrophoresis to Next-Generation Sequencing. *J. Mol. Diagn.* **2021**, *23*, 805–815. [\[CrossRef\]](#)
23. Martin, A.; Chahwan, R.; Parsa, J.Y.; Scharff, M.D. Chapter 20—Somatic Hypermutation: The Molecular Mechanisms Underlying the Production of Effective High-Affinity Antibodies. In *Molecular Biology of B Cells*, 2nd ed.; Alt, F.W., Honjo, T., Radbruch, A., Reth, M., Eds.; Academic Press: London, UK, 2015; pp. 363–388.

24. Nouri, N.; Kleinstein, S.H. Somatic hypermutation analysis for improved identification of B cell clonal families from next-generation sequencing data. *PLoS Comput. Biol.* **2020**, *16*, e1007977. [\[CrossRef\]](#)
25. Lefranc, M.P. IMGT databases, web resources and tools for immunoglobulin and T cell receptor sequence analysis, <https://imgt.cines.fr>. *Leukemia* **2003**, *17*, 260–266. [\[CrossRef\]](#)
26. Xu-Monette, Z.Y.; Li, J.; Xia, Y.; Crossley, B.; Bremel, R.D.; Miao, Y.; Xiao, M.; Snyder, T.; Manyam, G.C.; Tan, X.; et al. Immunoglobulin somatic hypermutation has clinical impact in DLBCL and potential implications for immune checkpoint blockade and neoantigen-based immunotherapies. *J. Immunother Cancer* **2019**, *7*, 272. [\[CrossRef\]](#)
27. Gupta, S.K.; Viswanatha, D.S.; Patel, K.P. Evaluation of Somatic Hypermutation Status in Chronic Lymphocytic Leukemia (CLL) in the Era of Next Generation Sequencing. *Front. Cell Dev. Biol.* **2020**, *8*, 357. [\[CrossRef\]](#)
28. Hauwel, M.; Matthes, T. Minimal residual disease monitoring: The new standard for treatment evaluation of haematological malignancies? *Swiss Med. Wkly.* **2014**, *144*, w13907. [\[CrossRef\]](#)
29. Fischbach, W.; Goebeler, M.E.; Ruskone-Fourmesttraux, A.; Wündisch, T.; Neubauer, A.; Raderer, M.; Savio, A. Most patients with minimal histological residuals of gastric MALT lymphoma after successful eradication of can be managed safely by a watch and wait strategy: Experience from a large international series. *Gut* **2007**, *56*, 1685–1687. [\[CrossRef\]](#)
30. Kiesewetter, B.; Simonitsch-Klupp, I.; Dolak, W.; Mayerhoefer, M.E.; Raderer, M. Depth of Remission Following First-Line Treatment Is an Independent Prognostic Marker for Progression-Free Survival in Gastric Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma. *Cancers* **2020**, *12*, 492. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Copie-Bergman, C.; Wotherspoon, A.C.; Capella, C.; Motta, T.; Pedrinis, E.; Pileri, S.A.; Bertoni, F.; Conconi, A.; Zucca, E.; Ponzoni, M.; et al. Gela histological scoring system for post-treatment biopsies of patients with gastric MALT lymphoma is feasible and reliable in routine practice. *Br. J. Haematol.* **2013**, *160*, 47–52. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Aguilar, C. Low-grade gastric MALT lymphoma as a second malignancy in chronic lymphocytic leukaemia. *Br. J. Haematol.* **2000**, *108*, 660–661. [\[CrossRef\]](#)
33. Wündisch, T.; Dieckhoff, P.; Greene, B.; Thiede, C.; Wilhelm, C.; Stolte, M.; Neubauer, A. Second cancers and residual disease in patients treated for gastric mucosa-associated lymphoid tissue lymphoma by *Helicobacter pylori* eradication and followed for 10 years. *Gastroenterology* **2012**, *143*, 936–942, quiz e13–4. [\[CrossRef\]](#)
34. Schuster, S.C. Next-generation sequencing transforms today's biology. *Nat. Methods* **2008**, *5*, 16–18. [\[CrossRef\]](#)
35. Shendure, J.; Ji, H. Next-generation DNA sequencing. *Nat. Biotechnol.* **2008**, *26*, 1135–1145. [\[CrossRef\]](#)
36. Pereira, M.I.; Medeiros, J.A. Role of *Helicobacter pylori* in gastric mucosa-associated lymphoid tissue lymphomas. *World J. Gastroenterol.* **2014**, *20*, 684–698. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Floch, P.; Megraud, F.; Lehours, P. *Helicobacter pylori* Strains and Gastric MALT Lymphoma. *Toxins* **2017**, *9*, 132. [\[CrossRef\]](#)
38. Hu, Q.; Zhang, Y.; Zhang, X.; Fu, K. Gastric mucosa-associated lymphoid tissue lymphoma and *Helicobacter pylori* infection: A review of current diagnosis and management. *Biomark. Res.* **2016**, *4*, 15. [\[CrossRef\]](#)
39. Wotherspoon, A.C.; Ortiz-Hidalgo, C.; Falzon, M.R.; Isaacson, P.G. *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. *Lancet* **1991**, *338*, 1175–1176. [\[CrossRef\]](#)
40. Gu, S.X.; Siddon, A.J.; Huntington, S.F.; Jain, D. *Helicobacter pylori*-negative mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach: A clinicopathologic analysis. *Am. J. Clin. Pathol.* **2023**, *160*, 612–619. [\[CrossRef\]](#)
41. Neubauer, A.; Thiede, C.; Alpen, B.; Ritter, M.; Neubauer, B.; Ehninger, G.; Morgner, A.; Bayerdörffer, E.; Wündisch, T.; Stolte, M. Cure of *Helicobacter pylori* infection and duration of remission of low-grade gastric mucosa-associated lymphoid tissue lymphoma. *J. Natl. Cancer Inst.* **1997**, *89*, 1350–1355. [\[CrossRef\]](#) [\[PubMed\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.