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Clinical activity and safety of combination immunotherapy with interferon-α2a and rituximab in patients with relapsed low grade non-Hodgkin's lymphoma

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Background and Objectives. To determine the clinical activity and safety of the combination immunotherapy of the chimeric anti-CD20 antibody, rituximab, and interferon (IFN)-α2a.

Design and Methods. Sixty-four patients with relapsed low-grade or follicular B-cell non-Hodgkin's lymphoma received 4 infusions of rituximab (375 mg/m^2 per infusion) after priming and simultaneous treatment with IFN-α2a.

Results. The overall response rate was 70% with 33% complete responses. The median duration of response is 19 months, after a median follow-up of 22 months. By univariate analysis none of the most common prognostic factors predicted for response to therapy. After treatment 10 patients became bcl-2 negative in the bone marrow, but no correlation between molecular and clinical response was found. Fifty-three patients (83%) had adverse events that were drug related or of unknown origin. The number of adverse events per patient varied from 1 to 21. Considering all 272 events, 231 (85%) were grade 1 or 2, 36 (13%) grade 3 and 5 (2%) grade 4. Twenty-three patients required a reduction in the dose and/or short discontinuation of IFN treatment, either during priming or subsequent treatment. The most frequent adverse events were leukopenia, fever, neutropenia, hypotension and thrombocytopenia.

Interpretation and Conclusions. This report shows that combination immunotherapy (rituximab + IFN-α2a) is active and relatively well tolerated. The overall response rate of 70% and the median duration of remission of 19 months compare favorably with the results obtained with rituximab alone in a similar subset of patients. Randomized trials investigating rituximab versus combination immunotherapy are needed.

Key words: clinical trials, hematologic malignancies, biological therapies.

The broad category of non-Hodgkin's lymphoma (NHL) includes a large number of distinct lymphoid neoplasms. The majority of NHL are derived from B-cells, with a sizable minority of about 15-20% arising from T-cells.[1]

Low-grade NHLs include small lymphocytic, follicular small cleaved cell, and follicular mixed categories in the Working Formulation.[2] These lymphomas are defined in the *Revised European American Lymphoma* (REAL) classification as indolent B-cell lymphomas.[3] They are considered to be those associated with a survival measured in years, independently of whether any therapy is applied. Because of their natural history and response to therapy, they have often been referred to as *good prognosis* or *favorable prognosis* lymphomas. The most common chemotherapies used are chlorambucil,[4] cyclophosphamide-doxorubicin-vincristine-prednisone (CHOP) or CHOP-like[5] and fludarabine-mitoxantrone-dexamethasone (FND).[6]

However, indolent lymphomas exhibit a tendency to relapse due to the incapacity of available

chemotherapeutic agents to eradicate the neoplastic clone and finally also exhibit a tendency to transform to a more aggressive large cell lymphoma. Patients eventually die from disease-related causes. Therefore, the development of novel therapeutic agents and strategies is required for this group of patients.

New approaches for indolent NHLs currently under investigation include (a) attempts to eradicate the disease using high-dose chemotherapy with stem cell rescue;[7] and (b) testing of new drugs or biological therapies. Among these, the use of rituximab appears a useful approach. Rituximab is a chimeric mouse/human anti-CD 20 antibody.[8] The CD20 antigen is expressed in the majority of B-cell lymphomas and in normal B-cells, but not in other normal tissues.[9-11] *In vitro*, rituximab binds human C1q, affecting both complement-dependent cytolysis (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC).[8,12] Also, *in vitro* experiments show that the antibody induces apoptosis.[13,14]

Rituximab alone has demonstrated significant clinical activity in the treatment of relapsed or refractory indolent lymphoma.[15-21]

The administration of interferon-α 2a (IFN-α 2a) before and during rituximab treatment could be effective in increasing CD-20 antigen surface expression;[22] furthermore, the immunomodulatory effects of IFN-α 2a, including stimulation of T-cell cytotoxicity and natural killer cell activity,[23-25] might synergize with rituximab to induce neoplastic clone suppression. Here we report the results of a recently completed phase II Italian study with IFN-α 2a plus rituximab for the treatment of relapsed indolent B-cell lymphomas.

Design and Methods

An open, non-comparative phase II trial was conducted at 16 centers in Italy from May 1997 through July 1999. The study was conducted according to the principles of the Declaration of Helsinki and had been approved by the Ethics Committee of the county of Modena and by local Ethics Committees at participating centers. All patients were required to provide written informed consent before entering the study.

Objectives of the study

Objectives of this study were to evaluate clinical activity and safety of repeated doses of rituximab after priming with IFN-α 2a. Secondary objectives were to evaluate duration of remission and the percentage of patients who became bcl-2 negative in bone marrow (BM) after treatment.

Table 1. Patient characteristics.

	n.	%
Age at study entry (year)	Median 54	Range 29-74
Sex		
Female	43	67
Male	21	33
Histological grade[a] at study entry		
Small lymphocytic	7	11
Follicular grade I	22	34
Follicular grade II	28	44
Follicular grade III	7	11
Relapses prior to study entry		
1	25	39
2	23	36
3	16	25
Disease stage at diagnosis		
II B	10	16
III	12	19
IV	42	65
Prior therapies		
Chemotherapy	64	100
IFN	10	16
Radiotherapy	6	9
ABMT	11	17
N° of CHT Regimens		
1 CHT regimen	31	48
2 CHT regimens	20	31
3 CHT regimens	12	19
4 CHT regimens	1	2

[a]*Based on the REAL classification.*[3]

Patient population

Sixty-four patients entered this trial and their clinical characteristics at the time of the study entry are listed in Table 1.

Inclusion criteria were: diagnosis of small lymphocytic or follicular center lymphoma according to the REAL classification[3] or categories A (only if total lymphocyte count at the time of diagnosis and relapse < 5000/mm^3), B, C, or D of the Working Formulation,[2] histologically confirmed at the time of relapse, expressing CD20 antigen; age 18-75 years; stage II B-IV; active phase of the disease, as demonstrated by the presence of at least one of the following signs or symptoms: lactate dehydrogenase (LDH) upper normal limit, B symptoms, bulky disease, leukemic phase, β_2-microglobulin upper normal limit, fast growing tumor, i.e. short doubling time of the tumor mass (<12 months); two-dimensionally measurable disease in at least one site; no more then 3 previous lines of chemotherapy; life expectancy > 6 months;

absence of renal, hepatic and respiratory failure; ECOG performance status 0-2; written informed consent. Exclusion criteria were: pregnancy, lactation, or refusal to employ an accepted method of birth control for fertile woman; bilirubin >2 mg/dL; serum glutamic-oxalacetic transaminase/serum glutamic-pyruvic transaminase (AST/ALT) > 2 × the upper limit of normal range; serum creatinine > 2 mg/dL; other chemotherapy or immunotherapy, concurrent or in the previous 15 days; serious concurrent diseases, i.e. cardiac (including clinically significant abnormalities of ECG, arrhythmias or acute myocardial infarction in the preceding 6 months); thromboembolic disease (concurrent); psychiatric or psychological conditions which could interfere with the patient's ability to understand the trial requirements, give informed consent and participate in the trial; other concomitant neoplasm with the exception of skin basal carcinoma or *in situ* carcinoma of the uterine cervix; history of repeated active severe infections or infections uncontrolled by treatment; alcoholism or drug addiction; HIV or HBs Ag positivity.

Treatment design

Treatment started with IFN-α 2a 1.5 MU/day × 5 s.c. for the first week and then at 3.0 MU/day × 5 s.c. for the second week. On day 15, patients received the first rituximab i.v. injection at a dose of 375 mg/m^2 that was repeated on days 22, 29, and 36. IFN-α 2a dose was maintained at 3 MU during the third week and then increased to 6 MU during the fourth and fifth weeks. The treatment schedule is detailed in Figure 1.

Roche S.p.a. (Milan, Italy) provided the IFN-α 2a and rituximab for the clinical trial. Rituximab was administered on an outpatient basis. The initial rate of infusion was 50 mg/h. The dose rate was increased by 50 mg/h every 30 minutes to a maximum of 300 mg/h, if no toxicity was observed. The infusion was interrupted if severe serious events occurred and then resumed at half the previous rate, after the event resolved. Patients at risk of tumor lysis syndrome were hydrated and treated with allopurinol before the first dose of rituximab. Oral pre-medication with acetaminophen and diphenhydramine hydrochloride was allowed.

Monitoring

Before entering the clinical trial, all patients underwent baseline evaluation that included history, physical examination, complete blood counts, chemistry profile, computerized tomography of the neck, chest, abdomen and pelvis, and bone marrow aspiration/biopsy.

Monitoring included frequent physical examinations, hematology and serum chemistry profiles, and full tumor re-staging evaluation one month following the fourth infusion, then every 3-6 months until relapse in responders.

Figure 1. Dosing schedule.

Polymerase chain reaction assay for t(14;18)

Paraffin-embedded, diagnostic lymph nodes specimens as well as mononuclear peripheral blood and bone marrow cells collected at the time of the study entry and during the post-treatment re-staging were subjected to nested polymerase chain reaction (PCR) for the bcl-2 major breakpoint region (MBR) and for the minor cluster region (mcr) with primers and conditions described by Gribben *et al.*[26] DNA from two follicular lymphomas with known t (14;18), one involving the MBR and the other the mcr, were run with each group of cases as positive controls. Serial dilutions of control PCR samples defined that the limit of sensitivity for the technique was one tumor cell in 10^5 normal cells.

Response criteria

Response criteria included complete remission (CR), complete disappearance of all previously detectable disease including complete disappearance of leukemic cells from the peripheral blood and absence of bone marrow leukemic cell infiltration for a period of at least 28 days, and no new lesions; partial

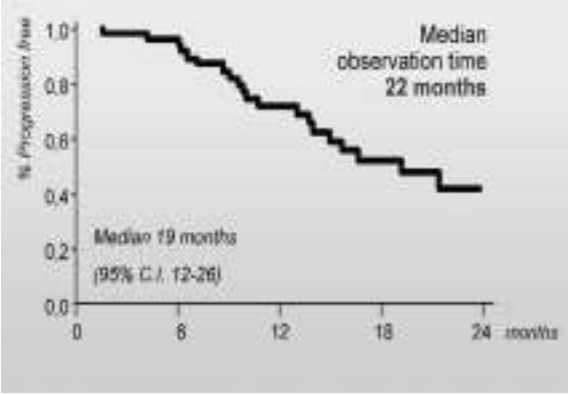

Figure 2. Duration of remission in 45 responders (21 CRs and 24 PRs).

Table 2. Clinical response.

Response	*Patients*	
	n.	*%*
CR	21	33
PR	24	37
SD	9	14
PD	2	3
Dropouts	8	13
Total	64	100

Table 3. Timing and reasons for withdrawal patients from study.

Patient	*Timing*	*Reason*	*Outcome*	*Specification*	*Relationship to treatment*
63	Week 1	Protocol violation	–	Lung cancer	–
1	Week 2	S.A.E.	Death	Bronchopneumonia	Not related
17	Week 2	S.A.E.	Death	Dehydration and oligoanuria	Not related
26	Week 3	S.A.E.	Recovery	Chills, dyspnea, nausea and fever during the first rituximab infusion	Related
29	Week 3	S.A.E.	Recovery	Abdominal and lumbar pain, muscular rigidity, chills, cyanosis, fever during the first rituximab infusion	Related
39	Week 3	S.A.E.	Recovery	Chills, cyanosis, fever, vomiting during the first rituximab infusion	Related
14	Week 5	S.A.E.	Recovery	Facial rash and mucosal congestion during the 3 rituximab administration. Successively fever and hypotension	Related
8	Week 9	S.A.E.	Death	Cardiac arrest	Of unknown origin

Abbreviation = SAE: serious adverse event.

remission (PR), more than 50% reduction in the sum of the products of the perpendicular diameters of all measurable disease for a period of at least 28 days, and no new lesions; stable disease (SD), less than 50% reduction and less than 25% increase in the sum of the products of the perpendicular diameters of all measurable disease and no new lesions; and progressive disease (PD), an increase in size of more than 25% of previously documented disease, or the appearance of disease at any sites.

Adverse events

Adverse events were classified as having the following relations with drugs: 1) unrelated (definitely not drug-related); 2) related (remotely, possibly, probably or definitely drug-related); 3) of unknown origin. Toxicity grade was evaluated using the WHO criteria. Investigators classified adverse events by relationship and severity.

Statistical methods

Overall survival (OS) and duration remission (DR) were analyzed by the Kaplan-Meier method.[27] Comparisons of clinical response data by individual prognostic variables were performed using Fisher's exact test. Clinical adverse events were analyzed by calculating the number and percentage of patients and events. If an individual event had more than one grade, the most severe grade was used to characterize this unified event.

Results

Clinical activity

The overall response (OR) rate for all 64 patients using intent-to-treat analysis was 70% of which 33% CRs. Stable disease and progressive disease occurred in 14% and 3% of the patients, respectively (Table 2). Thirteen percent of patients dropped out of the trial. A univariate analysis of baseline prognostic factors such as age, sex, disease duration, histologic type, bone marrow infiltration, bcl-2 status in bone marrow, peripheral blood and lymph node

Table 4. Bcl-2 status before and after treatment, clinical response and duration of remission.

Patient	bcl-2 status				Clinical response	Duration of remission (months)
	before		after			
	BM	PB	BM	PB		
7	ND	+	-	-	CR	19
19	+	+	-	-	CR	14
23	ND	+	ND	-	CR	23+
40	ND	+	ND	-	CR	7
9	+	+	-	-	PR	30+
18	+	ND	-	ND	PR	26+
34	+	+	-	-	PR	19+
37	+	+	-	-	PR	18+
47	+	+	-	-	PR	16
49	+	+	-	-	PR	14+
55	+	+	+	-	PR	12+
62	+	+	+	-	PR	8+
51	+	ND	-	ND	SD	13+
57	+	-	-	-	SD	11+

Abbreviation = BM: bone marrow; PB: peripheral blood; + : persistence of clinical response; ND: not done.

biopsies, clinical stage, previous autologous bone marrow transplantation (ABMT), number of previous chemotherapies, and number of relapses, demonstrated that none of these factors predicted for response to treatment (all p values ≥ 0.05). After a median follow-up of 19 months (21 months for patients alive), 8 patients have died and 56 are alive. The 3-year overall survival rate is 80%. With a median observation time of 22 months, the median duration of remission is 19.2 months (95% confidence interval, 12-26 months) (Figure 2). Five patients who had obtained PR after IFN + rituximab were subsequently treated with local radiotherapy (2 patients), short course IFN (2 patients) and 2 courses of CHOP regimen (1 patient). Two patients who had a partial response at the end of treatment, after 1 month obtained complete response (disappearance of lymph nodes) at the subsequent evaluation at 3 months.

Bcl-2 status

At study entry, paraffin-embedded, formalin-fixed biopsies of 35 out of 64 patients were analyzed for

Table 5. Adverse events, related and of unknown origin, reported during the treatment period in 53 patients.

	GRADE I		GRADE II		GRADE III		GRADE IV		TOTAL	
	Events	n*	Events	n.	Events	n	Events	n	Events	n
Any adverse event	114	9	117	24	36	16	5	4	272	53°
									n (%)	n (%)
Leukopenia	17	6	29	18	6	5	–	–	52 (19)	29 (55)
Fever	17	9	13	8	2	2	–	–	32 (12)	19 (36)
Neutropenia	3	1	12	7	9	5	–	–	24 (9)	13 (25)
Hypotension	11	6	9	7	1	1	–	–	21 (8)	14 (26)
Thrombocytopenia	5	3	5	4	3	2	2	2	15 (6)	11 (21)
Hepatic toxicity	7	2	6	3	1	1	–	–	14 (5)	6 (11)
Chills	3	1	9	6	–	–	1	1	13 (5)	8 (15)
Nausea	5	4	1	1	4	2	–	–	10 (4)	7 (13)
Asthenia	4	4	5	4	1	1	–	–	10 (4)	8 (15)
Vomiting	3	1	3	3	3	3	–	–	9 (3)	7 (13)
Anemia	6	4	2	2	1	1	–	–	9 (3)	7 (13)
Cutaneous rush	4	4	5	4	–	–	–	–	9 (3)	8 (15)
Headache	6	3	2	2					8 (3)	5 (9)
Myalgia	3	3	4	4			–	–	7 (2)	7 (13)
Pain	4	4	2	2	1	1	–	–	7 (2)	7 (13)
Tachycardia	2	2	1	1	–	–	–	–	3 (1)	3 (6)
Dyspnea	1	1	1	1	–	–	1	1	3 (1)	3 (6)
Diarrhea	3	2	–	–	–	–	–	–	3 (1)	2 (4)
Mucosal congestion	1	–	1	1	1	1	–	–	3 (1)	2 (4)
Angioedema	2	2	1	1	–	–	–	–	3 (1)	3 (6)
Depression	–	–	2	1	1	1	–	–	3 (1)	2 (4)
Paresthesiae	2	2	1	1	–	–	–	–	3 (1)	3 (6)
Cyanosis			2	2	–	–	–	–	2 (1)	2 (4)
Pruritus	1	1	–	–	1	1	–	–	2 (1)	2 (4)
Other categories#	4	2	1	1	1	1	1	1	6 (2)	4 (8)

**Number of patients; patients are counted only under worst grade experienced; °no adverse events were reported in 8 patients; 3 patients had not related adverse events; #other categories include adverse events reported once only.*

bcl-2 status: 19 (54%) were negative, and 16 (46%) were positive.

Before treatment, 39 patients were tested for bcl-2 status in peripheral and bone marrow blood: 18 (46%) and 24 (62%) were positive, respectively. After treatment, 14 patients changed bcl-2 status, 8 becoming negative either in BM or PB, 2 in BM without PB analyzed, 2 in PB, remaining positive in BM, 2 in PB without BM analysis. Table 4 compares bcl-2 negative status after treatment with clinical response and DR.

Adverse events

During the treatment period (the time interval between the first IFN injection and 1 month after the end of treatment) 309 adverse events were reported. Fifty-six (88%) patients out of 64 who started treatment had toxic effects. The number of events per patients varied from 1 to 21. Considering all 309 events 83% were grade 1 or 2, 14% grade 3 and 3% grade 4. Regarding the relation between treatment and toxicity, 37 (12%) were not related, 231 (75%) were related and 41 (13%) of unknown origin. Fifty-three patients (83%) had drug-related adverse events or events of unknown origin. The most frequent adverse events, related and of unknown origin, are summarized by grade in Table 5.

Five grade 4 events were reported in 4 patients and they were: thrombocytopenia (2), cardiac arrest (1), bronchospasm (1) and chills (1). During the phase of IFN priming before rituximab treatment (first and second weeks) patients had 51 adverse events (19%) of which 10 were grade 3 in 6 patients and 1 grade 4. Twenty-three patients required a reduction in the dose and/or a short discontinuation of IFN treatment, either during priming or subsequent treatment.

The timing and reasons for withdrawing patients from study are listed in Table 3.

Discussion

Interferon-α is a pleiotropic cytokine. Understanding the biological activity of IFN-α *in vivo* is complicated by the well-known redundancy and ambiguity of cytokine actions, as well as the capacity to induce the production of other cytokines. IFN exerts various effects on the immune system, including modulation of immunoglobulin production, inhibition of T-suppressor cell function, stimulation of T-cell cytotoxicity, monocyte/macrophage functions and natural killer cell activity, an integral part of ADCC.[23-25,28-30] IFN enhances expression of class I major histocompatibility (MHC) antigens, and *in vivo* can increase the frequency of blood leukocytes that express class II MHC antigens.[31,32] Furthermore, interferons can up-regulate neoplastic antigen expression on the surface of human carcinoma cells and can augment the localization of a radiolabeled monoclonal antibody to the tumor site.[33] In mice, IFN-α is able to enhance tumor uptake of an anti-melanoma monoclonal antibody.[34] Recently a study on B-CLL-cells *in vitro* showed that IFN-α is able to induce overexpression of CD20 antigen on cell surfaces.[22] Although the mechanism of action of rituximab is not completely defined, the antibody binds human C1q and affects both CDC and ADCC.[8,12]

The administration of IFN-α 2a before and during rituximab treatment may be effective in enhancing the efficacy of this treatment increasing the surface expression of CD20. Furthermore, the immunomodulatory effect of IFN-α, including stimulation of T-cell cytotoxicity and natural killer cell activity, might synergize with the rituximab-dependent cell-mediated cytotoxicity in inducing neoplastic clone suppression. The results obtained in our multicenter trial showed that combination immunotherapy with IFN and rituximab was active and relatively well tolerated. The vast majority of adverse events, whether related or of unknown origin, (231, 85%) were grade 1-2. Regarding the 36 grade 3 events, hematologic toxicity accounted for 19 events including, leukopenia, neutropenia, thrombocytopenia and anemia, mostly related to IFN. Four patients presented 5 grade 4 events, of which four completely resolved. Our efficacy results show an ORR of 70% (33% CR and 37% PR) and a median DR of 19 months, after a median follow-up of 22 months. In the pivotal study,[18] the ORR was 48% (6% CR and 42% PR), median TTP for responders was 13 months and 15% of patients presented 20 grade 3-4 related adverse events. Because the characteristics of the patients are substantially similar in the two series of patients, our results compare favorably with those obtained in the pivotal trial, even considering the limitation of a historical control.

A recently published study[35] with rituximab (375 mg/m^2 once weekly $\times$ 4) given in combination with IFN-α (5 MU s.c., 3 times weekly $\times$ 12 weeks) in 38 patients with relapsed low-grade or follicular NHL, demonstrated that this treatment was active and well tolerated. The median DR was 22 months, while ORR and toxicity were similar to those obtained in the pivotal study.[18] The improvement of DR is consistent with the results of combination trials of IFN and chemotherapy in low-grade NHL patients, in which IFN was demonstrated to prolong TTP.[36-38] In our study the molecular biology analysis results show that 10 out of 24 patients who were bcl-2$^+$ in the BM became negative after treatment, but no correlation between molecular and clinical response could be

established. Several patients reached BM clearance, obtaining only a clinical PR or SD, because of persistence of enlarged lymph nodes. Thus these results are consistent with a different efficacy of this treatment in different sites (PB, BM, and lymph nodes). No statistically significant differences between various prognostic factors and response was identified by univariate analysis. These results are in agreement with those of the other study in which a combination of rituximab and interferon was utilized,[35] but both are in contrast with the results of the pivotal study.[18] In our series patients of female gender, with follicular grade I and II, previous ABMT, and a low number of previous chemotherapies showed a trend for better response but the relationship was never statistically significant. The results of our trial show that combination therapy with IFN and rituximab is feasible and relatively safe. This association could increase both ORR and DR. In the other combination trial,[35] treatment appeared to be able to prolong the TTP. Thus we think that randomized trials investigating rituximab alone versus combination therapy are needed. If these promising results are confirmed, combination therapy with IFN and rituximab could be utilized in a number of new possibilities. In previously untreated patients, who may have difficulty tolerating chemotherapies, the association could be an excellent treatment option. In patients with minimal residual disease after chemotherapy, alone or associated with rituximab, combination treatment could be effective as a maintenance therapy, in prolonging DR. In conclusion, the results obtained with combination therapy provide the basis for further developments of targeted biological agents for patients with malignant lymphomas.

Contribution and Acknowledgments

SS, MF, MB and GD fully and directly participated in the conception and design of the study. SS and GV wrote the manuscript. UV, CB, DV, LB, MP, SR, FDR, FM, VL, AT and GS participated in the patients' care, data recording, interpretation and analysis. All authors contributed critically to the drafting of the article and gave final approval of the study. We would like to thank Maria Giovanna Vannini and Monica Civallero for their expert technical assistance.

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Disclosures

Conflict of interest: none.

Redundant publications: yes, <50% (The contents of this manuscript have not been published elsewhere, except for an abstract presented in Miami at the 1999 ASH Meeting and an interim analysis presented as a short report published in June 2000 in Biological Therapy of Lymphoma, which is a sponsored, non-peer-reviewed journal).

Manuscript processing

This manuscript was peer-reviewed by rttwo external referees and by Professor Pierre Solal-Celigny, who acted as an Associate Editor. The final decision to accept this paper for the publication was taken jointly by Prof. Solal-Celigny and the Editors. Manuscript received June 1, 2000; accepted August 23, 2001.

Potential implications for clinical practice

Our results show that combination immunotherapy with IFN and rituximab is active, relatively well tolerated and compares favorably with treatment of historical controls, providing the basis for further developments of targeted biological agents in malignant lymphomas. This combination could be utilized either as a first line treatment in patients who may have difficulties tolerating chemotherapy or as a maintenance therapy in patients with minimal residual disease after chemotherapy.[39,40]

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