

Achieving a Major Molecular Response at the Time of a Complete Cytogenetic Response (CCgR) Predicts a Better Duration of CCgR in Imatinib-Treated Chronic Myeloid Leukemia Patients

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Abstract Purpose: Most patients with chronic-phase chronic myeloid leukemia (CML) who receive imatinib achieve a complete cytogenetic remission (CCgR) and low levels of BCR-ABL transcripts. CCgR is durable in the majority of patients but relapse occurs in a subset.

Experimental Design: To determine the potential of quantitative reverse transcription-PCR of BCR-ABL to predict cytogenetic relapse, we serially monitored residual disease in 97 CML patients with an imatinib-induced CCgR. Patients with late chronic phase CML after IFN- α failure were treated with imatinib (400 mg daily).

Results: During the imatinib median follow-up time of 36 months (range, 12-54 months), disease monitoring occurred by cytogenetics and quantitative PCR. Twenty percent of patients experienced cytogenetic relapse at a median of 18 months after CCgR and a median of 24 months after starting imatinib. None of the possible prognostic factors studied in univariate and multivariate analyses seemed to predict for loss of cytogenetic response but the reduction of BCR-ABL transcript levels at the time of CCgR is an important prognostic factor.

Conclusions: In our study, we showed not only that achieving a major molecular remission at 12 months is predictive of a durable cytogenetic remission but also that patients who achieved a major molecular remission (expressed both as the BCR-ABL/ β 2 microglobulin ratio % <0.0005 and as a 3-log reduction from median baseline value) already at the time of first achieving a CCgR have significantly longer cytogenetic remission durations than those without this magnitude of molecular response ($P < 0.05$).

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The introduction of imatinib mesylate, a potent and selective tyrosine kinase inhibitor, into chronic myeloid leukemia (CML) therapy has marked a major advance in CML treatment with regard to efficacy and lack of adverse reactions. The beneficial effect of imatinib was shown in the chronic phase, advanced phase, and in blast crisis, as well as in Philadelphia-positive acute lymphatic leukemia. Imatinib induces complete cytogenetic response (CCgR) in ~50% of patients treated after failure of IFN- α and in at least 80% of patients who start treatment soon after diagnosis (1-5). In comparison with earlier treatments, imatinib seems to prolong survival or progression-free survival in both categories of patients (6). On the basis of survival data of IFN-treated patients with CML who achieved CCgR, a 10-year-survival rate of 70% to 85% was estimated for imatinib-treated patients (7, 8).

The proper follow-up of imatinib-treated patients is based on cytogenetic (conventional and fluorescence *in situ* hybridization, as appropriate) and molecular techniques. Particularly, once Ph negativity is achieved, residual leukemia can best be monitored by measuring the number of BCR-ABL transcripts, which presumably reflect the survival of a small number of leukemia cells through molecular quantification; results are

usually expressed as a percentage ratio related to an internal control transcript. The long-term molecular follow-up of these patients would make it possible to evaluate the overall and major molecular response rates and the prognostic effect of different levels of BCR-ABL transcript reduction, given the same complete cytogenetic result. The clinical significance of molecular response determined by PCR has been established in CML after bone marrow transplantation and IFN- α therapy (9). Patients with at least a 3-log reduction of BCR-ABL transcript levels after 12 months of therapy had a significantly better probability of disease-free survival compared with those in CCgR but with a <3-log reduction of BCR-ABL (10). In this study, we investigated the potential of quantitative reverse transcription-PCR (RT-PCR) of BCR-ABL transcript to predict cytogenetic relapse in 97 late chronic phase CML patients enrolled onto the CML/002/STI571. Our data showed that patients with a major molecular remission at the time of first achieving a CCgR had a significantly longer cytogenetic remission durations than patients without this molecular response.

Patients and Methods

Patients. The patients treated belonged to the study protocol CML/002/STI571 designed by the GIMEMA Working Party on CML. Patients were required to have Ph-positive chronic-phase CML after IFN- α failure because of hematologic or cytogenetic resistance or relapse or because of IFN- α toxicity. Chronic-phase CML was defined as the presence in the peripheral blood and bone marrow of blasts <15%, basophils <20%, blasts together with promyelocytes <30%, and platelets $>100 \times 10^9/L$. Failure of the hematologic response to IFN- α was defined as hematologic resistance (failure to achieve a complete hematologic response after at least 6 months of IFN- α) or relapse ($>30\%$ increase in Ph-positive metaphases on two occasions or $\geq 65\%$ increase in Ph-positive metaphases on one occasion). Intolerance of IFN- α therapy was defined as grade 3 or 4 nonhematologic toxicity.

Patients received 400 mg of imatinib alone, once daily at the same dosage until disease progression. Criteria for dose reductions and treatment discontinuation have been described in a previous study (11). The median age at the time of imatinib start was 55 years (range, 29-74 years).

Cytogenetic and molecular studies. Cytogenetic studies were done by standard banding techniques and at least 20 metaphases were analyzed. The cytogenetic response was rated according to the proportion of Ph⁻ metaphases as complete (Ph⁻ 100%), partial (Ph⁻ 66-99%), minor (Ph⁻ 34%-65%), and minimal or none (Ph⁻ $\leq 33\%$). Cytogenetic relapse (loss of CCgR) was defined by the detection of one or more Ph-positive marrow metaphases. For the cytogenetic analysis, bone marrow samples (5 mL) were collected at baseline, after 3 and 6 months, at the end of study treatment (12 months), and thereafter every 6 months. BCR-ABL transcripts were detected by real-time quantitative RT-PCR analysis on bone marrow aspirate and on peripheral blood. Samples were collected before treatment (baseline), after 3 and 6 months, and at the end of the study treatment period (12 months). Subsequent samples were obtained every 6 months only from the patients who were in CCgR. The frequency with which cytogenetic analysis and quantitative RT-PCR were done was equivalent for all patients. Total leukocytes were extracted from 3 to 5 mL of bone marrow aspirate and 10 to 20 mL of peripheral blood after separation on a Ficoll Hypaque gradient. Mononuclear cells were resuspended in 500 μ L of guanidinium thiocyanate and stored at -20°C . Total RNA was isolated using the RNeasy kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. RNA quality was assessed on an ethidium bromide-stained 2% agarose gel.

Minimal residual disease was detected during the follow-up by a standardized quantitative RT-PCR method that was established within

the framework of the EU Concerted Action (12, 13). The method independently measures in each sample by real-time PCR the copy number of mRNA encoding for the p210 BCR/ABL protein and for a control gene to verify sample-to-sample RNA quality variations. In this study, $\beta 2$ microglobulin was selected and used as a control gene. Real-time quantitative RT-PCR was done on an ABI Prism 7700 Sequence Detector (Perkin-Elmer, Foster City, CA). The quantification principles and procedure using the TaqMan probe have been previously described (11, 14). All real-time RT-PCR experiments were done in duplicate. The copy number of BCR-ABL and $\beta 2$ microglobulin transcripts was derived by the interpolation of threshold cycle (Ct) values to the appropriate standard curve, and the result for each sample was expressed as a ratio of BCR-ABL mRNA copies to $\beta 2$ microglobulin mRNA. The threshold was systematically set at 0.1 to avoid any particular problems of baseline creeping. The lowest level of detectability of the method is 10^{-5} .

Statistics. Complete cytogenetic remission duration was considered from the time a CCgR was first achieved to the time when any Ph-positive metaphases were first detected again. Survival was calculated from the time the treatment began until death of any cause or last follow-up. Univariate analyses to identify prognostic factors for cytogenetic relapse were carried out using the long-rank test. The probability of remission was estimated using the Kaplan-Meier product limit method and compared by means of the long-rank test. Comparison of means was made with the *t* test and comparison of frequencies with the χ^2 test or the Fisher's exact test, as appropriate. Median ranges between pairs of continuous variables were analyzed by the Wilcoxon rank test. The significance level for all statistical tests was 0.05. Overall cytogenetic and molecular responses were calculated on the basis of all patients recruited into the study; response at the specific time points refers to the number of sample analyzed at these time points. All statistical calculations were done using GraphPad Prism 4 (GraphPad Software, Inc., San Diego, CA).

Results

Cytogenetic response and its durability. Among 114 patients who obtained a CCgR, 97 patients were valuable for molecular analysis. The median time from start of imatinib therapy to achievement of CCgR was 6 months (range, 2-12 months). The median imatinib follow-up time was 36 months (range, 12-54 months). Median BCR-ABL/ $\beta 2$ microglobulin ratio % before the start of imatinib was 0.3264 (range, 0.0062-2.2236) on bone marrow and was 0.093 (range, 0.0001-1.472) on peripheral blood. At the time of first achieving CCgR, BCR-ABL RNA levels had decreased by a median of 2 logs below the median baseline level, both in bone marrow and in peripheral

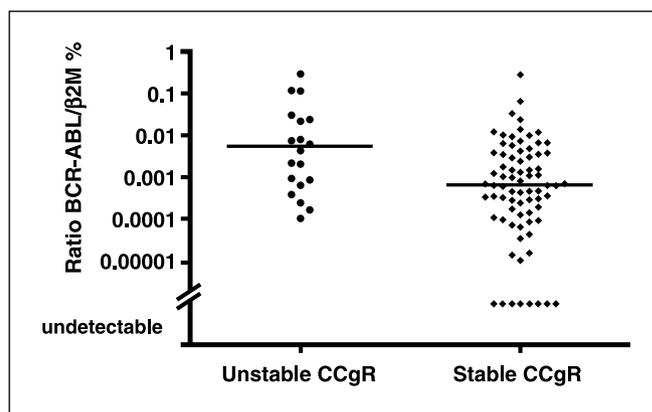


Fig. 1. Schematic representation of molecular responses on peripheral blood before starting imatinib and at the time of first achieving CCgR. The transcript level is expressed as the ratio of BCR-ABL to $\beta 2$ microglobulin $\times 100$. Values are median.

Table 1. Analysis of various clinical and biological characteristics at the beginning and during treatment to predict for a sustained CCgR

Variable	All patients	Patients who lost CCgR (n)	P
Sex			0.13
Female	41	12	
Male	56	7	
Age (y)			1.00
≤65	82	16	
>65	15	3	
Disease history			0.96
Intolerant to IFN	20	3	
Hematologic resistance to IFN	32	6	
Cytogenetic resistance to IFN	45	8	
Time from diagnosis to start of imatinib (mo)			1.00
≤24	12	2	
>24	85	17	
Time to CCgR (mo)			0.29
≤6	68	11	
>6	29	8	
Log reduction at the time of CCgR			0.006
≥3 log	37	1	
<3 log	60	18	
BCR-ABL/β2 microglobulin ratio % at the time of CCgR			0.01
≤0.0005	39	2	
>0.0005	58	17	

blood samples. Median BCR-ABL/β2 microglobulin ratio % at the time of CCgR was 0.0034 (range, 1×10^{-5} -0.8460) and 0.0009 (range, 5.7×10^{-6} -0.2855) in bone marrow and peripheral blood samples, respectively (Fig. 1). During subsequent follow-up among 97 patients, 19 (20%) developed cytogenetic relapse (defined as any Ph-positive metaphase cell) and the remaining (78, 80%) were still in CCgR at last contact. The median time to loss of CCgR was 18 months (range, 2-63 months) from CCgR and 24 months (range, 6-41 months) after starting imatinib. Among the patients with cytogenetic relapse, 3 proceeded to 100% Ph positivity, 5 patients presented with cytogenetic response becoming minor, and 11 patients achieved major cytogenetic remission (1-35% Ph-positive marrow metaphases). Three (16%) patients with relapse progressed to accelerated or blastic phase. We investigated various clinical and biological characteristics at the beginning of treatment and during it to predict for a sustained CCgR. As shown in Table 1, sex, median age at the time imatinib treatment was started, median time from diagnosis to the start of imatinib therapy, disease history (intolerance/hematologic resistance/cytogenetic resistance to prior IFN-α therapy), and the time to achieve CCgR were not able to predict for cytogenetic relapse.

Molecular response. The BCR-ABL transcript levels at the time of first achieving CCgR were significantly less ($P = 0.0006$) in

those patients with a stable cytogenetic response than in patients with cytogenetic relapse. In fact, median BCR-ABL/β2 microglobulin ratio % was 0.0007 (range, 0.116×10^{-6} - 5.7×10^{-6}) in patients with sustained CCgR and 0.0200 (range, 0.285-0.00004) in patients who lost CCgR (Fig. 2) on peripheral blood analysis.

We investigated the CCgR duration according to achievement of a major molecular response. For this analysis, only patients who had both a molecular analysis and were still on therapy and in CCgR are valuable. The definition of molecular responses is still evolving. We considered a major molecular response as reaching an absolute value of BCR-ABL/β2 microglobulin ratio ≤ 0.0005 (8, 15-17), a value that has been found predictive of duration of cytogenetic response by our group and others. At the time of first achieving CCgR, a major molecular response (ratio % ≤ 0.0005) was achieved in 39 of 97 (40%) patients. Other studies (10) used a 3-log reduction compared with a baseline value rather than an absolute value as a measure of molecular response for patients treated with imatinib. We thus repeated the analysis using a 3-log reduction as a measure of major molecular response. For this purpose, published reports have used the median calculated from measuring pretreatment values from a small number of patients as baseline levels. For our analysis, we calculated a 3-log reduction in two different ways. First, we used the median of the total population (0.093) as the baseline value. In this way, we obtained a major molecular response in 42% (41 of 97) of peripheral blood samples. We then used the pretreatment BCR-ABL value of each individual patients as their own baseline to determine log reduction and we found no difference between two ways. In fact, we observed a 3-log reduction in 38% (37 of 97) of patients. Patients who achieved a major molecular remission (expressed both as the ratio % and as a 3-log reduction) at the time of first achieving CCgR have significantly longer cytogenetic remission durations than those without this magnitude of molecular response ($P < 0.05$; Fig. 3). Only one of patients who have achieved a major molecular response (defined as a 3-log reduction) at the time of first achieving CCgR has lost CCgR compared with 18 patients not reaching these degrees of response. Table 2 shows the patterns of molecular response at the time of CCgR and its relationship with the loss of CCgR.

To further define the long-term prognostic implications of molecular response, we investigated the CCgR duration

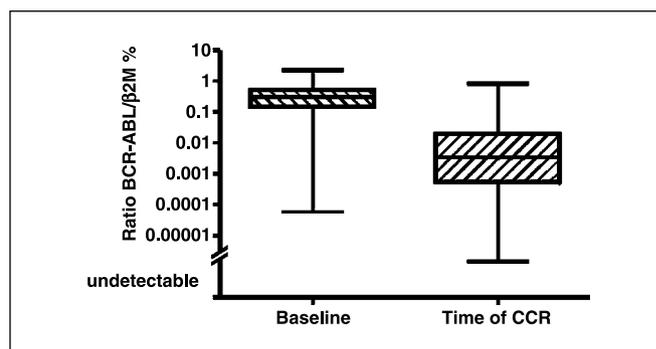


Fig. 2. Molecular responses on peripheral blood in patients with stable CCgR and in patients who lost CCgR at the time of CCgR. The lowest limit of detectability of the method is 0.00001. The BCR-ABL transcript level at the time of CCgR was significantly less in the patients with stable CCgR.

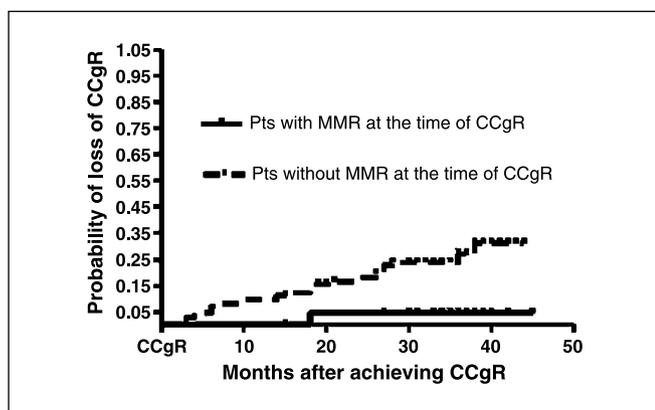


Fig. 3. Probability of loss CCgR. Patients (*Pts*) who achieved a major molecular remission (expressed both as the ratio % and as 3-log reduction) at the time of first achieving CCgR have significantly longer cytogenetic remission durations than those without this magnitude of molecular response ($P < 0.05$).

according to molecular response at 12 months of treatment. We observed that patients who achieved a 3-log reduction of BCR-ABL transcript level or a ratio % ≤ 0.0005 by 12 months after the start of therapy have significantly longer cytogenetic remission durations than those without this magnitude of molecular response ($P = 0.021$ and $P = 0.013$, respectively; Fig. 4). These results show that achieving a major molecular response at the time of first achieving CCgR or after 12 months of therapy is predictive of cytogenetic remission duration.

In the patients with sustained CCgR (78 of 97), the BCR-ABL transcript level continued to decrease over time until reaching a median of BCR-ABL/ $\beta 2$ microglobulin ratio % of 0.0002 at 48 months of treatment, whereas in patients who lost CCgR, we observed an increasing of the level of BCR-ABL during subsequent follow-up. In these patients, the median value of BCR-ABL/ $\beta 2$ microglobulin at 48 months was 0.0300. The probability of survival at 4 years of imatinib in these patients with late chronic phase disease is 60% for patients with relapse and increasing BCR-ABL transcript levels and 95% for patients with stable CCgR ($P = 0.0004$).

Discussion

The introduction of imatinib in the treatment of CML has determined a high frequency of CCgR. Among patients who failed prior therapy with IFN- α , 45% to 60% of patients achieve a CCgR and 80% of patients remain alive and free of progression after 4 years. CCgR is durable in the majority of

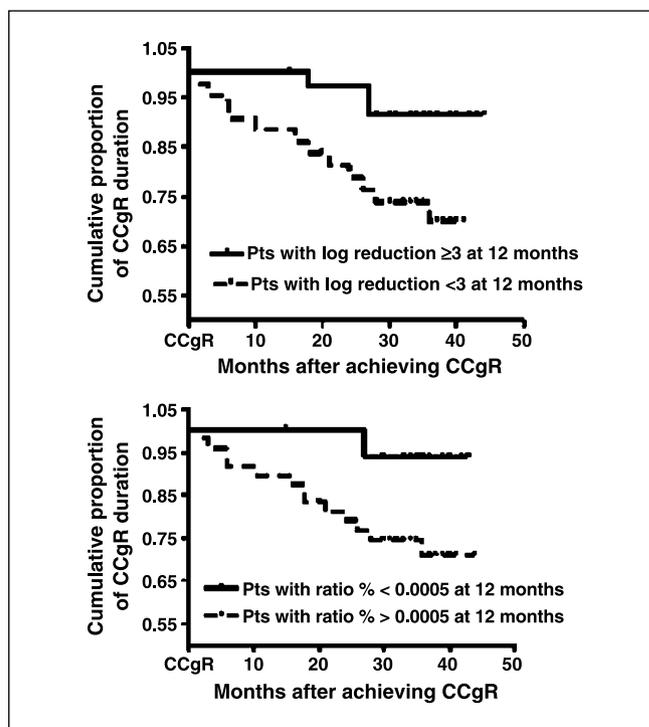


Fig. 4. Landmark analysis of duration of CCgR according to the major molecular response, defined both as a log reduction and as a ratio BCR-ABL/ $\beta 2$ microglobulin $\times 100 \leq 0.0005$, at 12 months.

patients but relapse occurs in a subset. Marin et al. (18) reported a cumulative incidence of cytogenetic relapse at 4 years after achieving CCgR of 26.4% without difference between patients previously treated with IFN- α and patients who receive imatinib as primary therapy. We observed that 20% of patients analyzed progressed to cytogenetic relapse after a median time of 18 months from CCgR. Molecular monitoring of BCR-ABL transcript levels with quantitative RT-PCR technology in patients with CCgR has become an important asset of long-term CML management. Reverse transcription-PCR is a sensitive method for supporting the diagnosis of the disease and for monitoring patients on treatment at a point of time when conventional cytogenetic fails to detect minimal residual disease. We investigated the potential of quantitative RT-PCR monitoring of the BCR-ABL transcript levels to predict cytogenetic relapse by monitoring the BCR-ABL transcript levels in 97 late chronic phase CML patients treated with

Table 2. Molecular response expressed as BCR-ABL/ $\beta 2$ microglobulin ratio % and as log reduction at the time CCgR is first achieved

	Ratio %		From baseline value of each patient			From median baseline value		
	>0.0005	≤ 0.0005	>2 -LR	2-LR	≥ 3 -LR	>2 -LR	2-LR	≥ 3 -LR
Total (<i>n</i>)	58	39	38	22	37	37	19	41
Lost (<i>n</i>)	14	5	13	5	1	12	3	3
Stable (<i>n</i>)	44	34	25	17	36	25	16	38

Abbreviation: LR, log reduction.

imatinib after IFN therapy failure. None of the possible prognostic factors studied in univariate and multivariate analyses seemed to predict for loss of cytogenetic response but the reduction of BCR-ABL transcript levels at the time of CCgR is an important prognostic factor and could possibly have helped to discriminate between patients with relapse and patients whose transcript numbers continue to decline. The importance of achieving an early molecular response was first reported from the International Randomised Study of Interferon versus STI571 (IRIS) Trial (10). Among patients who achieved a CCgR after 12 months of therapy, those with at least a 3-log reduction in BCR-ABL transcript levels had significantly better progression-free survival compared with those with <3-log reduction. Cortes et al. (19) reported that patients who achieved a major molecular remission by 12 months after the start of imatinib had an improved probability of a sustained CCgR. In our study, we showed not only that achieving a major molecular remission at 12 months is predictive of a durable cytogenetic remission but also that patients who achieved a major molecular remission (expressed both as the ratio % and as a 3-log reduction) already at the time of first achieving CCgR have significantly longer cytogenetic remission durations than those without this magnitude of molecular response ($P < 0.05$). Only 3% of patients who have achieved a major molecular response (defined as a 3-log reduction from a median baseline value) at the time of first achieving CCgR have lost their CCgR versus 30% of patients not reaching this degree of response. Another important question is the significance of increasing levels of BCR-ABL transcripts during therapy. There is a strong correlation between the risk of loss of cytogenetic remission and great increases of measurable molecular disease. Late chronic phase CML patients with cytogenetic relapse had a probability of survival at 4 years of imatinib of 60% compared with 95% of patients with stable CCgR ($P = 0.0004$). In summary, the present findings confirm that quantification of BCR-ABL transcripts is an essential noninvasive technique for the optimal management of CML patients who achieve CCgR on imatinib. Achieving a major molecular remission at the time of first achieving CCgR may be predictive of cytogenetic response and favorable clinical course in imatinib-treated chronic-phase patients with CML.

Appendix A

The following members of the GIMEMA Working Party on CML actively participated in this study: G. Lucarelli and G. Polimeno (Acquaviva delle Fonti); P. Galieni and C. Bigazzi (Ascoli Piceno); V. Liso and G. Specchia (Bari); V. Zampaglione

(Biella); P. Coser and R. Quaini (Bolzano); E. Abruzzese (Roma); M. Gobbi and M. Miglino (Genova); E. Pogliani, C. Gambacorti Passerini, and M. Miccolis (Monza); M. Lazzarino and E. Orlandi (Pavia); P. Bernasconi and R. Invernizzi (Pavia); R. Fanin and M. Tiribelli (Udine); D. Russo and M. Malagola (Brescia); G. Alimena (Roma); G. Rossi and A. Capucci (Brescia); F. Nobile, M. Martino, and E. Oliva (Reggio Calabria); L. Gugliotta and P. Avanzini (Reggio Emilia); P. Fattori (Rimini); G. Leone and S. Sica (Roma); L. Annino (Roma); M.C. Petti (Roma); E. Conte (Roma); A.M. Carella (Genova and San Giovanni Rotondo); M. Longinotti and S. Pardini (Sassari); E. Gottardi and M. Fava (Orbassano); L. Cavanna, D. Vallisa, and E. Trabacchi (Piacenza); A. Bacigalupo (Genova); B. Rotoli and L. Luciano (Napoli); F. Ferrara and E. Schiavone (Napoli); V. Mettievier (Napoli); A. Tabilio, C. Mecucci, and D. Falzetti (Perugia); G. Visani and G. Nicolini (Pesaro); T. Barbui and U. Giussani (Bergamo); V. Rizzoli and L. Mangoni (Parma); M. Bocchia (Siena); E. Volpe, F. Palmieri, and N. Cantore (Avellino); M.C. Michieli (Aviano); S. Amadori and A. Cantonetti (Roma); A. Levis and M. Pini (Alessandria); E. Angelucci and E. Usala (Cagliari); A. Cuneo and G.L. Scapoli (Ferrara); E. Curioni and F. Radaelli (Milano); R. Marasca and G. Leonardi (Modena); E. Morra and E. Pungolino (Milano); V. Montefusco (Milano); A. Peta and F. Iuliano (Catanzaro); P. Leoni and S. Rupoli (Ancona); A. Bosi and S. Santini (Firenze); R. Giustolisi, F. Stagno, and P. Guglielmo (Catania); F. Porretto (Palermo); A. Liberati and E. Dotti (Perugia); A. Zaccaria, E. Zuffa, and B. Giannini (Ravenna); P. Mazza and M. Cervellera (Taranto); D. Ferrero and C. Della Casa (Torino); M. Candela and G. Danieli (Ancona); S. Morandi and C. Bergonzi (Cremona); A. Gabbas and D. Noli (Nuoro); G. Semenzato and L. Trentin (Padova); S. Mirto, S. Tringali, and D. Turri (Palermo); V. Abbadessa, G. Marini, and Caracciolo (Palermo); M. Musso and F. Porretto (Palermo); A. D'Emilio (Vicenza); A. Bonati (Parma); M. Petrini, F. Parneschi, and R. Fazzi (Pisa); F. Ricciuti and M. Pizzuti (Potenza); E. Gallo and P. Pregnò (Torino); F. Gherlinzoni and C. Tecchio (Treviso); A. Ambrosetti and V. Meneghini (Verona); R. Di Lorenzo and G. Fioritoni (Pescara); G. Quarta and M. Girasoli (Brindisi); E. De Biasi (Castelfranco Veneto); M. Monaco and E. Capussela (Foggia); A. Gallamini and M.A. Pistone (Cuneo); A. De Blasio (Latina); C. Musolino (Messina); S. Luatti, C. Nicci, E. Montanari, G. Marzocchi, F. Buontempo, T. Grafone, E. Ottaviani, S. Colarossi, A. Gnani, M. Renzulli, and C. Terragna (Bologna).

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