2735 CML Patients In Clinical Trials Represent Fairly Well The General Population Of CML Patients: A Comparative Analysis Of 5803 Patients From The EUTOS Registry

Program: Oral and Poster Abstracts
Session: 632. Chronic Myeloid Leukemia: Therapy: Poster II
Sunday, December 8, 2013, 6:30 PM-8:30 PM
Hall E (Ernest N. Morial Convention Center)

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Introduction: The centerpiece of the European Treatment and Outcome Study (EUTOS) for Chronic Myeloid Leukemia (CML) is a registry collecting representative samples of CML patients in Europe. The In-Study section of the registry combines data of patients enrolled in investigator-sponsored prospective studies of treatment with imatinib-based regimens. The population-based (PB) section includes data of all newly diagnosed CML patients in specified regions of 27 European countries in an attempt to represent the general population of CML patients.

Aims*:* There is a common assumption that patients enrolled in prospective trials are highly selected, do not represent the 'typical' patient and that thus the results of such trials may not be easily generalized to all patients. Thus we analyzed possible differences in the baseline characteristics of the two patient groups. Available were age, sex, EUTOS score, phase of disease, spleen enlargement, platelets, leukocytes, and percentages of blasts, eosinophils, and basophils in peripheral blood.

Methods*:* For all analyzed factors we calculated distribution parameters or percentages depending on the scale of the factor. To identify significant differences we used χ^2-tests and Mann-Whitney U-tests. Level of significance was 0.05.

Results*:* The In-Study section included 2346 patients from study groups in Germany, Italy, France, Spain, the Nordic study group, the Netherlands, and the United Kingdom, newly diagnosed from 2002 to 2006. The PB section of the registry included 3457 patients newly diagnosed with CML from 2008 to 2012 in 27 European countries.

The median age at diagnosis of In-Study patients (51 years (18-88)) was significantly lower than the age of the general population newly diagnosed with CML (56 years (18-99), p<0.0001). Also, while in the PB section the percentage of male patients was 54%, in the In-Study section the percentage was significantly higher (60%, p<0.0001).

The median spleen size enlargement (cm below costal margin) did not differ significantly between the two groups (In-Study (1 cm (0-38), PB section 0 cm (0-40)).

While 7% of patients in the PB section were not in chronic phase, this was only true for less than 1% of patients included into the In-Study section. Accordingly, there were significant differences (both p<0.0001) regarding percentages of blast cells in peripheral blood (In-Study: 1% (0-14), PB 1% (0-92)) and leukocytes (In-Study: 74 $x10^9$/L (20-650), PB 85 $x10^9$/L (3-932)). There were no differences in percentage of basophils, eosinophils and in platelet count.

The EUTOS risk score was developed to predict the treatment success of patients in chronic phase and thus is calculated for patients in chronic phase only. In the In-Study section 10.5% of patients had a high EUTOS risk score while the percentage in the general population was 11.4%. The resulting difference was not significant (p=0.3374).

Conclusions*:* With a total of 5803 patients included in the two sections of the EUTOS registry analyzed for this work, the combined data allow a unique insight into the characteristics of CML patients in Europe. The comparison between the In-Study and the PB sections shows some important differences between the two populations, such as age and sex distribution. However, several other clinical and hematological factors which are known to be predictive for treatment outcome did not differ substantially. We conclude that patients enrolled in investigator-sponsored studies represent fairly well the general population of CML patients in Europe, with the exception of sex and age distribution, which may limit the value of the calculations of overall survival because those are affected by both age and gender.

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4030 Deep Molecular Responses In Patients With Newly Diagnosed Chronic Myeloid Leukemia Receiving Nilotinib As Assessed Within The EUTOS Laboratory Network In The ENEST1st Study

Program: Oral and Poster Abstracts
Session: 632. Chronic Myeloid Leukemia: Therapy: Poster III
Monday, December 9, 2013, 6:00 PM-8:00 PM
Hall E (Ernest N. Morial Convention Center)

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***Background**:* Nilotinib is a selective BCR-ABL tyrosine kinase inhibitor (TKI) that has been shown to be more active than imatinib at inducing earlier and deeper molecular

responses in the frontline treatment of chronic myeloid leukemia (CML; Larson, et al. *Leukemia.* 2012). ENEST1st (Evaluating Nilotinib Efficacy and Safety in clinical Trials as First-Line Treatment, NCT01061177) is the largest study ever in TKI-treated patients (pts) with CML. Its primary endpoint was the rate of MR^4 (defined as BCR-ABL ≤ 0.01% on the International Scale [$BCR\text{-}ABL^{IS}$] or undetectable BCR-ABL in cDNA with ≥ 10,000 ABL transcripts) at 18 mo. Molecular response (MR) was assessed by standardized quantitative polymerase chain reaction (RQ-PCR) in the 14-laboratory network of the European Treatment and Outcomes Study (EUTOS).

***Methods**:* ENEST1st is a phase 3b, open-label study of nilotinib 300 mg twice daily (BID) in adults with newly diagnosed BCR-ABL-positive CML in chronic phase. MR was assessed every 3 mo by peripheral blood RQ-PCR at 1 of 14 EUTOS laboratories. Following completion of the 18-mo primary efficacy time point, all pts continued to receive treatment and were followed for an additional 6 mo.

***Results**:* 1160 pts were screened, with 1086 eligible pts treated in 26 European countries. Overall, median age was 53 y (range, 18-91 y); 59.2% of pts were male, 96.9% had typical b2a2 and/or b3a2 BCR-ABL transcripts (intent-to-treat population for MR analyses), and 90.3% were Philadelphia chromosome–positive by bone marrow metaphase cytogenetics. Per protocol, efficacy and safety analyses were conducted in the initial 819 pts (75.4% of enrolled pts) who were on study for 24 mo or discontinued early. In this group, median age was 53 y (range, 18-91 y), and 58.5% were male; Sokal risk scores were low, intermediate, and high in 33.7%, 39.5%, and 18.7% of pts, respectively (8.1% missing). EUTOS scores were low in 83.8% and high in 9.2% of pts (7.0% missing).

A total of 658 pts (80.3%) completed 24 mo of treatment; 161 pts (19.7%) discontinued early. The most common reasons for discontinuation included adverse events (AEs; 10.5%), withdrawal of consent (2.7%), and disease progression (as indicated by the investigator; 1.7%).

MR^4 rate at 18 mo was 43.0% (95% CI, 39.3-46.8). Rates of major MR (MMR, $BCR\text{-}ABL^{IS}$ ≤ 0.1%), MR^4, and $MR^{4.5}$ ($BCR\text{-}ABL^{IS}$ ≤ 0.0032% or undetectable cDNA with ≥ 32,000 ABL transcripts) at 18 and 24 mo are shown in the Table. Cumulative incidence of MR^4 by 18 mo was 50.1%.

All pts were followed up for progression or death for 24 months after start of treatment: 7 pts (0.85%) experienced progression to accelerated phase/blast crisis (AP/BC) while on core treatment or after discontinuation. Overall, 12 pts (1.5%) died; 2 of them died following progression to AP/BC.

The safety profile of nilotinib was similar to that observed in other frontline studies. The most common AEs of any relationship were rash (21.4%), pruritus (16.8%), and headache (15.0%); most AEs were grade 1-2. Rates of grade 3-4 hematological AEs were low, with thrombocytopenia and neutropenia reported in 8.2% and 2.9% of pts, respectively. Peripheral arterial occlusive disease (PAOD) occurred in 13 pts (1.6%), ischemic heart disease in 31 (3.8%), and ischemic cerebrovascular conditions in 4

(0.5%). Three cases of pain in extremities are under investigation for potential relationship to PAOD.

***Conclusions**:* ENEST1st confirms that frontline nilotinib results in high rates of deeper and earlier MR and very low rates of progression to AP/BC. By 18 mo, half of pts achieved a response of MR^4. This MR^4 rate supports the use of frontline nilotinib as a treatment of choice on which to develop treatment-free remission studies. Finally, the molecular response rates measured in a standardized and validated network of 14 EUTOS laboratories in ENEST1st provide prospective confirmation of the centrally reviewed molecular response rates reported in the ENESTnd trial.

	Nilotinib 300 mg BID (n = 819)	
	18 mo	24 mo
Response at 18 mo (n = 674)[a] or 24 mo (n = 624),[a] %		
MMR	75.2	69.2
MR^4	43.0	44.2
$MR^{4.5}$	23.4	26.4
MR^4 by 18 or 24 mo (n = 797),[a] %	50.1	56.5
Low EUTOS score (n = 669)[b]	51.1	58.3
High EUTOS score (n = 72)[b]	36.1	38.9
Low Sokal score (n = 270)[b]	57.0	63.3
Intermediate Sokal score (n = 314)[b]	47.5	53.8
High Sokal score (n = 148)[b]	38.5	47.3

[a] n = number of pts with typical transcripts evaluable at or by 18 or 24 mo.

[b] n = number of pts in the indicated risk group evaluable for MR^4.

Disclosures: Giles: *Novartis:* Consultancy, Research Funding. Baccarani: *Novartis:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Bristol Myers Squibb:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Ariad:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Pfizer:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Speakers Bureau. Brümmendorf: *Novartis:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties, Research Funding; *Bristol Myers Squibb:* Consultancy, Honoraria; *Pfizer:* Consultancy, Honoraria; *Ariad:* Consultancy. Mahon: *Novartis:* Consultancy, Honoraria, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Brisol Myers Squibb:* Consultancy, Honoraria; *Ariad:* Honoraria; *Pfizer:* Honoraria. Rosti: *Novartis:* Consultancy, Speakers Bureau; *Bristol Myers Squibb:* Consultancy, Speakers Bureau; *Ariad:* Consultancy, Speakers Bureau; *Roche:* Speakers Bureau; *Pfizer:* Speakers Bureau. Saglio: *Novartis:* Consultancy, Honoraria; *Bristol Myers Squibb:* Consultancy, Honoraria; *Ariad:* Consultancy, Honoraria; *Celgene:* Consultancy, Honoraria. Steegmann: *Novartis:* Membership on an entity's Board of Directors or advisory committees, Research

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1475 NK-Cells In Dasatinib-Treated Chronic Myeloid Leukemia Patients Display a Unique Phenotype Associated With Cytotoxic Potential

Program: Oral and Poster Abstracts
Session: 631. Chronic Myeloid Leukemia: Biology and Pathophysiology, excluding Therapy: Poster I
Saturday, December 7, 2013, 5:30 PM-7:30 PM
Hall E (Ernest N. Morial Convention Center)

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Background. Dasatinib is a second-generation tyrosine kinase inhibitor (TKI), which is used successfully in the treatment of chronic myeloid leukemia (CML). Dasatinib has two unique features when compared to other TKIs (imatinib, nilotinib); first, dasatinib has a significantly shorter half-life in the plasma and second, dasatinib inhibits a wider spectrum of kinases, including several kinases known to be important in the function of the immune system (src, tec, and syk families), which are not affected by the other TKIs. Interestingly, it has been recently shown that both short-term exposure to dasatinib *in vivo* and long-term treatment with dasatinib improves NK-cell cytotoxicity, however, the mechanisms are not known.

To study the improved cytotoxicity observed in dasatinib-treated patients, we aimed to perform a complete NK-cell phenotyping in these patients. Finally, our goal is to correlate NK-cell phenotype with NK-cell cytotoxicity, and to study the possible correlation between phenotypical changes, NK-cell function and clinical outcome.

Methods. This study included 19 dasatinib-treated (DA) CML patients, both first-line (n=7) and second-line (n=12). To investigate the specificity of the immunomodulatory effects of dasatinib, a control group of 9 CML patients treated with imatinib (IM) and another group of 12 healthy donors (HD) were included. Peripheral blood samples obtained before the patients took their daily drug dose were phenotyped with a comprehensive 8-color flow cytometry panel (total 32 antibodies, table 1).

To study the correlation between phenotypical changes and NK-cell cytotoxicity, we performed a standard CD107 degranulation assay. Mononuclear cells were incubated for 6 hours in the presence of the target cell line K562 and a CD107 antibody. CD107 positive NK-cells were then phenotyped with the same panel of 32 antibodies.

Results. All results are summarized in table 1. In brief, DA- and IM-treated CML patients and HD had equal proportions of NK-cells (CD3negCD56+) of total lymphocytes. Regarding trafficking molecules, NK-cells in both DA- and IM-patients had a lower frequency of the chemokine receptor CCR7 when compared to HD. This suggests a reduction in the NK-cell population that is able to migrate to lymph nodes, and is likely caused by the disease or TKIs in general. Moreover, DA-treatment specifically decreases the expression of the homing molecule CD62L in NK-cells.

In addition, NK-cells in DA-patients, when compared to IM and HD, expressed less CD11b and significantly more often CD11c and HLA-DR, which reproduce the immunophenotypic changes that typically occurs in recently activated NK-cells and has been shown to associate with improved clinical benefits. Conversely, increased expression of CD57 together with a lower frequency of CD27 and CD28 were observed in both groups of patients and were similar to those typically observed in conditions of chronic NK-cell stimulation.

In contrast, DA-patients had a lower frequency of most of the studied NK-receptors (Nkp30, Nkp46, NKG2D, CD94, CD161, KIR2DL1/S1) when compared to IM and HD. This suggests that NK-cells in DA-treated patients have a more mature phenotype, which is caused by the treatment.

Conclusions. NK-cells in TKI-treated CML patients display a mature phenotype, which is often observed after chronic stimulation suggesting that TKIs have immunomodulatory effects on NK-cells or the disease itself causes the changes. Interestingly, NK-cells in DA-treated patients express a highly differentiated phenotype characterized by high expression of CD57, and decreased expression of Nkp30, Nkp46 and CD161. Similar changes were not seen in IM-patients or HD. It is possible that NK-cells expressing this phenotype might also represent those NK-cells that have previously been driven into clonal expansion by encounters with pathogens because of the specific immunomodulatory effects of dasatinib. This phenotype of highly mature NK-cells, which is associated with high cytolytic potential, could be responsible for the previously described enhanced NK-cytotoxicity caused by dasatinib. In accordance, our preliminary results suggest that these unique phenotypic changes observed in DA-treated patients correlates with the cytotoxic potential. Studies to correlate these results with therapy outcome are ongoing.

	Median, range (%)			DA vs. (*P*)		
	DA (n=19)	IM (n=9)	HD (n=12)	IM	HD	All
NK cells	13,1 (3,6-57,7)	18,4 (5,9-31,0)	14,0 (5,6-20,0)	0.50	0.95	0.80
Trafficking						
CCR7	7,4 (0,1-22,9)	9,0 (0,6-31,7)	19,7 (0,0-75,4)	0.25	***0.011***	***0.025***
CD62L	35,6 (3,1-74,10)	43,8 (23,3-66,3)	38,6 (15-87,1)	***0.015***	***0.028***	*0.09*
Activation and proliferation						
CD7	95,6 (85,2-99,4)	97,5 (83,4-99,4)	94,8 (80,2-98,8)	0.52	0.46	0.59
CD11b	31,5 (0-96,4)	76,7 (8,4-92,4)	68,5 (2,9-90,2)	0.16	***0.006***	***0.008***
CD11c	84,7 (66,8-99,8)	74,4 (46,4-91,5)	74,35 (46,4-96,4)	0.27	*0.06*	0.13
CD25	0,7 (0-17,9)	25,3 (0,7-59,0)	3,5 (0,5-27,2)	***0.0003***	*0.056*	*0.07*
CD27	3,6 (0,2-21,2)	4,8 (2,4-11,8)	8,4 (1,9-46,3)	0.50	***0.029***	*0.08*
CD28	0,3 (0-28,9)	0,2 (0-5,3)	2,1 (0,2-10,3)	0.80	***0.018***	*0.056*
CD38	90,5 (43,2-99,8)	93 (83,8-98,2)	83,6 (71,7-92,7)	0.49	0.54	0.34
CD45RA	97,6 (88,6-99,5)	99,1 (96,4-99,5)	95,8 (81,2-99,2)	***0.023***	0.11	0.29
CD45RO	1,9 (0-8,1)	3,0 (0,5-22,5)	2,1 (0-34,3)	0.42	*0.076*	0.19
CD57	63,6 (12,5-91,6)	70,6 (34,3-78,7)	45,0 (0,1-68,7)	0.86	***0.040***	*0.057*
HLA-DR	20,4 (0-56,4)	3,8 (0,5-31,7)	13,2 (4,7-27,6)	***0.007***	0.25	0.44
NK receptors						
DNAM	89,5 (77,9-97,9)	85,3 (68-93,3)	87,7 (73,6-96,4)	0.35	0.43	0.61
Nkp30	28,8 (0-92,6)	57,7 (33,1-78,8)	79,1 (41,2-95,1)	0.32	***0.012***	***0.027***
Nkp46	62,8 (3,7-94,6)	89,9 (67,1-99,3)	78,3 (51,9-96,8)	***0.011***	0.13	0.30
NKG2A	25,2 (0,1-76,7)	31,4 (14,9-45,6)	34,8 (1,2-90,2)	0.31	*0.09*	0.21
NKG2C	24,3 (4,4-78,3)	43,6 (4,4-54,7)	10,2 (0-96,9)	0.89	0.19	0.33
NKG2D	79,1 (24,9-91,4)	97,7 (91,8-98,4)	87,6 (70,1-96,2)	***0.0003***	***0.033***	***0.049***
CD94	70,1 (36,1-94,2)	89,0 (51,1-97,7)	85,2 (49,7-90,7)	***0.010***	***0.019***	0.39
CD161	30,4 (2,6-95,3)	71,8 (62,4-94,1)	62,5 (47,6-91,8)	*0.07*	***0.011***	***0.016***
KIR2DS4	8,0 (0,5-84,5)	2,7 (0-54,2)	20,9 (0-96,1)	0.50	0.88	0.87
KIR2DL1/S1	8,9 (0,1-72,2)	41,5 (12,3-71,9)	16,9 (0-31,0)	***0.045***	0.73	0.73
KIR2DL2/L3	34.4 (4.3-71.2)	52,5 (16,0-70,1)	52.2 (16,0-70,1)	0.33	0.76	0.81
KIR2DL4	0,4 (0-7,4)	0,1 (0-1,5)	1,8 (0-6,1)	0.11	*0.078*	0.14
KIR3DL1	7,8 (0-64,8)	6,3 (0,4-48,4)	13,9 (5,5-43,5)	0.80	0.21	0.40

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